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PLANT POLYPHENOLS
IX. THE GLYCOSIDIC PATTERN OF ANTHOCYANIN PIGMENTS*

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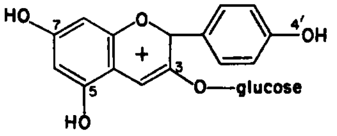
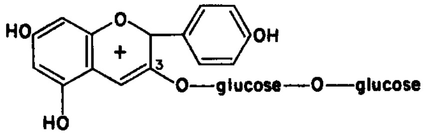
Abstract—The anthocyanins present in colour varieties of *Lathyrus odoratus* and *Streptocarpus* have been identified; the sugars of some forty other anthocyanins have also been examined. In all, twenty-two new anthocyanins have been found; they include the first representatives of the following glycosidic classes: 3-rhamnosides, 3-(xylosylgalactosides), 5-glucoside-3-rhamnosides, 5-glucosides-3-sophoroside, 3-sophoroside-7-glucosides and 5-glucoside-3-sambubiosides. Each of the two unusual disaccharides sophorose and sambubiose occurs combined in the anthocyanins of several plant genera. Structures previously proposed for pigments of *Matthiola incana* flowers and of red cabbage have been revised. This work brings the number of known classes of anthocyanins to seventeen.

INTRODUCTION

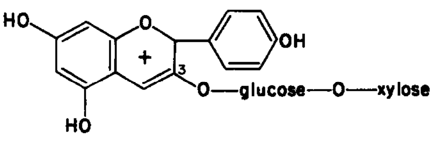
UNTIL recently, relatively little information was available regarding the nature and number of sugars present in anthocyanins. Thus, although several anthocyanin surveys of higher plants have appeared (e.g.[1-3]), the authors were concerned mainly with the identification of the aglycones. The few pigments that were examined in greater detail by earlier workers, such as Robinson and his school,[4] were found to belong to one of four classes†, namely: "3-monosides" (usually 3-glucosides (I)) "3-biosides" (i.e. 3-glucosylglucosides (II)), "3-pentoseglycosides" (i.e. 3-xylosylglucosides (III)), or 3,5-dimonosides" (e.g. 3,5-diglucosides (IV)).



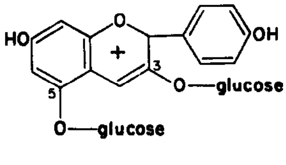
(I)



(II)



(III)



(IV)

* Part VIII. J. B. HARBORNE, *Phytochemistry*, **1**, 203 (1962).

† The numbering (3-, 5- or 7-) always refers to the position in the anthocyanin molecule occupied by the sugar. Only the 1- position of the sugar is involved in anthocyanin linkages; this is usually a β-linkage, although an α-linked anthocyanin, cyanidin 3-α-arabinoside, is known.

Most of the terms used by the Robinsons to describe the 4 classes of glycoside are no longer required. The form 3,5-diglucoside is retained for 3-glucoside 5-glucoside because of its convenience. However, 3-diglucoside is always given in full as 3-(glucosylglucoside) if the nature of the disaccharide is not known.

[1] W. J. C. LAWRENCE, J. R. PRICE, G. M. ROBINSON and R. ROBINSON, *Phil. Trans.* B., **230**, 149 (1939).
[2] H. REZNIK, *Abh. heidelberg Akad. Wiss.* 125 (1956).
[3] W. G. C. FORSYTH and N. W. SIMMONDS, *Proc. Roy. Soc.* B. **142**, 549 (1954).
[4] G. M. ROBINSON and R. ROBINSON, *Biochem. J.* **25**, 1687 (1931).

The present study of the sugars of anthocyanins was initiated after suitable methods of determining the glycosidic pattern on a microscale had been developed.[5,6] While most attention has been paid to the anthocyanins in plants of known genotypes, the pigments of a variety of other plants have been examined. The identification of the anthocyanins present in colour forms of *Solanum*,[7] *Primula*[8] and *Rosa*,[9] and in *Plumbago*[10] and *Rhododendron*[10] species has already been described. The purpose of this paper is to describe the identification of the anthocyanins of two cultivated plants, *Lathyrus* and *Streptocarpus*, which are notable for the complex mixtures of glycosidic types present in their petals. A number of other anthocyanins, which have been found while searching plants for new glycosidic classes are also described; the structures of some of them have been mentioned in preliminary reports.[11,12]

RESULTS

Identification of pigments

Spectral and chromatographic methods[5,6] were used to detect acyl groups which were then removed by alkali treatment. All the identifications described in this paper were therefore carreid out on anthocyanins having only sugar substituents. The nature and position of the attachment of these sugars were determined by published procedures.[5-8] Identifications of known pigments were confirmed by direct comparisons with authentic compounds.

The properties of new pigments or pigments of which R_F values and spectra have not been previously recorded are shown in Tables 1, 2 and 3. New pigments were identified by means of their R_F values, spectral and colour properties and from the results of studies of their hydrolysis products. Disaccharides were obtained from all those pigments which appeared, from R_F values and other data, to have such sugars attached to them. These disaccharides were identified on the basis of chromatographic and electrophoretic comparisons with authentic sugars (Table 4). The identity of sophorose was rigorously established by comparing it directly not only with authentic sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) but also with the other seven known glucosylglucoses.

The fact that 3-glycosides and 3,5-di(glycosides) can be distinguished by means of spectral characteristics in the 400–450 mμ region has already been reported;[5] an additional method of distinguishing them has now become apparent. It has been found that the short wavelength peak (at about 265–280 mμ) of 3,5-di(glycosides) is consistently less intense than that of 3-glycosides. The optical densities at the low wavelength have been measured for most of the new pigments and these values are shown in Tables 1, 2 and 3 as percentages of the relevant optical densities at the long wave-length peak. The average for 3,5-di(glycosides) is 42 per cent (range ± 7) and for 3-glycosides is 63 per cent (range ± 6). These measurements do not appear to depend either on the nature of the aglycone or on the number of sugar residues. Apigeninidin and luteolinidin 5-glucosides give slightly low values, but this is to be expected of anthocyanidins which lack a 3-hydroxyl group.

