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PLANT POLYPHENOLS—XIII.

THE SYSTEMATIC DISTRIBUTION AND ORIGIN OF ANTHOCYANINS CONTAINING BRANCHED TRISACCHARIDES*

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Abstract—Cyanidin 3-*O*-β-(2^{G}-xylosylrutinoside) and cyanidin and pelargonidin 3-*O*-β-(2^{G}-glucosylrutinoside) have been found in flowers and leaves of species of *Begonia* and *Clivia* and in the fruits of *Prunus*, *Ribes* and *Rubus*. They co-occur with the related 3-sambubioside, 3-rutinoside and 3-sophoroside and appear to be formed when the appropriate systems for transferring rhamnose, xylose and glucose to cyanidin or pelargonidin 3-glucoside co-exist in the same plant. Their distribution is limited so that they are of systematic interest at the generic and specific levels. They are accompanied in leaves and flowers of *Begonia* by rutin, kaempferol 3-glucoside, quercetin 3-glucoside and quercetin 3-xyloside. Three *Begonia* species are exceptional in having, in addition, glucosides of kaempferol and quercetin 3-*O*-methyl ethers. 1-Caffeoyl- and 1-feruloyl-glucose have also been identified in this genus.

INTRODUCTION

WHILE surveying the anthocyanins of higher plants, ten new glycosidic types were found,[1] bringing the total of known classes to sixteen.[2] Five of these new types were triglycosides, which, because of their relatively limited natural distribution, were of phytochemical interest.[2] In continuing this survey, two further triglycosides of cyanidin were found in leaves and flowers of *Begonia*. They were both found to contain branched trisaccharides[3] and, since flavonoids of this type have not previously been found, their natural distribution was examined in more detail and the anthocyanins and flavonol glycosides occurring with them were identified. A survey of fruits showed they were present in raspberries, currants and cherries, and a related pelargonidin derivative was also discovered. The chemical identifications of these three new anthocyanins and of two new flavonols from *Begonia* are described in this paper and the results of the various surveys are also presented.

RESULTS

Anthocyanins and Related Phenols in Begonia

In 1957, Bopp[4] carried out an exhaustive survey of the anthocyanins in the leaves of *Begonia* and found seven cyanidin glycosides to be variously present. He did not characterize them beyond recording their R_f values but noted that the main flavonol glycoside appeared to be rutin. At about the same time, the major leaf pigment of *Begonia rex* was isolated in this laboratory and identified as cyanidin 3-xylosylglucoside.[5] Later, a comparison of the

* Part XII. J. B. HARBORNE and E. HALL, *Phytochemistry* **3**, 421 (1964).

[1] J. B. HARBORNE, *Phytochemistry* **2**, 87 (1963).

[2] J. B. HARBORNE in *Chemical Plant Taxonomy* (Edited by T. SWAIN), pp. 359–88, Academic Press, London (1963).

[3] J. B. HARBORNE and E. HALL, *Biochem. J.* **88**, 41P (1963).

[4] M. BOPP, *Planta* **48**, 631 (1957).

[5] J. B. HARBORNE and H. S. A. SHERRATT, *Experientia* **13**, 486 (1957).

properties of this pigment and its disaccharide (produced by H_2O_2 oxidation) with those of cyanidin 3-sambubioside and sambubiose (2-xylosyl-β-D-glucose), from *Sambucus nigra* berries,[6] showed them to be identical.[1]

In a limited survey of thirteen species and three *Begonia* cultivars, five anthocyanins besides cyanidin 3-sambubioside, were noted (Table 1). Three of these were readily identified,

Table 1. Distribution of flavonoids in *Begonia*

Anthocyanins*	*Begonia* species†	Other flavonoid glycosides
Cy 3-sambubioside Cy 3-xylosylrutinoside Cy 3-sophoroside Cy 3-glucosylrutinoside	cv. "President Carnot" (L, F) × *lucerna* (L, F)	rutin (L, F)
Cy 3-sophoroside Cy 3-glucosylrutinoside	*coccinea* (F)	none observed
Cy 3-sambubioside Cy 3-xylosylrutinoside	*metallica* (L, F) *haageana* v. *drostii* (L, F) *glaucophylla* (L, F)	rutin (F), quercetin and kaempferol 3-glucosides (F)-*glaucophylla* has, in addition, quercetin 3-xyloside and glucosides of flavonol 3-methyl esters
	argento-guttata (L) *rex* (L)	rutin (L)
	× *richmondensis* (L, F) cv. "Woodruff's Scarlet" (F)	none observed
	cv. "Courbeille de Feu" (F)	unidentified fluorescent yellow-green compd.
Cy 3-sambubioside	*pearcei* (L) *manicata* (L)	rutin (F, L) quercetin and kaempferol 3-glucosides and glucoside of flavonol 3-methyl esters (see Table 3)
Cy 3-sophoroside	*fuchsioides* v. *rosea* (F)	unidentified fluorescent yellow-green compd.
Pg 3-sophoroside	*sutherlandii* (F)	none
Cy 3-glucoside	*martiana*‡ (L, F)	rutin, quercetin 3-glucoside and glucosides of flavonol 3-methyl esters (F)

