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THE CHEMISTRY OF ANTHOCYANINS, ANTHOCYANIDINS AND RELATED FLAVYLIUM SALTS

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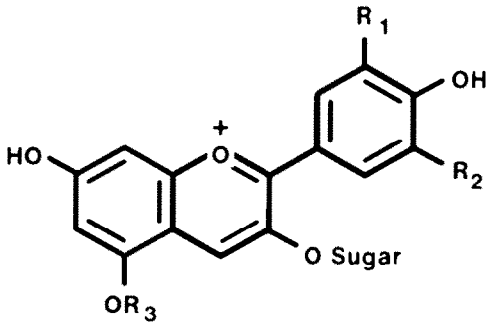
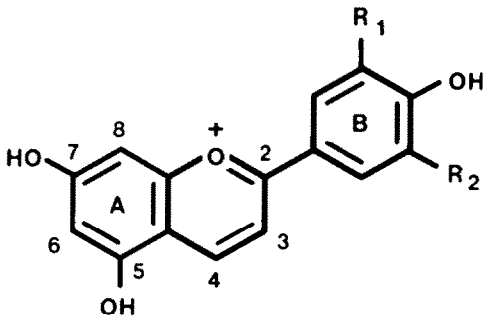
CONTENTS

I. INTRODUCTION

The last decade has seen the development of an increased interest in the chemistry of anthocyanins, a family of glycosidic pigments represented by the general structure **1**. The chromophoric aglycones (anthocyanidins) are red polyhydroxylated flavylium salts, which due to their instability are seldom found in their free form in plant tissues. The yellow 3-deoxyanalogs, however, are quite stable[1] in acidic media and have been isolated as such from mosses and ferns, as well as from members of the *Gramineae* and *Gesneriaceae* families of flowering plants. The compounds apigeninidin (**2**), luteolinidin (**3**) and tricetinidin (**4**) are important representatives of this restricted group of anthocyanidins, as they exist in food plants like corn[2], sorghum[3] (**2**, **3**), and black tea leaves (**4**).[4] The red to blue glycosides **1**, on the other hand, are widely distributed in plants, where they contribute to the coloration of flowers, fruits and leaves.





1. R_1 = H, OH, OCH_3
   R_2 = H, OH, OCH_3
   R_3 = H, Sugar

2. $R_1 = R_2$ = H
3. R_1 = H, R_2 = OH
4. $R_1 = R_2$ = OH

The reasons behind this interest in anthocyanins are several; the most important perhaps is the need to understand their elusive role in the photobiology of flowering plants. Towards the applied end, there is an interest in industrial circles to ascertain (a) whether the anthocyanins can be used profitably for the coloration of processed foods, and (b) how they can be made available by synthesis for such purposes.

The use of synthetic natural products as food additives is a widespread, highly accepted practice. For reasons of purity and consistency in composition and function, it is the preferred option over the alternative use of crude extracts of natural origin. This is the case for all the vitamins of value in human nutrition, as well as for the carotenoid pigments β-carotene, *apo*-carotenal and canthaxanthin which are finding increasing application in food formulations.

Although the recovery of anthocyanin-enriched materials from the skins of grapes and other by-products of wine manufacture is being practiced in Europe (France, Italy, Spain, Bulgaria), Australia and the American continent (USA, Argentina), the possibility of isolating pure, individual pigments out of these complex polyphenolic mixtures seems rather bleak. The alternative use of fermentation techniques for pigment production is also ruled out at the present time, as anthocyanins are not microbial metabolites.[5] The use of microorganisms for flavonoid biosynthesis should not be completely ignored, however, as there is one well-documented instance on the synthesis of a flavonol by the mold *Aspergillus candidus*.[6] This metabolite, chlorflavonin, is biosynthesized from acetate and benzoate precursors by a pathway differing from that found in higher plants.[7]

Another relevant area of research related to the intended food use of anthocyanins is the full understanding of their biological effects in mammals. Although metabolic studies on pure anthocyanins are yet to be reported, considerable information exists on the toxicology and metabolic disposition of several of the more abundant, related flavonoids: quercetin, citrus flavanones, catechins and biflavans (condensed tannins). This body of information has been reviewed recently,[8] and will not be dealt with in this work. Also outside the scope of this review are the technological applications of anthocyanins, which have been discussed at length in several monographs of recent date.[9–15] Instead, we have restricted our discussion to the chemical basis of anthocyanin performance, the structural factors influencing their reactivity in solution, and the synthetic methods available for their preparation.

II. SYNTHESIS

(a) *Synthesis of anthocyanins and anthocyanidins*

This section reviews the several approaches reported for the synthesis of flavylium salts bearing the hydroxylation patterns found in the natural pigments. The emphasis of the discussion is on the more recent results, as earlier work is collected in a series of previous reviews.[9,16–22] Emphasis has also been placed on discussing the relative preparative merits of the various alternatives available for the synthesis of these natural products.

The structure of anthocyanidins is characterized by a phloroglucinoid ring A, reflecting its polyketide

origin, a shikimate-derived ring B showing either p-coumaric, ferulic or gallic-type hydroxylation, and an electron-deficient pyrilium ring that does (1) or does not (2–4) bear a hydroxyl group at C3. The synthetic procedures summarized in Table 1 are arranged on the basis of the various ring A synthons used for the construction on the ring system. The several alternatives listed are of course of variable relevance. The procedures developed by Sir Robert Robinson and coworkers at the University of Manchester are epochal, as they opened the road to the first synthesis of natural glycosides. Remarkably, the 80 year-old work of Bulow is still of current importance, as it provides the simplest access to the flavylium ring system. It has been extensively used for the synthesis of 3-deoxyanthocyanidins (Table 1), and of several flavylium salt analogs bearing substituents at C4 (Table 3).

An alternative approach to the synthesis of anthocyanidins and anthocyanins is provided by procedures based on the reduction of flavanones, dihydroflavonols or flavonols. These approaches take advantage of a preconstructed ring system, having the necessary hydroxylation patterns in place. The chemical operations are performed on the heterocyclic ring, in order to reduce the carbonyl present at C4 and to adjust the level of oxidation to that of the flavylium cation. Due to the polyphenolic nature of the precursors, the procedures for their proper protection/deprotection require careful consideration, both in terms of chemical compatibility and selectivity. The results in this area are summarized in Table 2, where the data is arranged according to the chemical nature of the compound considered to be the key intermediate in the synthetic sequence.

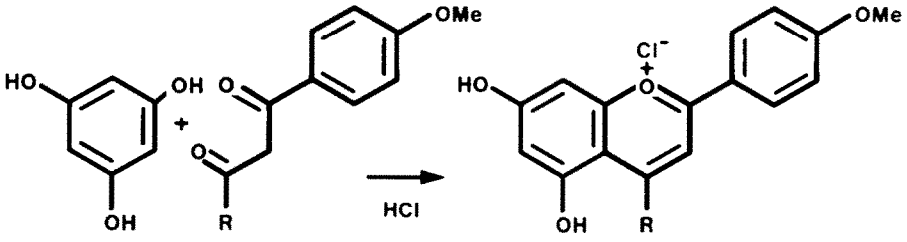
Flavanones and 2,3-dihydroflavonols are widely distributed plant metabolites, that are intermediates in the biosynthesis of anthocyanins and flavonols in higher plants.[7] Practical interest in flavanones, however, is restricted to those few which are available in quantity as agricultural by-products, like the compounds naringenin and hesperetin present in the albedo of orange and grapefruit peels.[59] Among the 2,3-dihydroflavonols, taxifolin (2,3-dihydroquercetin) and aromadendrin (2,3-dihydrokaempferol) are available from Douglas fir bark[20] and from the kino of *Eucalyptus corymbosa*,[50] respectively.

Table 1. Total synthesis of anthocyanins and anthocyanidins

RING A SYNTHON

A. *Phloroglucinol*

1. Bulow and Wagner:[23,24]



Good yields (90–95%) for R = CH_3, COOH, p-anisyl. Not operative for R = H. Flavylium chloride usually crystallized directly out from reaction mixture. Alternative regioisomers are usually not formed for above values of R. In the case of R = phenyl, a mixture of flavylium salts was observed[25] to form, which was not easily separable by fractional crystallization.

2. Malkin and Robinson:[26]

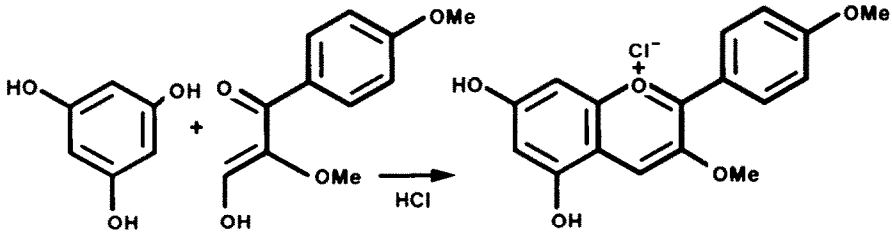
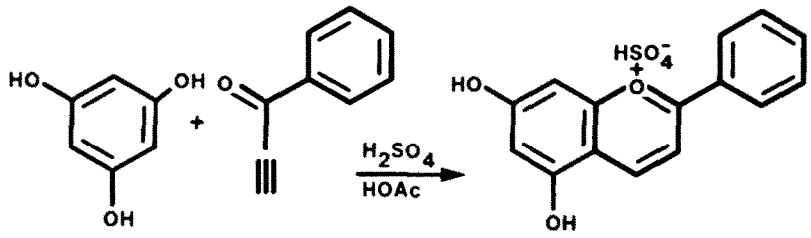


Table 1. *Contd*

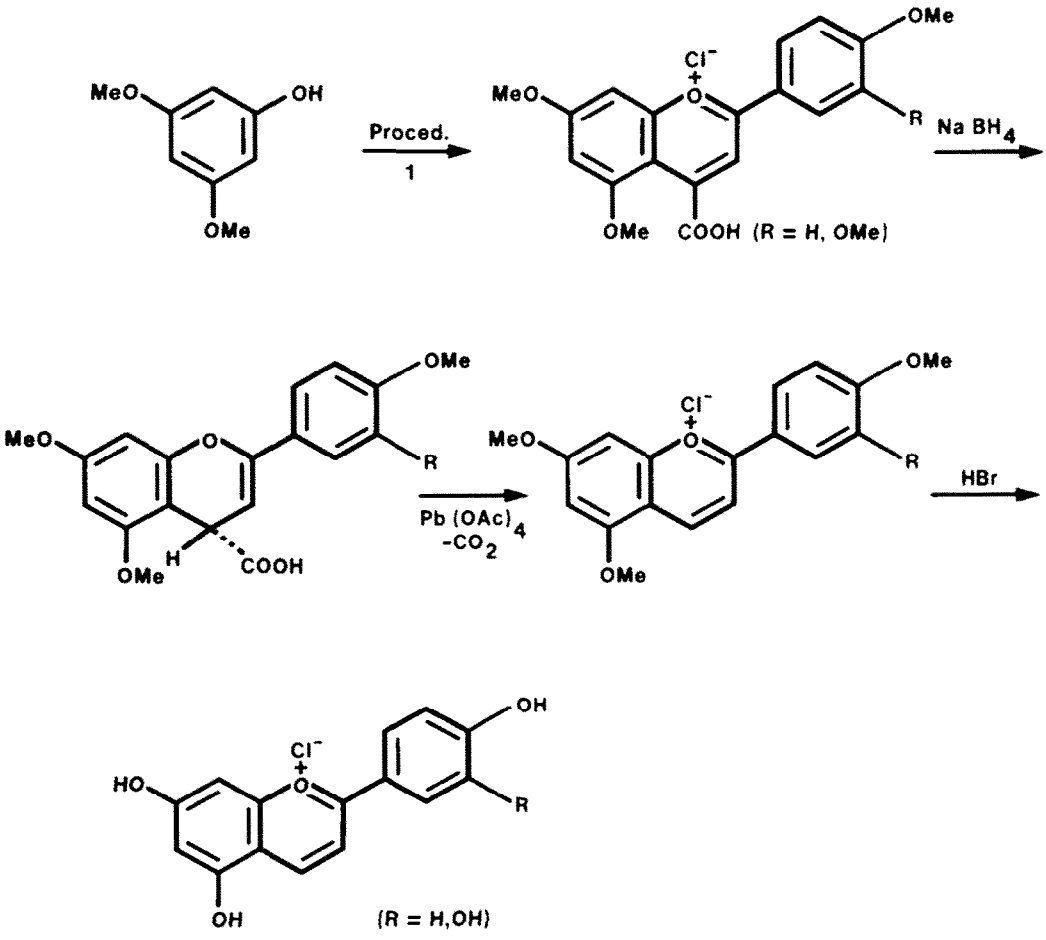
3,4'-0-Dimethylpelargonidin chloride formed found mixed with alternate regioisomer. Both isomers separable with difficulty by fractional crystallization. For that reason, this procedure was early abandoned by Robinson in preference to the one starting with phloroglucinaldehyde (see below) for his synthesis of anthocyanins.

3. Johnson and Melhuish:[27]



Procedure restricted to the synthesis of 3-deoxyanthocyanidins. Example illustrated is reported with a yield of 37%. No indication of the formation of mixtures of salts, as often happens in Procedure 1. This procedure has been used[28] for the synthesis of apigeninidin and luteolinidin-0-methyl ethers.

4. Sweeny and Iacobucci:[29]

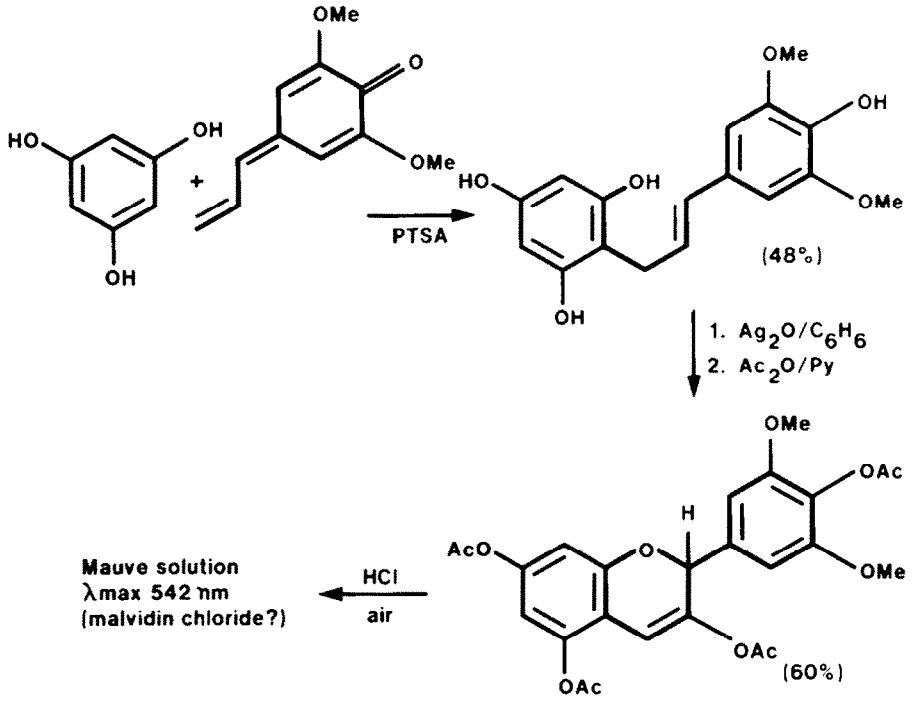


Procedure applied to the synthesis of apigeninidin 2 and luteolinidin 3 chlorides. Good (50% overall) yield based on phloroglucinol dimethyl ether.

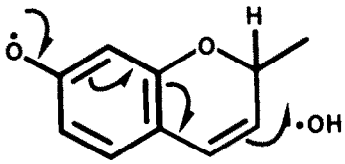
The Bulow intermediate resisted all attempts at direct decarboxylation. The oxidative decarboxylation described was seen to proceed also in electrochemical (anodic) oxidation experiments with the dihydroderivative. Dehydrogenation of this derivative with chloranil, however, gave back the original 4-carboxyanthocyanidin in high yield.