[5] J. B. Harborne, *Biochem. J.* **70**, 22 (1958).
[6] J. B. Harborne, *J. Chromatog.* **1**, 473 (1958).
[7] J. B. Harborne, *Biochem. J.* **74**, 262 (1960).
[8] J. B. Harborne and H. S. A. Sherratt, *Biochem. J.* **78**, 298 (1961).
[9] J. B. Harborne, *Experientia* **17**, 72 (1961).
[10] J. B. Harborne, *Arch. Biochem. Biophys.* **92**, 171 (1962).
[11] J. B. Harborne and H. S. A. Sherratt, *Experientia* **13**, 486 (1957).
[12] J. B. Harborne, *Nature, Lond.* **187**, 240 (1960).

TABLE 1. PROPERTIES OF NEW PELARGONIDIN GLYCOSIDES

Designation*	Source	Colour in u.v. light§	λ max. in MeOH–HCl (in mμ)† Band I	Band II	Ratios (as %) $E_{\text{Band I}}/E_{\text{Band II}}$	$E_{440}/E_{\text{Band II}}$	R_F values‡ in BAW	BuHCl	1% HCl
Pg 1	*Fagus*	dOR	278	505	57	39	0·39	0·37	0·13
Pg 2	*Streptocarpus*	dOR	277	508	69	38	0·34	0·34	0·30
Pg 3	*Lathyrus*	dOR	271	507	62	35	0·32	0·31	0·34
Pg 4	*Papaver*	dOR	268	505	66	39	0·33	0·30	0·38
Pg 5	*Raphanus*	flY	265	503	—	21	0·25	0·10	0·60
Pg 6	*Papaver*	dOY	279	499	66	40	0·18	0·04	0·73
Pg 7	*Lathyrus*	flY	268	505	42	18	0·46	0·24	0·39
Pg 8	*Matthiola*	flY	280	508	49	17	0·24	0·18	0·43
Pg 9	from Pg 6	dOY	279	499	69	42	0·28	0·10	0·38
Pg 10	from Pg 6	dOR	270	510	51	42	0·46	0·51	0·15

Designation	Products of partial and complete hydrolysis	Identified as
Pg 1	Pg (1·0) and galactose (1·02)	3-galactoside
Pg 2	Pg 3-glucoside, Pg (1·0), xylose (1·36), glucose (1·32) and sambubiose	3-sambubioside
Pg 3	Pg 3-galactoside, Pg (1·0), xylose (0·93), galactose (0·84) and xylosylgalactose	3-(xylosylgalactoside)
Pg 4	Pg 3-glucoside, Pg (1·0), glucose (2·05) and sophorose	3-sophoroside
Pg 5	Pg 3-glucoside, Pg 5-glucoside, Pg 3-sophoroside, Pg 3,5-diglucoside, Pg (1·0) and glucose (3·30)	5-glucoside-3-sophoroside
Pg 6	Pg 3-glucoside, Pg 9, Pg 10, Pg 3-sophoroside, Pg, glucose and sophorose	7-glucoside-3-sophoroside
Pg 7	Pg 3-rhamnoside, Pg (1·0), glucose (0·83) and rhamnose (0·98)	5-glucoside-3-rhamnoside
Pg 8	Pg 3-glucoside, Pg 5-glucoside, Pg 3,5-diglucoside, Pg (1·0), glucose (2·03), xylose (0·87) and sambubiose	5-glucoside-3-sambubioside
Pg 9	Pg 3-glucoside, Pg 10, Pg and glucose	3,7-diglucoside
Pg 10	Pg and glucose	7-glucoside

* Following abbreviations are used for anthocyanidins in this and subsequent Tables:
Pg, pelargonidin; Cy, cyanidin; Pn, peonidin; Rs, rosinidin; Dp, delphinidin; Pt, petunidin; Mv, malvidin; Hs, hirsutidin; Ap, apigeninidin; Lt, luteolinidin.

† All pigments had low absorption (less than 10% of that at visible max.) in the 300–330 mμ region, indicating the absence of acyl groups.

‡ Solvents are: BAW, butan-1-ol–acetic acid–water (4 : 1 : 5, top layer); BuHCl, butan-1-ol–2*N*.HCl (1 : 1, top layer); 1% HCl, water-conc. HCl (97 : 3).

§ Key: O = orange, R = red, Y = yellow, d = dull, fl = fluorescent.

Anthocyanins of Lathyrus odoratus

Although the genetics and chemistry of colour variation in the sweet pea, *Lathyrus odoratus*, have been much investigated,[13] it is only recently that complete identification of the anthocyanins has been attempted, nineteen different ones having been detected.[12] Contemporary varieties can be divided into three main classes represented by the bright scarlet "Air Warden", the carmine "Harrow" and the purple "Jupiter". The pigments isolated from these varieties are shown in Table 5. The 3-rhamnosides and 5-glucoside-3-rhamnosides which are present form two new series of anthocyanidin glycoside. The 3-rhamnosides of pelargonidin, cyanidin and delphinidin were subsequently found also in *Plumbago rosea.*[10] In the light of these results, it seems likely that the incompletely characterized delphinidin rhamnoside found by Karrer and Widmer[14] in 1927 was the 3-rhamnoside.

[13] G. H. BEALE, G. M. ROBINSON, R. ROBINSON and R. SCOTT-MONCRIEFF, *J. Genet.* 37, 375 (1939).
[14] P. KARRER and R. WIDMER, *Helv. chim. Acta* 10, 67, 83 (1927).

