THE FLAVONOIDS: ADVANCES IN RESEARCH SINCE; V.1.

1980

The Flavonoids

ADVANCES IN RESEARCH SINCE 1980

Edited by

J.B. HARBORNE

London
CHAPMAN AND HALL
New York

First published in 1988 by
Chapman and Hall Ltd
11.New Fetter Lane, London EC4P 4EE
Published in the USA by
Chapman and Hall
29 West 35th Street, New York NY 10001

Printed in Great Britain by
J.W. Arrowsmith Ltd., Bristol

ISBN 0 412 28770 6

British Library Cataloguing in Publication Data

The flavonoids: advances in research.
Since 1980
1. Flavonoids
I. Harborne, J.B.
547.7 QP925.F5

ISBN 0-412-28770-6

Library of Congress Cataloging in Publication Data

The Flavonoids: advances in research since 1980.

1. Flavonoids. I. Harborne, J.B. (Jeffrey B.)
QK898.F5F554 1988 582'.019'218 88-11879
ISBN 0-412-28770-6

Contributors

B.A. Bohm
Department of Botany, University of British Columbia, Vancouver, Canada

R. Brouillard
Institut de Chimie, Université Louis Pasteur, Strasbourg, France

J. Chopin
Laboratoire de Chimie Biologie, Université Claude Bernard, Villeurbanne, France

G. Dellamonica
Laboratoire de Chimie Biologie, Université Claude Bernard, Villeurbanne, France

P.M. Dewick
Department of Pharmacy, University of Nottingham, UK

D.M.X. Donnelly
Department of Chemistry, University College, Dublin, Eire

G. Forkmann
Biologisches Institut II, Tubingen, West Germany

H. Geiger
Windhalmweg 14, D 7000 Stuttgart 70, West Germany

D.E. Giannasi
Department of Botany, University of Georgia, Athens, USA

R.J. Grayer
Plant Science Laboratories, University of Reading, UK

J.B. Harborne
Plant Science Laboratories, University of Reading, UK

W. Heller
Institut für biochemisches Pflanzenpathologie, Neuherberg, West Germany

M. Jay
Institut für Botanik, Technische Hochschule, Darmstadt, West Germany

K.R. Markham
Chemistry Division, DSIR, Petone, New Zealand

G.J. Niemann
Botanisch Laboratoire, Rijksuniversiteit, Utrecht, The Netherlands

L.J. Porter
Chemistry Division, DSIR, Petone, New Zealand

C.J. Quinn
School of Botany, University of New South Wales, Australia

M. Helen Sheridan
Department of Chemistry, University College, Dublin, Eire

E. Wollenweber
Institut für Botanik, Technische Hochschule, Darmstadt, West Germany

Preface

The major purpose of this third volume in *The Flavonoids* series is to provide a detailed review of progress in the field during the five years, 1981–1985 inclusive. It thus continues the comprehensive coverage of the literature on these fascinating and important plant pigments which began in 1975 with the publication of *The Flavonoids* and which was followed in 1982 with *The Flavonoids: Advances in Research*. As with the two previous volumes, this one is entirely self-contained and where necessary tabular data and references from earlier volumes are included and expanded here. A unique feature is the complete listing in the Appendix of all known flavonoids, which now number over 4 000 structures; in this list, structures newly reported during the period 1981–1985 are so indicated.

The first ten chapters of this book provide a critical review of the new substances that have been discovered among each of the main classes of flavonoid during the period under review. Again, the number of new isoflavonoids reported outweighs that of other classes and a hundred pages are needed to describe all the novel findings. Neoflavonoids, which were omitted in the first supplement, have been included again and a special chapter on miscellaneous flavonoids has been introduced to cope with those structures (e.g. homoisoflavonoids) which do not fit in easily anywhere else. Although there have been advances in flavonoid methodology, these have not been as spectacular as in earlier years. Hence, literature reports on new chromatographic and spectral procedures are included here in the individual chapters under the different flavonoid classes.