Table 1. *Contd*

5. Zanarotti:[30,31]

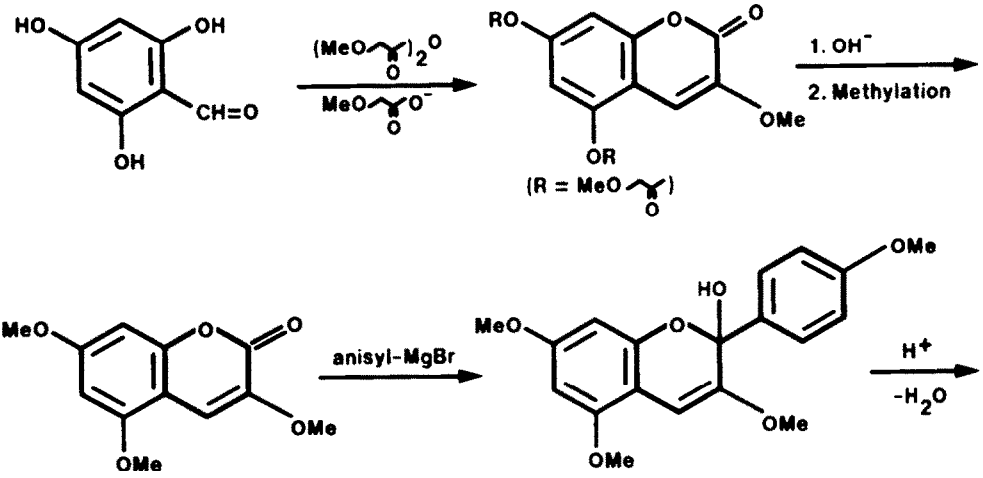


The preparation of C-[illegible]-phloroglucinols and their oxidative cyclization to flav-3-enes find precedence in the work of Ollis,[32,33] Jurd,[34,35,36,37,38] and [illegible]. The procedure has achieved the elusive hydroxylation of a flav-3-ene to a flav-3-ene-3-ol, by using Ag_2O in benzene. The product, isolated as the tetraacetate, was found by ^{1}H-NMR, IR, MS and UV to have the structure shown. A similar enolacetate has been prepared before through the reductive acetylation of quercetin (Table 2, Procedure 8). The flav-3-ene-3-ol is believed to be formed through a $^{\cdot}$OH attack at C3, perhaps involving the quinone methide radical.



B. Phloroglucinaldehyde

6. Willstätter and Zechmeister:[41,42]



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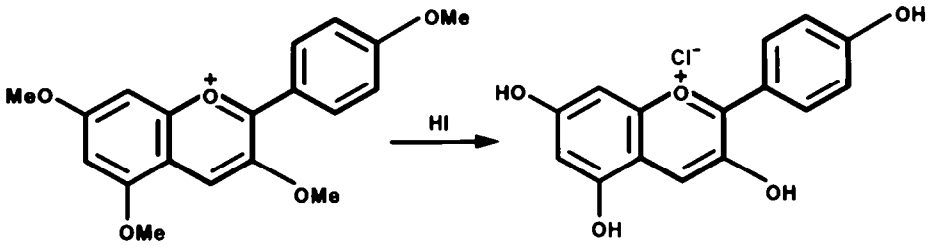
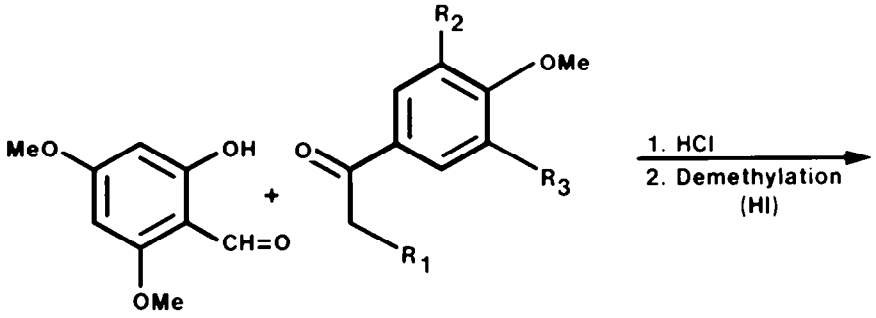
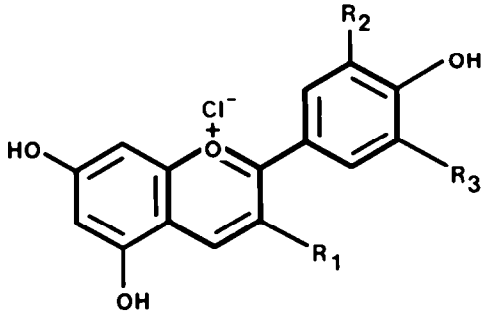


Extension to a 3-methoxycoumarin of a Grignard reaction previously practiced on coumarin by Decker and Fellenberg.[43,44]

First reported instance of use of phloroglucinaldehyde as synthon for ring A. The synthesis of pelergonidin chloride illustrated represents the first synthesis of a natural flavylium salt.

7. Pratt and Robinson:[45–48]





$R_1 = R_2 = R_3 = H$: apigeninidin
$R_1 = R_2 = H$; $R_3 = OH$: luteolinidin
$R_1 = R_2 = R_3 = OH$: delphinidin

This procedure for the synthesis of anthocyanidins was outlined first in notes by Decker[49] and Perkin and Robinson[50], and later fully described by Decker and Fellenberg[43,44] and Perkin, Robinson and Turner.[51]

Besides the illustrated anthocyanidins, it was used also for the synthesis of several flavylium cations: 5,7-dihydroxy-; 3,5,7-trihydroxy-; 2',4',5,7-tetra-hydroxy- and 2',4',3,5,7-pentahydroxyflavylium chlorides.

The condensation of phloroglucinaldehyde with acetophenones represents the classic Robinson synthesis. With slight variations (Procedures 8, 9 and 10) it was used extensively by him and co-workers at the University of Manchester for the synthesis of natural anthocyanins.

8. Bradley, Robinson and Schwarzenbach:[52]

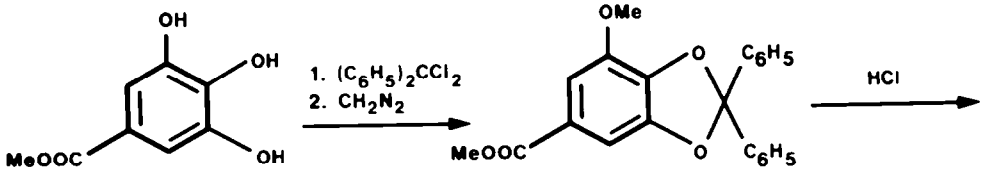
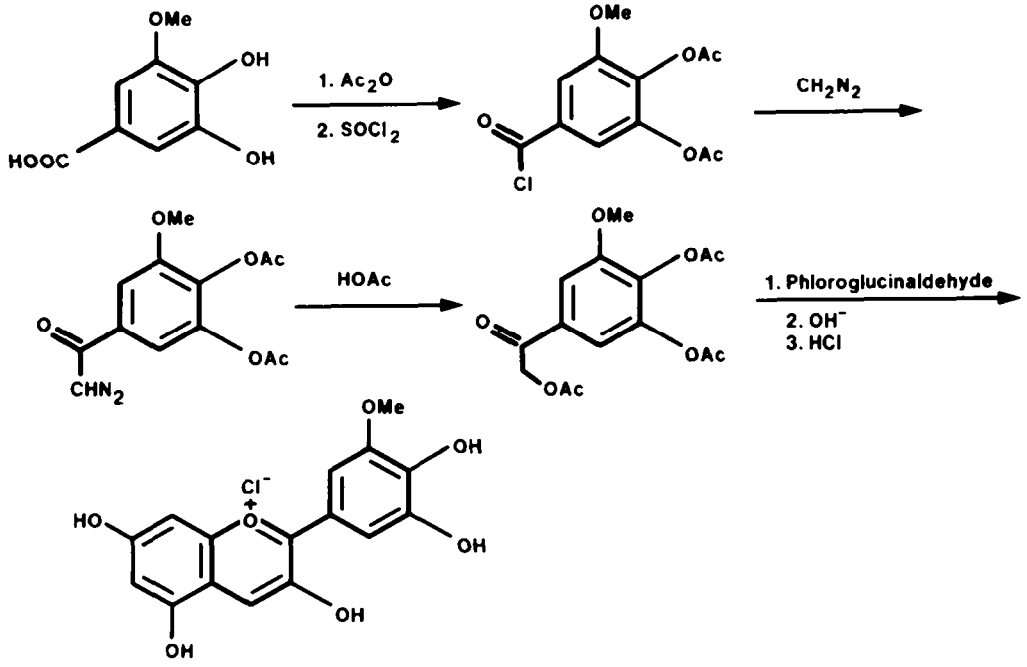


Table 1. *Contd*



Prior use (Procedure 7) of methoxylated intermediates, followed by demethylation with HI, did not allow for the elaboration of the partially methylated B-rings of petunidin and malvidin. The use of acyl groups, generally acetyl, as reversible protecting groups made possible the synthesis of petunidin chloride, as illustrated.

9. Robertson and Robinson:[53–56]

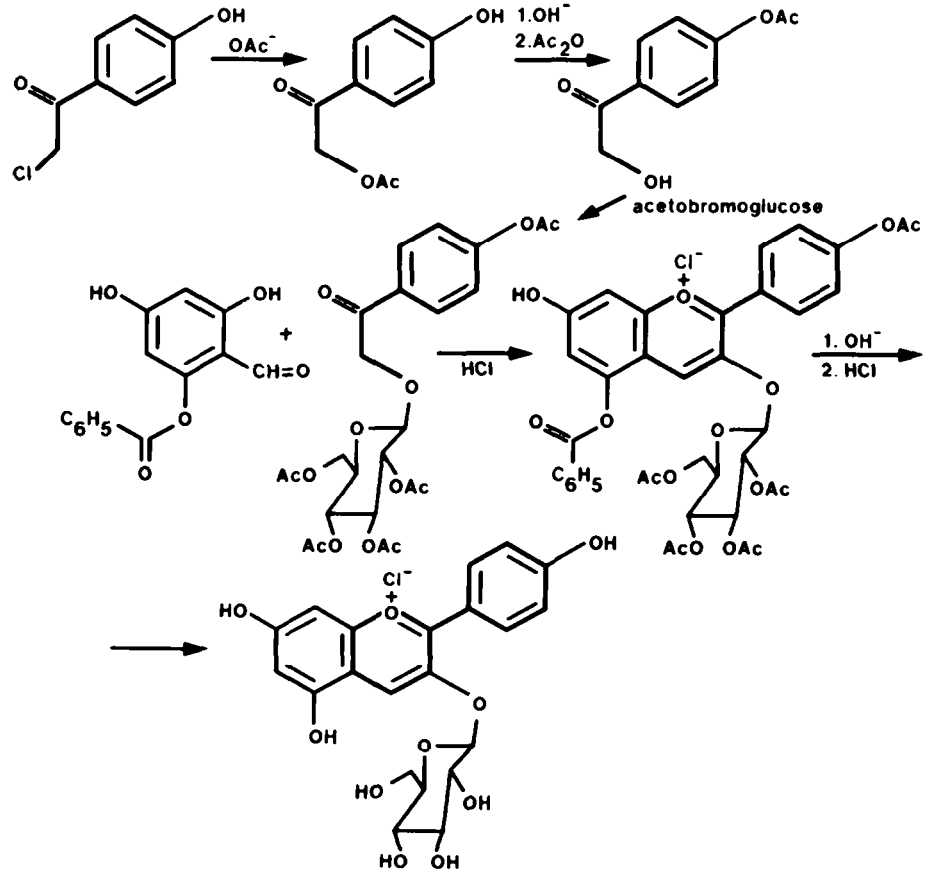
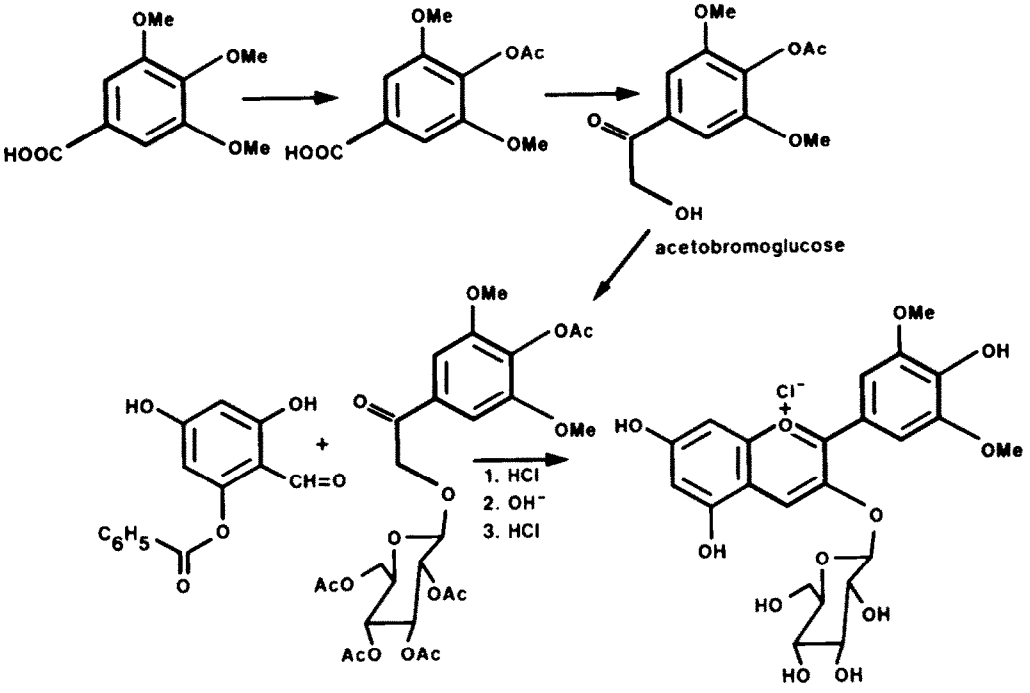


Table 1 *Contd*

First synthesis of anthocyanins based on the procedures discussed above, the glucosides prepared by the use of 1-α-bromo-tetraacetyl-D-glucose. The general method is outlined for the synthesis of pelargonidin-3-β-glucoside.[56]

This work also includes the first use of the 2-benzoylphloroglucinaldehyde as an improved version of this reactant, a development of importance for increasing the yields of these condensations. The recollection of this development by Sir Robert Robinson is worth reading.[22]

10. Levy, Posternak and Robinson:[57]



Preparation of oenin chloride (malvidin-3-β-glucoside) by an extension of the above procedure. For the synthesis of 3,5-diglucosides, the 2-benzoylphloroglucinaldehyde is replaced by 2-0-(tetraacetyl)glucoside.

These procedures remain today the only way known to approach the synthesis of anthocyanins. The only exception is keracyanin chloride (cyanidin-3-rutinoside) that is available[82] through the reduction of rutin (see Table 2).