TABLE 2. PROPERTIES OF NEW CYANIDIN AND PEONIDIN GLYCOSIDES

Designation	Source	Colour in u.v. light*	λ max. in MeOH–HCl (in mμ) Band I	Band II	Ratios (as %) $\frac{E_{Band\ I}}{E_{Band\ II}}$	$\frac{E_{440}}{E_{Band\ II}}$	R_F values in BAW	BuHCl	1% HCl
Cy 1	*Lathyrus*	dM	282	527	61	25	0·31	0·15	0·29
Cy 2	*Papaver*	dM	282	523	58	23	0·33	0·22	0·34
Cy 3	*Lathyrus*	bR	278	526	48	13	0·34	0·21	0·36
Cy 4	*Matthiola*	bR	278	526	37	10	0·19	0·10	0·41
Cy 5	*Brassica*	bR	278	524	49	12	0·17	0·09	0·54
Pn 1	*Lathyrus*	dM	278	528	60	19	0·67	0·69	0·18
Pn 2	*Lathyrus*	flP	279	524	42	11	0·44	0·23	0·39
Pn 3	*Lathyrus*	dM	279	526	—	19	0·34	0·25	0·38
Rs 1	*Primula*	flP	278	519	—	12	0·21	0·10	0·36

Designation	Products of partial and complete hydrolysis	Identified as
Cy 1	Cy 3-galactoside, Cy, xylose, galactose and xylosylgalactose	3-(xylosylgalactoside)
Cy 2	Cy 3-glucoside, Cy (1·0), glucose (1·79) and sophorose	3-sophoroside
Cy 3	Cy 3-rhamnoside, Cy (1·0), glucose (1·0) and rhamnose (0·82)	5-glucoside-3-rhamnoside
Cy 4	Cy 3-glucoside, Cy 5-glucoside, Cy 3,5-diglucoside, Cy (1·0), glucose (2·10) and xylose (1·25)	5-glucoside-3-sambubioside
Cy 5	Cy 3-sophoroside, Cy 5-glucoside, Cy 3-glucoside, Cy 3,5-diglucoside and sophorose	5-glucoside-3-sophoroside
Pn 1	Pn (1·0) and rhamnose (1·17)	3-rhamnoside
Pn 2	Pn 3-rhamnoside, Pn (1·0), glucose (0·75) and rhamnose (0·96)	5-glucoside-3-rhamnoside
Pn 3	Pn 3-galactoside, Pn (1·0), xylose (0·97), galactose (1·32) and xylosylgalactose	3-(xylosylgalactoside)
Rs 1	Rs and glucose	3,5-diglucoside

* Key: M = magenta, R = red, P = pink, b = bright, d = dull, fl = fluorescent.

TABLE 3. PROPERTIES OF NEW GLYCOSIDES OF DELPHINIDIN AND RELATED DERIVATIVES

Designation	Source	Colour in u.v. light*	λ max. in MeOH–HCl (in mμ) Band I	Band II	Ratios (as %) $\frac{E_{Band\ I}}{E_{Band\ II}}$	$\frac{E_{440}}{E_{Band\ II}}$	R_F values in BAW	BuHCl	1% HCl
Dp 1	*Lathyrus*	dPu	274	533	40	13	0·21	0·10	0·26
Pt 1	*Lathyrus*	dPu	278	533	54	19	0·40	0·42	0·10
Pt 2	*Lathyrus*	bPu	274	535	42	11	0·28	0·09	0·31
Mv 1	from Mv 2	dPu	278	536	54	23	0·39	0·40	0·11
Mv 2	*Lathyrus*	flC	276	533	44	11	0·31	0·10	0·34
Hs 1	*Primula*	flC	273	532	49	9	0·25	0·02	0·32
Ad 1	*Gesneria*	flY	274	477	37	14	0·46	0·47	0·26
Lt 1	*Gesneria*	bO	277	493	28	6	0·42	0·30	0·14

Designation	Products of partial and complete hydrolysis	Identified as
Dp 1	Dp 3-rhamnoside, Dp (1·0), glucose (1·0) and rhamnose (1·07)	5-glucoside-3-rhamnoside
Pt 1	Pt (1·0) and rhamnose (1·11)	3-rhamnoside
Pt 2	Pt 3-rhamnoside, Pt (1·0), glucose (1·0) and rhamnose (1·2)	5-glucoside-3-rhamnoside
Mv 1	Mv (1·0) and rhamnose (0·87)	3-rhamnoside
Mv 2	Mv 1, Mv (1·0), glucose (1·2) and rhamnose (1·4)	5-glucoside-3-rhamnoside
Hs 1	Hs and glucose	3,5-diglucoside
Ad 1	Ad (1·0) and glucose (1·2)	5-glucoside
Lt 1	Lt (1·0) and glucose (1·25)	5-glucoside

* Key: Pu = purple, C = cerise, O = orange, Y = yellow, b = bright, d = dull, fl = fluorescent.

Some of the galactose-containing glycosides in *Lathyrus* are also new, and were not detected during the preliminary investigation[12] because they were mistaken for the related glucosides. The replacement of glucose by galactose does not alter appreciably the R_F value of anthocyanins.[6] However, the 3-galactosides can be distinguished from the 3-glucosides by extending the normal period of development with butanol–acetic acid–water (18 hr) to 36 hr. Also the 3-(xylosylgalactosides) yield, on oxidation, a xylosylgalactose, with different R_F values from the common xylosylglucose, sambubiose (see below). A recent report on the anthocyanins of the scarlet red "Valencia" sweet pea by Reichel and Hiller[15] fails to mention any rhamnosides or galactosides. It is probable that the various cyanidin and peonidin "glucosides" described by these authors are, in fact, the same pigments that have been obtained from "Harrow" during the present investigation.

TABLE 4. PROPERTIES OF DISACCHARIDES ATTACHED TO ANTHOCYANINS

Disaccharide (linkage)	R_G value in* BAW	BEW	BBPW	M_G† value	Hydrolysis by β-glucosidase
Sambubiose (β1→2) and xylosylglucoses from pigments Pg 2, Pg 8 and Cy 4‡	0·65	0·76	0·60	0·25	+
Xylosylgalactose (β1→?) from pigments Pg 3, Cy 1 and Pn 3	0·64	0·65	0·54	—	—
Sophorose (β1→2) and glucosylglucoses from pigments Pg 4, Pg 6, Cy 2 and Cy 5	0·58	0·71	0·61	0·25	+
Laminaribiose (β1→3)	0·69	0·74	0·71	0·58	+
Cellobiose (β1→4)	0·50	0·58	0·51	—	+
Gentiobiose (β1→6)	0·42	0·50	0·38	0·63	+
Maltose (α1→4)	0·54	0·67	0·60	0·29	—
Kojibiose (α1→2)	—	—	—	0·30	—
Nigerose (α1→3)	—	—	—	0·58	—

* On Whatman No. 1 paper. BEW is butan-1-ol-ethanol-water (4 : 1 : 2·2) and BBPW is butan-1-ol-benzene-pyridine-water (5 : 1 : 3 : 3). Mixtures of isolated and authentic disaccharides did not separate; mixtures with other sugars did.