Major developments have taken place in flavonoid biosynthesis with the description of many new enzymes of the pathway and a separate chapter by W. Heller and G. Forkmann covering the 1981–1985 literature is therefore provided. Significant advances have also occurred in our understanding of the way that anthocyanins provide *in vivo* colour in flower petals and R. Brouillard reviews these advances in the last chapter. The dominant themes of the remaining four chapters in the second half of the book are the natural distribution and the evolution of the flavonoids. The first really critical and comprehensive listing of flavonoid occurrences in algae, bryophytes and ferns is provided by K.R. Markham in his chapter on their distribution in lower plants, while G.J. Niemann likewise provides a detailed account of their presence in gymnosperms. The most abundant and prolific sources of flavonoids are the flowering plants and so much is known here that separate books would be required to provide comprehensive

coverage of flavonoid occurrences in the angiosperms. Here, we have two contrasting chapters outlining the relationship between flavonoid patterns and plant evolution, first in the dicotyledons and then in the monocotyledons.

Inevitably, some areas of recent flavonoid research have had to be omitted in order to keep the book within the limit set by the publishers. For example, flavonoids have recently been shown to have a signalling function in nitrogen fixation through their ability to regulate the nodulating genes of the *Rhizobium* bacterium. Other recent studies have shown that flavone glycosides are involved as oviposition stimulants to swallowtail butterflies. I hope to review these and other developments in the ecology, physiology and biochemistry of flavonoids in a third supplement. In the meantime I would welcome comments, criticisms and suggestions from readers about this series. As editor, I am most grateful to all the contributors who have once again carried out their taxing assignments on time. Because of other commitments, my co-editor, T.J. Mabry was unable to contribute to this volume but he hopes to take part in future developments. I would personally like to thank my two co-workers at Reading, who have joined me in writing three of the chapters, and also the many students and visiting scientists who have worked with me on flavonoid projects in recent times. Finally, I am most grateful to the publishers for their continued support and interest in this endeavour.

December, 1987

JEFFREY B. HARBORNE
Reading

Contents

1

The anthocyanins

JEFFREY B. HARBORNE and RENEE J. GRAYER

1.1 INTRODUCTION

Anthocyanin pigmentation is almost universal in the flowering plants and provides scarlet to blue colours in flowers, fruits, leaves and storage organs. It continues to provide a challenge to plant biochemists because of the intricate chemical variation and the complexity of biosynthesis, metabolism and regulation. The two most important recent advances in the structural characterization of anthocyanin pigments have been the application of high performance liquid chromatography (HPLC) and of fast atom bombardment mass spectrometry (FAB-MS) to their analyses. Both these procedures have proved of value in studying zwitterionic anthocyanins, a relatively new class of acylated anthocyanin recently recognized to be widespread in the plant kingdom (Harborne and Boardley, 1985). These anthocyanins, which are acylated through sugar by such acids as malonic, are labile in solution and when such pigments are isolated using solvents containing mineral acid, they are rapidly degraded to the corresponding unacylated glycoside. They are, however, stable in the solid state and molecular ions can be obtained by means of FAB-MS (Saito *et al.*, 1983). Work has continued on anthocyanins substituted by hydroxycinnamic acids and extensive proton NMR studies have allowed the characterization of the complex pigment of *Ipomoea purpurea*, a peonidin glycoside which contains six glucose and three caffeyl substituents (Goto, 1984). Studies of the blue pigment of *Commelina communis* have finally shown, after some earlier controversy, that this anthocyanin probably occurs *in vivo* as a dimagnesium complex in which six anthocyanin and six flavone molecules are linked either covalently or by hydrogen bonding (Goto *et al.*, 1986).

Our knowledge of the natural distribution of anthocyanins has been enhanced during the period 1981–1985 by surveys in families such as Araceae, Bromeliaceae, Commelinaceae and Polygonaceae (See Section 1.4). Only one new anthocyanidin has been reported, 6-hydroxycyanidin, but a variety of new glycosides have been described, including several with sugars unusually substituted in the B-ring. Such substitution can cause a hypsochromic shift in flower colour and a bright scarlet pigment from bromeliads has been recognized as cyanidin 3, 5, 3′-triglucoside (Saito and Harborne, 1983).