A recent reexamination of this synthesis of oenin chloride has shown an overall yield of about 0.6% from gallic acid methyl ether.[58]

Table 2. Synthesis of anthocyanins and anthocyanidins by chemical conversion of flavonoid precursors

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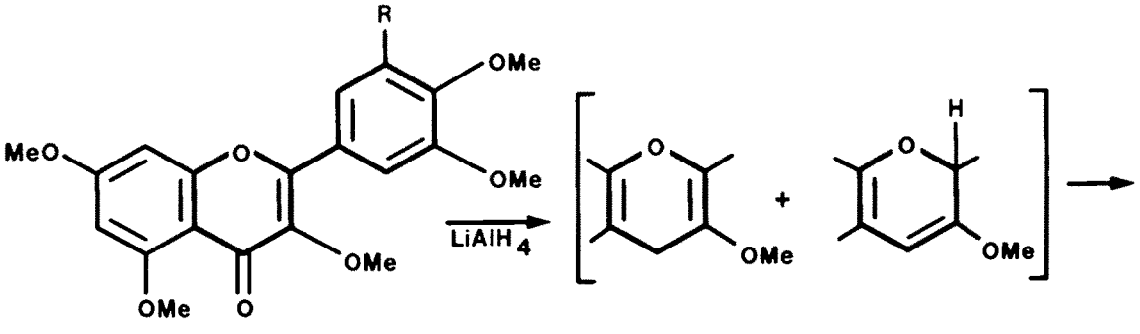
KEY INTERMEDIATE

A. *Flavenes*

(a) From flavonol methyl ethers

1. Jurd:[61,62]



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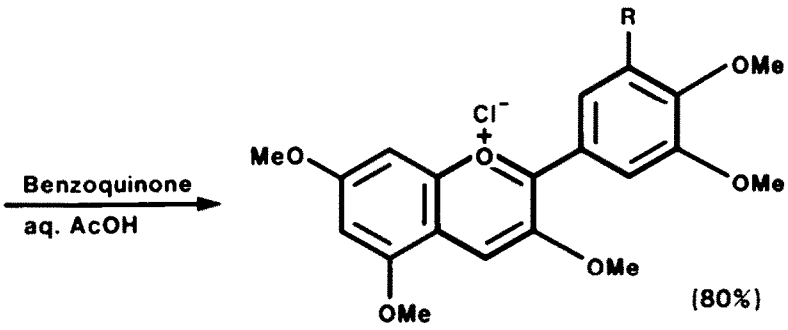


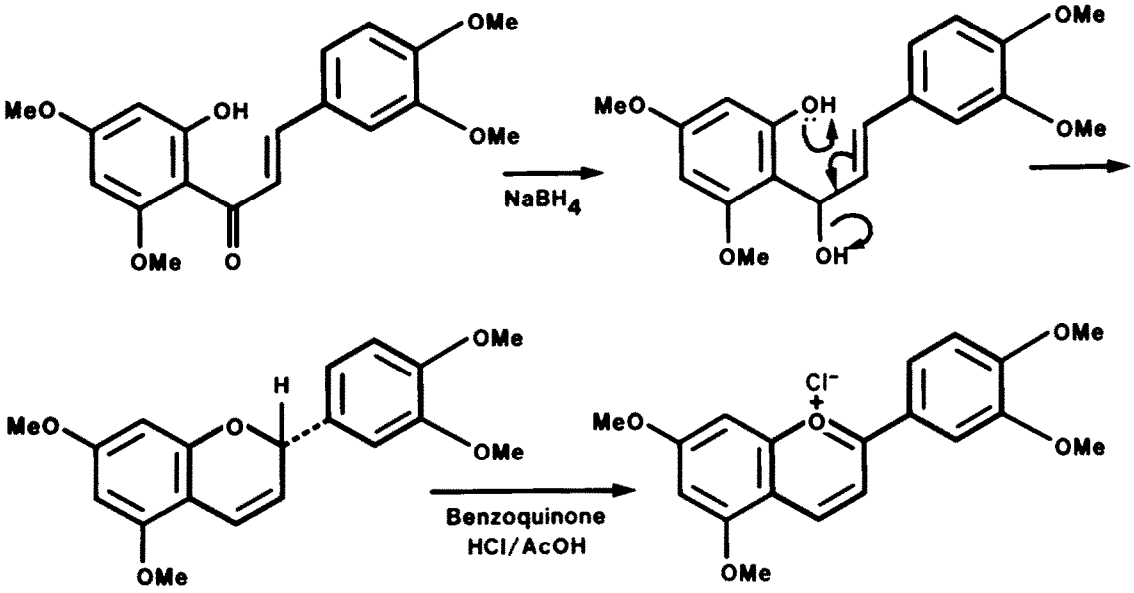
R = H: cyanidin pentamethylether chloride
R = OMe: delphinidin hexamethylether chloride.

A mixture of flavenes is prepared by LiAlH4 reduction of flavonol methyl ethers, as described previously.[63]

The use of benzoquinone for the oxidation of flavenes was introduced by Jurd, who reported yields up to 80%. The reduction with LiA1H4 finds precedence in the work of Mirza and Robinson,[64] and the use of benzoquinone in the observations of Robinson and Walker[65] (*cf* Table 3, Procedure 2).

(b) From 2'-hydroxy-chalcones.

2. Clark-Lewis and Jemison:[66]

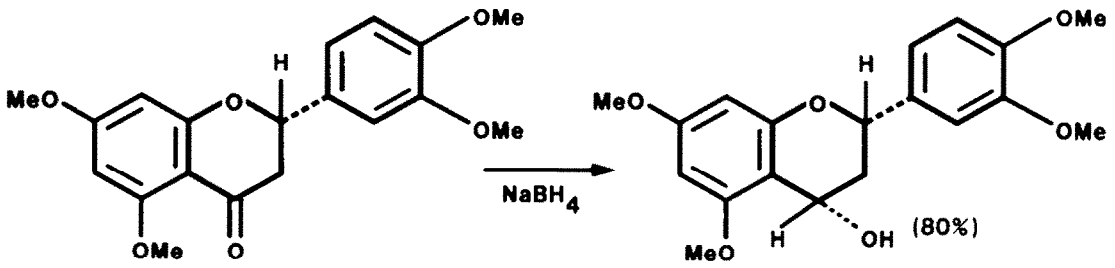


Although applicable to natural 2'-hydroxychalcones,[9] the procedure takes advantage of chalcones prepared by condensation of o-hydroxyacetophenones with substituted benzaldehydes.[67-69]

This reductive cyclization introduced by Clark-Lewis[70] gives flav-3-enes in 60–80% yields. The dehydrogenation step is practiced according to Jurd above.

(c) From flavan-4-ols methyl ethers

3. Sweeny and Iacobucci:[71]



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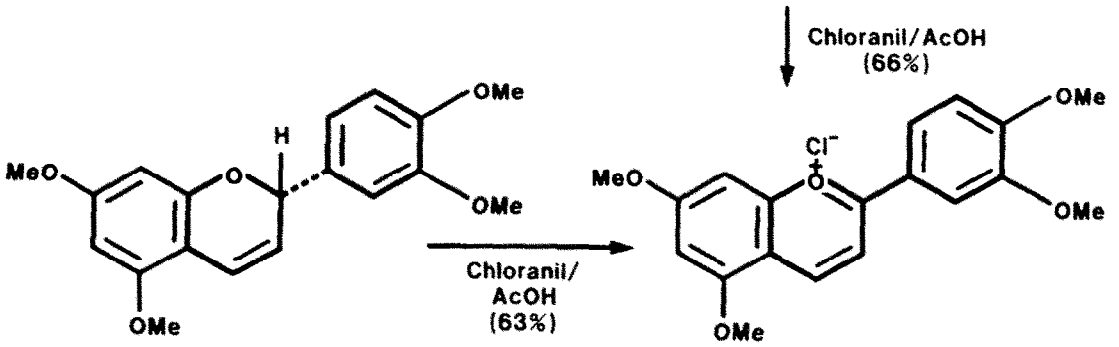
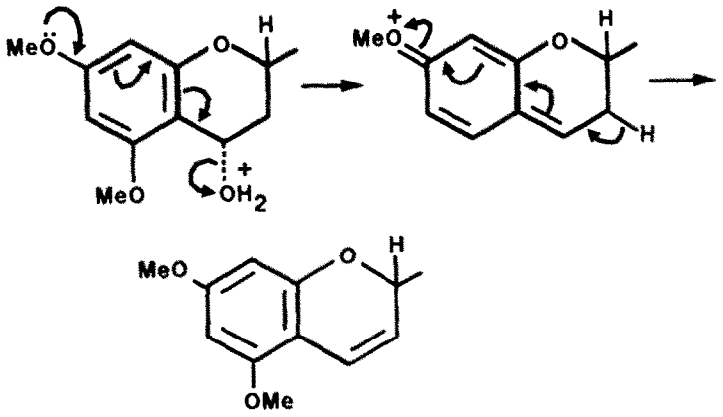


4-Hydroxy-3',4',5,7-tetramethoxyflavan was prepared from 3,'4',5,7-tetra-0-methyleriodictyol by known procedures.[72] Oxidation with chloranil proceeds through a flavene intermediate, as shown. Anthocyanidin yields with chloranil (60–70%) are better than those obtained with benzoquinone (20–40%). The dehydration step requires oxygen substituent at C7, suggesting intermediacy of a quinone methide in flavene formation:



Apigeninidin trimethyl ether and luteolinidin tetramethyl ether chlorides were prepared by this procedure.

(d) From flavan-3,4-diols methyl ethers

4. Sweeny and Iacobucci:[71]

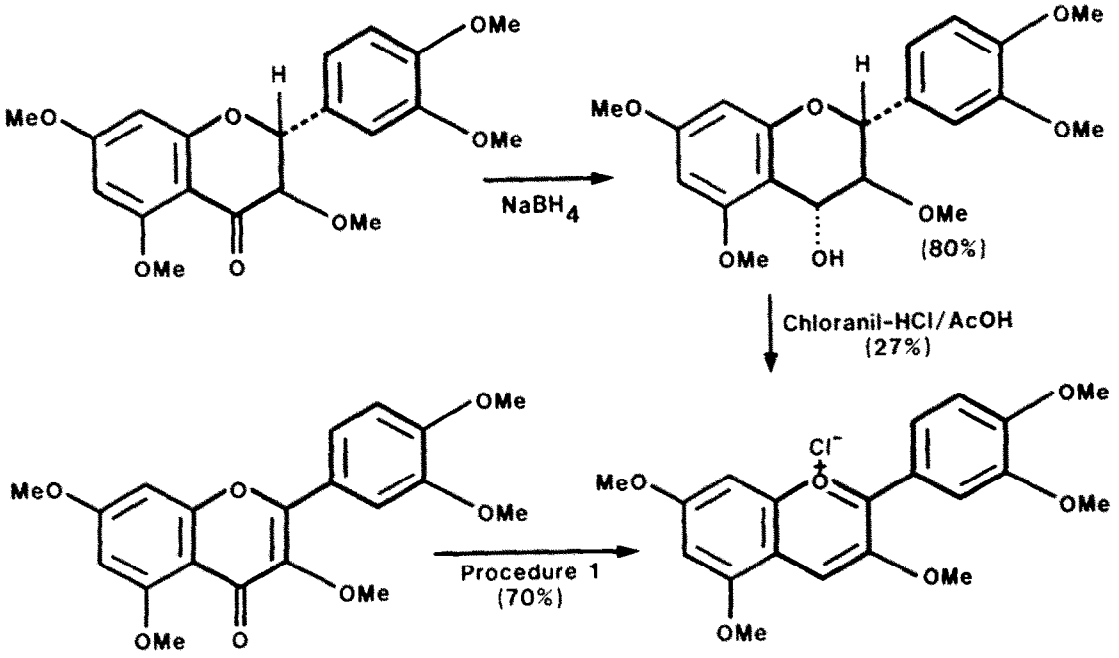


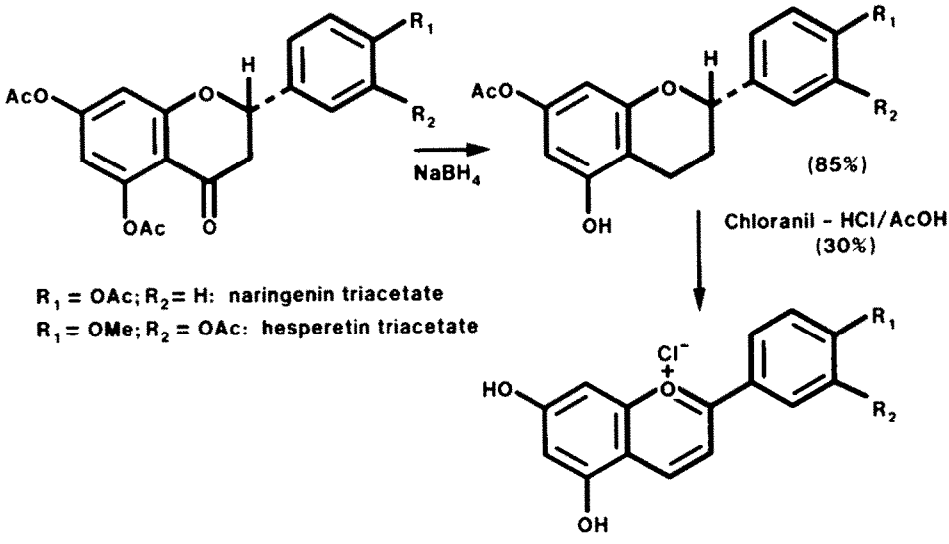
Table 2. *Contd*

Borohydride reduction of taxifolin-3,3',4',5,7-pentamethyl ether gives the pentamethylflavan-3,4-diol shown. Chloranil oxidation to pentamethylcyanidin chloride requires addition of HCl to facilitate the dehydration to the intermediate flav-3-ene. The product was identical to the one prepared from pentamethylquercetin using Procedure 1.

The chloranil oxidation yields in this case were lower than those observed in the oxidation of flavan-4-ols (Procedure 3), and the benzoquinone oxidation of the 3',4',5,7-tetramethoxy-3,4-dihydroxyflavan.[61]

(e) From flavan acetates

5. Sweeny and Iacobucci:[73]



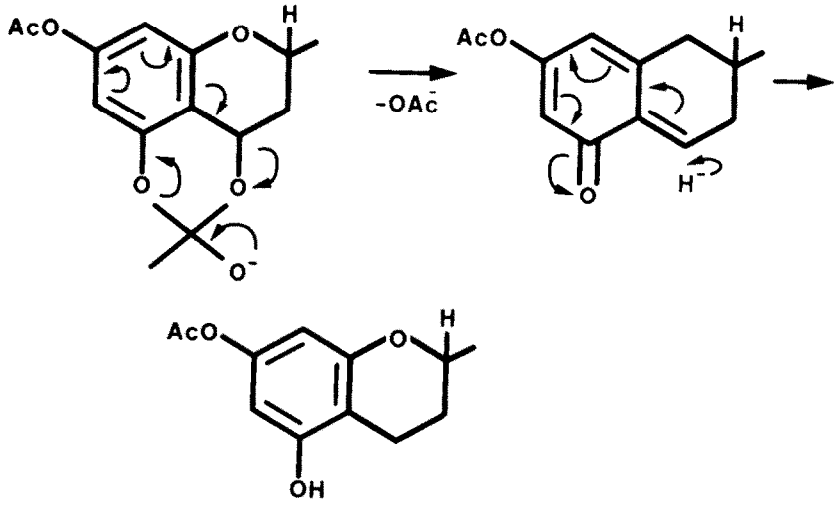
R_1 = OAc; R_2 = H: naringenin triacetate

R_1 = OMe; R_2 = OAc: hesperetin triacetate

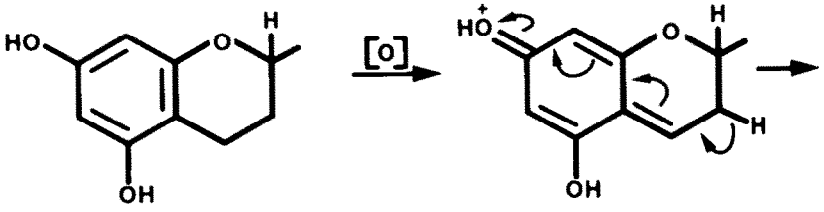
R_1 = OH; R_2 = H: apigeninidin chloride

R_1 = OMe; R_2 = OH: 4'-O - methylluteolinidin chloride

The NaBH4 reduction of flavanone acetates gives flavans with the simultaneous loss of acetyl from the C5-OH. This reaction finds precedence in the reduction of *o*-acetoxyacetophenones to *o*-hydroxy-ethylbenzenes.[74,75] The reduction proceeds through a cyclic ester intermediate, that eliminates acetate from C4 through formation of a quinone methide, that is subsequently reduced by hydride:



The oxidation step requires the prior hydrolysis of the C7(C4')-OAc to the free phenol, and proceeds through the flavene resulting from phenol oxidation at C7(C4'), as illustrated in the following expression for C7:



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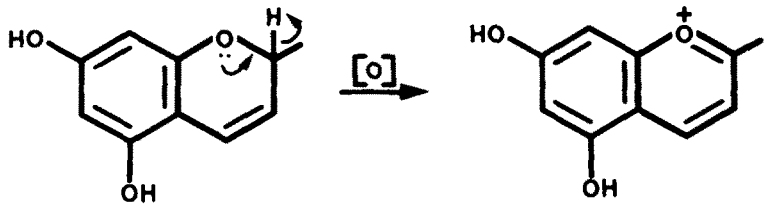
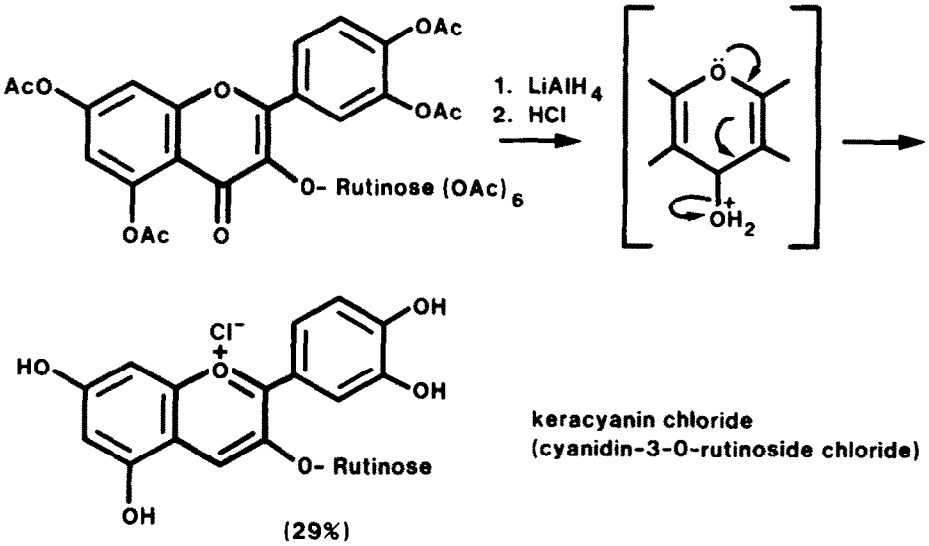


This interpretation is supported by the work of Merlini[76,77] and Jurd[37] on flavene synthesis via phenol oxidation.

