Phytochemistry, 1981, Vol. 20, pp. 297–303. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0201-0297 $02.00/0

STRUCTURAL ASPECTS OF ANTHOCYANIN–FLAVONOID COMPLEX FORMATION AND ITS ROLE IN PLANT COLOR

LAO-JER CHEN and GEZA HRAZDINA

Department of Food Science and Technology, Cornell University, Geneva, NY 14456, U.S.A.

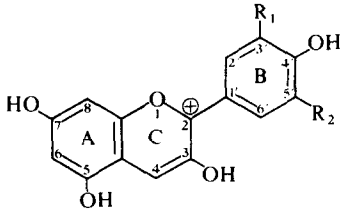
(*Received* 28 *April* 1980)

Key Word Index—Anthocyanin; flavonoid glycosides; synthesis; complex formation; plant color; spectral properties.

Abstract—The complex formation of flavonoids with anthocyanins, resulting in increase in both absorbance and in a bathochromic shift of the visible absorption maximum of the latter, is based mainly on hydrogen bond formation between the carbonyl group of the anthocyanin anhydrobase and aromatic hydroxyl groups of the complex-forming flavonoids. The larger the number of hydroxyl groups in the flavonoid molecule, the stronger the complex formation. The presence of a 3-hydroxyl group in the flavonoid molecule has little effect on the complex-forming ability. The nature of the sugar substituent of the complex-forming flavonoid compound has no influence on the reaction. The 5-hydroxyl group of flavonoids is strongly bound by intramolecular hydrogen bond to the 4-carbonyl and does not participate in the complex formation. The most important hydroxyl group in the flavonoid molecule is the one in the 7-position. Unsaturation at C_2–C_3 in the heterocyclic ring is an important factor for complex formation. Aromatic hydroxyl groups in the flavonoid system alone cannot account for all the complex-forming ability, suggesting additional involvement by electrostatic forces and configurational or steric effects.

INTRODUCTION

Anthocyanins, in addition to other pigments, play an important role in expression of plant color. Anthocyanins are usually isolated in acidic environment as the orange-red colored flavylium salts. The most commonly occurring are the glycosides of pelargonidin **1**, cyanidin **2** and its monomethyl ether peonidin **3**, and delphinidin **4**, its monomethyl ether petunidin **5**, and the dimethyl ether malvidin **6** (Fig. 1). All these pigments show a visible absorption maximum at 505–530 nm. Because of the minimal changes in color caused by the different B-ring substituents, the diversity of plant color ranging from orange to blue cannot be explained by the chemical properties of anthocyanins alone.



1 $R_1 = R_2 = H$
2 $R_1 = OH$; $R_2 = H$
3 $R_1 = OMe$; $R_2 = H$
4 $R_1 = R_2 = OH$
5 $R_1 = OMe$; $R_2 = OH$
6 $R_1 = R_2 = OMe$

Fig. 1. Structure of the most commonly occurring anthocyanins. **1**, pelargonidin; **2**, cyanidin; **3**, peonidin; **4**, delphinidin; **5**, petunidin; **6**, malvidin.

It was suggested by the Robinsons in the 1930s [1–3], that plant colors in the orange to blue region are caused by co-pigmentation of anthocyanins with other natural products. Blue colors in the Plant Kingdom were thought to be caused by anthocyanin–metal complexes [4, 5], or by a resonance-stabilized anion at slightly alkaline pH values [6]. It has been shown unequivocally that anthocyanins, similarly to other secondary plant metabolites, accumulate solely in the vacuole of plant cells [7]. The vacuolar content of plant cells is acidic with an average value of 3.70–4.15 in rose petals [8] and 2.6–3.2 in grape epidermal tissue (Moskowitz, A. H. and Hrazdina, G., unpublished data). In this pH range the anthocyanins undergo reversible structural transformation reactions (Fig. 2). The anthocyanins are present, at pH values of 1 and below, quantitatively in the red-colored flavylium salt form **1**. Upon increase in pH to 6, the flavylium salt converts into the purple-colored anhydrobases **7** and **7a**. The anhydrobases are labile and upon nucleophylic attack of water transform to the colorless carbinol base **8** [9–14]. In the pH range of *ca* 4–4.5, the general pH range of plant vacuoles, these pigments are in the pure state colorless.

Asen's work [15–18] showed that among the natural plant products investigated, flavonoids formed strong complexes with anthocyanins in this pH range, and that most flower color and visible spectrum could be duplicated by forming various complexes between anthocyanins and flavonoids. This complex formation results in both a bathochromic shift in the visible λ_{max} and in an increase in absorbance of the pigment solutions. Stabilization of the anhydrobase forms **7** and **7a** was shown to be responsible for the complex formation [19, 20].