An important discovery about the biosynthetic pathway to anthocyanins has been the recognition of flavan-3, 4-diols (leucoanthocyanidins) as the immediate precursors of the flavylium cations (see Chapter 11). Genetic studies have also enhanced our knowledge of biosynthesis: the structure of the gene for chalcone synthase in *Antirrhinum* has been elucidated (Sommer and Saedler, 1986) and the *c* locus in *Zea mays*, which regulates anthocyanin synthesis, has been cloned by transposon tagging (Paz-Ares *et al.*, 1986).

In this review of anthocyanin research of the period 1981–1985, advances in analytical procedures will be described first. New aglycones, glycosides and acylated glycosides will then be enumerated. This will be followed by a review of the new reports on the natural distribution of these pigments in plants. This review updates three earlier listings (Harborne, 1967; Timberlake and Bridle, 1975; Hrazdina, 1982). These four listings together provide a complete record of anthocyanin occurrences in plants to the generic, and in most cases, to the specific level. A book on anthocyanins as food colours was published by Markakis (1982), and the industrial and medical applications of anthocyanin research will be considered in the final section. The role of anthocyanins in

The Flavonoids. Edited by J.B. Harborne
Published in 1988 by Chapman and Hall Ltd,
11 New Fetter Lane, London EC4P 4EE.

flower coloration will not be mentioned here, since this is the subject of a separate review later in this volume (see Chapter 16).

1.2 ANALYTICAL PROCEDURES

1.2.1 New chromatographic techniques

The HPLC of anthocyanins, which was pioneered during the 1970 s (see Hrazdina, 1982), has now become routine in most laboratories. It has the advantage over other chromatographic procedures of sensitivity, rapidity and easy quantification but the apparatus is expensive to set up and maintain. When coupled with a diode array detector, HPLC provides an almost ideal procedure for accurately analysing, quantitatively and qualitatively, the complex mixtures of pigments present in cultivated flowers and fruits.

Asen, who first applied the HPLC of anthocyanins to cultivar identification in the case of poinsettias, has described its use in the case of geraniums (Asen and Griesbach, 1983) and of *Gerbera* flowers (Asen, 1984). Akavia *et al.* (1981) have used HPLC to separate and quantify the nine anthocyanins variously present in *Gladiolus* cultivars. Van Sumere and van de Casteele (1985) have similarly applied the HPLC of anthocyanins to the differentiation of *Rhododendron* cultivars.

HPLC is also useful in many other ways. It can be applied to the detection of intermediates in the partial hydrolysis of anthocyanins with more than one sugar residue and may reveal compounds which are not apparent when PC or TLC is used (Strack *et al.*, 1980). Its greater sensitivity means that it is the method of choice when only small amounts of fresh plant tissue are available for anthocyanin analysis. Schram *et al.* (1983) were able to detect cyanidin 3-glucoside and 3-diglucoside in a rare flower mutant of *Petunia hybrida*, when only 9 mg wet weight of flower were available. Again in plant tissue culture, HPLC has proved useful for comparing pigmentation in callus or suspension culture with the pigments of the parent plant. Such analyses, in the case of *Petunia*, have shown mainly malvidin glycoside with traces of petunidin glycoside in the intact plant, with these pigment concentrations being reversed in tissue culture (Colijn *et al.*, 1981).

Typical HPLC retention times for some of the more common anthocyanins are shown in Table 1.1. From these data, it appears that glycosylation increases mobility on a reversed phase column, with 3, 5-diglycosides moving off the column faster than 3-diglycosides. Increasing hydroxylation of the anthocyanidin improves the mobility, and *O*-methylation reverses this trend so that malvidin glycosides are generally eluted after the other anthocyanins. Acylation with either aromatic acids (e.g. *p*-coumaric) or aliphatic acids (e.g. malonic) increases the retention time. Some typical values for acylated anthocyanins and their unacylated analogues are shown in Table 1.2.

TLC continues to be widely employed for anthocyanin separation, because it is a highly effective, convenient and inexpensive technique. Andersen and Francis (1985) have shown that it is possible to analyse anthocyanins and anthocyanidins simultaneously on cellulose layers, if the solvent system conc. HCl–formic acid–water (24.9:23.7:51.4) is used.