B. *flav-2-ene-4-ols*

(a) From rutin decaacetate

6. Bauer, *et al.*[79]



This reaction has been carefully reexamined recently.[80] The keracyanin yields obtained after chromatography on Polyclar AT were only 8–10%. The same reduction conducted on the 3',4',5,7-0-tetramethylrutin hexaacetate, however, gave tetramethylkeracyanin chloride in 35% yield. As the intermediate flav-2-ene-4-ol is easily reducible to the flav-2-ene by hydride (see Procedure 1), the observed yields of anthocyanin might result from the protective effect of the bulky disaccharide at C3, preventing further reduction of the intermediate vinyl alcohol.

Similar results are recorded in the patent literature,[81] where the reduction of deca-(trimethylsilyl) rutin with $LiAlH_4$ yielded keracyanin chloride (after chromatography on Polyclar AT) in 25% yield.

(b) From rutin

7. Wakihira *et al.*:[82]

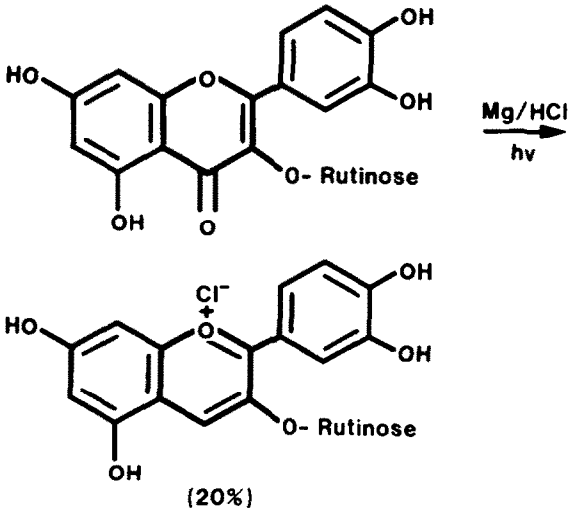


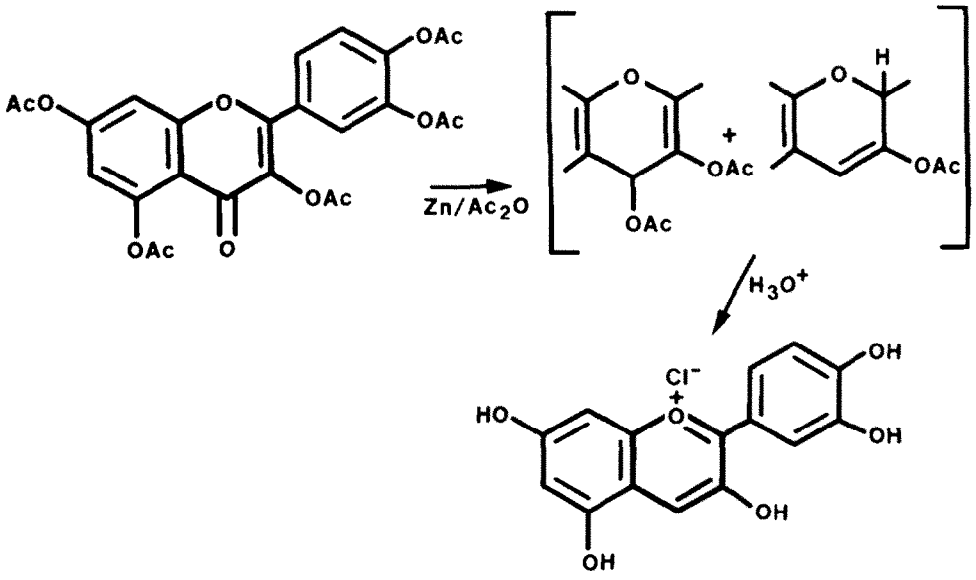
Table 2. *Contd*

The success of this simple method seems to reside in the irradiation (incandescent or ultraviolet lamp) of the reaction mixture while the reaction proceeds. Yield of recrystallized keracyanin chloride was 20%, but only 5% in the absence of irradiation.

A similar procedure using Mg/H_2SO_4 without irradiation, is documented in a German patent.[83]

(c) From flavonol acetates and flavonol glycoside acetates

8. Robertson and Robinson:[55]



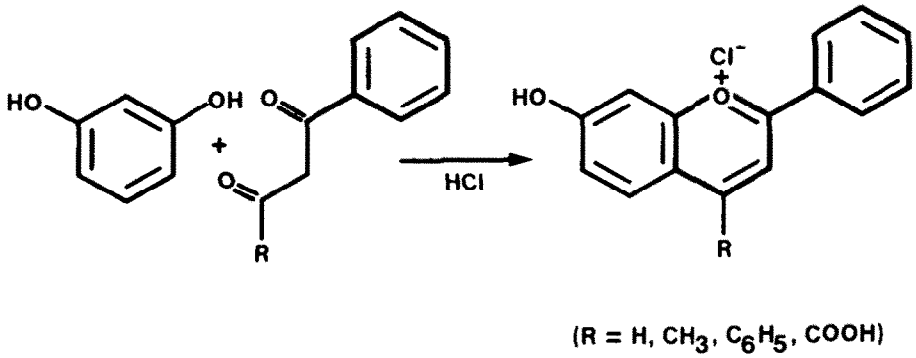
These pioneering experiments on the reductive acetylation of flavonols produced anthocyanidins in low yields. This procedure was developed on the basis of the original observations of Willstätter[84] on the reduction of quercetin with Mg and Zn dust in methanolic HCl (*cf* procedure 7 above) to cyanidin chloride. Subsequent work by King and White[85] improved on the preparative conditions and raised the yields of cyanidin chloride from quercetin up to 25%. The most recent papers published on the subject[86–88] review the history of this reaction, and provide NMR evidence that the cyanidin precursors are a mixture of the flav-2-ene and flav-3-ene derivatives.

Table 3. Synthesis of flavylium salts related to anthocyanidins

RING A SYNTHON

A. *Resorcinol*

1. Bulow:[23,24,89]

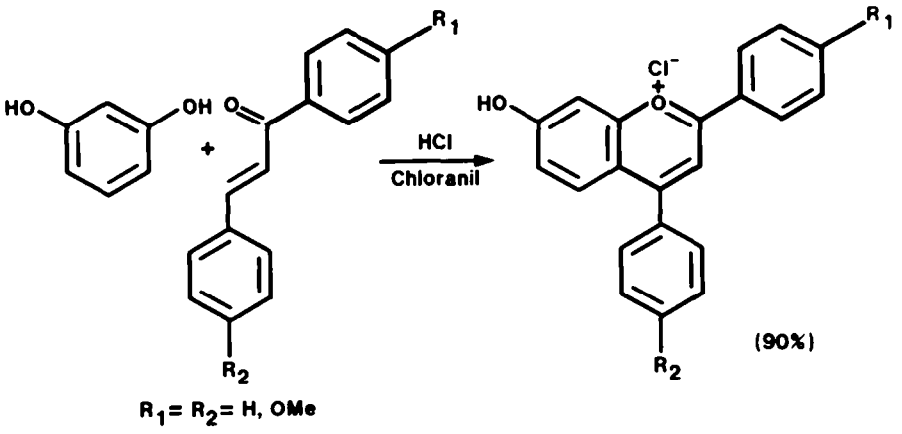


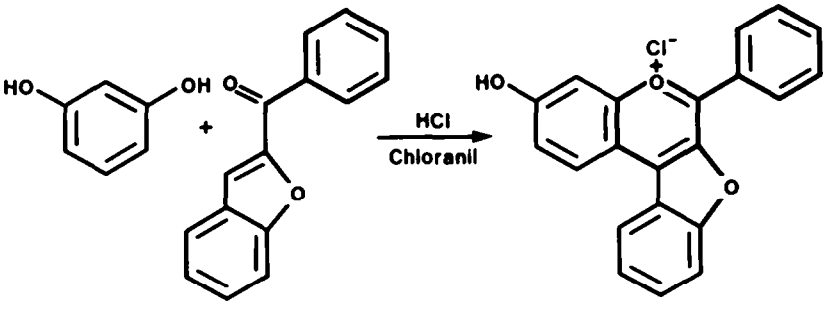
Extension to resorcinol of the procedure 1 of Table 1 described for phloroglucinol.

Benzoylacetaldehyde (R = H) gives 7-hydroxyflavylium chloride with resorcinol, although it failed to react with phloroglucinol.[89] *Timberlake*[90,91] has utilized this reaction for the synthesis of 7-hydroxy-4-methyl- and 7-hydroxy-4-*p*-anisylflavylium chlorides with rings B of varied hydroxylation patterns.

Table 3. *Contd*

2. Robinson and Walker:[65]

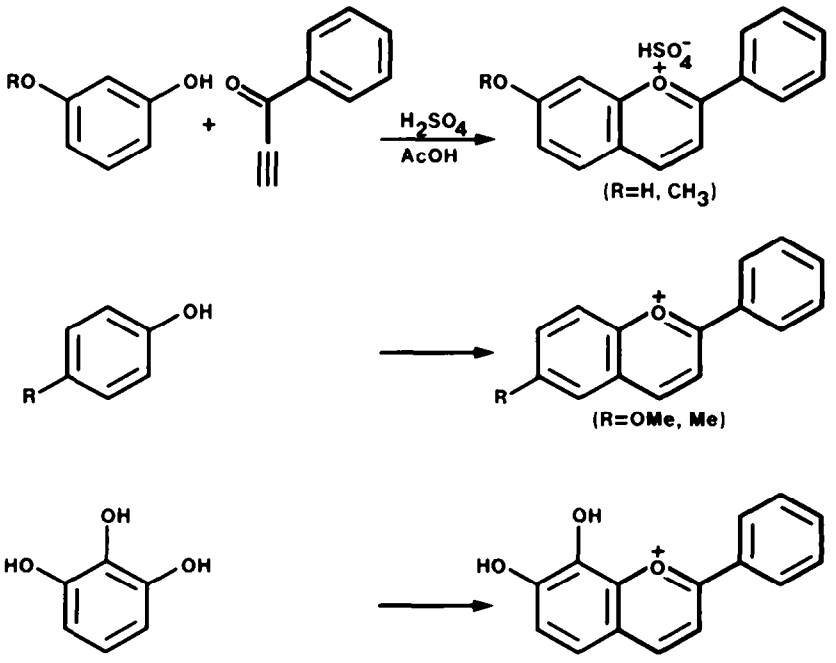




Resorcinol condenses well with a variety of α,β-unsaturated ketones of the type illustrated, to give 7-hydroxy-4-phenyl-substituted flavyliums.

Chloranil is used for the first time for the dehydrogenation of the intermediate flavenes.

3. Johnson and Melhuish:[27]

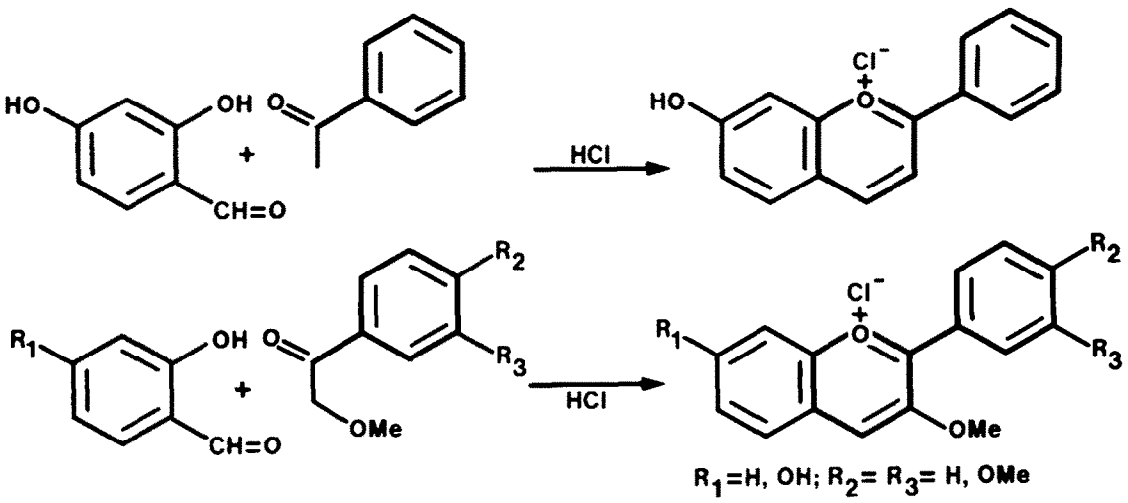


This procedure already discussed for phloroglucinol (Table 1, Procedure 3) can be extended to resorcinol and other reactive phenols equally well.

Table 3. *Contd*

B 2-*Hydroxybenzaldehydes, via* 2-*hydroxychalcones*

4. Perkin and Robinson:[50,51]



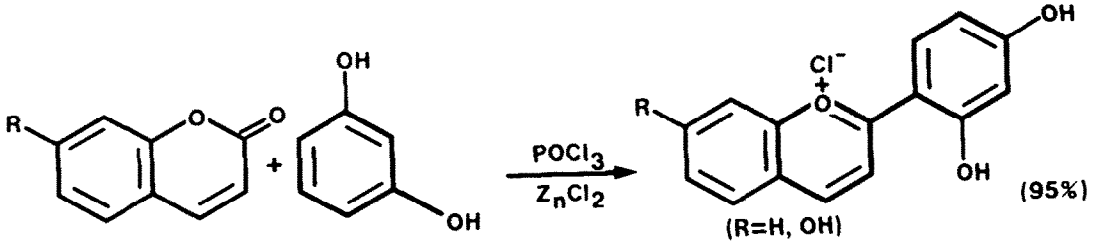
First successful preparation of a flavylium salt by the condensation of acetophenone with a 2-hydroxybenzaldehyde, followed by acid catalyzed cyclisation of the chalcone.

These simple model reactions opened the way to the more definitive work by Pratt and Robinson[45-48] on the synthesis of anthocyanidins (*cf* Table 1, Procedure 7).

The same procedure was utilized by Jurd[92-94] for the preparation of a family of 7-hydroxyflavylium salts, investigated as potential food colorants.

C. *Coumarins*

5. Goswami and Chakravarti:[95]



A variation of the original procedure of Decker and Fellenberg[43,44] on the 2-phenylation of coumarin using Grignard reagents (*cf* Table 1, Procedure 6). This method, as revised by Wizinger[96], proceeds in excellent yields. It is convenient for the preparation of flavylium salts having unusual substitutions in ring B, starting from readily available coumarin and 7-hydroxycoumarin.

The reaction between coumarin and phloroglucinol was reported by Jurd[97] to proceed in 20% yield only.