* Abbreviations: Cy = cyanidin, Pg = pelargonidin.
† Anthocyanin-containing organs are shown in parentheses: L = leaf, F = flower.
‡ Also contains a cyanidin 3-pentoside, possibly the 3-xyloside, in the flowers.

by comparison with authentic pigments,[1] as the 3-glucoside and 3-sophoroside of cyanidin and the 3-sophoroside of pelargonidin. From their very high R_f values in 1% aqueous hydrochloric acid, it was clear that the other two pigments were cyanidin triglycosides, which did not correspond with any known derivatives. They were therefore isolated from suitable sources (CyT1 from flowers of *B. coccinea* and CyT2 from leaves of *B. metallica*), purified and analysed by standard procedures (Table 2). Quantitative analyses showed that each contained three

[6] L. Reichel and W. Reichwald, *Naturwiss.* **47**, 40 (1960).

TABLE 2. PROPERTIES OF ANTHOCYANINS CONTAINING BRANCHED TRISACCHARIDE

Pigment	R_f values in*				Colour in u.v. light
	BAW	BuHCl	1% HCl	HOAc–HCl	
CyT1 (*Beg. coccinea*)	0·26	0·11	0·61	0·73	dull magenta
CyT2 (*Beg. metallica*)	0·28	0·15	0·47	0·68	dull magenta
Cy 3-sophoroside	0·18	0·09	0·40	0·62	dull magenta
Cy 3-sambubioside	0·28	0·11	0·20	0·46	dull magenta
Cy 3-rutinoside	0·29	0·12	0·19	0·43	dull magenta
Cy 3-glucoside	0·29	0·11	0·03	0·18	dull magenta
PgT1 (*Rubus* "September")	0·33	0·15	0·63	0·73	dull orange
Pg 3-sophoroside	0·33	0·14	0·45	0·65	dull orange
Pg 3-rutinoside	0·39	0·19	0·21	0·44	dull orange

Spectra

Pigment	λ_{max} in MeOH–HCl, mμ		$AlCl_3$ shift	Ratios (as %)		
	Band I	Band II		$E_{440}/E_{band\ II}$	$E_{310}/E_{band\ II}$	$E_{band\ I}/E_{band\ II}$
CyT1	283	530	+43	22	12	58
CyT2	283	528	+45	23	13	56
PgT1	275	508	0	38	12	68

Pigment	Products of acid hydrolysis (ratios)	Identified as
CyT1	cyanidin (1·09), glucose (2·0), rhamnose (0·91) Cy 3-sophoroside, Cy 3-rutinoside and Cy 3-glucoside	Cy 3-(2^G-glucosylrutinoside)
CyT2	Cyanidin, glucose (1·0), xylose (1·02), rhamnose (0·80) Cy 3-sambubioside, Cy 3-rutinoside and Cy 3-glucoside	Cy 3-(2^G-xylosylrutinoside)
PgT1	pelargonidin, glucose, rhamnose, Pg 3-sophoroside Pg 3-rutinoside and Pg 3-glucoside	Pg 3-(2^G-glucosylrutinoside)

Properties of oligosaccharides

	R_g value in*			Aniline hydrogen phthalate	Colours† with	
	BAW	BEW	BBPW		diphenyl-amine/aniline	Diphenyl-amine/urea
Trisaccharide‡ ex CyT1 and PgT1	0·46	0·53	0·42	light brown	brown	purple
Trisaccharide ex CyT2	0·55	0·61	0·57	red-brown	brown	purple
Sophorose	0·62	0·69	0·59	light brown	brown	brown
Rutinose	0·74	0·78	0·77	brown	blue	mauve
Sambubiose	0·75	0·76	0·77	red-brown	brown	purple

* Solvent abbreviations, see experimental.