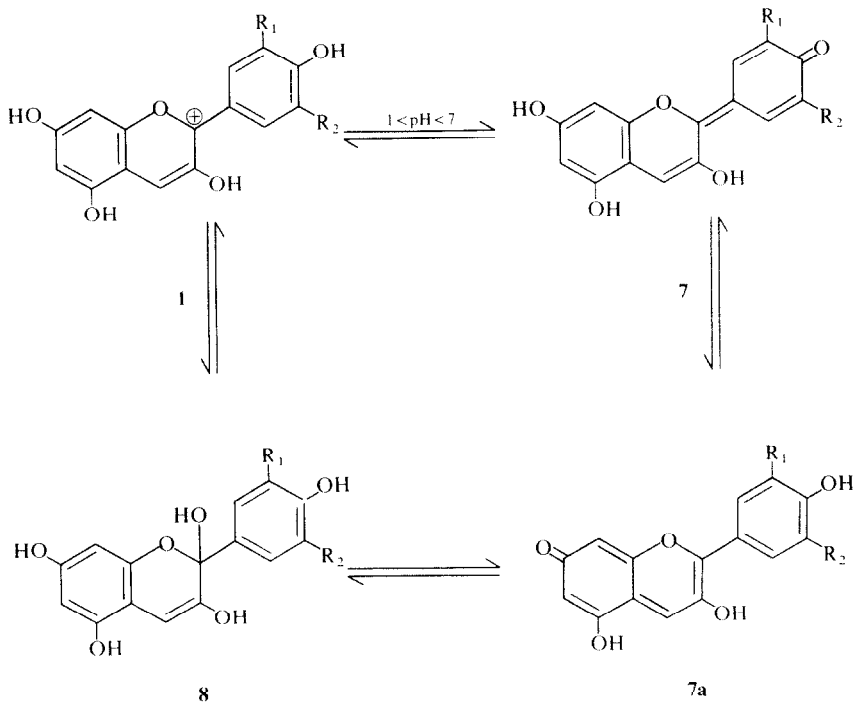


Fig. 2. Structural transformations of anthocyanidins in the pH range 0–6. The red-colored flavylium salt **1** converts into the purple anhydro base forms **7** and **7a**; these, upon nucleophilic attack of H_2O, are transformed to the colorless carbinol base **8**.

In this paper we report the effect of various substituents and unsaturation at C_2–C_3 in the heterocyclic ring of flavonoids on the complex formation with anthocyanins.

RESULTS

The structures of flavonoids used in this investigation, are shown in Fig. 3. Complex formation with these flavonoids results both in a bathochromic shift of the absorption maxima and increase in absorbance of the pigment solutions. Therefore, the effects of the complex formation are expressed as $\Delta\lambda_{max}$ (nm) and ΔA/mm path length, as shown in Table 1.

The major factor in the complex formation at pH 3.2 is hydrogen bonding between the carbonyl group of the anthocyanin anhydrobases and the aromatic hydroxyl groups of the flavonoids. Myricetin, **9N**, a hexahydroxyflavone gave the strongest response, followed by quercetin, **9L**, a pentahydroxyflavone and fisetin, **9M**, a tetrahydroxyflavone. There is no significant difference in the response between quercetin and fisetin, compounds differing only in one hydroxyl group in the 5-position. Apparently a strong intramolecular hydrogen bond between this hydroxyl and the adjacent 4-carbonyl prevents it from participation in the reaction.

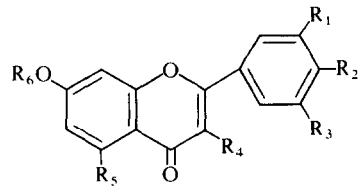
The 3-hydroxyl group of flavonoids is not involved in the complex formation with anthocyanins as the data with apigenin glycoside **9B** and kaempferol glycoside **9D** show. The nature of the sugar substituent plays no role in the reaction (compare data with **9A**, **9B** and **9F**, **9G**, **9H**). Saturation of the C_2–C_3 bond (as in flavanones **10A**–**10E**, flavanonols **11A** and flavanols **11B**) markedly decreases the complex-forming properties of the compounds.

Ring opening (compare poncirin, **10C** to poncirin chalcone **12**) increases the ability of flavanone glycosides to form complexes. Unsaturation between Cα and Cβ increases the complex-forming ability of chalcone derivatives. Chemical precursors, such as phloroacetophenone 4-neohesperidoside, **14A**, show slight activity. Simple phenols, such as phloroglucinol **14B**, are not effective.

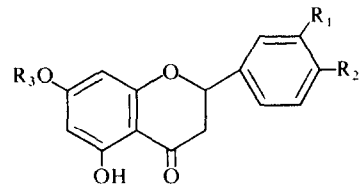
Although the complex formation of flavonoids has been investigated with the 3,5-diglucosides of malvidin **1**, cyanidin **2**, and petunidin **5**, with malvidin 3-glucoside and malvidin 3-(6-*O*-*p*-coumarylglucoside)-5-glucoside, only the data obtained with malvidin 3,5-diglucoside are shown here. The flavonoids investigated here behaved similarly in reactions with other anthocyanins. However, the anthocyanins other than malvidin 3,5-diglucoside formed complexes to a lesser degree, in decreasing order: cyanidin 3,5-diglucoside, petunidin 3,5-diglucoside, malvidin-3-(6-*O*-*p*-coumarylglucoside)-5-glucoside and malvidin 3-glucoside.

DISCUSSION

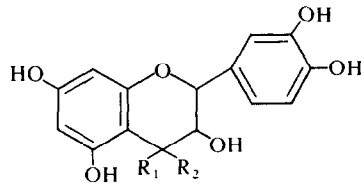
While the complex formation of anthocyanins with metals occurs only with pigments containing an *ortho*-dihydroxyl system [21–23], flavonoids form complexes with all anthocyanins. Spectral studies on structural transformation of anthocyanins and flavylium salts [9–14], and on the properties of flavonoid–anthocyanin complexes [16, 19, 20] show that the changes in absorbance and absorption maximum of the pigment solutions are caused by the stabilization of the anhydrobase forms **7** and **7a**. The nature of the reaction seems to be physical rather than chemical. Chromatographically, no new product can be detected in the reaction mixture. The complex formation is