† In borate buffer, pH 10 on Whatman 3 mm paper at 15 volts/cm. The identity of the isolated sophorose was confirmed by molybdate electrophoresis of the sodium borohydride reduction product, which separated from isomaltitol (α1→6). These experiments were kindly carried out by Dr. J. B. Pridham, University of London.

‡ The xylosylglucose present in the major anthocyanin of *Begonia* leaves is also identical with sambubiose.

Examination of the pigments in the flowers of a reputedly wild form of *L. odoratus*, collected in Palermo, and of *L. latifolius* shows that the 5-glucoside-3-rhamnosides of malvidin, petunidin and delphinidin are present in each instance; it seems likely that these three pigments occur in most wild *Lathyrus* species which have coloured flowers. These anthocyanins have also been studied by Pecket; he originally considered them to be 3-glucosides,[16] but his later observations[17] agree with the above suggestion. The only aberrant species (among the nineteen examined) is *L. hirsutus*, which contains a pigment, described by Pecket as free malvidin but now provisionally identified as malvidin 3-rhamnoside (Table 7). Although no sugar analysis has yet been carried out on this pigment, its relatively high R_F value in 1 per cent HCl and its general stability show that it cannot be malvidin; it is chromatographically identical with a sample of malvidin 3-rhamnoside, prepared by enzymic hydrolysis of the related 5-glucoside-3-rhamnoside.

[15] L. REICHEL and W. HILLER, *Naturwissenschaften* 47, 83 (1960).
[16] R. C. PECKET, in *Plant Phenolics in Health and Disease*, p. 119. Pergamon Press, Oxford (1960).
[17] R. C. PECKET, *New Phytol.* 59, 138 (1960).

TABLE 5. ANTHOCYANINS IN COLOUR VARIETIES OF *Lathyrus Odoratus*

Pigment	R_F in BAW*	Relative† amount	Occurrence in colour varieties
Pg 3-rhamnoside	0·57	+	All in "Air Warden" (scarlet cerise); all, except the 3-rhamnoside, in other scarlet shades ("Topper", "Mary Malcolm", "Princess Elizabeth") and in some pink shades ("Mrs. R. Bolton", "Judy" and "Monty")‡
Pg 3-galactoside	0·33	++	
Pg 5-glucoside-3-rhamnoside	0·33	+++	
Pg 3-(xylosylgalactoside)	0·25	++	
Pg 3-galactoside-5-glucoside§	0·20	+	
Pn 3-rhamnoside	0·47	+	All in "Harrow" (deep carmine); all (except the 3-rhamnosides and Pn 3-galactoside-5-glucoside) in the pink shades ("Skylon", "Gaiety", Reconnaissance" and "Rosy Frills")
Cy 3-rhamnoside	0·43	++	
Pn 5-glucoside-3-rhamnoside and Pn 3-galactoside	0·29	+++	
Cy 5-glucoside-3-rhamnoside and Cy 3-galactoside	0·24	++	
Cy 3-(xylosylgalactoside) and Pn 3-(xylosylgalactoside)	0·20	++	
Pn 3-galactoside-5-glucoside§	0·18		
Pt 3-rhamnoside and Dp 3-rhamnoside	0·39	+	All in "Jupiter" (deep purple); all (except the 3-rhamnosides) in violet shades ("Gertrude Tingay", "Stylish", "Fortune", "Stella", "Pall Mall"); some pale lilac shades ("Limelight", "Mandy", "Mabel Gower") only have the Mv pigment
Mv 5-glucoside-3-rhamnoside	0·32	++	
Pt 5-glucoside-3-rhamnoside	0·26	+++	
Dp 5-glucoside-3-rhamnoside	0·21	+++	

* On Whatman No. 3 paper in preliminary separations of pigments of the first mentioned colour variety.
† +++ = strong, ++ = present, + = trace.
‡ These three varieties also contain small amounts of Pn 5-glucoside-3-rhamnoside.
§ Tentative identifications; these pigments have the same R_F values as 3,5-diglucosides, give on hydrolysis galactose and glucose but are not present in sufficient amount for further studies.

Anthocyanins of Streptocarpus

The many flower colour forms of *Streptocarpus*, the cape primrose, have been studied extensively by Lawrence and his co-workers.[18,19] Not only have the pigments of the common garden hybrids, mainly derived from *S. rexii* and *S. dunnii*, been examined but also those present in many wild species and their hybrids. In Lawrence's work, the Robinson colour and distribution tests[4] were used so that identification was necessarily incomplete. The results of chromatographic analysis are summarized in Tables 6 and 7. Most of the pigments are known compounds, the 5-glucoside-3-rutinosides,* for example, having previously been found (in acylated form) in the potato.[7] The cyanidin 3-sambubioside of *Streptocarpus* is chromatographically identical with the major pigment of *Sambucus nigra* berries[20,21] and of *Begonia* leaves;[11,22] the pelargonidin 3-sambubioside is a new pigment (Table 1). The xylosylglucoses obtained by oxidation of the pigments present in *Begonia*, *Streptocarpus* and *Matthiola* (see below) are all chromatographically identical with sambubiose, the disaccharide present in *Sambucus* (Table 4). This sugar, originally thought to be primeverose (6-*o*-β-D-xylofuranosyl-D-glucose),[20] has recently been characterized as 2-*o*-β-D-xylofuranosyl-D-glucose by Reichel and Reichwald.[21]

* Rutinose is 6-*o*-α-L-rhamnosyl-D-glucose.

[18] W. J. C. LAWRENCE and V. C. STURGESS, *Heredity* **11**, 303 (1957).
[19] W. J. C. LAWRENCE, *Heredity* **11**, 337 (1957).
[20] L. REICHEL, H. STROH and W. REICHWALD, *Naturwissenschaften* **44**, 468 (1957).
[21] L. REICHEL and W. REICHWALD, *Naturwissenschaften* **47**, 40 (1960).
[22] M. BOPP, *Planta* **48**, 631 (1957).