† None reacted with triphenyltetrazolium chloride or 3,5-dinitrobenzoic acids, tests which are specific for disaccharides with $\beta 1 \rightarrow 4$ and $\beta 1 \rightarrow 6$ links.

‡ β-Glucosidase hydrolysis gives rutinose and glucose; the other trisaccharide is unaffected.

monosaccharide residues and the spectral properties indicated that these sugars were attached only to the 3-hydroxyl group of cyanidin in each case. Thus, the spectra of both pigments gave a positive shift with aluminium ion, indicating that the 3',4'-dihydroxylic groups were free, and had E_{440}/E_{max} ratios indicating that the 5-hydroxyls were free; further, the positions of the visible maxima showed that the 7-hydroxyls were free.

Each of these pigments gave, as intermediates of controlled acid hydrolysis, two isomeric cyanidin 3-diglycosides and cyanidin 3-glucoside. Both pigments must, therefore, bear branch trisaccharides because an anthocyanin with a linear trisaccharide gives only one intermediate 3-diglycoside on hydrolysis.[7] The intermediate glycosides obtained from CyT2 were identified as the 3-sambubioside and 3-rutinoside and the appropriate disaccharides, obtained by oxidizing the 3-diglycosides with H_2O_2, were compared directly with authentic samples of sambubiose and rutinose (6-rhamnosyl-α-D-glucose). The trisaccharide, itself, was isolated from H_2O_2 oxidation of CyT2 and this had the R_f values and gave colours with a range of sugar reagents expected of an oligosaccharide with $\beta 1 \rightarrow 2$ and $\alpha 1 \rightarrow 6$ linkages;[8] furthermore, it gave rutinose and sambubiose as the only detectable intermediates of acid hydrolysis. This trisaccharide is thus identified as *O*-β-D-xylopyranosyl-(1→2)-*o*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranose, abbreviated for convenience as 2^G-xylosylrutinose; the original pigment, CyT2, is then cyanidin 3-*O*-β-(2^G-xylosylrutinoside).

The second pigment, CyT1, gave the 3-sophoroside and 3-rutinoside of cyanidin on hydrolysis; on oxidation with H_2O_2 it gave a branched trisaccharide which was hydrolysed by β-glucosidase to rutinose and glucose. The trisaccharide of CyT1 is thus *O*-β-D-glucopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranose, or 2^G-glucosylrutinose. CyT1 is therefore cyanidin 3-*O*-β-(2^G-glucosylrutinoside) and analogous in structure to CyT2.

The flavonol glycosides of *Begonia* were next studied, in order to see if their glycosidic pattern was related to that of the anthocyanidins. Rutin was found in nine out of sixteen species or hybrids and the 3-glucosides of kaempferol and quercetin were found in leaves of *B. manicata* (Table 3) and in four other species (Table 1). Quercetin 3-xyloside was isolated from flowers of *B. glaucophylla*. In addition, two unusual flavonol glycosides were, unexpectedly, found in *B. manicata* and chemical studies (Table 3) showed them to be 7-(glucosylglucosides) of 3-*O*-methylkaempferol and 3-*O*-methylquercetin. While the latter aglycone has previously been found in flowers of *Nicotiana tabacum*,[9] the former, kaempferol 3-methyl ether, is a new plant constituent.

These two methyl ethers are not characteristic only of *B. manicata* but they also occur in glucosidic form in *B. martiana* and *B. glaucophylla*. Another interesting (but unidentified) substance was found only in *B. fuchsioides* and in the cultivar "Courbeille de Feu", a hybrid known to be derived from *B. fuchsioides*; this compound had a striking yellow-green fluorescence in u.v. light and R_f values 0·37 in BAW and 0·00 in water. The chromatographic survey of leaf and petal extracts also showed that several simpler phenolic constituents present in *Begonia* were of limited distribution. 1-Caffeoyl- and 1-feruloylglucose were identified in *B. manicata* leaves; the former appeared to occur also in eleven other species. A *p*-coumaric ester, different from *p*-coumaroylglucose, was noted in petals of *B. manicata* but was not further examined.