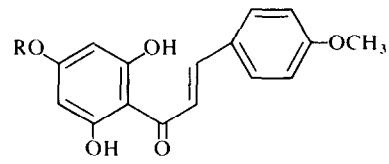
9



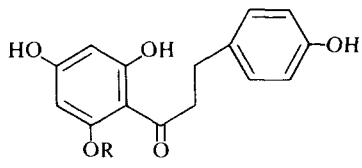
10



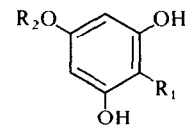
11



12



13



14

Flavone glycosides	R_1	R_2	R_3	R_4	R_5	R_6
9A Apigenin 7-glucoside	H	OH	H	HOH	H	Glc
9B Apigenin 7-neohesperidoside	H	OH	H	H	OH	Glc–Rha
9C Diosmetin 7-rutinoside	OH	OMe	H	H	OH	Glc–Rha
Flavonol glycosides and flavonols						
9D Kaempferol 7-neohesperidoside	H	OH	H	OH	OH	Glc–Rha
9E Kaempferol 3-robinobioside 7-rhamnoside (Robinin)	H	OH	H	O–Gal–Rha	OH	Rha
9F Quercetin 3-glucoside	OH	OH	H	O–Glc	OH	H
9G Quercetin 3-rhamnoside	OH	OH	H	O–Rha	OH	H
9H Quercetin 3-rutinoside	OH	OH	H	O–Glc–Rha	OH	H
9I Quercetin 7-neohesperidoside	OH	OH	H	OH	OH	Glc–Rha
9J Isorhamnetin 3-glucoside	OMe	OH	H	O–Glc	OH	H
9K Tamarixetin 7-rutinoside	OH	OMe	H	OH	OH	Glc–Rha
9L Quercetin	OH	OH	H	OH	OH	H
9M Fisetin	OH	OH	H	OH	H	H
9N Myricetin	OH	OH	OH	OH	OH	H

Flavanone glycosides	R_1	R_2	R_3
10A Pinocembrin 7-neohesperidoside	H	H	Glc–Rha
10B Naringenin 7-neohesperidoside	H	OH	Glc–Rha
10C Isosakuranetin 7-neohesperidoside	H	OMe	Glc–Rha
10D Eriodictyol 7-neohesperidoside	OH	OH	Glc–Rha
10E Hesperetin 7-rutinoside	OH	OMe	Glc–Rha

Flavanonols and flavanols	
11A Taxifolin	$R_1, R_2 = O$
11B (+)-Catechin	$R_1 = R_2 = H$

Chalcone and dihydrochalcone glycosides	
12 Poncirin chalcone	R = Glc–Rha
13 Phloridzin	R = Glc

Phloroglucinol derivatives	R_1	R_2
14A Phloroacetophenone 4-neohesperidoside	Ac	Glc–Rha
14B Phloroglucinol	H	H

Fig. 3. Structures of flavanones, flavonols, flavones, chalcones, phloroglucinol derivatives and their glycosides synthesized and used in this investigation.

Table 1. Complex formation of flavonoid compounds and phloroglucinol derivatives (4×10^{-3} M) with malvidin 3,5-diglucoside (1×10^{-3} M)

Flavonoids	$\Delta\lambda_{max}$ (nm)	ΔA/mm at λ_{max}
9A Apigenin 7-glucoside	31	1.29
9B Apigenin 7-neohesperidoside	32	1.31
9D Kaempferol 7-neohesperidoside	29	1.22
9E Kaempferol 3-robinobioside-7-rhamnoside (robinin)	27	1.17
9F Quercetin 3-glucoside (isoquercitrin)	30	1.48
9G Quercetin 3-rhamnoside (quercitrin)	29	1.49
9H Quercetin 3-rutinoside (rutin)	30	1.52
9I Quercetin 7-neohesperidoside	27	1.35
9J Isorhamnetin 3-glucoside	30	1.20
9K Tamarixetin 7-rutinoside	30	1.61
9L Quercetin	28	1.67
9H Fisetin	29	1.72
9N Myricetin	34	2.07
10A Pinocembrin 7-neohesperidoside	4	0.27
10B Naringenin 7-neohesperidoside (naringin)	6	0.46
10C Isosakuranetin 7-neohesperidoside (poncirin)	7	0.28
10D Eriodictyol 7-neohesperidoside	12	0.52
11A Taxifolin	9	0.42
11B (+)-Catechin	6	0.35
12 Poncirin chalcone	29	0.65
13 Phloridzin	7	0.27
14A Phloracetophenone 4-neohesperidoside	5	0.18
14B Phloroglucinol	0	0

concentration dependent, and the complexes are dissociated upon heating or upon dilution with alcohol or dimethylformamide [16].

The intensity of complex formation depends mainly on the number of free aromatic hydroxyl groups in the flavonoid molecule: hexahydroxyflavones form stronger complexes than pentahydroxyflavones (see **9N** and **9L**); the same holds true for their glycosides (**9H**, **9D**); the 5-OH in flavonoids does not participate in the complex formation, probably due to strong intramolecular hydrogen bonding with the adjacent carbonyl group; the nature of the sugar residue has no effect on the complex formation.

The most important hydroxyl group for complex formation is the one in the 7-position. The number of OH groups in the B-ring is an important factor. Methylation of 3′- and/or 4′-OH groups decreases the complex-forming ability of the compounds, as shown with quercetin 3-glucoside **9F** and isorhamnetin 3-glucosides **9J**, and naringin **10B** and poncirin **10C**. The role of the 4′-hydroxy group is not clear: while there is indication (see **10A**, **10B** and **10C** in Table 1) that it also participates in the complex formation, its derivatization in tamarixetin 7-rutinoside, **9K**, enhances the compound's complex-forming properties. At concentrations higher than those reported in Table 1 this effect is diminished. The same holds true for hesperetin 7-rutinoside **10E**, and diosmin, **10C**, whose properties are not reported in Table 1 because of problems encountered in solubilization.