TABLE 6. ANTHOCYANINS IN COLOUR FORMS OF *Streptocarpus*

Pigment	R_F in BAW†	Colour forms and genotypes‡
Pg 3-glucoside	0·35	pink (*oorrDD*) and salmon (*oorrdd*)
Pg 3-sambubioside	0·33	pink and salmon
Pg 3,5-diglucoside	0·29	pink and salmon
Pg 5-glucoside-3-rutinoside	0·24	pink
Pn 3-glucoside	0·38	rose (*ooRRdd*)
Cy 3-glucoside	0·34	rose
Cy 3-sambubioside*	0·31	salmon and rose
Pn 3,5-diglucoside, Cy 3,5-diglucoside	0·28	rose
Pn 5-glucoside-3-rutinoside	0·26	magenta (*ooRRDD*)
Cy 5-glucoside-3-rutinoside	0·23	magenta
Mv 3-glucoside	0·32	mauve (*OORRdd*)
Mv 3,5-diglucoside	0·24	mauve
Mv 5-glucoside-3-rutinoside	0·22	blue (*OORRDD*)

* Occurs in stems and leaves of all genotypes.
† On Whatman No. 3 paper.
‡ Genes *o* and *R* control the hydroxylation of the anthocyanins. Gene *D* controls the addition of rhamnose to the 3,5-diglucosides. For further genetic details, see Lawrence and Sturgess.[18]

Anthocyanins of other plants

The results of a search of a variety of plants for anthocyanins with unusual glycosidic patterns are presented in Table 7. The survey was directed mainly towards members of the families Cruciferae, Gesneriaceae, Leguminosae, Iridaceae, Papaveraceae and Primulaceae.

The anthocyanins of the several crucifers studied were particularly difficult to characterize, partly because they occur naturally as rather complex mixtures of acylated derivatives and partly because they are accompanied by (and difficult to separate from) the well-known mustard oils.[23] Much work has been devoted in the past to the pigments of red cabbage, *Brassica oleracea*, but it is only recently that a convincing structure has been put forward for the single glycoside, obtained when the sinapoyl residues are removed from the original pigment mixture. Stroh[24] found that it gave cyanidin and glucose in the molecular proportions of 1 to 3 on hydrolysis and proposed that it was the 3-(glucosylglucosylglucoside). Since his evidence for the allocation of the sugar residues did not rule out the possibility of the pigment having other structures, the pigment was re-examined and found to be cyanidin 5-glucoside-3-sophoroside. Thus, it shows the characteristic spectral properties of a 3,5-di(glycoside)[5] (Table 2) and gives four (rather than two) intermediate glucosides on partial hydrolysis. Confirmation was obtained by oxidizing the pigment with hydrogen peroxide, when it gave a disaccharide (sophorose; see Table 4), not a trisaccharide. This reagent is known to remove specifically the 3-substituted sugar of flavonoids, without splitting other sugar linkages.[25] Preliminary studies of two of the original pigments in red cabbage indicate that they are the mono- and di-sinapoyl esters of cyanidin 5-glucoside-3-sophoroside.

Another crucifer, the garden stock, *Matthiola incana*, has been reported by Seyffert[26] to contain in its flowers a large number of acylated cyanidin mono-, di- and triglucosides. In order to compare Seyffert's "triglucoside" with that of red cabbage, the anthocyanins of a range of commercial colour varieties of *Matthiola* were studied; the pelargonidin

[23] A. KJÆR, *Fortschr. Chem. org. Naturst.* **18**, 122 (1960).
[24] H. H. STROH, *Z. Naturf.* **14B**, 699 (1959).
[25] B. V. CHANDLER and K. A. HARPER, *Aust. J. Chem.* **14**, 586 (1961).
[26] W. SEYFFERT, *Z. Pflanzenz.* **44**, 4 (1960).

glycosides of orange and scarlet forms (not examined by Seyffert) were also included in this survey. Results (Table 2) show that the cyanidin glycoside of *Matthiola* is different from that of red cabbage and, further, that it does not have the structure indicated by Seyffert, since it contains xylose (one equivalent) as well as glucose (two equivalents). Seyffert, indeed, found xylose among the products of hydrolysis but erroneously assumed that it had arisen as an artifact of paper chromatographic separation. It is known, however, that

TABLE 7. SURVEY OF ANTHOCYANINS

Plant	Organ	Pigment(s)
Caprifoliaceae		
Sambucus nigra	berry	Cy 3-glucoside, Cy 3-sambubioside Cy 5-glucoside-3-sambubioside
Cruciferae		
Brassica oleracea var. rubra	leaf	Cy 5-glucoside-3-sophoroside (Ac)*
Matthiola incana	orange petal	Pg 3-glucoside
	carmine petal	Pg 5-glucoside-3-sambubioside (Ac)
	blue petal	Cy 5-glucoside-3-sambubioside (Ac)
Raphanus sativus	scarlet root	Pg 5-glucoside-3-sophoroside (Ac)
	red root	Cy 5-glucoside-3-sophoroside (Ac)
Fagaceae		
Fagus sylvatica var. purpurea	leaf	Pg and Cy 3-galactoside
Gesneriaceae		
Gesneria cardinalis	petal	Ad and Lt 5-glucoside
Kohleria eriantha	petal	Ad 5-glucoside, Pg 3-rutinoside
	sepal	Lt 5-glucoside
Saintpaulia ionantha	petal	Mv 5-glucoside-3-rutinoside
Streptocarpus rexii and *S. caulescens*	mauve petal	Mv 5-glucoside-3-rutinoside
S. dunnii	red petal	Cy 3-glucoside, Cy 3-sambubioside
Streptocarpus garden hybrids		see Table 6
Iridaceae		
Tritonia cv. "Prince of Orange"	petal	Pg 3-gentiobioside
Watsonia meriana, *W. rosea* and *W. tabularis*	orange petal	Pg 7-glucoside-3-sophoroside, Pg 3-sophoroside and Pg 3-glucoside
Leguminosae		
Lathyrus hirsutus	blue petal	Mv 3-rhamnoside
L. latifolius	mauve petal	Dp, Pt and Mv 5-glucoside-3-rhamnoside
L. odoratus wild	purple petal	Dp, Pt and Mv 5-glucoside-3-rhamnoside
cultivated		See Table 5
Phaseolus multiflorus	scarlet petal	Pg 3-sophoroside
Liliaceae		
Fritillaria meleagris	purple petal	Cy 3-rutinoside Cy 3,5-diglucoside
Myrsinaceae		
Ardisia crispa	berry	Cy and Pn 3-galactoside
Papaveraceae		
Papaver orientale	petal	Pg 7-glucoside-3-sophoroside and Pg 3-sophoroside
P. nudicaule	petal	Pg 7-glucoside-3-sophoroside and nudicaulin†
P. rhoeas	petal	Pg and Cy 3-sophoroside
P. argemone *P. pilosum*	petal	Pg 3-sophoroside 7-glucoside
Punicaceae		
Punica granatum	orange petal	Pg 3,5-diglucoside
Primulaceae		
Primula auricula	petal	Mv 3-glucoside
P. cashmiriana		Hs 3,5-diglucoside
P. obconica		Dp, Pt and Mv 3,5-diglucoside
P. rosea		Rs 3,5-diglucoside
Tropaeolaceae		
Tropaeolum majus	orange petal	Pg 3-sophoroside