Anthocyanins of Rubus, Prunus *and* Ribes

Although no systematic survey of the fruit pigments of *Rubus* appears to have been carried out, some information on the anthocyanins present is available. Thus, Huang[10] confirmed an earlier report[11] that cyanidin 3-glucoside occurred in the blackberry, *R. fruticosus*, and

[7] J. B. HARBORNE and H. S. A. SHERRATT, *Biochem. J.* **78**, 298 (1961).
[8] R. W. BAILEY, *J. Chromatog.* **8**, 57 (1962).
[9] C. H. YANG, D. D. BRAYMER, E. L. MURPHY, W. CHORNEY, N. SCULLY and S. H. WENDER, *J. Org. Chem.* **25**, 2063 (1960).
[10] H. T. HUANG, *Nature* **177**, 39 (1955).
[11] P. KARRER and B. PIEPER, *Helv. Chim. Acta* **13**, 1067 (1930).

TABLE 3. PROPERTIES OF FLAVONOL GLYCOSIDES OF *Begonia*

Glycosides	Source	R_f value in BAW	BEW	H_2O	PhOH	Colours in u.v. light: Alone	+ Ammonia
F_1	*B. manicata* flowers	0·66	0·68	0·15	0·70	dull purple-brown	yellow-green
F_2	*B. manicata* flowers	0·55	0·54	0·08	0·51	dull purple-brown	yellow
F_3	*B. manicata* leaves	0·44	0·43	0·27	0·77	dull purple-brown	yellow-green
F_4	as F_1	0·35	0·30	0·09	0·61	dull purple-brown	yellow
F_5	*glaucophylla* flowers	0·69	0·77	0·10	0·63	dull purple-brown	
F_6	*glaucophylla* flowers	0·41	0·56	0·27	0·47	dull purple-brown	

Aglycones*	BAW	Forestal	50% HOAc	PhOH	Alone	+ Ammonia
F_3A† and Km 3-methyl ether	0·92	0·84	0·70	0·91	dark purple	dull brown
F_4A and Qu 3-methyl ether	0·93	0·84	0·76	0·71	dark purple	dull brown
demethylated F_3A and Km	0·86	0·58	0·44	0·40	bright yellow	bright yellow
demethylated F_4A and Qu	0·71	0·42	0·31	0·22	bright yellow	bright yellow

Flavonol	Ultraviolet spectra (λ_{max}, mμ): 95% EtOH Band I	95% EtOH Band II	EtOH/NaOAc band I	EtOH/NaOAc/H_3BO_3 band II	EtOH/$AlCl_3$ band II	EtOH/NaOH band II
F_1	267	353	272	355	347, 396	400
F_2	258	362	268	383	365, 399	420
F_3	268	352	268	352	346, 402	393
F_4	257	360	257	381	362, 407	400
F_5	258	361	262	378	360, 395	408
F_6	260	362	269	378		415
F_3A	268	352	271	353	347, 398	402
F_4A	257	362	260	379	362, 403	415
demethylated F_3A	268	368		368	348, 419	unstable
demethylated F_4A	255	374		389	360, 428	unstable

Glycoside	Acid hydrolysis products	Identified as
F_1	Km and glucose	Km 3-glucoside
F_2	Qu and glucose	Qu 3-glucoside
F_3‡	F_3A, glucose and a 7-monoglucoside	3-*O*-methyl Km 7-(glucosylglucoside)
F_4‡	F_4A, glucose and a 7-monoglucoside	3-*O*-methyl Qu 7-(glucosylglucoside)
F_5	Qu and xylose	Qu 3-xyloside
F_6	Qu, rhamnose and glucose	Qu 3-rutinoside

* Abbreviations: Km = kaempferol, Qu = quercetin. Demethylations were carried out with pyridinium chloride at 120–140° for 3 hr.

† This aglycone had m.p. 272–276° (Km 3-methyl ether, lit. m.p. 270–272°) and analysed as follows. (Found OMe: 10·1%. $C_{15}H_9O_5$ (OMe) requires OMe: 10·3%.)

‡ Both these glycosides were relatively resistant to β-glucosidase, and were also only slowly hydrolysed by acid. The intermediate 7-monoglucosides were obtained in highest yield from ½-hr heatings at 100° with 2 N HCl.

Keranen and Suomalainen[12] found that cyanidin 3-glucoside and a cyanidin 3-diglucoside pigmented the berry of the arctic bramble, *R. arcticus*. More recently, Nybom[13] examined red raspberries, *R. idaeus*, and found four cyanidin glycosides: the 3-glucoside, the 3-rutinoside, a 3-diglucoside and a 3-diglycosylrhamnoside. He also found a xylose-containing glycoside in *R. occidentalis*, the black raspberry.