However, hydrogen bonding of aromatic hydroxyl groups of flavonoids cannot account alone for all the complex-forming properties of the compounds. Unsaturation at C_2–C_3 in the heterocyclic ring is an important factor in complex formation (compare structures **9A**–**N** to **10A**–**E** and **11A**, **B** and their data in Table 1). Flavanones and flavanols, not having unsaturation between carbons 2 and 3 form much weaker complexes than do flavones, suggesting that electrostatic forces and configurational or steric effects are additionally involved in the reaction.

The flavonoid–anthocyanin complex formation is an important factor in the color expression of plants in the red–blue color region. However, these complexes are significant only in plants containing *both* anthocyanins and flavone glycosides within their central vacuoles. Plant tissues containing anthocyanins in higher concentration, but little or no flavone glycosides, can also appear bluish because of the high absorbance of their vacuoles and their light-scattering effect.

EXPERIMENTAL

Mps are uncorr. NMR spectra were recorded on a Varian HA-100D spectrometer using $CDCl_3$ as solvent and TMS as int. standard, and reported as δ values. Elemental analyses were carried out by Micro-Analysis, Inc., Wilmington, DE. TLC was performed on Eastman Chromagram sheets (cellulose and Si gel). Column chromatography was carried out on Si gel (70–230 mesh, Brinkman Instruments Inc.), MN-polyamide SC-6 (Brinkman Instruments, Inc.) and Polyclar AT powder (General Anilin and Film Co., New York). Acetylation was carried out at room. temp in pyridine–Ac_2O overnight.

Synthesis of flavonoids. Quercetin 3-glucoside (isoquercitrin). Rutin (5 g, recrystallized 2× from 50% MeOH) was dissolved in 120 ml boiling cyclohexanol [24] and 40 ml HCO_2H was added through a reflux condenser. The mixture was refluxed for 15 hr. Small samples were withdrawn at regular intervals and chromatographed on cellulose TLC in 15% aq. HOAc. The hydrolysis mixture contained rutin, quercetin 3-glucoside and quercetin (R_fs in 15% HOAc: 0.51, 0.29 and 0.04 respectively). The solvent was evapd to dryness, the residue dissolved in Me_2CO and filtered through a Si gel column (2 × 8 cm). The filtrate was concentrated and diluted with MeOH, whereupon quercetin pptd. More quercetin was removed after addition of H_2O to the filtrate. The filtrate was concentrated and a small amount of MeOH added. The resulting soln was chromatographed on a polyamide column (3.5 × 30 cm), sequentially eluting with 40%, 50%, 80% and 100% MeOH. Fractions containing quercetin 3-glucoside were combined and evapd to give a yellow powder. Recrystallized from MeOH–H_2O, 263 mg chromatographically and spectroscopically pure quercetin 3-glucoside was obtained: mp 216–218° (lit: 217–218° [25], 228° [24]); UV (MeOH) λ_{max} 258 (4.24), 360 (4.23) nm; lit: [22] 258 (4.41), 360 (4.32) nm. R_f (cellulose) 0.70 (1-BuOH: HOAc–H_2O (BAW) = 4:1:5, upper phase), 0.41 (BAW = 4:1:5, lower phase), 0.07 (H_2O), 0.20 (15% HOAc). Hydrolysis of the prepared quercetin 3-glucoside yielded one sugar, glucose, and quercetin, both identified by TLC.

Isorhamnetin 3-glucoside. (a) *Quercetin pentaacetate.* From quercetin (8.0 g), recrystallized twice from Me_2CO–EtOH to give fine needles. Yield: 11.17 g (86%), mp 199–201° (lit. [26] 192–194°); NMR ($CDCl_3$) δ 2.34 (*s*, 12H, aromatic OAc), 2.54 (*s*, 3H, 3-OAc), 6.88 (*d*, J = 2.2 Hz, 6-H), 7.33 (*d*, J = 8.5 Hz, 5′-H), 7.36 (*d*, J = 2.2 Hz, 8-H), 7.66–7.80 (*m*, 2′-H, 6′-H).

(b) *7,4′-Dibenzylquercetin.* Synthesized from quercetin pentaacetate (18.5 g), KI (2.0 g), benzyl chloride (20 ml), dry K_2CO_3 (50 g) and dry Me_2CO (300 ml) according to [28]. 7,4′-Dibenzylquercetin (2.7 g) was recrystallized from Me_2CO–MeOH, mp 182–183° (lit. [28] 181°).

7,4′-Dibenzylquercetin triacetate from 7,4′-dibenzylquercetin (3.0 g). Colorless needles, recrystallized from MeOH–Me_2CO: mp 157–158° (lit. [28] 156°); NMR ($CDCl_3$) δ 2.32 (*s*, 3H) and 2.34 (*s*, 3H) for 3′,5′-OAc, 2.44 (*s*, 3H, 3-OAc), 5.14 (*s*, 2H) and 5.17 (*s*, 2H) for 7 and 4′-O$\underline{CH}_2C_6H_5$, 6.71 (*d*, J = 2.2 Hz, 6-H), 6.91 (*d*, J = 2.2 Hz, 8-H), 7.08 (*d*, J = 8.5 Hz, 5′-H), 7.40 and 7.42 (two singlets with equal area, 10 H, aromatic protons), 7.59 (*d*, J = 2.2 Hz, 2′-H), and 7.69 (*dd*, J = 8.5 and 2.2 Hz, 6′-H).