6. Mercier *et al.*:[98]

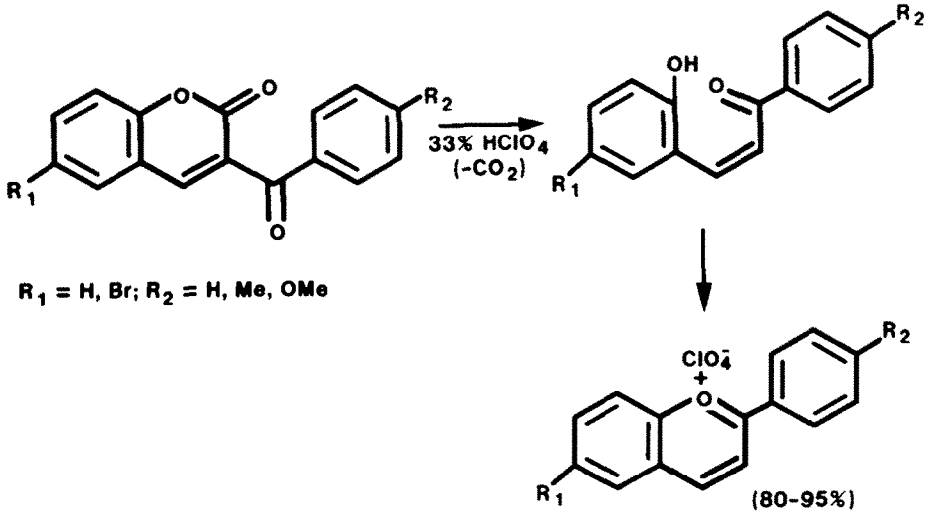


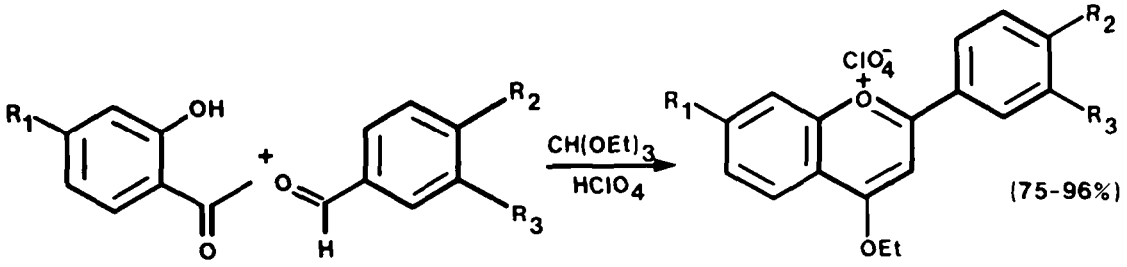
Table 3. *Contd*

Procedure practiced satisfactorily with the series of 3-benzoylcoumarins indicated. The reaction involves hydrolysis of the lactone ring, decarboxylation of the intermediary cinnamic acid, and reclosure of the 2-hydroxychalcone formed to the flavylium salt.

3-Benzoyl coumarins are easily accessible from the acid catalyzed condensation of salicylaldehydes with ethyl benzoylacetate.[99] So far, no natural product has been made in this way.

D. 2-*Hydroxy acetophenones, via 2'-hydroxychalcones*

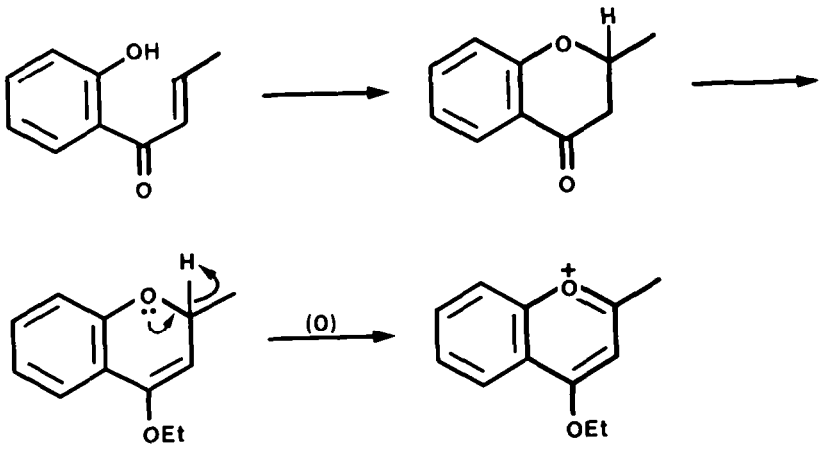
7. Dorofeyenko and Tkachenko:[100-102]



R_1 = H, OH, OMe
R_2 = H, OH, OMe
R_3 = H, OMe

Procedure for the preparation of 4-ethoxyflavylium salts, that are equivalent to flavone enol ethers. Best prepared by one-pot condensation of acetophenones and benzaldehydes with ethyl ortho-formate, in 70% perchloric acid.

This reaction probably proceeds through a flavanone diethyl acetal intermediate:



Upon boiling with water, the 4-ethoxyflavylium hydrolyses to the flavone quantitatively. 18 different 4-ethoxy-flavylium salts have been prepared by this procedure.

E. *Miscellanea*

Reactions of little synthetic value, or of questionable interpretation.

(a) Isomerization of 3-hydroxyflavanones into anthocyanidins.

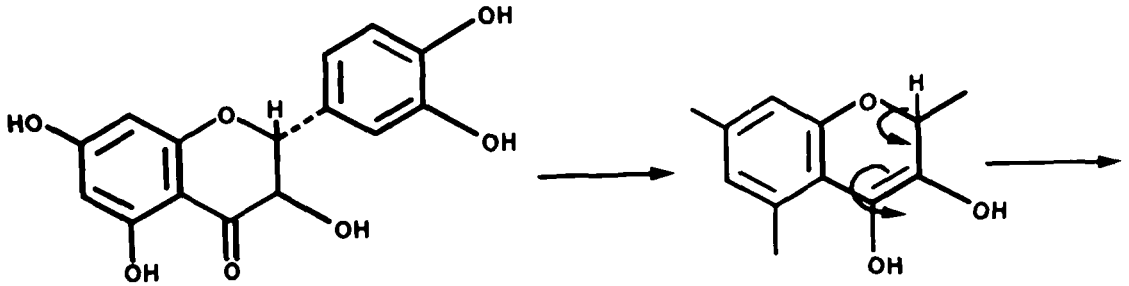
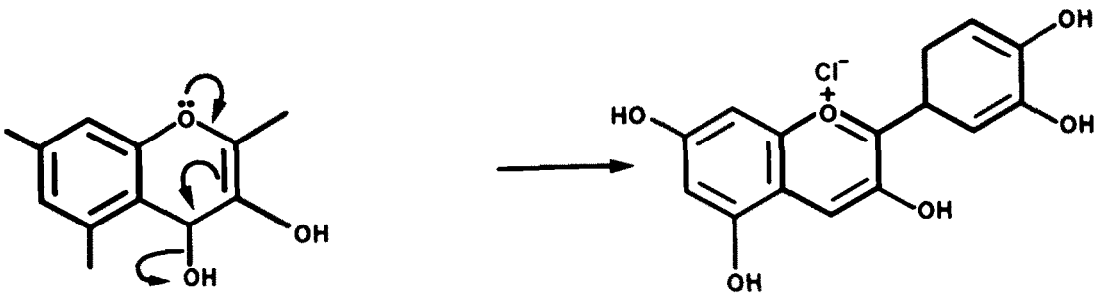


Table 3. *Contd*

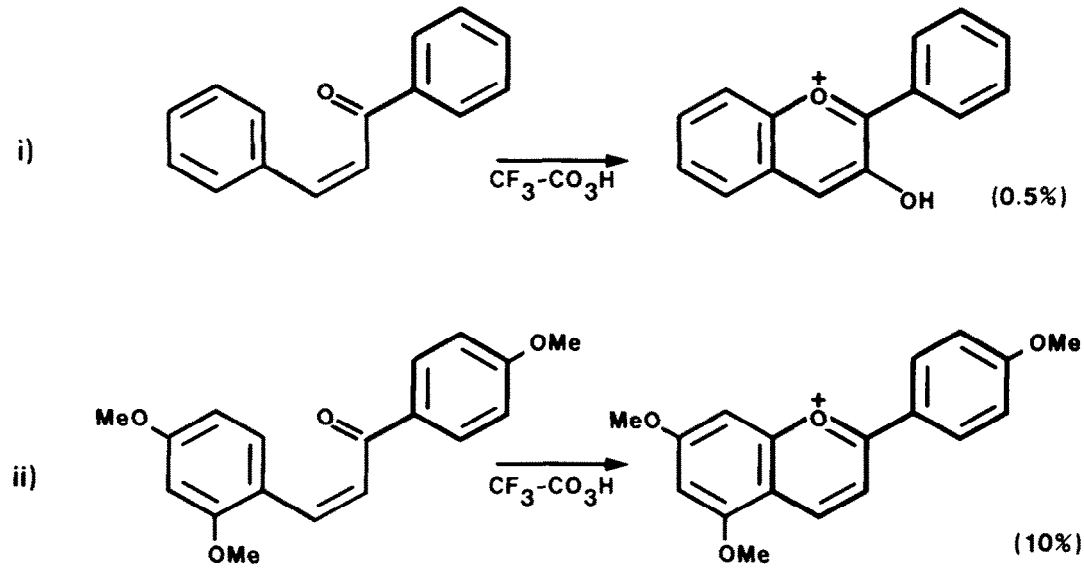


Independent reports by Seshadri[103] and Jurd[104] have noted the appearance of a red color when flavanonols were heated etiher with bases (acetate, pyridine) or acids (EtOH/HCl), respectively.

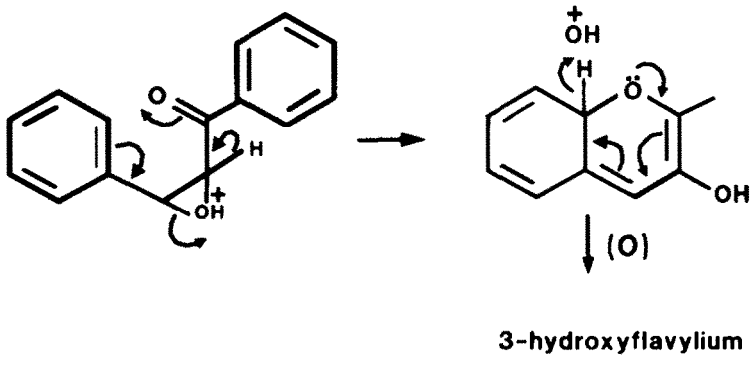
The suspicion that those colors are due to anthocyanidins has not been satisfactorily documented, nor the isolation and characterization of the products attempted.

The conversion presumably proceeds under acid or base catalysis, as both reactant and product are at the same oxidation level.

(b) Oxidative cyclization of chalcone and 2,4,4'-trimethoxy-chalcone to flavylium salts.



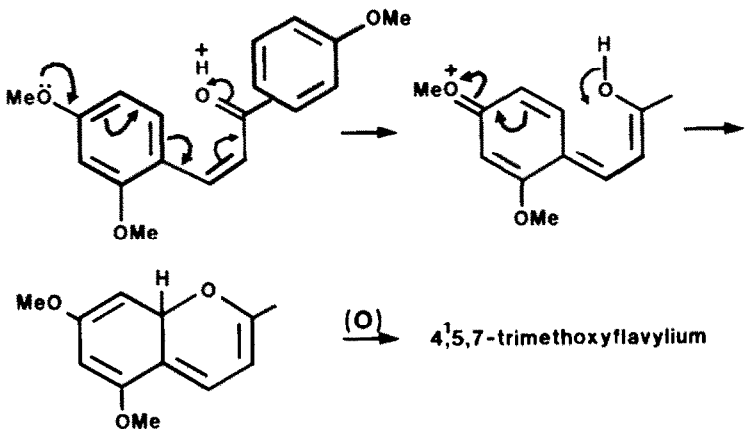
Both reactions have been reported by Brown *et al.*[105] Reaction (i) could be considered as driven by the concerted opening of an intermediate epoxide, followed by dehydrogenation to the flavylium:



3-hydroxyflavylium

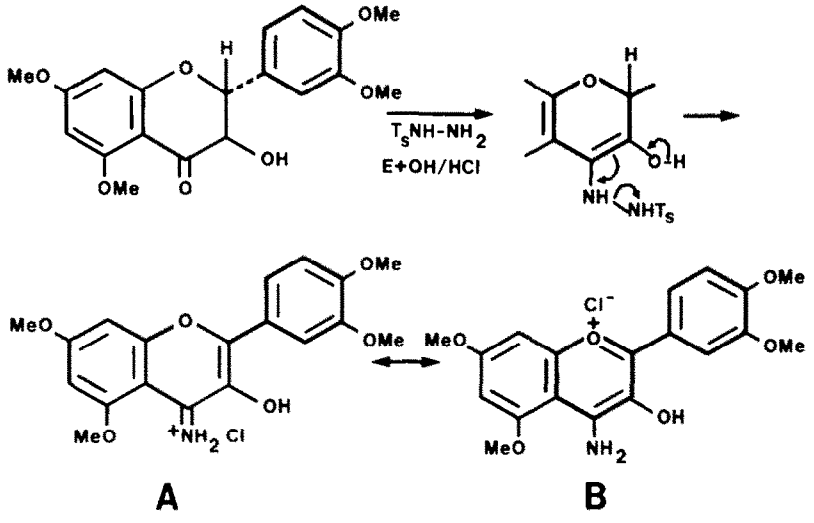
Reaction (ii) follows a different course, perhaps under the influence of the 4-OMe group:

Table 3. *Contd*



The low yields, and the disparity in course of both reactions, suggest they should be carefully reexamined.

(c) The nature of 3-hydroxy-4-aminoflavylium salts.



This reaction was reported by Kallay[106–108] as giving 92% of a monochloride assigned the flavylium structure B. No electronic spectra was shown, although the chemical reactivity (base hydrolysis to the flavonol; acetylation to the iminiflavonol-O-N-diacetate) is more directly interpreted by the resomer A.

We have repeated the preparation[80] and the product (yellow needles, m.p. 205–7°) had λ_{max}(MeOH): 211, 275, 438 nm (ϵ = 30,000, 21,800, 24,400), as compared to 3',4',5,7-tetramethyl quercetin (λ_{max} 360 nm; ϵ = 20,200) and to 3',4',5,7-tetra-methyl cyanidin chloride (λ_{max} 535 nm, ϵ = 32,000). An attempted $NaBH_4$ reduction in EtOH/H_2O with 20 fold excess of reductant yielded unchanged starting material, as expected from structure A. Consequently, it is suggested that these compounds be named as derivatives of 4-iminoflavonol hydrochloride, instead of the 4-aminoflavylium chloride nomenclature proposed by Kallay.

Quercetin is by far the most abundant of the flavonols; it is commercially available in the form of rutin (quercetin-3-O-rhamnoglucoside), present as such in high concentrations (3–5%) in the flowers of *Sophora japonica*, and the leaves of buckwheat (*Fagopyrum esculentum*).[20] Rutin is of importance because (a) it is a glycoside and (b) the point of attachment of the sugar moiety at C3 is typical of anthocyanins and is critical for securing the chemical stability of the resulting anthocyanin. The several documented attempts to convert rutin into cyanidin-3-rutinoside (keracyanin) are listed in Table 2. The commercial availability of rutin of high purity has stimulated further work in that direction, since it was first shown by Birch[79] that this conversion was feasible.

(b) *Synthesis of flavylium salts other than anthocyanidins*

This section comprises an extensive list of anthocyanidin analogs whose structures differ from the natural pigments either in the hydroxylation pattern of ring A or in the presence of unusual substituents attached to the pyrilium ring.

The most common variation of the hydroxylation pattern in flavyliums involves the lack of hydroxyl at C5. These flavyliums are prepared from resorcinol using the procedures of Bulow or Robinson, in yields higher than those usually obtained from phloroglucinol (Table 1). Methods of this kind were extensively practiced by Jurd[92-94] and Timberlake[90,91] during the decade 1960–70 to investigate simple flavylium salts that could function in foods with advantage over the more elusive anthocyanidins and anthocyanins. These expectations have not been quite fulfilled, as more recent work on the correlation between chemical structure and stability[1,114,120,125] has shown.

The various structures synthesized are given in Table 3.