For preparative work, droplet counter current chromatography (DCCC) has been used with the anthocyanins of

Table 1.1 Retention times (t_R) of anthocyanins on a Lichrosorb RP-18 column eluted with a water–acetic acid–acetonitrile gradient

Pigment		t_R *(min)**
3-Rhamnosylglucoside-5-glucoside	Dp†	15.4
	Cy	19.1
	Pt	22.0
	Pg	22.2
	Pn	25.8
	Mv	28.4
3, 5-Diglucoside	Dp	18.2
	Cy	21.7
	Pt	25.0
	Pn	25.0
	Pg	25.3
	Mv	32.1
3-Rhamnosylglucoside	Dp	27.0
	Cy	31.1
	Pt	33.6
	Pg	34.8
	Pn	39.0
	Mv	42.8

†For key to anthocyanidin abbreviations, see Table 1.3.
*Data from Akavia *et al.* (1981).

Table 1.2 Effect of acylation on anthocyanin HPLC retention times

Pigment	RR_t
Pg 3, 5-diglucoside	1.00*
Pg 3-(6″-malonylglucoside)-5-glucoside	1.87
Pg 3, 5-di(malonylglucoside)	2.67
Cy 3-glucoside	1.00*
Cy 3-malonylglucoside	2.14
Cy 3-dimalonylglucoside	2.96
Mv 3-rhamnosylglucoside-5-glucoside	1.00†
Mv 3-(*p*-coumarylrhamnosylglucoside)-5-glucoside	4.00

*On a Spherisorb-hexyl column at 35 °C eluted with a 0.6% aq. $HClO_4$–MeOH gradient (Takeda *et al.*, 1986a).
†On a Lichrosorb 10 RP-18 column eluted with a MeOH–HCO_2H–H_2O gradient (Schram *et al.*, 1983).

blackcurrants and the solvent system: butan-1-ol–acetic acid–water (4:1:5) (Francis and Andersen, 1984). However, HPLC can also be used for semi-preparative separations, with a wider column than in analytical work, and this probably has the edge over DCCC. A technique for the automated HPLC separation of anthocyanins in blackberry and cranberry on the preparative scale has been described by Hicks *et al.* (1985).

1.2.2 Spectral methods

The first application of FAB-MS to anthocyanin identification was that of Saito *et al.* (1983) on the known pigment violanin from *Viola* and on a novel pigment platyconin from *Platycodon grandiflorum*. Since then the method has been widely used for the characterization of acylated anthocyanins, especially zwitterionic pigments carrying malonyl substitution (Bridle *et al.*, 1984; Takeda *et al.*, 1986a) (see Section 1.3.4). Proton and ^{13}C NMR spectroscopy also continue to be applied with success to anthocyanin structural elucidation, together with the newer ^{1}H–^{1}H correlated spectroscopy (COSY) (Tamura *et al.*, 1983; Bridle *et al.*, 1984; Kondo *et al.*, 1985). Further details of the application of spectral techniques to anthocyanin studies are discussed by R. Brouillard in Chapter 16.

1.3 CHEMISTRY

1.3.1 New anthocyanidins

To the 16 known anthocyanidins, one new structure has to be added, namely 6-hydroxycyanidin (Table 1.3). This has been found in the red flowers of *Alstroemeria* (Alstroemeriaceae) where it occurs as the 3-glucoside and 3-rutinoside (Saito *et al.*, 1985b). Its structure was established by spectral measurements and comparison with the literature data. The related 6-hydroxypelargonidin (aurantinidin) is known (Clevenger, 1964; Jurd and Harborne, 1968) but the delphinidin analogue has yet to be described.