(c) *3′-Methyl-7,4′-dibenzylquercetin.* 7,4′-Dibenzylquercetin triacetate (2.96 g) was refluxed with Me_2SO_4 (0.73 g), dry K_2CO_3 (5.0 g), Me_2CO (60 ml) and MeOH (20 ml) for 2 hr. The yellow undissolved K-salts were collected and suspended in dil. aq. HCl. 7,4′-Dibenzylquercetin pptd as a yellow solid, recrystallized from Me_2CO–MeOH as yellow needles: mp 172–174°. The Me_2CO–MeOH filtrate from above was treated with 10% HCl (15 ml), concentrated and diluted with H_2O. 3′-Methyl-7,4′-dibenzylquercetin was recrystallized from Me_2CO–MeOH as yellow needles (735 mg), mp 188–190° (lit. [28] 187°).

(d) *7,4′-Dibenzylisorhamnetin 3-glucoside.* 7,4′-Dibenzylisorhamnetin (0.50 g), acetobromoglucose (0.45 g), pyridine (6 ml) and Ag_2O (1.2 g) was stirred at room temp. for 4 hr. The silver salt was filtered off and washed with a small amount of MeOH. The filtrate was poured into 100 ml 15% HOAc whereupon a brown ppt. formed. This was collected, washed with H_2O and dissolved in Me_2CO. The brown soln was centrifuged to remove the remaining silver salts, diluted with MeOH and adjusted to a basic pH with 10% KOH. The soln was allowed to stand for 30 min at room temp. and then acidified with 10% HOAc. The solvents were evapd, the residue washed with H_2O and dissolved in Me_2CO–MeOH. The mixture was chromatographed on a Si gel column (3.5 × 30 cm) with EtOAc–MeOH–H_2O (20:4:3). 7,4′-Dibenzylisorhamnetin 3-glucoside (404 mg) was obtained and recrystallized twice from 95% EtOH: mp 185–187° (lit. [27] 182–183°).

(e) *Isorhamnetin 3-glucoside.* 7,4′-Dibenzylisorhamnetin 3-glucoside (140 mg) was suspended in 100 ml abs. MeOH in the presence of a small amount of 5% Pd–charcoal. The mixture was hydrogenated with stirring for 4 hr, the catalyst removed and the filtrate evapd to dryness under red. pres. Isorhamnetin 3-glucoside was crystallized (× 3) from MeOH–H_2O to give fine yellow needles, 77 mg (76%): mp 177–179° (lit. [27] 175–176°); UV (MeOH) λ_{max} 256 (4.28), 268 (sh), 303 (sh), 357 (4.18) nm; +$AlCl_3$: 268, 300, 362, 402 nm; +$AlCl_3$/HCl: 267, 298, 358, 398 nm; R_f (cellulose): 0.35 (15% HOAc), 0.25 (BAW = 6:1:2).

Apigenin 7-neohesperidoside. (a) *Naringin octaacetate.* From naringin (2 g), recrystallized from EtOH–H_2O. Yield: 2.7 g colorless needles, mp 132–135°; NMR ($CDCl_3$), δ 1.28 (*d*, 3H, J = 6.0 Hz, rhamnose-Me), 2.00–2.20 (*m*, 18H), 2.35 (*s*, 3H, 4′-OAc), 2.41 (*s*, 3H, 5-OAc), 2.90 (*m*, 2H, 3-H_{cis}, H_{trans}), 3.52 (*dd*, J = 3 and 10 Hz, 1H, 2-H), 3.86–4.30 (*m*, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 4.96–5.45 (*m*, 7H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4), 6.41 (*d*, J = 2.2 Hz, 6-H), 6.62 (*d*, J = 2.2 Hz, 8-H), 7.18 (*d*, J = 8.5 Hz, 3′-,5′-H), 7.50 (*d*, J = 8.5 Hz, 2′-,6′-H).

(b) *Apigenin 7-neohesperidoside.* Naringin octaacetate (0.60 g), HOAc (6 ml), KOAc (0.60 g), Ac_2O (1.5 ml) and I_2 (0.30 g) were heated under reflux for 16 hr. The cold reaction mixture was poured into 150 ml 0.5% ice-cold KI solution. After 2 hr the ppt. was filtered and dissolved in 75 ml EtOH. The reddish-brown soln was decolorized with $NaHSO_3$ and diluted with water. The white ppt. so formed was filtered and dried (516.5 mg). The above material was treated with 20 ml 1.8 N NaOMe at room temp. for 30 min, and then acidified with 10% HCl. The soln was refrigerated (4°) overnight and apigenin 7-neohesperidoside crystallized (200 mg). Recrystallized twice from pyridine–H_2O: mp 203–204° (lit. 202–205° [29], 198–200° [30]); UV (MeOH) λ_{max} 268 (4.29), 336 (4.23) nm; R_f (cellulose) 0.46 (15% HOAc), 0.66 (BAW = 4:1:5, upper phase), 0.77 (BAW = 4:1:5, lower phase).

Apigenin 7-neohesperidoside octaacetate. Colorless needles from 95% EtOH: mp 221–223° (lit. 220–244 [29], 215–217° [30]); NMR ($CDCl_3$) δ 1.28 (*d*, J = 6.0 Hz, rhamnose-Me), 1.98–2.20 (*m*, 18H), 2.37 (*s*, 3H, 4′-OAc), 2.46 (*s*, 3H, 5-OAc), 3.90–4.32 (*m*, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 4.95–5.50 (*m*, 7H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4), 6.61 (*s*, 3-H), 6.73 (*d*, J = 2.2 Hz, 6-H), 7.08 (*d*, J = 2.2 Hz, 8-H), 7.26 (*d*, J = 8.5 Hz, 2′-,6′-H) and 7.90 (*d*, J = 8.5 Hz, 3′-,5′-H).