III. THE CHEMISTRY OF FLAVYLIUM SALTS IN AQUEOUS SOLUTIONS

(a) *pH Change effects*

Anthocyanins are notoriously unstable under neutral or basic conditions. The effect of increasing pH on anthocyanin structure was first investigated in the sixties by Jurd,[109,110] Timberlake and Bridle[111] and Harper and Chandler,[112,113] but it was not until Brouillard's work in the late seventies using pH jump techniques that the structural transformations involved on increasing the pH of an anthocyanin solution were finally clarified.[114-120]

At pH's below 2, the anthocyanin exists primarily in the form of the red (R_1 = 0-sugar) or yellow (R_1 = H) flavylium cation (AH^+). As the pH is increased a rapid proton loss occurs to afford the red or blue quinoidal forms A (Scheme 1). The quinoidal form usually exists as a mixture, as the pK_a's of the 4',7- and (if present) 5-OH groups are very similar.[114,78] On standing, a further reaction occurs, that is hydration of the flavylium cation (AH^+) to give the colorless chromenol B. This in turn can, at an even slower rate, equilibrate to the open chalcone form C, which is also colorless.

The alternative structure for the chromenol, that is attack of H_2O at the 4 position to give the chrom-2-ene-4-ol, has also been considered. McClelland and Gedge[121] have shown that although nucleophilic attack at the 4 position of 5-unsubstituted flavylium cations is the kinetic product in agreement with MO calculations,[122] the thermodynamic product is the chromen-2-ol by a factor of at least 10^2.

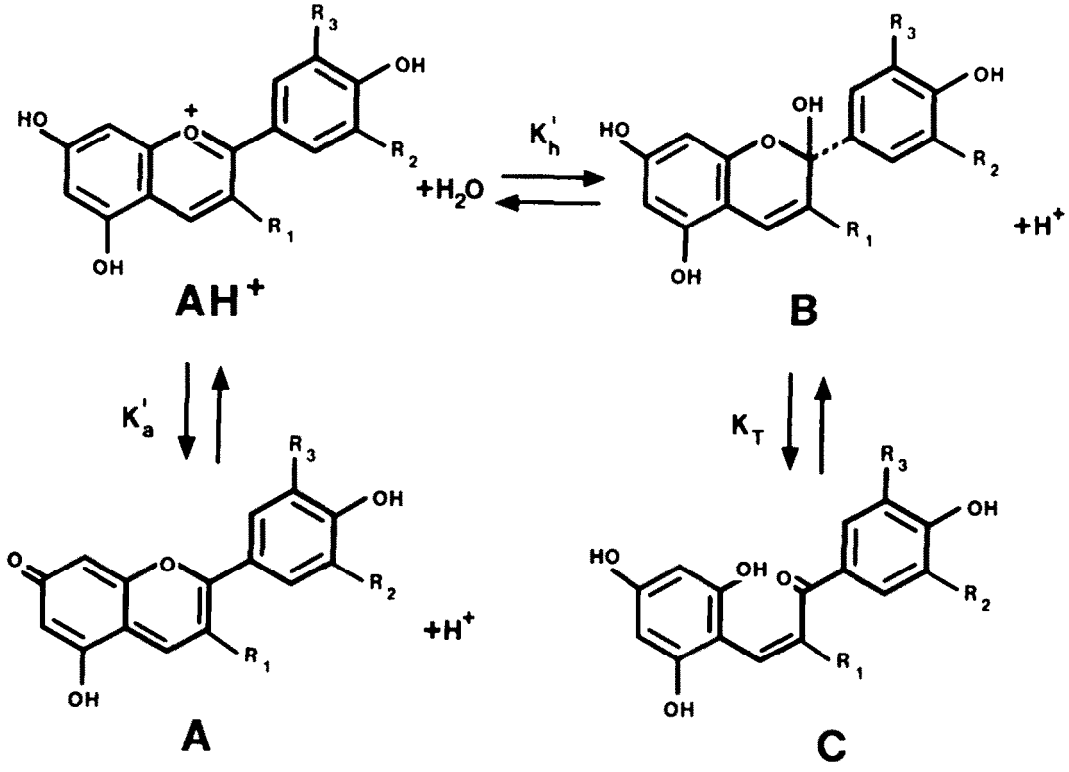
The relative amounts of cation (AH^+) , quinoidal forms (A), chromenol (B) and chalcone (C) at equilibrium vary with both pH and the structure of the anthocyanin. For the common anthocyanin 3-0-glycosides or 3,5-di-0-glycosides, the principal product formed on raising the pH above 3 is the colorless chromenol B as shown in Fig. 1 for malvidin-3-glucoside.[116]

By varying the substitution pattern of the flavylium ring, anthocyanidins which exist primilarly in the quinoidal form A or the chalcone form C can be prepared.[120] This is exemplified by 4',7-dihydroxyflavylium chloride (Fig. 2) which forms the chalcone at pH's above 3, and 4-methyl-7-hydroxy-4'-methoxyflavylium chloride (Fig. 3) which exists as the quinoidal isomer at pH's above 5. Of course, many flavylium salts can form mixtures of the quinoidal, chalcone and chromenol forms as is shown for 4',5,7-trihydroxyflavylium chloride (apigeninidin, Fig. 4). Very recently, McClelland and McGall have shown that 4'-hydroxyflavylium chloride also exists as a mixture of chromenol, chalcone and quinoidal base at pH 7.[123]

During his kinetic studies, Brouillard[114] also found that each of the reactions shown in Scheme 1 was endothermic. On heating an anthocyanin solution, therefore, the equilibria are driven toward the chalcone form C and a resulting decrease in colored forms occurs. Cooling will reverse the change, although as long as one hour may be required to re-establish equilibrium.[124] This time lag for reversion of the chalcone to the anthocyanin form has been exploited by Preston and Timberlake[124] to isolate malvidin-3-glucoside chalcone and malvidin-3,5-diglucoside chalcone for the first time via high pressure liquid chromatography of a rapidly cooled anthocyanin solution.

(b) *Effects of structure on the stability of the color of anthocyanins*

Assuming that an anthocyanin is kept at an adequate acidic pH (2–3), it should be quite stable at room temperature. For instance, the half-life of a typical anthocyanin, cyanidin-3-rutinoside, is about 65 days at room temperature in 0.01 M citric acid, pH 2.8.[1] The corresponding free anthocyanidin, cyanidin, however, has a half-life of only 12 hr. The slow hydrolysis of the 3-0-sugar units of anthocyanins under



Scheme 1.

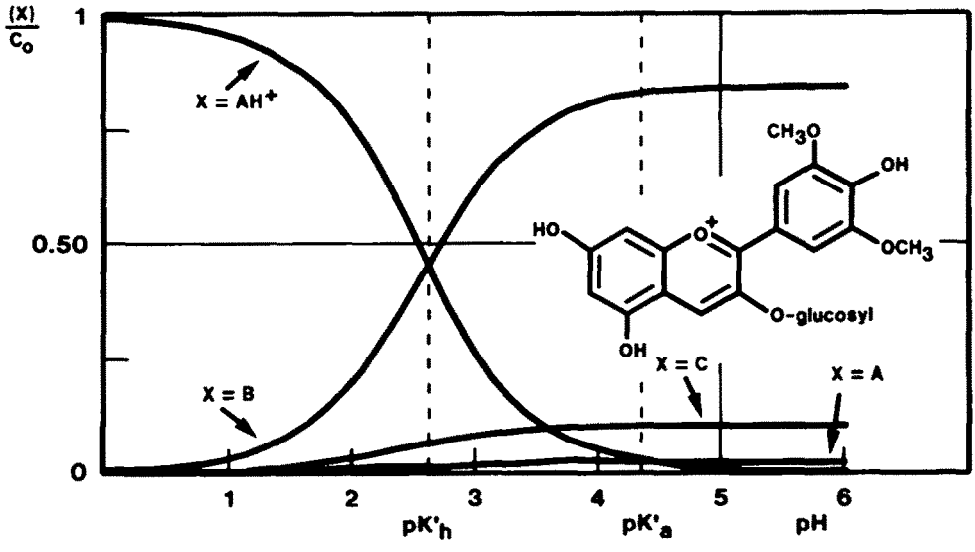


Fig. 1. Equilibrium distribution of AH^+, A, B and C for malvidin-3-glucoside as a function of pH (from Ref. 116, by permission of Elsevier).

acidic conditions is presumably responsible, therefore, for the long-term degradation of these pigments. Attempts to improve the stability by methylation of free phenolic hydroxyl groups had the opposite effect instead (Table 4).

In a related study on the stability of 4 anthocyanins and 13 synthetic 3-deoxyanthocyanidins, Ohta *et al.*[125] found poenidin and malvidin to be much less stable than their corresponding 3-glucosides at pH 2.5 and 4.5. The results of this study led to two conclusions: (a) the presence of a 7-OH group greatly enhanced stability, and (b) the addition of OH groups in the B ring decreased stability.

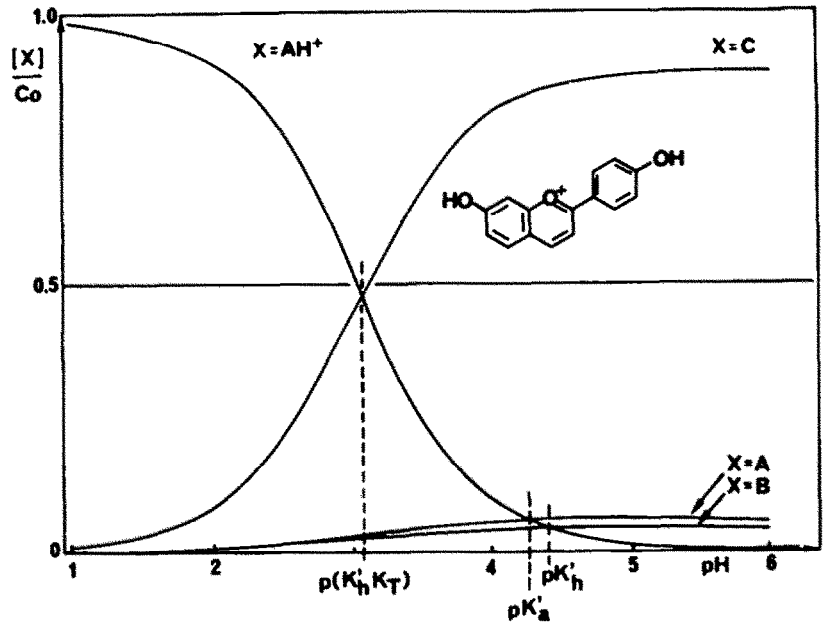


Fig. 2. Equilibrium distribution of AH^+, A, B and C forms for 4',7-dihydroxy flavylium chloride as a function of pH. (From Ref. 120, by permission of the American Chemical Society).

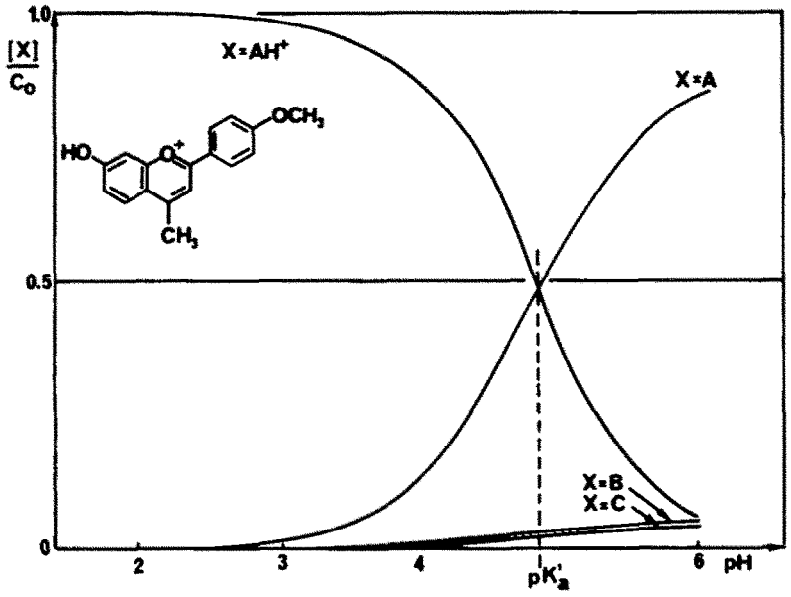


Fig. 3. Equilibrium distribution of AH^+, A, B and C forms for 4'-methoxy-4-methyl-7-hydroxy flavylium chloride. (From Ref. 120, by permission of the American Chemical Society).

Our own studies[1] with synthetic 3-deoxyanthocyanidins are summarized in Table 4. From this table it is apparent that the presence of either a 4'-OH or a 7-OH confers stability while methylation of these hydroxyls decreases it. Introduction of a vinyl group between C2 and the B ring to give a red 3-deoxyanthocyanidin[126] also results in loss of stability. Interestingly, of all the synthetic flavylium salts tested, the naturally occurring apigeninidin showed the greatest stability.

The nature of the products formed during long term standing in solution remains unknown. Kinetic studies have shown that the reaction is first order with respect to the anthocyanin in the pH range 2.5–4.5.[127] The most likely reaction mechanisms would involve (a) hydrolysis of the anthocyanin to the

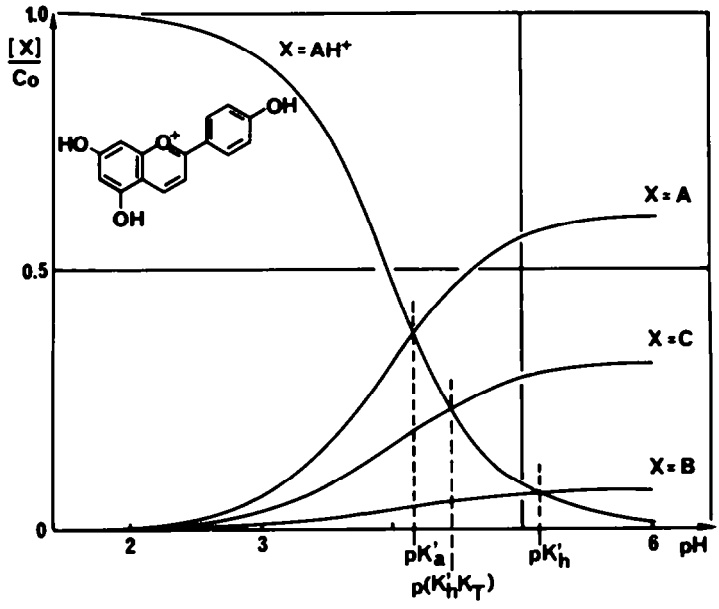
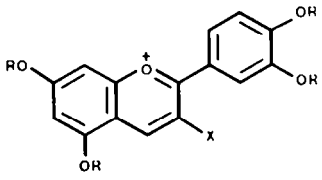
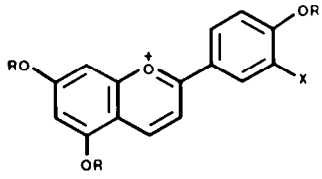


Fig. 4. Equilibrium distribution of AH', A, B and C forms for apigeninidin chloride. (From Ref. 120, by permission of the American Chemical Society).

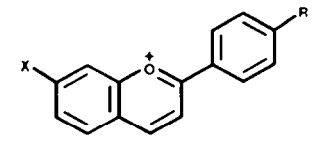
Table 4. Effects of structure on the half-life (τ) of flavylium colors, measured at their λ_{max} in 0.01M citric acid, pH 2.8, at room temperature



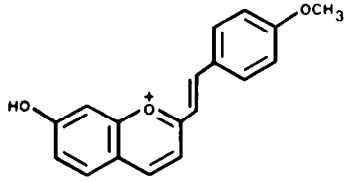
X	R	τ(days)	λmax (nm)
OH	CH_3	0.04	512
OH	H	0.50	512
OCH_3	CH_3	6	512
O-Rutinose	CH_3	13	512
O-Rutinose	H	65	512



OCH_3	CH_3	170	488
H	H	400	475



OH	OH	400	458
H	OH	400	436
H	OCH_3	35	437
OH	H	300	428
OCH_3	H	8	427



		50	506

anthocyanidin (free 3-OH), which in turn could be hydrolytically cleaved at the α,β-diketo function, and (b) reaction with oxygen to give degradation or phenolic coupling products. Phenolic oxidation as a possible mechanism is also supported by the acceleration of decomposition with increasing pH[127,128] and the general browning usually observed.