Examination of a range of raspberry varieties and related hybrids and species has now confirmed the presence of cyanidin triglycosides in the genus. In fact, the red raspberry triglycoside was found to be identical with cyanidin 3-*O*-β-(2^G-glucosylrutinoside), first found in *Begonia* (see above). The diglucoside in raspberries was identified as cyanidin 3-sophoroside and the presence of cyanidin 3-glucoside and 3-rutinoside was also confirmed. One raspberry variety, "September", contained these four cyanidin pigments and the four related pelargonidin derivatives as well. The pelargonidin triglycoside, a new pigment, PgT1, was characterized as pelargonidin 3-*O*-β-(2^G-glucosylrutinoside), by methods similar to those used for the cyanidin derivatives (Table 3). Pelargonidin is apparently rare in the genus for only two other occurrences were noted in the present survey: berries of *R. odoratus* are entirely pigmented by the 3-glucoside and 3-rutinoside of pelargonidin, and *R. parviflorus* contains a mixture of pelargonidin and cyanidin 3-glucosides. It is, perhaps, significant that these two species are both placed in the same section of the genus, *Anoplobatus*.

Cyanidin 3-*O*-(2^G-glucosylrutinoside), besides occurring in most raspberry varieties, is present in seven raspberry–blackberry hybrids and in two related wild species (Table 4). Cyanidin 3-*O*-(2^G-xylosylrutinoside) was identified in the black raspberry *R. occidentalis* (cf. Nybom[13]) and appears to be characteristic of this species. An F_1 hybrid with *R. idaeus* did not contain it, so that the gene (or genes) determining its occurrence appears to be recessive.

The four patterns of anthocyanidin glycosylation present in *Rubus* are shown in Table 4. It will be seen that the triglycosides and the sophoroside occur exclusively in raspberry types (section *Ideobatus*) and that all blackberry types (section *Rubus*) contain only the rutinoside and glucoside. The present survey has been limited mainly to edible fruit, so that it is too early to say what taxonomic significance these glycosylation patterns have in the genus as a whole. However, within the edible group, this information about the anthocyanins might well prove of value in identifying fruits of unknown origin.

The discovery of triglycosides in *Rubus* suggested that fruits of other rosaceous genera might also contain them. And, indeed, cyanidin 3-(2^G-glucosylrutinoside) was at once noted in the cherry (Table 5). It is present in the sour cherry, *P. cerasus*, (seven varieties examined) but is uniformly absent from sweet cherries (*P. avium*). These results agree with earlier studies of Nybom[13] and of Li and Wagenknecht,[14,15] who found the 3-glucoside and a 3-rhamnosylglucoside in the sweet variety "Windsor" and a 3-rhamnosylglucoside and a 3-diglucoside in the sour "Montmorency". The present studies show that the 3-rhamnosylglucoside in cherries is cyanidin 3-rutinoside and the 3-diglucoside, cyanidin 3-sophoroside.

In contrast to the cherry, the plum, *P. domestica*, and the apple, *Malus pumila*, lack triglycosides. A survey of fifteen common plum varieties showed that cyanidin 3-glucoside and 3-rutinoside, accompanied by traces of the related paeonidin derivatives, are uniformly present. Indeed, the same four pigments were found in fruit of both parents of *P. domestica*,

12 A. J. A. KERANEN and H. SUOMALAINEN, *Suomen Kem.* **33**, 155 (1960).
13 N. NYBOM, *Ann. Rep. Balsgård Fruit Inst. Sweden*, p. 31 (1960).
14 K. C. LI and A. C. WAGENKNECHT, *J. Amer. Chem. Soc.* **78**, 979 (1956).
15 K. C. LI and A. C. WAGENKNECHT, *Nature* **182**, 657 (1958).

TABLE 4. DISTRIBUTION OF ANTHOCYANINS IN *Rubus* BERRIES

Rubus material	Pigments present
Raspberry (*R. idaeus*) cultivars: "September",* "Tweed" and "Preussen" Raspberry hybrids: "Bedford Giant", Loganberry, Mertonberry, Lowberry, Youngberry, Boysenberry F_1 hybrid *R. idaeus* × *occidentalis* Species: *strigosus, gracilis, frondosus*	Cy 3-glucoside Cy 3-rutinoside Cy 3-sophoroside and Cy 3-glucosylsophoroside
Species: *R. occidentalis*	Cy 3-glucoside Cy 3-rutinoside Cy 3-sambubioside and Cy 3-xylosylrutinoside
Raspberry cultivars: "October", "Golden Everest" and "Cascade"	Cy 3-glucoside Cy 3-sophoroside
Raspberry cultivar: "Malling Landmark" Blackberry cultivars: "Merton Early" and "John Innes" Species: *parviflorus*†, *odoratus*‡, *caesius, ulmifolius, schleicheri, leucostachys, dasyphylloides, bellardii* and *inopertus*	Cy 3-glucoside and Cy 3-rutinoside§