Diosmetin 7-rutinoside (Diosmin). (a) *Hesperidin octaacetate.* From hesperidin (1.6 g), yielded 2.10 g octaacetate: NMR ($CDCl_3$) δ 1.23 (*d*, J = 6.0 Hz, rhamnose-Me), 2.00–2.20 (18H), 2.38 (*s*, 3H), and 2.44 (*s*, 3H) for 4′- and 5-OAc, 2.75–2.92 (*m*, 3-H_{cis}, H_{trans}), 3.92 (*s*, 4′-OMe), 3.54–4.10 (*m*, glucose-H-5,6,6 and rhamnose-H-5), 4.78 (*d*, J = 1 Hz, rhamnose-H-1), 5.20–5.58 (*m*, 7H, glucose-H-1,2,3,4 and rhamnose-H-2,3,4), 6.37 (*d*, J = 2.2 Hz, 6-H), 6.55 (*d*, J = 2.2 Hz, 8-H), 7.15 (*d*, J = 8.5 Hz, 5′-H), 7.21 (*d*, J = 2.2 Hz, 2′-H), 7.36 (*dd*, J = 2.2 Hz and 8.5 Hz, 6′-H).

(b) *Diosmetin 7-rutinoside.* A mixture of hesperidin octaacetate (1.05 g) was treated as described under apigenin 7-neohesperidoside. Yield: 881 mg. The above material was deacetylated in 30 ml 5% NaOMe for 25 min at 0°. The reaction mixture was acidified with 10% HCl in the cold to give 373 mg yellow powder. Diosmetin 7-rutinoside (272 mg) was recrystallized twice from

DMSO–MeOH: mp 288–289° (lit. [30] 290–292°) to give pale yellow needles.

Acetylation of diosmetin 7-rutinoside (30 mg) yielded 38 mg octaacetate, recrystallized from EtOH–H_2O: mp 135–137 (lit. [30] 129–130°); NMR ($CDCl_3$) δ 1.21 (*d*, $J = 6.0$ Hz, rhamnose-Me), 1.98–2.20 (18H), 2.24 and 2.49 (*s*, each for 3H, 3′- and 5-OAc), 3.96 (*s*, 4′-OMe), 3.60–4.30 (*m*, 4H, glucose-H-5,6,6 and rhamnose-H-5), 4.78 (*d*, $J = 1.0$ Hz, rhamnose-H-1), 4.48–5.42 (*m*, 7H, glucose-H-1,2,3,4 and rhamnose-H-2,3,4), 6.55 (*s*, 1H, 3-H), 6.69 (*d*, $J = 2.2$ Hz, 6-H), 7.01 (*d*, $J = 2.2$ Hz, 8-H), 7.12 (*d*, $J = 8.5$ Hz, 5′-H), 7.59 (*d*, $J = 2.2$ Hz, 2′-H), 7.76 (*dd*, $J = 2.2$ and 8.5 Hz, 6′-H).

Phloroacetophenone 4-neohesperidoside. Prepared from naringin (8.5 g) by alkaline hydrolysis according to ref. [31]. Colorless needles from H_2O (3.1 g) were obtained: mp 162–165 (lit.: 164–166° [31], 163–165° [33]). UV (MeOH) λ_{max} 281 (4.22), 326 (sh, 3.44) nm; R_f (cellulose) 0.83 (15% HOAc), 0.87 (2% HOAc). Octaacetate: NMR ($CDCl_3$) δ 1.22 (*d*, $J = 6.0$ Hz, rhamnose-Me), 1.96 (*s*, 3H), 2.03 (*s*, 6H), 2.05 (*s*, 3H), 2.12 (*s*, 3H), 2.14 (*s*, 3H), 2.26 (*s*, 6H, 2-,6-OAc), 2.44 (*s*, 3H, 1-COMe), 3.85–4.25 (*m*, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 4.94–5.46 (*m*, 7H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4), 7.79 (*s*, 2H, aromatic protons).

Poncirin chalcone. Obtained from condensation of phloroacetophenone 4-neohesperidoside (2.4 g) with anisaldehyde (1.27 ml) in the presence of 25% ethanolic KOH [32]. Orange crystals from EtOH (340 mg): mp 198–200 (lit. [32]: 198–201°); UV (MeOH) λ_{max} 363 (3.49) nm; R_f 0.36 (Si gel, EtOAc–MeOH–H_2O, 80:14:10), 0.47 (cellulose, 15% HOAc).

Isosakuranetin 7-neohesperidoside (poncirin). A soln of poncirin chalcone (242 mg) in 10 ml pyridine and 40 ml H_2O was allowed to stand at room temp. overnight. The solvents were removed and the yellow residue was recrystallized from abs. EtOH. Isosakuranetin 7-neohesperidoside (160 mg) was obtained: mp 210–212 (lit. [32] 211–212°); UV (MeOH) λ_{max} 282 (4.33), 330 (infl., 3.61) nm; R_f (cellulose) 0.78 (15% HOAc), 0.59 (H_2O).

Acetylation gave the heptaacetate, recrystallized from EtOH–H_2O: mp 120–123° (lit. [32]: 122–123°). NMR ($CDCl_3$) δ 1.25 (*d*, $J = 6.0$ Hz; rhamnose-Me), 1.90–2.20 (18H), 2.38 (*s*, 3H, 5-OAc), 2.90 (*m*, 2H, 3-H_{cis}, H_{trans}), 3.70–4.30 (*m*, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 3.84 (*s*, 3H, 4′-OMe), 4.90–5.50 (*m*, 8H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4 and 2-H), 6.36 (*d*, $J = 2.2$ Hz, 6-H), 6.58 (*d*, $J = 2.2$ Hz, 8-H), 6.94 (*d*, $J = 8.5$ Hz, 3′-, 5′-H), 7.37 (*d*, $J = 8.5$ Hz, 2′-, 6′-H).