Table 1.3 Known anthocyanidins

Name	*Abbreviation*	*Structure*
Apigeninidin	Ap	5,7,4′-TriOH
Luteolinidin	Lt	5,7,3′,4′-TetraOH
Tricetinidin	Tr	5,7,3′,4′,5′-PentaOH
Pelargonidin	Pg	3,5,7,4′-TetraOH
Aurantinidin	Au	3,5,6,7,4′-PentaOH
Cyanidin	Cy	3,5,7,3′,4′-PentaOH
5-Methylcyanidin	5MCy	5-Methyl ether
Peonidin	Pn	3′-Methyl ether
Rosinidin	Rs	7,3′-Dimethyl ether
6-Hydroxycyanidin	6OHCy	3,5,6,7,3′,4′-HexaOH
Delphinidin	Dp	3,5,7,3′,4′,5′-HexaOH
Petunidin	Pt	3′-Methyl ether
Malvidin	Mv	3′,5′-Dimethyl ether
Pulchellidin	Pl	5-Methyl ether
Europinidin	Eu	5,3′-Dimethyl ether
Capensinidin	Cp	5,3′,5′-Trimethyl ether
Hirsutidin	Hs	7,3′,5′-Trimethyl ether

The first report of 5-methylcyanidin as a new anthocyanidin in *Egeria densa* (Elodeaceae) (Momose *et al.*, 1977) was inadvertently omitted from the text of the last review (Hrazdina, 1982) but is now included in Table 1.3. Europinidin, which was found in petals of *Plumbago europaea* (Harborne, 1966), was not analysed at the time because of shortage of material. Its structure as the 5,3′-dimethyl ether of delphinidin has now been confirmed by FAB-MS, which gave the required molecular ion $[M+H]^+$, at 331 (Harborne and Self, unpublished results).

Several other anthocyanidins have been partly described (i.e. carajurin from *Arrabidaea*, carexidin from *Carex*, columnidin from *Columnea*, purpurinidin from *Salix* and margicassidin from *Cassia*) (cf. Timberlake and Bridle, 1975) but these still await complete characterization. X-ray crystallography of pelargonidin bromide monohydrate has shown that this anthocyanidin is nearly planar in the solid state (Saito and Ueno, 1985). The phenyl ring makes a dihedral angle of only 3.8° with the benzopyrylium moiety. A new total synthesis of apigeninidin and luteolinidin has been described (Sweeny and Iacobucci, 1981). It involves the oxidative decarboxylation of the corresponding 4-carboxyflav-2-enes with lead tetra-acetate and the yields are much higher than in the traditional method starting from 2-*O*-benzoylphloroglucinaldehyde.

1.3.2 New glycosides

The most unusual feature of the new anthocyanidin glycosides reported over the last five years (Table 1.4) is the regular presence, in nine pigments, of B-ring hydroxyl groups which are linked to glucose. Such compounds are not completely new; for example, an acylated derivative of delphinidin 3-rutinoside-5,3′,5′-triglucoside was reported in *Lobelia* by Yoshitama in 1977. However, such substances have been reported rarely; it is now apparent that they may occur in a range of plants and have a variety of substitution patterns. Major sources are members of the Bromeliaceae (Saito and Harborne, 1983) but they have also been found in plants of the Commelinaceae, Compositae, Gentianaceae, Leguminosae, Liliaceae and Lobeliaceae.

Substitution of a B-ring hydroxyl by sugar causes a hypsochromic shift in the visible spectrum which makes it easy to recognize such glycosides. For example, while cyanidin 3,5-diglucoside has a visible maximum at 526 nm in methanolic HCl, the 3,3′-diglucoside has a maximum at 519 nm and the 3,5,3′-triglucoside at 518 nm. This has an

Table 1.4 New glycosides of anthocyanidins

Pigment	*Source*	*References*
Cy 3-rhamnosylarabinoside	*Cissus sicyoides*	Toledo *et al.* (1983)
Cy 3,3′-diglucoside Cy 3,5,3′-triglucoside Cy 3-rutinoside-3′-glucoside Cy 3-rutinoside-5,3′-diglucoside	From Bromeliaceae in leaf, sepal, bract or petal	Saito & Harborne (1983)
Cy 3,7,3′-triglucoside*	*Senecio cruentus* flowers	Yoshitama (1981)
Cy 3-glucuronosylglucoside*	*Helenium autumnale* flower rays	Takeda *et al.* (1986a)
6OHCy 3-glucoside 6OHCy 3-rutinoside	*Alstroemeria* cvs	Saito *et al.* (1985b)
Pn 3-arabinoside-5-glucoside	*Polygonum longisetum* sepal	Yoshitama *et al.* (1984)
Dp 3,7-diglucoside	*Aristotelia chilensis* fruit	Diaz *et al.* (1984)
Dp 3-rhamnosylglucoside-7-xyloside	*Olea europaea* fruit	Tanchev *et al.* (1980a,b)
Dp 3,5,3′-triglucoside*	*Gentiana makinoi* petals	Goto *et al.* (1982).
Dp 3,7,3′-triglucoside†	*Puya* petals	Scogin (1985)
Dp 3,3′,5′-triglucoside*	*Clitoria ternatea* petals	Saito *et al.* (1985a)
Pt 3-(2^G-glucosylrutinoside)-5′-glucoside‡	*Ophiopogon jaburan* seed coat	Yoshitama (1984)
Mv 3-xyloside-5-glucoside*	*Tibouchina granulosa* petals	Francis *et al.* (1982)
Mv 3,7-diglucoside	*Aristotelia chilensis* fruit	Diaz *et al.* (1984)