* Also contains four related pelargonidin glycosides (see text).
† Also contains pelargonidin 3-glucoside.
‡ Contains only pelargonidin derivatives (the 3-glucoside and 3-rutinoside).
§ The 3-rutinoside is sometimes present in much smaller quantities than the 3-glucoside and is occasionally absent altogether (e.g. in *R. parviflorus*).

TABLE 5. DISTRIBUTION OF ANTHOCYANINS IN FRUITS OF *Prunus*

Anthocyanins present	*Prunus* material
Cy 3-glucosylrutinoside, Cy 3-sophoroside, Cy 3-rutinoside and Cy 3-glucoside	Sour cherries: "Wye Morello", "Olivet", "Empress Eugenie",* "Late Duke",* "Archduke"* and "Ostheimer Weichsel"
Cy 3-rutinoside and Cy 3-glucoside	Sour cherry: "Morello A" Sweet cherries: "Late Black Bigarreau", "Hedelfingen", "Napoleon" Species: *P. cerasifera, P. spinosa*,† *P. domestica* (15 named varieties)

* These varieties are claimed to be hybrids of *cerasus* × *avium*.
† 40% of the pigment in the sloe is paeonidin (as the 3-glucoside and 3-rutinoside); paeonidin is also present in traces in *P. cerasifera* and in most domestic plums.

i.e. in the sloe *P. spinosa* and in *P. cerasifera*. Also, a survey of *Malus* (14 species, 5 species hybrids and 10 cultivars) showed that cyanidin 3-galactoside, earlier identified in "Grimes Golden" apple,[16] is consistently present in these fruits.

Since the family Saxifragaceae is fairly closely allied to the Rosaceae, it seemed reasonable to seek for the triglycosides there as well, and the currants, *Ribes*, were chosen for investigation.

TABLE 6. DISTRIBUTION OF ANTHOCYANINS IN *Ribes*

Anthocyanins*	*Ribes* material
Cy 3-(2^G-glucosylrutinoside) Cy 3-(2^G-xylosylrutinoside) Cy 3-sambubioside Cy 3-sophoroside Cy 3-rutinoside Cy 3-glucoside	Red currant: "Earliest of the Fourlands" Species and species hybrids: × *futurum*, *davidii*, × *robustum*, × *koehneanum*, and *warszewiczii*
Cy 3-(2^G-xylosylrutinoside) Cy 3-sambubioside Cy 3-rutinoside Cy 3-glucoside	Red currant: "Fay's Prolific" Species and species hybrids: *sativum*, × *holosericeum*, × *gondouini* and *rubrum*
Cy 3-(2^G-glucosylrutinoside) Cy 3-sophoroside Cy 3-glucoside	Red currant: "Red Lake" Species: *multiflorum*
Dp 3-glucoside Dp 3-rutinoside Cy 3-glucoside Cy 3-rutinoside	Species and species hybrids: *nigrum* (black currant), *hirtellum*, × *rusticum*, *divaricatum*, *cynosbati* and × *fuscescens*
Cy 3-rutinoside Cy 3-glucoside	Species and species hybrids: *cereum*, *odoratum*, × *utile*, *alpinum*, *coeleste*, × *culverwellii*, *watsonianum*, *sanguineum*, *leptanthum*, *fasciculatum*, *aureum*, *caucasicum*, *nevadense* and × *carrierei* Red currant: "Rondom" Gooseberries: "Lancashire Lad" and *uva-crispa* (wild)

* All pigments in each group were not always present in every specimen listed in left-hand column. In particular, the red currant "Red Lake" lacks triglycoside.

Working with *R. nigrum*, Reichel and Reichwald[17] and Chandler and Harper[18] have both recorded the presence of a mixture of delphinidin and cyanidin 3-glucosides and cyanidin 3-rutinoside in this fruit. A survey now shows that both the cyanidin triglycosides of *Begonia* are present in red currants. Of twenty-nine species and four cultivars examined (Table 6), eleven contained the triglycosides; the cyanidin xylosylrutinoside was very common, being present in ten of these. No less than eight of the ten species having triglycosides are included in the same section (*Ribes*) and sub-section (*Ribes*) of the genus. The presence of delphinidin

[16] C. E. SANDO, *J. Biol. Chem.* **117**, 45 (1937).
[17] L. REICHEL and W. REICHWALD, *Naturwiss.* **47**, 41 (1960).
[18] B. V. CHANDLER and K. A. HARPER, *Austral. J. Chem.* **15**, 114 (1962).