Pinocembrin 7-neohesperidoside. Phloroacetophenone 4-neohesperidoside (2 g) in 20 ml EtOH was treated with 20% ethanolic KOH soln (30 ml). Benzaldehyde (0.5 g) was added under N_2 and stirred at room temp. for 112 hr, diluted with water (250 ml) and acidified to pH 4 with 3 N HCl. Pyridine (40 ml) was added and heated on a steam bath for 8 hr. Upon concentration a yellow ppt. formed. Recrystallization from aq. EtOH gave 250 mg pinocembrin 7-neohesperidoside. The mother liquid was chromatographed on polyamide (3.5 × 30 cm) with a linear gradient of H_2O–MeOH. Additional 213 mg pinocembrin 7-neohesperidoside was obtained: mp 250–252° (lit. 263–266 [33], 238–239 [34]); UV (EtOH) λ_{max} 284 (4.15), 330 (3.49) nm; R_f (cellulose) 0.63 (H_2O), 0.73 (2% HOAc), 0.80 (15% HOAc).

Heptaacetate. Mp 122–125°, NMR ($CDCl_3$) δ 1.24 (*d*, $J = 6.0$ Hz, rhamnose-Me), 1.95–2.16 (18H), 2.38 (*s*, 3H, 5-OAc), 2.76–3.10 (*m*, 2H, 3-H_{cis}-H_{trans}), 3.80–4.25 (*m*, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 4.90–5.40 (*m*, 7H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4), 5.30–5.65 (*m*, 1H, 2-H), 6.36 (*d*, $J = 2.2$ Hz, 6-H), 6.59 (*d*, $J = 2.2$ Hz, 8-H), 7.43 (*s*, 5H, B-ring aromatic protons).

Kaempferol 7-neohesperidoside. Naringin (5 g) in 30 ml 2 N NaOH and 30% H_2O_2 (1.5 ml) was stored at 0° for 48 hr with repeated addition of H_2O_2 (1.5 ml) after 24 hr. It was acidified to pH 6 with conc HOAc, stored at 4° overnight, $NaHSO_3$ (3.7 g) added and refluxed for 2 hr. After chromatography on a polyamide column (3.5 × 25 cm) with H_2O, 40% and 80% MeOH, kaempferol 7-neohesperidoside (1.35 g) was isolated. Recrystallized from MeOH–H_2O: mp 258–260° (lit.: 255° [35], 252–255° [30]); UV (MeOH) λ_{max} 252 (infl., 4.22), 266 (4.23), 326 (infl., 4.03) and 368 (4.29) nm; R_f (cellulose) 0.34 (15% HOAc), 0.65 (BAW = 4:1:5), 0.67 (60% HOAc).

Nonaacetate. From aq. EtOH, mp 138–140 (lit. [30] 144–145°); NMR δ 1.23 (*d*, $J = 6.0$ Hz, rhamnose-Me), 1.85–2.20 (18H), 2.32 and 2.35 (*s*, each for 3H, 4′- and 5-OAc), 2.44 (*s*, 3H, 3-OAc), 3.80–4.30 (*m*, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 4.85–5.50 (*m*, 7H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4), 6.73 (*d*, $J = 2.2$ Hz, 6-H), 7.03 (*d*, $J = 2.2$ Hz, 8-H), 7.26 (*d*, $J = 8.5$ Hz, 2′-, 6′-H).

Tamarixetin 7-rutinoside. Hesperidin (2.5 g) in 50 ml 2 N NaOH was reacted with 30% H_2O_2 (5 ml) acidified to pH 6 as above, $NaHSO_3$ (6 g) added, refluxed for 2 hr and the yellow ppt. formed was collected. Recrystallized from MeOH–H_2O, tamarixetin 7-rutinose was obtained as yellow needles (535 mg): mp 259–261 (lit.: 255–257 [30], 288 [35]); UV (MeOH) λ_{max} 256 (4.13), 272 (infl.), 372 (4.21) nm; R_f (cellulose) 0.21 (15% HOAc), 0.57 (BAW = 4:1:5).

Nonaacetate. Mp 129–131°; NMR ($CDCl_3$) δ 1.19 (*d*, $J = 6.0$ Hz, rhamnose-Me), 1.90–2.18 (18H), 2.34 and 2.38 (*s*, each for 3H, 4′,5-OAc), 2.45 (*s*, 3H, 3-OAc), 3.93 (*s*, 3H, 3′-OMe) 3.60–4.15 (*m*, 4H, glucose-H-5,6,6 and rhamnose-H-5), 4.75 (*d*, $J = 1$ Hz, rhamnose-H-1), 4.90–5.50 (*m*, 7H, glucose-H-1,2,3,4 and rhamnose-H-2,3,4), 6.70 (*d*, $J = 2.2$ Hz, 6-H), 6.97 (*d*, $J = 2.2$ Hz, 8-H), 7.09 (*d*, $J = 8.5$ Hz, 5′-H), 7.57 (*d*, $J = 2.2$ Hz, 2′-H), 7.74 (*d*, $J = 2.2$ and 8.5 Hz, 6′-H).

Quercetin 7-neohesperidoside and eriodictyol 7-neohesperidoside. Quercetin 7-neohesperidoside was synthesized from eriodictyol 7-neohesperidoside. Procedure and properties of these compounds are described in ref. [36].

Source and purification of other flavonoids. Rutin was obtained from NBC, Cleveland, OH; (+)-catechin, hesperidin, naringin, phloridzin and quercetin from Sigma; myricetin from Aldrich; apigenin 7-glucoside and quercitrin from ICN, (K&K), Plainview, NY; phloroglucinol from Eastman. Taxifolin, fisetin and robinin were from our laboratory collection. All above compounds were recrystallized from H_2O or aq. alcoholic solns.