*Only occurring in acylated form (see Tables 1.5 or 1.6 for details).
†Previously known in acylated form; now reported as the simple glycoside.
‡First reported in 1976–1982 period, but omitted from the 1982 list.

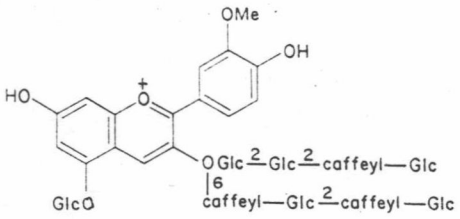
effect *in vivo* on flower colour, and, in the Bromeliaceae, these unusual cyanidin glycosides are found in bracts or petals which are scarlet rather than crimson in colour (Saito and Harborne, 1983). Pigments such as cyanidin 3,5,3′-triglucoside can be characterized by partial hydrolysis, since they produce all the expected intermediates before complete hydrolysis to the aglycone. Their structures can be confirmed by FAB-MS, when intense molecular ions are obtainable, with fragment ions caused by sequential loss of the different glucose moieties.

The only new monosaccharide reported since 1982 in association with anthocyanidins is glucuronic acid. Cyanidin 3-glucuronosylglucoside has been found in malonated form (see Section 1.3.4) in flowers of *Helenium autumnale* (Takeda *et al.*, 1986a). As expected, it is zwitterionic in character and is highly resistant to acid hydrolysis.

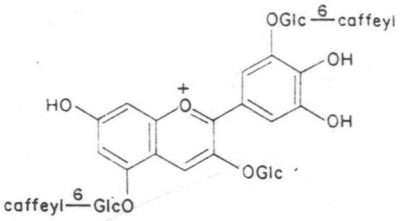
In addition to the glycosides listed in Table 1.4, there have been several tentative reports of 'new' glycosides which need further investigation. Bobbio *et al.* (1983) provisionally detected 3-maltosides in the seed and skin of *Cyphomandra betacea* in spite of an earlier finding of 3-rutinosides in the same plant cultivated in New Zealand (Wrolstad and Heatherbell, 1974). Again, Tsukui *et al.* 1983 describe an acylated cyanidin 3-glucosylfructoside-5-xyloside as a tuber skin pigment of the sweet potato *Ipomoea batatas*. This is at variance with earlier identifications of acylated 3-sophoroside-5-glucosides in *Ipomoea* (see Hrazdina, 1982, and Table 1.8). Since fructose is so rarely found as a glycosidic component of flavonoids, this work needs confirmation before it can be accepted.

1.3.3 Anthocyanins with aromatic acylation

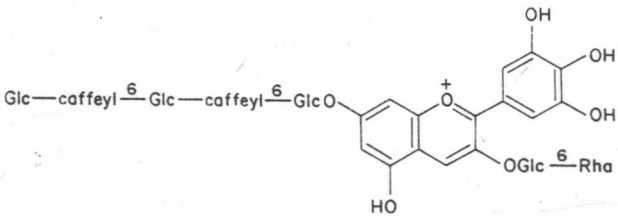
An example of the structural complexity that can be present in anthocyanins acylated with hydroxycinnamic acids is provided by the pigment 'heavenly blue anthocyanin' (HBA) from the flowers of *Ipomoea purpurea*. This is now recognized as having structure (*1.1*) and is probably the largest anthocyanin so far reported with a molecular weight of 1759. Its structure was established by extended proton NMR studies on the original pigment and on two partly deacylated derivatives (Goto, 1984). It is a peonidin 3-sophoroside-5-glucoside substituted through sugar with three caffeylglucose residues. Two other similar pigments have also recently been characterized: gentiodelphin (*1.2*) from *Gentiana makinoi* and platyconin (*1.3*) from *Platycodon grandiflorum*. Other related pigments that still await full characterization are present in flowers of members of the Commelinaceae (Stirton and Harborne, 1980), other than in the genus *Commelina*.