* (Ac) indicates that the pigment occurs naturally in acylated form.
† The structure of this pigment has yet to be determined.

arabinose is the only pentose sugar produced by the action of acid solvents on filter paper.[6] The xylosylglucose combined in these pigments is probably sambubiose (see above). The two main pigments of *Matthiola* are thus pelargonidin and cyanidin 5-glucoside-3-sambubioside. The cyanidin 5-glucoside-3-sambubioside of *Matthiola* has also been found to occur in trace amounts in the berries of *Sambucus*, where it is accompanied by larger quantities of cyanidin 3-glucoside and 3-sambubioside.[20,21] It seems likely that at least part of the variation in acylation of the anthocyanins of *Matthiola* detected by Seyffert may be due to partial hydrolysis occurring during isolation. In the course of the present survey, only a few acyl derivatives were found; they will be described elsewhere.

Four acylated anthocyanins are present in the roots of another crucifer, *Raphanus sativus*. As already reported,[11] "Scarlet Globe" and other bright red varieties of radish contain pelargonidin 5-glucoside-3-sophoroside, acylated either with ferulic or with *p*-coumaric acid. The properties of the deacylated pigment are given in Table 1. A purple form, obtained in the F_1 progeny of crosses between scarlet and white skinned radishes, contains the corresponding cyanidin derivative, again in association with ferulic or *p*-coumaric acid. The cyanidin 5-glucoside-3-sophoroside, produced by deacylation, is identical with the pigment from red cabbage. The purple radish, which is not available commerically in this country, was apparently used by Cho[27] for biosynthetic studies, since he reports the presence of a cyanidin "diglucoside" in his material.

Only one pigment of *Papaver* was completely characterized in the earlier studies of Robinson and his co-workers.[28] This was mecocyanin, obtained from the corn poppy, *Papaver rhoeas*, and described as the 3-gentiobioside. The recent isolation from *Primula sinensis* of a cyanidin 3-gentiobioside with different properties from mecocyanin indicated that the structure of mecocyanin might require revision.[8] In fact mecocyanin gives sophorose (and not gentiobiose) on hydrolysis (Table 4) and is therefore the 3-sophoroside (Table 2). The pelargonidin derivative, present in *Papaver rhoeas* and *P. orientale*, is also the 3-sophoroside (Table 1); chromatographic identity has been established between this pigment and those present in flowers of the garden nasturtium, *Tropaeolum majus*, and the runner bean, *Phaseolus multiflorus*.

The most interesting new pigment of Papaveraceae is orientalin, first isolated from *P. orientale*. Its provisional identification as pelargonidin-7-glucoside-3-sophoroside[5,8] has now been confirmed by comparing the 7-glucoside, obtained as an intermediate product of hydrolysis, with synthetic material, obtained by reductive acetylation[29] of dihydrokampferol 7-glucoside. This is the first 3,7-di(glycoside) to be found in nature; it also occuis in three other *Papaver* species and in *Watsonia* (family Iridaceae) (Table 7).

A number of rare anthocyanins occur in *Primula*; the identification of various 3-glucosides, 3-gentiobiosides and 3-(glucosylglucosylglucosides) in *Primula sinensis* has already been described.[8] Details of two other pigments are now given. The first is hirsutidin 3,5-diglucoside (hirsutin), which was isolated from *Primula hirsuta* by Karrer and Widmer[30] in 1927. Since material of this species does not seem to be available at the present time, the pigment was sought among other members of the genus. *Primula cashmiriana* proved to be a good source, which yielded the *Rf* values and spectral characteristics of hirsutin given here (Table 3). Comparison of the properties of hirsutin with rosininin, a second rare pigment isolated from *P. rosea*,[31] indicates that both compounds have the same glycosidic pattern.

[27] D. H. Cho, *Univ. Seoul* **8**, 13 (1959).
[28] K. E. Grove, M. Inubuse and R. Robinson, *J. Chem. Soc.* 1608 (1934).
[29] H. Pacheco, *Bull. Soc. Chim. biol., Paris* **39**, 971 (1957).
[30] P. Karrer and R. Widmer, *Helv. chim. Acta* **10**, 14, 758 (1927).

Since rosinin gives rosinidin (7-methylpeonidin)[31] and glucose on hydrolysis, and has the spectral characteristics of a 3,5-di(glycoside), it is probably, therefore, the 3,5-diglucoside. Owing to the scarcity of plant material, it has not been possible to obtain sufficient rosinin for more exhaustive analysis.

The presence of petunidin 3,5-diglucoside (petunin) in *Primula obconica* is of some interest, since attempts to find it in *Petunia* cultivars, in which it was first reported to occur,[32] were unsuccessful. The only other reliable source of this pigment is the grape, *Vitis vinifera*.[33] The occurrence of malvidin 3,5-diglucoside in mauve and blue forms of *Primula obconica*, which is reported here, has also been observed by Reznik.[34]

A third anthocyanin, delphinidin 3,5-diglucoside, is present in red forms of *P. obconica* (Table 7). 3,5-Diglucosides of cyanidin, peonidin and pelargonidin have not yet, however, been discovered in *Primulas*. The only plant found (Table 7) to contain the common pelargonidin 3,5-diglucoside is *Punica granatum*. The original identification[14] required confirmation because of a melting point discrepancy; spectral and chromatographic comparison with authentic material show that the original identification was correct.