Anthocyanins. Malvidin, cyanidin and petunidin 3,5-diglucosides, malvidin 3-(6-*O*-*p*-coumarylglucoside)-5-glucoside and malvidin 3-glucoside were isolated from DeChaunac and Concord grapes [37, 38]. Stock solns. of flavonoids and anthocyanins were prepared in 0.08 N NaOH and 1% H_3PO_4 respectively and used immediately in disodium citrate buffer, pH adjusted to 3.2 with HCl.

Complex formation between anthocyanins and flavonoids. The reaction mixture consisted of two parts flavonoid stock solution, one part anthocyanin stock solution and one part buffer in a total vol. of 200 or 400 μl, depending on the spectrophotometer cell used (0.102 or 1.0 mm path length). The pH of these solns was usually 3.2 after mixing. The spectrum of the reactions mixture was recorded immediately.

Acknowledgment—This investigation was supported by a Research Grant from Welch Foods Inc., Westfield, NY.

REFERENCES

1. Robinson, G. M. and Robinson, R. (1931) *Biochem. J.* **25B**, 1687.
2. Robinson, R. and Robinson, G. M. (1939) *J. Am. Chem. Soc.* **61**, 1605.

3. Robinson, R. and Robinson, G. M. (1939) *J. Am. Chem. Soc.* **61,** 1605.
4. Shibata, K., Shibata, Y. and Kashiwaga, I. (1919) *J. Am. Chem. Soc.* **41,** 208.
5. Shibata, K., Hayashi, K. and Isaka, T. (1949) *Acta Phytochim. (Tokyo)* **25,** 17.
6. Kuhn, H. and Sperling, W. (1960) *Experientia* **16,** 237.
7. Wagner, G. and Siegelman, H. W. (1975) *Science* **190,** 1298.
8. Asen, S., Norris, K. H. and Stewart, R. N. (1971) *J. Am. Hortic. Soc. Sci.* **96,** 770.
9. Sondheimer, E. (1953) *J. Am. Chem. Soc.* **75,** 1507.
10. Jurd, L. (1963) *J. Org. Chem.* **28,** 987.
11. Jurd, L. and Geissman, T. A. (1963) *J. Org. Chem.* **28,** 2394.
12. Harper, K. A. (1967) *Aust. J. Chem.* **20,** 2691.
13. Harper, K. A. and Chandler, B. V. (1967) *Aust. J. Chem.* **20,** 731.
14. Sperling, W., Werner, F. C. and Kuhn, H. (1966) *Ber. Dtsch. Bunsenges. Phys. Chem. (Weinheim)* **70,** 530.
15. Asen, S., Stewart, R. N. and Norris, K. H. (1971) *Phytochemistry* **10,** 171.
16. Asen, S., Stewart, R. N. and Norris, K. H. (1972) *Phytochemistry,* **11,** 1139.
17. Asen, S., Norris, K. H. and Stewart, R. N. (1972) *Phytochemistry* **11,** 2739.
18. Asen, S., Stewart, R. N. and Norris, K. H. (1975) *Phytochemistry* **14,** 2677.
19. Scheffeldt, P. and Hrazdina, G. (1978) *J. Food Sci.* **43,** 517.
20. Williams, M. and Hrazdina, G. (1979) *J. Food Sci.* **44,** 66.
21. Geissman, T. A., Jorgenson, E. C. and Harborne, J. B. (1953) *Chem. Ind.* 1389.
22. Bayer, E. (1959) *Chem. Ber.* **92,** 1062.
23. Bayer, E., Egeter, H., Fink, A., Neither, K. and Wegmann, K. (1966) *Angew. Chem. Int. Ed. Engl.* **5,** 791.
24. Fox, D. W., Savage, W. L. and Wender, S. H. (1953) *J. Am. Chem. Soc.* **75,** 2504.
25. Hoerhammer, L., Wagner, H., Arndt, H. G., Dirscherl, R. and Farkas, L. (1968) *Chem. Ber.* **101,** 450.
26. Underhill, E. W., Watkin, J. E. and Neish, A. C. (1957) *Can. J. Biochem. Physiol.* **35,** 219.
27. Hoerhammer, L., Wagner, H., Arndt, H. G., Kraemer, H. and Farkas, L. (1966) *Tetrahedron Letters* **6,** 567.
28. Jurd, L. (1962) *J. Org. Chem.* **27,** 1294.
29. Wagner, H., Aurnhammer, G., Hoerhammer, L. and Farkas, L. (1969) *Chem. Ber.* **102,** 2083.
30. Roesler, H., Mabry, T. J., Cranmer, M. F. and Kagan, J. (1965) *J. Org. Chem.* **30,** 4346.
31. Horowitz, R. M. and Gentili, B. (1963) *Tetrahedron* **19,** 773.
32. Wagner, H., Aurnhammer, G., Hoerhammer, L., Farkas, L. and Nogradi, M. (1969) *Chem. Ber.* **102,** 785.
33. Aurnhammer, G., Wagner, H., Hoerhammer, L. and Farkas, L. (1970) *Chem. Ber.* **103,** 3667.
34. Chopin, J. and Dellamonica, G. (1966) *C. R. Acad. Sci.* **262,** 1712.
35. Pacheco, H. and Grouiller, A. (1966) *Bull. Soc. Chim. Fr.* **10,** 3212.
36. Chen, L.-J. and Hrazdina, G. (1980) *Experientia* (in press).
37. Hrazdina, G. (1975) *Lebensm. Wiss. Technol.* **8,** 111.
38. Hrazdina, G. and Franzese, A. J. (1974) *Phytochemistry* **13,** 225.