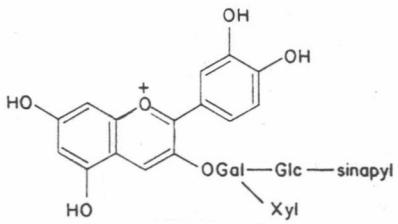
Heavenly blue anthocyanin (*1.1*)



Gentiodelphin (*1.2*)



Platyconin (*1.3*)



Carrot anthocyanin (*1.4*)

Cyanidin and delphinidin 3, 7, 3′-triglucosides are present variously trisubstituted with caffeic and/or ferulic acid residues but the positions of attachment have yet to be determined.

A list of recently characterized anthocyanins of this general type are shown in Table 1.5. Pigments which have both aromatic and aliphatic acyl substituents are discussed in the next section. Apart from pigments (*1.1*)–(*1.3*), most of the anthocyanins in the table are still incompletely characterized in as much as the position of acylation on the sugar is often unknown. One of these acylated pigments deserves further comment, namely cyanidin 3-(sinapylxylosylglucosylgalactoside) (*1.4*), one of the constituents of *Daucus carota* (Harborne, 1976). In 1982, Hemingson and Collins described four simple glycosides of cyanidin from tissue cultures of the same plant. Since none of these pigments occurs in the intact carrot, their presence in tissue culture was unexpected. Re-examination of carrot tissue culture showed, however, that pigment (*1.4*) was the only major constituent (Harborne *et al.*, 1983). The erroneous report of Hemingson and Collins arose mainly from the fact that these authors inadvertently partly hydrolysed the pigment during purification. Recent FAB-MS studies on (*1.4*) (Harborne and Self, unpublished results) have indicated that it has a branched trisaccharide with the sinapyl residue located on the glucose residue, but further work is needed to determine the exact modes of linkage. A report by Canbas (1985) based only on spectral measurements of malvidin and peonidin 3-glucosides in the purple–black carrot root can be dismissed in view of the earlier detailed characterization of acylated cyanidin glycosides from the same source (Harborne, 1976).

It is remarkable how many reports appear in the anthocyanin literature, especially on food plants, where the investigators appear to completely overlook previous studies on the same or on a closely related species. If the results agree with the previous work, as in the case of Lu (1985) reinvestigating radish pigments, there is no harm done. However, if the results are completely at variance with earlier investigations, then there can be considerable confusion. For example, anthocyanins with aromatic acylation have recently been described in raspberries (Joo and Park, 1983) and in elderberries (Shin and Ahn, 1980) in spite of the fact that earlier investigators only found simple glycosides in these fruits (cf. Timberlake and Bridle, 1975; Hrazdina, 1982). Since acyl groups are labile and can be lost during isolation and purification (see next section), it is always possible that acylated pigments may have been overlooked during the earlier work. However, in these two cases, the new data are ambiguous and it is our view that further work is needed before it can be accepted that acylated pigments occur in these two fruits.

A report of vanillic acid as an acyl group in pigments of *Helianthus annuus* achenes (Vaccari *et al.*, 1981) is based on incorrect spectral evidence: a peak at 240 nm in one of the pigments is assumed to be due to the vanillyl substituent in spite of the fact that vanillic acid actually has a maximum at 258 nm. Again, further investigation is necessary before this phenolic acid is added to the list of known acylating groups.

1.3.4 Zwitterionic anthocyanins

Until recently, acylated anthocyanins were known to be substituted by hydroxycinnamic acids (*p*-coumaric,