In complex phenolic mixtures like wines, condensation with co-occurring tannins can also proceed in anthocyanin extracts high in these materials. In red wine, for instance, the molecular weight of the colored fraction increases slowly with time due to the anthocyanin-tannin condensation reaction.[129–132]

One suggested method of increasing the stability of anthocyanidins involves substitution of the 4-position with a methyl or phenyl group.[91] Although these compounds show enhanced resistance to fading in the presence of SO_2 or ascorbic acid, their behavior in aqueous solutions is similar to that of the 4-unsubstituted parent compounds.[1]

The substitution of a carboxy group at the 4-position also gives a compound whose stability at pH 2.8 is similar to that of apigeninidin.[1] The 4-carboxy anthocyanidin does, however, possess the interesting property of being stable at neutral pH.[1]

(c) *Oxidation reactions*

(1) *Reactions with ascorbic acid/O_2.* Anthocyanins as a class have long been known to be particularly unstable in the presence of ascorbic acid.[133–135] The role of O_2 in this reaction was recognized early and explained on the basis of the autoxidative properties of ascorbic acid.[133] The importance of O_2 was further indicated by the inhibitory effect on anthocyanin bleaching exhibited by phenolic anti-oxidants like quercetin.[136–137] Later observations[138] have reaffirmed the essential role of O_2 in the bleaching of anthocyanidins by ascorbic acid. After rigorous exclusion of air, through repeated evacuations and displacements with argon, ascorbic acid was unable to decolorize apigeninidin during 10 days standing, but did it in a few hours after air was admitted.[138] That the bleaching is the result of an oxidative cleavage of the pyrilium ring of apigeninidin was evidenced by the GC/MS characterization of phloroglucinol, hydroquinone and p-hydroxybenzoic acid as degradation products.[138] Suggestions that the fading results from the direct attack of ascorbic acid on the flavylium cation[139,140] can apparently be discounted. In fact, the blocking of the autoxidizable ene-diol function of ascorbate by methylation or acylation, as in the case of the 3-0-methyl and 2-0-isovaleryl derivatives shown in Fig. 5, suppressed the

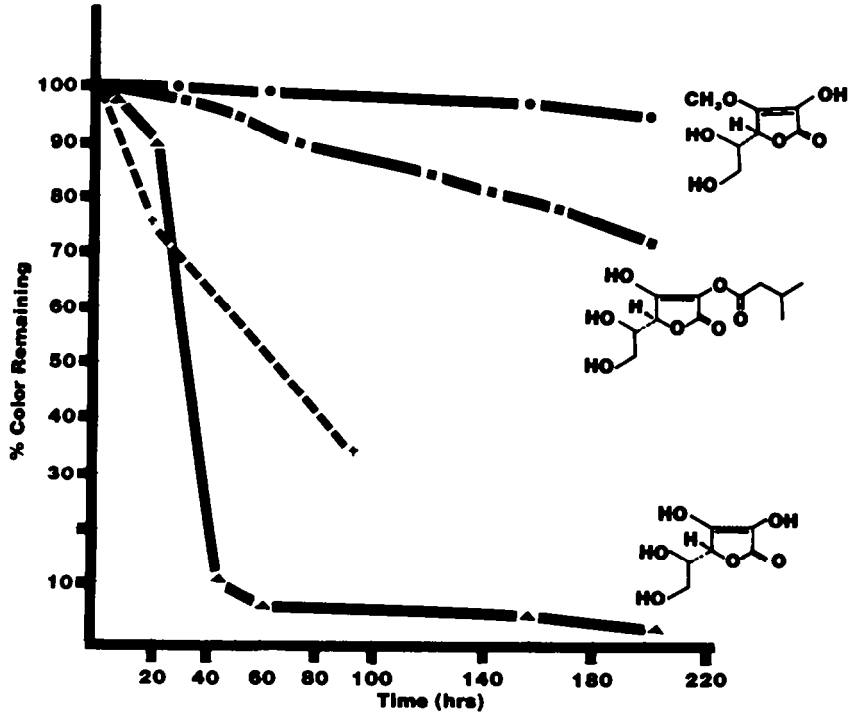
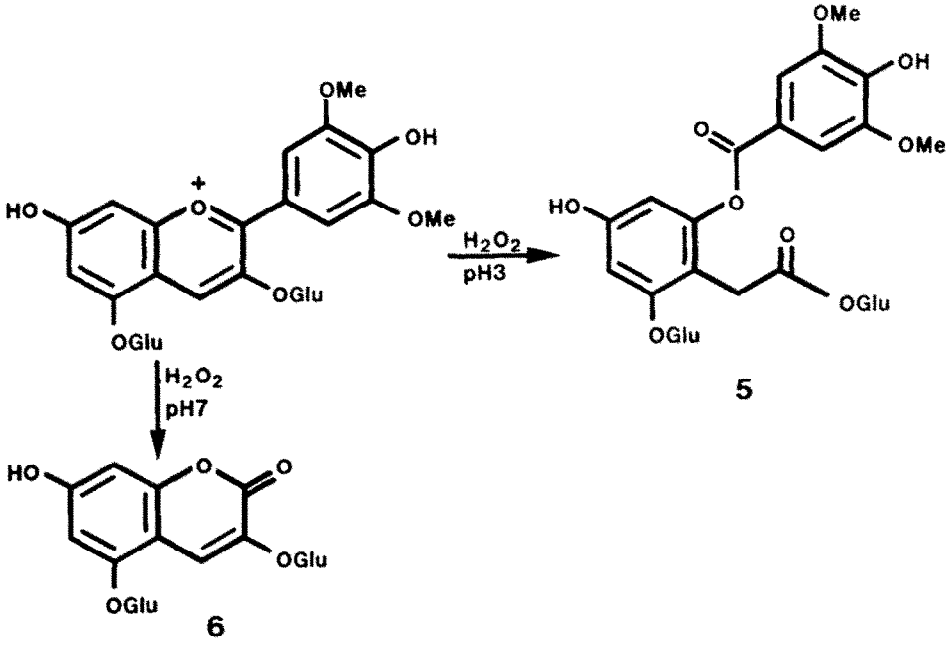


Fig. 5. Decoloration of apigeninidin chloride (20 ppm) by ascorbic acid (--, 500 ppm) and by equivalent concentrations of 3-0-methyl ascorbic acid (●--●) and 2-0-isovaleryl ascorbic acid (--), in 0.01 M citric acid (pH 2.8) saturated with air, at room temperature. (+---+) % Hydrolysis of 2-0-isovaleryl ascorbic acid as followed by HPLC. (From Ref. 138).

decolorization of apigeninidin in the presence of air.[138] This observation has led to the development of anthocyanin rich preparations of improved color stability containing ascorbic acid 2-O-phosphate as a source of vitamin C.[141]

The exact nature of the activated oxygen species responsible for the bleaching is still a subject of investigation. It is known, however, that the autoxidation of ascorbic acid proceeds to yield hydrogen peroxide and dehydroascorbic acid. The intermediacy of the perhydroxyl radical HOO· has been demonstrated[142,143] but it is not certain whether this species causes the bleaching or whether it reacts further with H_2O_2 (the Haber–Weiss reaction[144]) to give HO· as the reactive intermediate. Support for the later mechanism comes from the finding[138] that a similar range of oxidation products are formed from treatment of apigeninidin chloride with Fenton reagent[145] (a classic source of ·OH) when compared to those obtained with ascorbic acid–oxygen.

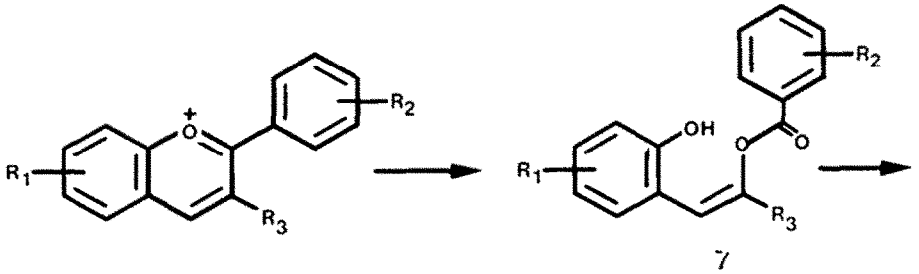
(2) *Reactions with hydrogen peroxide.* The reaction of anthocyanins with H_2O_2 was first investigated by Karrer, who found that malvin reacted to give malvone 5.[146,147] In malvone one sugar residue is

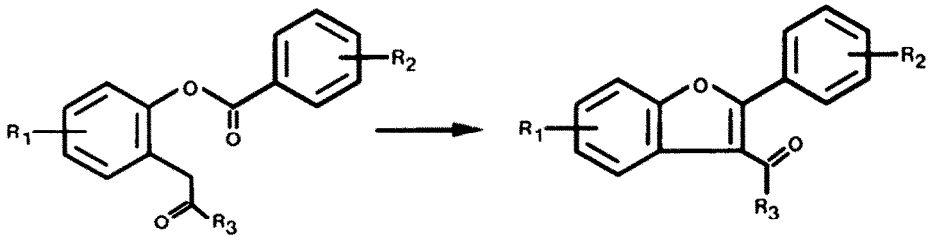


attached as an ester and can be easily removed by NH_4OH; the C5 sugar unit requiring acid hydrolysis for removal. Hydrogen peroxide oxidation has therefore been suggested as a method to determine the nature of the C3 and C5 sugar units.

In more recent studies, Hrazdina and Franzese[148] have found that the reaction takes a different course if conducted at pH 7 instead of pH 3 as described by Karrer. At pH 7 the anthocyanin exists almost exclusively as the chromenol and gives the coumarin derivative **6** as the major product.

Following upon an early report by Dilthey and Qunit,[149] Jurd[150-154] has elucidated the course of the H_2O_2 oxidation of simple flavylium salts. When the flavylium cation contains a 3-substituent, oxidation occurs in a series of steps depending on the pH. In aqueous acetic acid, the C ring is cleaved to give the Baeyer–Villiger product **7**. At higher pH (5–7) acyl transfer occurs to afford ester **8**, which in turn can yield the acyl-benzofuran **9**. A benzofuran of type **9** has also been isolated by Niebes and Janot from the carefully controlled oxidation of cyanidin-3-glucoside-5-[6-O-p-coumaryl-glucoside].[155]





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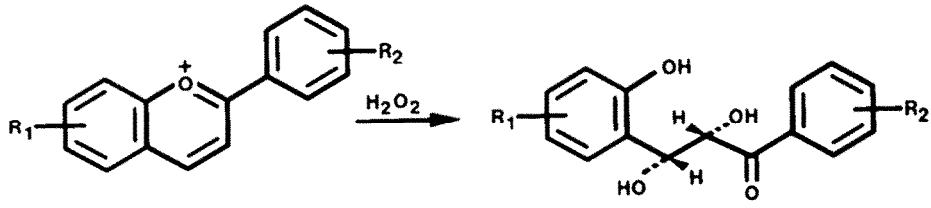
Simple flavylium salts unsubstituted in the 3-position follow a different oxidation path, affording *threo*-dihydroxyketones **10**.[154]



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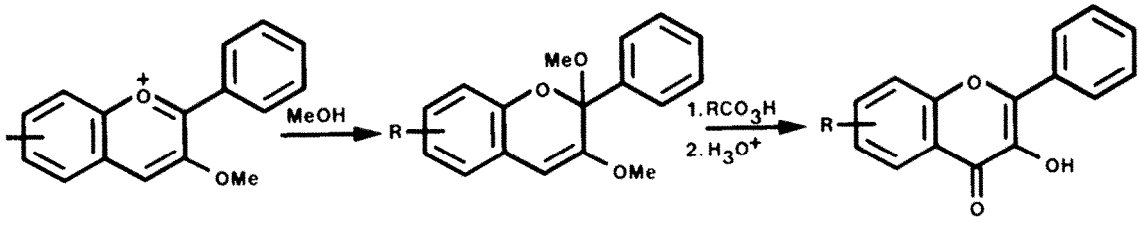
(3) *Other oxidation reactions.* In the early 1940s Karrer[156-159] found that oxidation with perphthalic acid of the methanol adduct **11** of simple 3-OCH_3 flavylium salts afforded the flavonol after hydrolysis.



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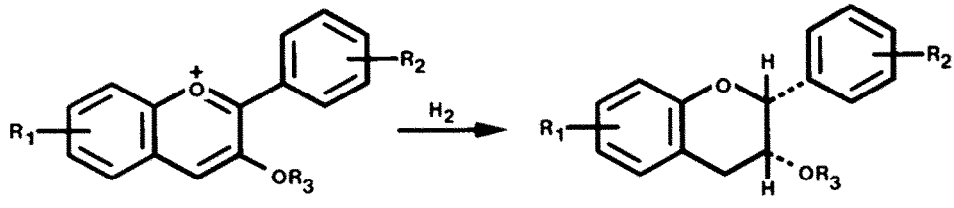
More recently, simple flavylium salts have been converted directly to the corresponding flavone with CrO_3 (Sarett reagent)[160] or, in better yield with $Tl(NO_3)_3$.[161]

(d) *Reduction reactions*

The catalytic hydrogenation of anthocyanins or flavylium salts results in the formation of the corresponding *epi*-catechin derivatives **12**, or the flavans in the case of 3-deoxyflavylium cations.[162-168]



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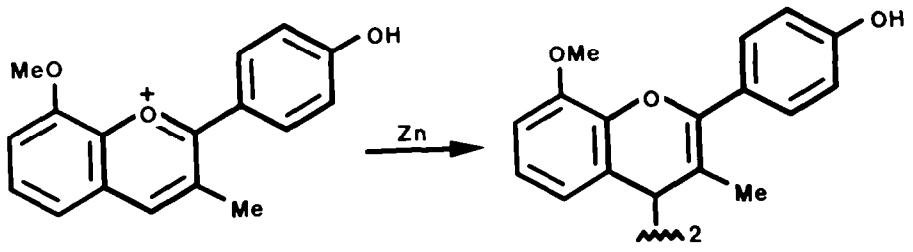
Reduction of simple flavylium salts with Zn takes a more complex course, yielding *bis*-flavenes such as **13**.[169,170]



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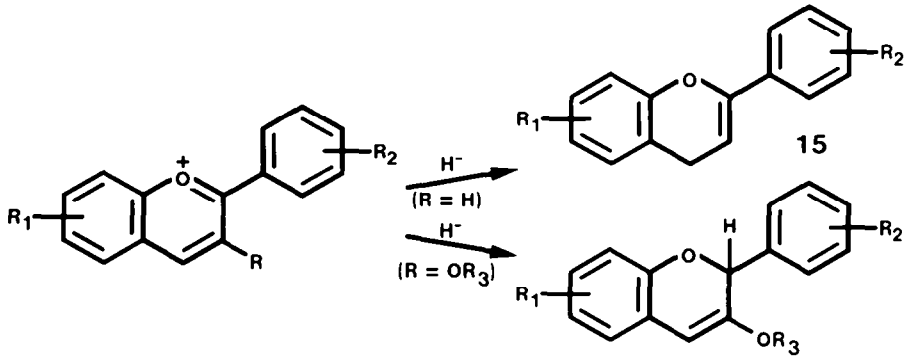
The course of the reduction of flavylium salts with complex metal hydrides was the subject of some confusion[28,62,63,171-176] until 1971 when the structures of the flavene products were clarified by ^{1}H-



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NMR.[177] In general, it was found that flavylium salts bearing an OR group at the 3-position afforded the flav-3-enes **14**, while flavylium salts unsubstituted in the 3-position gave flav-2-enes **15**.

