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THE REDUCTIVE ACETYLATION OF QUERCETIN

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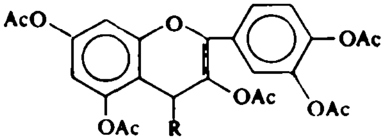
Abstract—The reductive acetylation of quercetin with zinc dust in acetic anhydride has been shown to give a mixture of acetylated products, only two of which give cyanidin chloride on hydrolysis with ethanolic hydrochloric acid. These cyanidin precursors have been isolated and identified as 3,3′,4′,5,7-*pentaacetoxyflav-3-ene* and 3,3′,4,4′,5,7-*hexaacetoxyflav-2-ene*. On acid hydrolysis, the flav-3-ene undergoes disproportionation to yield cyanidin and catechin.

CONVERSION of flavones and flavonols to the corresponding flavylium salts is most conveniently effected by reductive acetylation, followed by hydrolysis of the amorphous product with ethanolic hydrochloric acid.[2] Although the reaction has been used frequently for the preparation of anthocyanidins and their glycosides,[3] the nature of the intermediate reduction product has remained in doubt for almost 40 years, owing to the fact that homogeneous, crystalline compounds have never been obtained. The recent publication[4] in which the isolation of a crystalline intermediate is described for the first time, prompts us to report the results of our examination of this reaction.

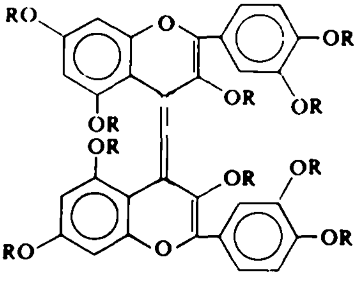
The reduction of quercetin with zinc dust and sodium acetate in acetic anhydride was investigated by Robinson and Robinson[3] who suggested that the major cyanidin-forming constituent of the crude product was 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene (I, R = OAc) although they did not isolate a single definitive compound.



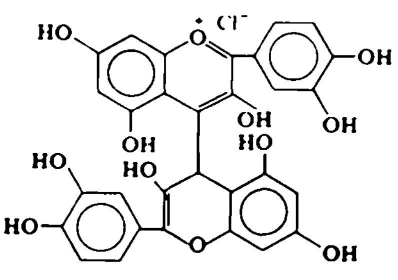
I

Malkin and Nierenstein,[5] however, claimed that the reduction of quercetin with zinc and acetic anhydride gave the bisflavenylidene, quercetylene acetate (II, R = Ac), which on acid hydrolysis gave quercetylene chloride (III) rather than cyanidin. This conclusion was based on the chloride analysis of the flavylium salt, which was one-half the value expected for cyanidin chloride and by analogy with simpler pyrone systems which form dimeric products on reduction in acid solution.

[1] Abstracted in part from the M.S. thesis submitted to Oregon State University, 1966.
[2] H. G. C. King and T. White, *J. Chem. Soc.* 3901 (1957).
[3] G. M. Robinson and R. Robinson, *Biochem. J.* 27, 206 (1933).
[4] B. J. Bergot and L. Jurd, *Tetrahedron* 21, 657 (1965).
[5] T. Malkin and M. Nierenstein, *J. Am. Chem. Soc.* 52, 2864 (1930).

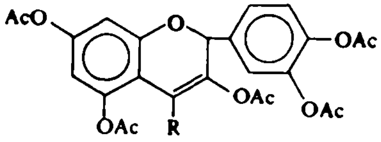


II



III

Recently, King and White,[5] employing qualitative tests and paper chromatographic comparisons have shown conclusively that cyanidin chloride can be produced from quercetin, in reasonable yield, by a modification of the Robinson procedure. Shortly thereafter, Laumas and Seshadri[6] assumed that the material which they obtained from the reductive acetylation of dihydroquercetin was substantially the same as the crude intermediate obtained from quercetin by King and White, both being impure forms of 3,3′,4′,5,7-pentaacetoxyflav-3-ene (IV, R = H).