3-glucoside in black currant was confirmed and this pigment, as might be expected, is characteristic of *Ribes* species having dark-coloured fruit. The majority of *Ribes* material studied had only two pigments, cyanidin 3-glucoside and cyanidin 3-rutinoside. The wild gooseberry, *R. uva-crispa*, and its cultivated forms (e.g. "Lancashire Lad") are also in this latter group. Broadly, the distribution of anthocyanins in *Ribes* is remarkably similar to that of *Rubus* and is, again, related to the systematics of the group.

Finally, it should be mentioned that, during a survey of anthocyanins in monocotyledons, cyanidin 3-(2^G-glucosylrutinoside) was found in *Clivia miniata* petals, together with cyanidin 3-rutinoside.

DISCUSSION

The discovery of three anthocyanins containing branched trisaccharides is noteworthy, because such sugars have not previously been encountered in association with flavonoids.

TABLE 7. GLYCOSIDIC PATTERNS OF THE ANTHOCYANINS OF THE ROSACEAE

Genus	Glycosidic pattern	Anthocyanidins*
Fragaria (Strawberry)	3-glucoside	Pg, Cy
Malus (Apple)	3-galactoside	Cy
Prunus (Cherry, plum)	3-glucoside, 3-rutinoside 3-sophoroside and 3-(2^G-glucosylrutinoside)	Cy, Pn
Pyrus (Pear)	3-galactoside	
Rubus (Raspberry, Blackberry)	3-glucoside, 3-rutinoside 3-(2^G-glucosylrutinoside) and 3-(2^G-xylosylrutinoside)	Cy, Pg
Rosa (Rose)	3-glucoside and 3,5-diglucoside	Cy, Pg, Pn

* Abbreviations: Pg = pelargonidin; Cy = cyanidin; Pn = paeonidin.

Indeed, branched oligosaccharides are relatively rare in nature,[19] and other examples are only known as components of steroidal alkaloids, such as solanine and demissine,[20] and of saponins, such as digitonin.[21]

These three anthocyanins with branched trisaccharides (which are more characteristic of fruits than leaves and flowers) have been found in five genera: in the monocotyledon *Clivia* (Amaryllidaceae) and in the four dicotyledons *Begonia* (Begoniaceae), *Prunus* and *Rubus* (Rosaceae) and *Ribes* (Saxifragaceae). Their broad distribution, therefore, has little relation to taxonomy although, as noted above, their distribution within the Rosaceae is at least suggestive of a systematically correlated occurrence. The results of this and earlier work (Table 7) show that a remarkable diversity of glycosidic patterns are present in the Rosaceae.

A study of the flavonol glycosides occurring with the anthocyanidin triglycosides in *Begonia* has shown that the glycosidic patterns of the two types of pigment are not closely

19 D. J. BELL in *Comparative Biochemistry* (Edited by M. FLORKIN and H. S. MASON), Vol. III, p. 755, Academic Press, New York (1962).
20 K. SCHREIBER, *Chem. Tech. (Berlin)* **6**, 648 (1954).
21 R. TSCHECHE and G. WULFF. *Tetrahedron* **19**, 621 (1963).

related (compare the situation in *Primula sinensis*[7]). Thus, flavonols with branched trisaccharides attached to them were not detected. Nevertheless, the occurrence of rutin is of biogenetic significance in relation to the anthocyanins (see below) and the quercetin 3-xyloside found in *B. glaucophylla* probably provides another link because a cyanidin 3-pentoside has been provisionally identified in one species (see Table 1). The unexpected appearance of two 3-*O*-methylated flavonols in the genus is noteworthy, since 3-*O*-methylation of flavonols appears to occur only rarely in plants. However, such compounds may be more widely distributed. Their presence could easily be missed in general plant surveys, since, in their chromatographic behaviour and colour reactions, they are not very different from the commonly occurring flavones (e.g. luteolin).