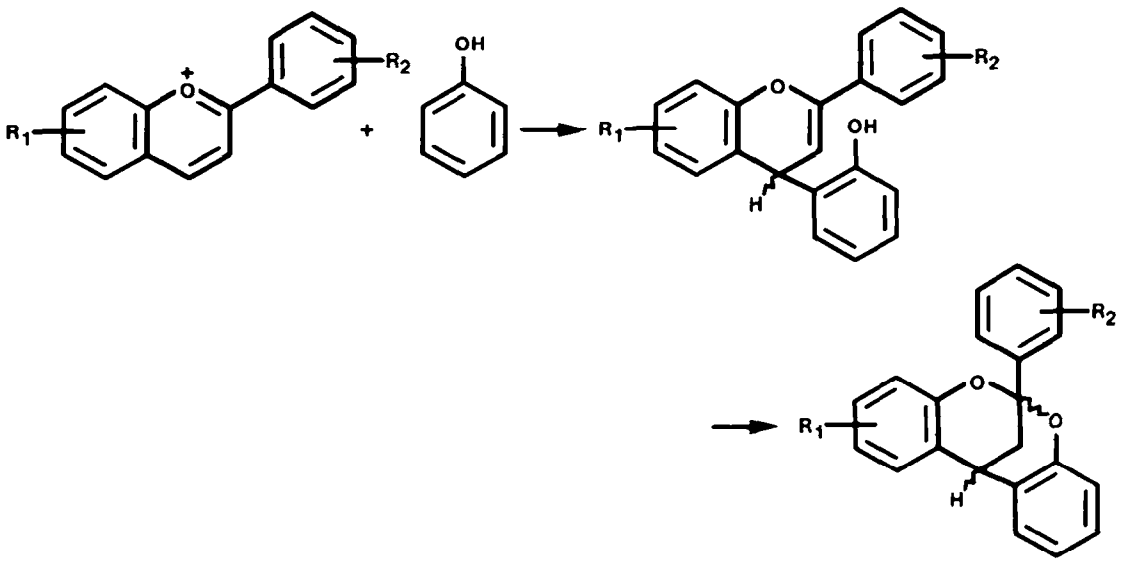
These assignments have been confirmed since by Andrieux *et al.*[178] Anthocyanidins, which have a free OH group in the 3-position, afford the corresponding *epi*-catechins when reduced with $NaBH_4$.[179]

In a series of qualitative experiments, Hurst and Harborne[180] have recommended the use of Na-amalgam as a reductant for anthocyanins and other flavanoids. This reagent cleaves the C ring to yield phenolic fragments similar to those obtained on heating with aqueous base.

(e) *Reactions with nucleophiles*

(1) *Bisulfite.* Anthocyanins are rapidly decolorized by the addition of SO_2, an antiseptic agent used extensively in the wine industry to control microbial growth. The bleaching reaction is reversible and pH dependent; a covalent adduct at the C2 or C4 positions of the flavylium ring being formed.[181] The equilibrium constants for the addition of HSO_3^- to several flavylium salts, $K_s = [A{\cdot}HSO_3]/[AH^+][HSO_3^-]$ have been determined, as well as the rates at which the reaction occurs.[181,182] Whether the adduct is the 2- or 4-isomer is not known; the 4-adduct has been favored, however, based on the inhibition of the reaction by substitution of the flavylium cation with methyl or phenyl groups at the C4 position.[91]

(2) *Other nucleophiles.* The reaction of flavylium salts with nucleophiles proceeds readily in those cases where the 5-position of the flavylium is unsubstituted. For carbon nucleophiles attack occurs at C4 to give the 4-substituted flav-2-ene. Examples include dimedone,[183,184] acetylacetone,[185] phloroglucinol,[186,187] catechin[188,189] phenol,[189] dimethylaniline[190] and 4-hydroxycoumarin.[191] In several cases, further reaction occurred between the phenolic OH and the flav-2-ene to afford a bicyclic structure **16**.



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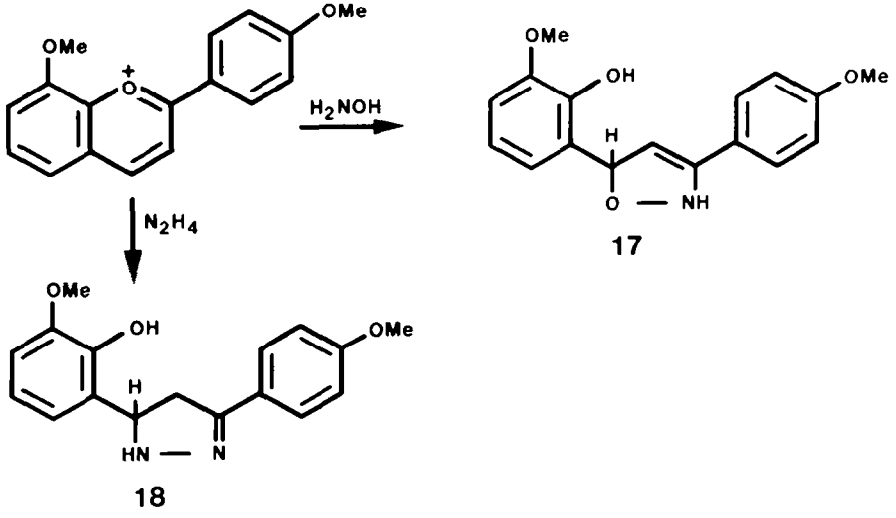
Grignard reagents have also been shown to give a normal 4-substituted flav-2-ene.[192,193]

Nitrogen nucleophiles have been less extensively studied than the carbon ones. Amino acids react to give the C4 substituted flav-2-ene.[194] Hydroxylamine and hydrazine would appear, however, to react first at the 2-position as the products have the structures shown in **17** and **18**.[195]



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(f) *Reactions with electrophiles*

The positive charge inherent in the flavylium ring renders these molecules resistant to electrophilic attack. Flavylium perchlorate has, however, been nitrated;[196] substitution occurring in the B ring at position 3′. More recently, Timberlake and Bridle[197] have found that anthocyanins react with catechin and acetaldehyde to give a product of enhanced color. Although the structures of these products remain to be determined, the authors have suggested that reaction occurs at C8 of the anthocyanin bridging to C8 of the catechin, through a CH_3–CH group. This would be in keeping with the preponderant C8 bromination of catechin[265] and the formation of a 8–8′ CH_2-bridged dimer on reacting pentamethylquercetin with formaldehyde.[80]

IV. COPIGMENTATION

(a) *Self-association*

In 1972, Asen *et al.*[198] reported that the λ_{max} absorbances of anthocyanin solutions do not increase linearly with concentration. At 10^{-2} M concentration and pH 3.14 the absorption is 3 times that expected from Beer's law. Asen *et al.* attributed this effect to the self-association of the anthocyanin molecules at high concentrations. This effect was further described by Scheffeldt and Hrazdina[199] in conjunction with work on flavonoid copigmentation. The exact nature of the complex formed at pH $\approx$ 3 remains unknown, but recent studies by Hoshino *et al.*[200–203] at pH 7.0 have provided very strong evidence that a vertical stacking of the anthocyanin quinoidal bases is occurring at this pH. The stacking would seem related to that shown by nucleosides, as the aggregates give strongly intensified CD curves with increasing concentration. For cyanidin-3,5-diglucoside, a θ value of +260,000 was observed at a concentration of 5×10^{-4} M (pH 7). Both left handed and right handed stacking is possible; pelargonidin and cyanidin-3,5-diglucosides show positive first Cotton peaks, while peonidin, delphinidin and malvidin-3,5-diglucosides show negative ones.[202] Hydrogen bonding appears to play no role in the self-association, as 7-O-methyl malvidin-3,5-diglucoside, which has no free OH groups in the quinoidal form, shows a typical association CD effect.

In their most recent paper, Hoshino *et al.*[203] report a ^{1}H-NMR study of malvidin-3,5-diglucoside quinoidal base, in which it was shown that the chemical shifts of the aromatic protons move upfield with increasing concentration. This is due, as would be expected, to the diamagnetic anisotropy caused by the ring current of the associated aromatic molecules.

Finally, Hoshino *et al.*[202] in work to be reported later in more detail indicate that flavylium cations also form chiral stacked aggregates similar to those of the quinoidal bases.

(b) *Complexation with flavonoids*

It has long been known that the wide range of colors found in various fruits and flowers are not due to the anthocyanins alone. As pointed out by Robinson and Robinson in 1931,[204] the presence of co-occurring flavonoids causes a bathochromic shift in the spectrum of the anthocyanin to give the typical purple to blue colors. Since that time, several investigations of the phenomenon have been undertaken,[198,199,205–208] mainly to determine the flavonoid structure which gives the best color augmentation and spectral shift. Among the flavonoids tested, flavones and flavonols proved superior to chalcones, aurones, flavanones and catechins. A double bond in the 2,3 position of the flavonoid C ring would appear to be a requirement. Both C- and O-glucosylation of the flavone also have proven beneficial. Flavonolsulfonic acids are exceptionally good copigments,[208] presumably due to the added attraction of the negative charge of the sulfonic acid groups to the flavylium cation (Fig. 6).

The structure of the anthocyanin/copigment complex has been the subject of debate, with some workers favoring a horizontal (end to end) hydrogen bonded stabilization of the quinoidal base,[199,206,207] while others[208] support the vertical stacking complex first proposed by Goto.[209,210] The recent results by Hoshino *et al.*[200–203] on anthocyanin self-association discussed above would indicate that the vertical stacking concept is correct. In particular, in their studies on the complex between the anthocyanin

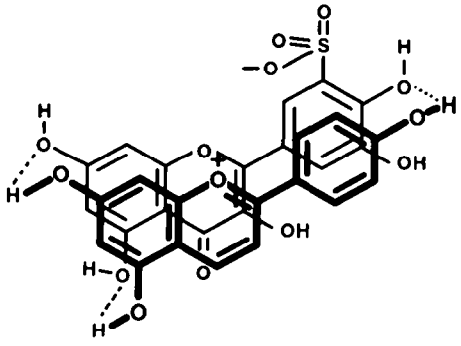


Fig. 6. Representation of the (1:1) molecular complex between apigeninidin (2) and quercetin-5'-sulfonic acid (20). (From Ref. 208, by permission of the American Chemical Society).

awobanin and the C-glucosyl flavone flavocommelin,[209] it was found that mixing equimolar quantities resulted in a large increase in the CD curve at 600 nm, an effect very similar to the self-association experiments. It should also be noted that pentamethylcyanidin chloride, which is incapable of forming a quinoidal form, shows a very large shift in its λ_{max} upon addition of quercetinsulfonic acid i.e. a typical copigment effect is observed.[208] It has also been found that the copigmentation of anthocyanins can lead to an increase in their resistance to photochemical bleaching, as discussed below.

(c) *Intramolecular copigmentation*

In recent years several blue anthocyanins which do not contain associated flavonoid copigments have been found.[211-224] In each case, the color shift is due to the presence of at least two cinnamyl type esters attached to the sugar moiety. These added ester groups also impart added stability to the pigment; they remain colored even at pH 7. This stability has been shown by Brouillard[225] to be due to inhibition of the anthocyanin hydration reaction which gives the colorless chromen-2-ol. Presumably the aromatic ester groups form an intramolecular π-π complex with the anthocyanins which hinders the water addition of the 2 position.

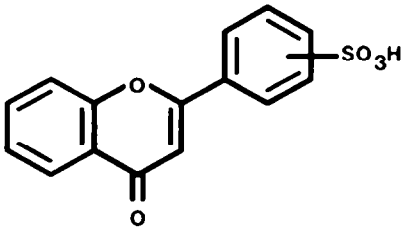
Several authors have noted the presence of metal ions (Mg^{2+}, Fe^{3+}) in the highly acylated anthocyanins,[214] but in most cases these do not appear to be required for the color stability.[226] Of course it has also been known for years that anthocyanins containing a vicinal dihydroxy group in the B ring give a diagnostic 30–50 nm blue shift on addition of Al^{3+} ions.[227-229] These complexes are unstable and decompose (precipitate) with time.

(d) *Photochemical effects of copigmentation*

Although anthocyanins are unstable when exposed to direct sunlight in solution, little is known about the reactions involved. Preliminary studies[80] with apigeninidin chloride in citric acid buffer pH 2.8 have shown that the decomposition is mainly photooxidative, as p-hydroxybenzoic acid has been isolated as a minor product by preparative HPLC.

It has also been found that the addition of copigments to the anthocyanin solution can either retard or accelerate the decomposition, depending on the nature of the copigment.[208] Polyhydroxylated flavone, flavonol, aurone and isoflavone sulfonates tend to protect the pigment while the corresponding non-hydroxylated flavonoid sulfonates accelerate the photodecomposition.

The reasons for these competing effects are not clear, but the interesting observation has been made that the enhancement or quenching of the photochemistry parallels the effects the copigments have on the anthocyanin fluorescence.[231] Anthocyanins containing substitution on the 5-OH are known to fluoresce.[230] The addition of quercetin-5′-sulfonic acid **20** to solutions of several synthetic anthocyanidins quenches their fluorescence,[231] and their photochemical decomposition[208] is similarly retarded. On the other hand, addition of flavonesulfonic acid **19** greatly enhances their fluorescence while simultaneously accelerating their photodecomposition. One possible explanation for these effects would involve a complexation between the copigment and the anthocyanin (Fig. 6). The quercetin-5′-sulfonic acid could quench the anthocyanidin excited state via electron transfer. Flavonesulfonic acid could not do this and hence might protect the excited state from radiationless decay and enhance both fluorescence and reactivity. Anthocyanin photochemistry is obviously an area in which much remains to be explored.



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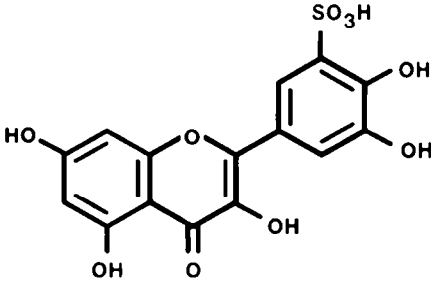

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V. STRUCTURE DETERMINATION OF COMPLEX ANTHOCYANINS

The use of advanced chromatographic and spectroscopic techniques in the determination of the structures of anthocyanins has begun only in recent years. Until the early 1970s, anthocyanins were separated from their natural sources primarily by paper chromatography.[232,233] The anthocyanins were then hydrolyzed and the anthocyanidins and sugars compared with standards, also through paper chromatography.[234] More recently, two new techniques bi-dimensional cellulose TLC[240,241] and polyamide column chromatography,[235-239] were introduced, the latter being especially valuable for the large scale isolation of pure anthocyanins.

In the 1970s the use of reversed phase high performance liquid chromatography for the separation of anthocyanins was described.[242-247] This has proven to be a powerful tool; in *Vitis lambrusca* for instance, 18 of a mixture of 20 anthocyanins could be separated in one 2 hr run.[245] The technique is non-destructive and the separated peaks can be readily collected for further spectroscopic analysis. Applications in the area of plant genetics have already been reported and should continue to gain in importance.[248-252]

Aside from an early report on the ^{1}H-NMR spectra of several simple flavylium salts by Nilsson,[253] Goto[203,212,215,254] was the first to use ^{1}H-NMR to determine the structures of several complex polyacylated anthocyanins and, as mentioned above, to study self-association effects. A further study has also been described by Cornuz *et al.*[255] ^{13}C NMR spectra of anthocyanins have not as yet been reported. This is more likely due to the difficulty of obtaining purified anthocyanins in quantities sufficient for study by this technique.

The infrared spectra of several anthocyanidins as nujol mulls were reported by Ribereau-Gayon and Josien in 1960.[256] The information available by this technique is limited and it has therefore not been generally adopted. Recently, however, Statoua *et al.*[257] have determined the resonance-Raman spectra of several anthocyanins in methanolic solution. The technique has even been applied to the direct identification of malvidin-3-glucoside in grape skin extracts.

With the exception of a few simple flavylium salts,[258] the mass spectra of anthocyanins have not been described. The basic problems, low volatility and poor stability, have recently been overcome by the use of fast atom bombardment mass spectrometry. With this technique the spectra of violanin (MW 919) and platyconin (MW 1421) have been determined.[259]

Anthocyanins have also been converted to the corresponding trimethylsilylquinoline derivatives by reaction with trimethyl-chlorosilane and hexamethyldisilizane in THF-DMSO. These derivatives were sufficiently volatile for conventional GC/MS analysis.[260,261]

Analyses by X-ray crystallography of anthocyanidins have been done only on apigeninidin chloride (4',5,7-trihydroxyflavylium chloride),[262] 4',6,7-trihydroxyflavylium chloride[263] and cyanidin bromide (3,3',4',5,7-pentahydroxyflavylium bromide),[264] and their crystal and molecular structure determined. The apigeninidin cation was quasi planar, and measured bond lengths and angles suggested an electronic conjugation extended to the three rings. The chloride anion was found tricoordinated through H-bonds to the 5-OH, 4'-OH and to one molecule of water of crystallization.

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