Among the Gesneriaceae, the occurrence of two anthocyanins lacking 3-hydroxyl groups (i.e. apigeninidin and luteolinidin 5-glucoside) in *Gesneria* and *Kohleria* has already been briefly reported;[35,36] the properties of the pigments are given in Table 3. Luteolinidin 5-glucoside has since been isolated by Bendz and Martensson[37] from a red moss, *Bryum cryophilum*. Two other unusual pigments, one in flowers of *Columnea banksii* and the other in leaves of a *Kohleria* cultivar, have also been found but not yet identified.

Finally, the anthocyanins of twenty-three *Fritillaria* species were surveyed, following the discovery by Shibata[38] of a quite exceptional cyanidin glycoside, a 3-(xylosylrhamnoside) in *F. kamchatchensis*. However, all the species examined had one major pigment, which was chromatographically identical with the well-known 3-rutinoside of cyanidin. A more detailed examination of the pigment of *F. meleagris* confirmed this finding; some cyanidin 3,5-diglucoside was also detected. Thus *F. kamchatchensis* remains the only *Fritillaria* species to have the unusual dipentoside.

DISCUSSION

The generally accepted view (see e.g.[39]) that glycosylation of the anthocyanins is virtually restricted to four classes clearly needs modification in the light of recent studies. There are six points that seem to be worth making in this connexion; they are set out in the following paragraphs.

1. The glycosidic pattern of anthocyanins is remarkably variable and is matched in this respect among plant products only by the flavonol glycosides. Six new glycosidic classes have been found in this survey, bringing the total number known to seventeen (Table 8).
2. Anthocyanins having a disaccharide in the 3- position and glucose in the 5- position (a type not recognized in the earlier studies[4]) are of wide occurrence. By contrast,

[31] J. B. HARBORNE, *Nature* **181**, 27 (1958).
[32] R. WILLSTÄTTER and C. L. BURDICK, *Liebigs Ann.* **412**, 217 (1917).
[33] A. H. BOCKIAN, R. F. KEPNER and A. D. WEBB, *Agric. Food Chem.* 3, 695 (1955).
[34] H. REZNIK, *Flora* **150**, 454 (1961).
[35] G. M. ROBINSON, R. ROBINSON and A. R. TODD, *J. Chem. Soc.* 809 (1934).
[36] J. B. HARBORNE, *Chem. & Ind.* 229 (1960).
[37] G. BENDZ and O. MARTENSSON, *Acta chem. scand.* **15**, 1185 (1961).
[38] M. SHIBATA, *Sci. Rep. Tohoku Univ.* **24**, 89 (1958).
[39] K. KARRER, *Konstitution und Vorkommen der Organischen Pflanzenstoffe*, p. 673. Birkhäuser-Verlag, Basel (1958).

3-glucosylglucosylglucosides are rare and only occur in *Primula*; reports of similar pigments in red cabbage[24] and *Lathyrus*[15] have not been substantiated.

3. A 3,7-di(glycoside), another type not found in the earlier work,[4] does exist in nature. However, the pelargonidin-7-glucoside-3-sophoroside found in *Papaver* and *Watsonia* is the sole representative of this class. No other 3,7-di(glycosides) have been reported, though their distinctive spectral and colour properties would make them conspicuous if they occurred widely.
4. The relative frequencies with which the various monosaccharides occur in bound form in anthocyanins are as follows: glucose>galactose>rhamnose>xylose>arabinose. That glucose is the most common sugar is apparent from the facts that (*a*) 3-glucosides are wide-spread, (*b*) nearly all known 3-biosides contain glucose as a constituent, (*c*) the 5-sugar in all the known 3,5-di(glycosides) is glucose and (*d*) the only known 3,7-di(glycoside) contains glucose. That arabinose is the least common sugar follows from the fact that it has been found in the anthocyanins of only three plant genera.[10,40,41]

TABLE 8. LIST OF GLYCOSIDIC CLASSES AND THEIR DISTRIBUTION IN NATURE

With regard to the distribution of the common types (7 classes), this list is not comprehensive. Genera listed refer only to results obtained during the present studies; reference may be made to Karrer[39] for details of earlier work. For other types, all known genera are given.

Glycosidic class	Distribution (aglycones in parentheses)
3-glucoside	Common; e.g. *Streptocarpus* (Pg, Cy, Pn), *Matthiola* (Pg), *Primula* (Mv)
3-galactoside	Common; e.g. *Fagus* (Pg), *Ardisia* (Cy, Pn), *Lathyrus* (Pg, Cn, Pn)
3-rhamnoside	*Lathyrus* (Pg, Cy, Pn, Dp, Pt), *Plumbago* (Pg, Cy, Dp, Cp)[10]
3-arabinoside	*Rhododendron* (Cy),[10] *Theobroma* (Cy),[41] *Hordeum* (Cy)[40]
3-xylosylglucoside (sambubioside)	Common; e.g. *Streptocarpus* (Pg, Cy)
3-xylosylgalactoside	*Lathyrus* (Pg, Cy)
3-xylosylrhamnoside*	*Fritillaria* (Cy)[38]
3-rhamnosylglucoside (rutinoside)	Common; e.g. *Fritillaria* (Cy)
3-glucosylglucoside (sophoroside)	Common; e.g. *Papaver* (Pg, Cy), *Tropaeolium* (Pg), *Watsonia* (Pg), *Phaseolus* (Pg)
3-glucosylglucoside (gentiobioside)	*Primula* (Pg, Cy, Pn, Pt, Mv),[8] *Tritonia* (Pg)
3-glucosylglucosylglucoside	*Primula* (Pg, Pn, Pt, Mv)[8]
3,5-diglucoside	Common; e.g. *Punica* (Pg), *Streptocarpus* (Pg, Cy, Pn, Mv), *Primula* (Dp, Pt, Mv, Hs, Rs)
5-glucoside-3-rhamnoside	*Lathyrus* (Pg, Cy, Pn, Dp, Pt, Mv)
5-glucoside-3-rutinoside	Common; e.g. *Streptocarpus* (Pg, Cy, Pn, Mv)
5-glucoside-3-sambubioside	*Matthiola* (Pg, Cy), *Sambucus* (Cy)
5-glucoside-3-sophoroside	*Raphanus* (Pg, Cy), *Brassica* (Cy)
7-glucoside-3-sophoroside	*Papaver* (Pg), *Watsonia* (Pg)

* Or 3-rhamnosylxyloside; the order of the sugars in this pigment has not been determined.