The natural distribution of anthocyanins with branched trisaccharides, and their co-occurrence with related diglycosides, has some bearing on a possible mode of biosynthesis. The results of these surveys indicate that there is no special enzyme system for synthesis of the trisaccharides; instead, it seems that they are formed whenever the two appropriate disaccharide synthesizing systems are present. Thus, the 2^{G}-glucosylrutinoside is formed in plants, which are able to make *both* sophoroside *and* rutinoside, and similarly for the 2^{G}-xylosylrutinoside. This would mean that the enzyme which transfers glucose $\beta 1 \rightarrow 2$ to cyanidin 3-glucoside from a suitable sugar source (e.g. uridinediphosphate glucose) is not completely substrate specific and is able to make the same transfer to cyanidin 3-rutinoside. The evidence in favour of this hypothesis is as follows:

1. The triglycosides only occur in genera in which systems for making rutinoside and sophoroside *or* rutinoside and sambubioside are present together.
2. The triglycosides almost always co-occur with the appropriate diglycosides (e.g. 2^{G}-glucosylrutinoside with rutinoside and sophoroside). This is evident in *Rubus*, *Ribes* and *Prunus* (Tables 4–6), and is also probable in *Begonia* (Table 1), although the intermediate rutinoside could not there be detected. Instead, however, quercetin 3-rutinoside is of common occurrence in the genus, and it has already been shown[1] that enzyme systems controlling flavonol glycoside formation can, on occasion, operate with anthocyanidin substrates.
3. One plant (*Solanum stoloniferum*) is already known[22] in which the enzyme system for transferring glucose to quercetin 3-glucoside to give the sophoroside is also apparently capable of transferring glucose to the rutinoside to give a linear triglycoside, the 3-glucosylrutinoside.

Studies on the substrate specificities of the enzymes involved in branched trisaccharide synthesis are clearly needed to substantiate this hypothesis and are in progress.

EXPERIMENTAL

Plant material. Most of the plants studied are grown in this Institute. The black raspberries were generously supplied by Dr. R. L. Knight, East Malling Research Station.

Authentic pigments. Sources of authentic anthocyanins are described in an earlier paper.[1] The 3-monomethyl ethers of kaempferol and quercetin were synthesized by the methylation procedure of Simpson,[23] and were separated from the other methyl ethers produced by paper chromatography in 50% HOAc.

[22] J. B. HARBORNE, *Biochem. J.* **84**, 100 (1962).
[23] T. H. SIMPSON and J. L. BETON, *J. Chem. Soc.* 4065 (1954).

Paper chromatography. Solvents used were: BAW, *n*-butanol–acetic acid–water (4:1:5, top layer); BuHCl, *n*-butanol–2N HCl (1:1, top layer); BEW, *n*-butanol–ethanol–ethanol–water (4:1:2·2); BBPW, *n*-butanol–benzene–pyridine–water (5:1:3:3); and HOAc–HCl, acetic acid–conc. HCl–water (15:3:82).

Flavonoid identification. Known pigments were identified by (*a*) spectral measurements, (*b*) identification of the aglycone and sugar produced by acid hydrolysis, and (*c*) co-chromatography in at least four systems with authentic material. Details of the identification of new pigments are given in Tables 2 and 3.

Flavonoid surveys. These were carried out by paper chromatography in a range of solvent systems of direct methanolic-HCl extracts of fruits, leaves or flowers. For separating and detecting the anthocyanins, chromatography in 1% aq. HCl was most valuable.

Hydroxycinnamic acid esters. These were isolated, purified, identified by standard procedures and compared directly with authentic substances.[24] 1-Caffeoylglucose, from *Begonia manicata* leaves, had R_f 0·62 in BAW, 0·56 in BEW and 0·60 and 0·68 in H_2O (the two spots in this solvent are due to the separation of the cis- and trans-isomers), λ_{max} 95% EtOH, 220, 248 and 334 mμ, λ_{max} NaOEt 387 mμ and gave caffeic acid and glucose on alkaline hydrolysis. 1-Feruloylglucose, also from *B. manicata* leaves, had R_f 0·71 in BAW, 0·26 in *n*-butanol–NH_4OH (1:1), 0·64 and 0·73 in 2% HOAc, λ_{max} 95% EtOH, 232 and 328 mμ, λ_{max} NaOEt 377 mμ, and gave ferulic acid and glucose on alkaline hydrolysis. The unidentified *p*-coumaroyl ester from *B. manicata* flowers had R_f 0·63 in BAW and 0·50 in BEW.

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[24] J. B. Harborne and J. J. Corner, *Biochem. J.* **81**, 242 (1961).