5. All anthocyanins have a sugar of some kind attached to the 3-hydroxyl group. The recent report[42] that *Bladhia sieboldii* (Myrsinaceae) contains delphinidin 7-galactoside (together with malvidin 3-galactoside) is inconsistent with this finding. There are, however, good reasons for believing that the so-called 7-galactoside is really delphinidin 3-galactoside. Thus the known instability of 7-glycosides makes it unlikely that such pigments occur naturally; furthermore, a related plant, *Ardisia crispa* (also Myrsinaceae) contains 3-galactosides of cyanidin and peonidin (Table 7).

[40] M. METCH and E. URION, *C. R. Acad. Sci., Paris* **252**, 356 (1961).
[41] W. G. C. FORSYTH and V. C. QUESNEL, *Biochem. J.* **65**, 177 (1957).
[42] P. YEN and P. HUANG, *Tetrahedron* **12**, 181 (1961).

6. It is apparent that only a few of the many theoretically possible isomeric 3-biosides occur in nature. Thus only one rhamnosylglucose (rutinose), one xylosylglucose (sambubiose), one xylosylgalactose and two glucosylglucoses (gentiobiose and sophorose) have so far been observed as disaccharide units in anthocyanins. Three of these (rutinose, sambubiose and sophorose) are of common occurrence. The fact that biosides with $\beta 1 \rightarrow 2$ links (i.e. sophorosides and sambubiosides) occur so frequently is remarkable, since such linkages have rarely been found among other plant glycosides or in polysaccharides.

This is not the place to explore fully the botanical implications of this work. There are, however, three general points which may usefully be made.

1. Glycosidic pattern is related to systematics. In particular, plants of the same genus tend to contain a glycosidic type characteristic of that genus. Several examples are recorded in Table 7; a more complete list and discussion is presented elsewhere.[43]
2. Plants containing mixtures of more than three glycosidic types are exceptional. Only two (*Streptocarpus* and *Lathyrus*) have been found during these studies. The presence of four glycosidic types in *Streptocarpus* can be traced to the hybrid nature of the garden forms, which are known to have originated from crosses between two species having different pigments (i.e. *S. rexii* and *S. dunnii*, Table 7). The occurrence of galactose-containing glycosides in red and orange (but not purple) forms of sweet pea is more difficult to account for, since it is not of hybrid origin and wild species contain only rhamnosides (3-rhamnosides or 5-glucoside-3-rhamnosides). The recent identification[44] of kampferol 7-rhamnoside-3-(xylosylgalactoside) in all garden forms of sweet pea indicates that a system for xylosylgalactoside synthesis is available in this plant. The production of anthocyanidin 3-galactosides and 3(xylosylgalactosides) in mutant forms may therefore be due to a simple alteration in enzyme specificity.
3. Synthesis of mono-, di- and triglycosides is a stepwise process involving separate additions of monosaccharides to appropriate precursors. Convincing evidence of this is provided by *Streptocarpus* in which genes are known[18] for controlling every step in the synthesis of the dominant 5-glucoside-3-rhamnosylglucoside.

EXPERIMENTAL

Authentic compounds. Sources of authentic pigments have already been described.[5,7] A very generous supply of pure cyanidin 3-rutinoside (antirrhinin) from Mrs. O. Meares is gratefully acknowledged. Sophorose and laminaribiose were kindly provided by Drs. W. J. Whelan and J. B. Pridham; sambubiose was obtained from elderberry pigment;[20,21] the other disaccharides were obtained commercially.

Plant material. Most plants were grown from seed or were available at this Institute. Seeds of *Lathyrus* species were kindly provided by Dr. R. C. Pecket and *Streptocarpus* plants weie made available through the courtesy of Mr. W. J. C. Lawrence. Seeds of an F_1 progeny of radishes, which segregated into purple, scarlet and white forms, were donated by Mr. B. J. Harrison. Fresh blooms of a wide range of sweet pea colour varieties were put at our disposal by Messrs. R. Bolton and Sons.

Isolation of anthocyanins. General procedures have been described before.[5,6,7,8] Details of the separation of anthocyanins from most plants are omitted, because it is usually clear

[43] J. B. Harborne, *Fortschr. Chem. org. Naturst.* **20**, 165 (1962).
[44] J. B. Harborne, *Chem. & Ind.* 222 (1962).

(from the Tables of R_F values) which band in a preliminary separation in BAW provides which pigment. A large number of pigments are present in *Lathyrus* and *Streptocarpus* and in these instances, R_F values obtained during the preliminary separation are given in Tables 4 and 5. Pairs of pigments which did not separate in BAW usually did so when chromatographed in 1% HCl. The 5-glucoside-3-rhamnosides and 3-(xylosylgalactosides) of pelargonidin, cyanidin and peonidin in *Lathyrus* were particularly difficult to obtain pure but they were successfully separated from each other by increasing the time of development with BAW from 16 to 30 hr. A few pigments were obtained as almost black needles or prisms by crystallization from aqueous HCl, after chromatographic purification.

Anthocyanin identification. Except in the case of the 5-glucoside-3-rhamnosides of *Lathyrus*, controlled acid hydrolysis of the various di- and triglycosides gave all the expected intermediate products. Treatment of the 5-glucoside-3-rhamnosides with excess anthocyanase for 1–2 hr gave good yields of the corresponding 3-rhamnosides. The disaccharides of di- and triglycosides were obtained by treating the relevant pigment with either (*a*) boiling 10 per cent acetic acid for 2–4 hr, or (*b*) hydrogen peroxide in methanol for 6 hr at room temperature.[25]