IV

Bayer and Kramer[7] re-examined the reductive acetylation of quercetin and obtained an impure product which they also formulated as the flav-3-ene (IV, R = H). They also concluded that bisflavenylidenes were not formed either from quercetin or from simpler flavones which they studied. In contrast to this, Bergot and Jurd[4] have shown that flavones unsubstituted in the 3 and 5 positions give crystalline bisflavenylidenes under reductive acetylation conditions. On treatment of quercetin under the same conditions, they obtained a crystalline product which melted over a wide range, even after repeated recrystallizations, and which was shown by its NMR spectrum to be a mixture of the flav-3-ene (IV, R = H) and the isomeric flav-2-ene (I, R = H) in a ratio of approximately 4 to 1. They did not entirely exclude the possibility that some quercetylene acetate was formed in this reaction, since acid hydrolysis of the crude reduction product gave traces of a second anthocyanidin-like compound on paper chromatographic analysis, having an R_f value distinctly different from that of cyanidin. Subsequent isolation by Chadha[8] of the bisflavenylidene (II, R = Me) in very low yield from the reductive acetylation of pentamethylquercetin indicated that the formation of such dimers is not absolutely precluded by substitution in the 3 and 5 positions of the flavone, although the probability that they are formed in significant amounts is low.

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[6] K. R. Laumas and T. R. Seshadri, *Proc. Indian Acad. Sci.* **49A**, 47 (1959).

[7] E. Bayer and B. Kramer, *Chem. Ber.* **97**, 1057 (1964).

[8] J. S. Chadha, *Chem. & Ind.*, 1263 (1965).

Our analysis of the crude reaction product, obtained from the reductive acetylation of quercetin with zinc dust in acetic anhydride, using TLC on silicic acid, showed it to be a mixture of at least seven compounds (Table 1). One of the minor products was quercetin pentaacetate identified by chromatographic comparison with an authentic sample. Two cyanidin precursors were detected by the development of the blue color characteristic of anthocyanidin pseudo-bases upon spraying the chromatogram with dilute sodium hydroxide. The differences in R_f values for these compounds facilitated their separation from the crude reduction product by column chromatography on silicic acid.

TABLE 1. TLC ANALYSIS OF THE CRUDE REACTION MIXTURE FROM THE REDUCTIVE ACETYLATION OF QUERCETIN

Compound	R_f Value in solvent		Spray reagent		Ultraviolet	Comments
	A	B	1	2		
Quercetin pentaacetate	0·33	0·63	Y	N	Y	
Catechin pentaacetate	0·46	0·79	O	N	N	
Dihydroquercetin pentaacetate	0·50	0·74	pB	N	N	
Pentaacetoxyflav-3-ene (IV)	0·45	0·79	Bl	R	N	
Hexaacetoxyflav-2-ene (I)	0·08	0·37	Bl	R	N	
Reaction mixture						
spot 1	0·08	0·37	Bl	R	N	A major component
spot 2	0·14	0·52	N	N	fB	A major component
spot 3	0·27	0·75	—	—	N	Trace, red before and after spraying with 1 or 2
spot 4	0·35	0·64	pY	N	pY	Trace
spot 5	—	0·72	N	N	pY	Trace
spot 6	0·43	0·79	N	N	fY	A major component
spot 7	0·45	0·78	Bl	R	N	Major product

Chromatography Solvents:
A. Benzene, Acetone (90:10)
B. Chloroform, Acetone (95:5)

Spray Reagents:
1. 10% NaOHaq
2. 10% NaOHaq followed immediately with 10% HClaq

Legend: f = fluorescent, p = pale, Bl = blue, O = orange, R = red, Y = yellow, B = brown, N = no color visible

The first compound giving a deep blue color with base was eluted from the column with benzene–acetone (9:1 v/v) and crystallized readily from methanol as colorless needles, m.p. 138–139° (λ_{max} 273 and 302 mμ, log ϵ = 3·97 and 3·76). Elemental analysis and acetyl determination for this compound were consistent with values expected for 3,3′,4′,5,7-pentaacetoxyflav-3-ene (IV, R = H). The NMR spectrum confirmed the assignment of this structure to the compound. The acetate groups appear as singlets at 7·70, 7·74, 7·78 and 7·98 τ integrating for 3,3,6 and 3 protons respectively; the high field signal being assigned to the enolic acetate group at the 3 position. The aromatic protons occur as two one-proton doublets at 3·51 and 3·42 τ, J = 2·2 c/s (A ring) and a three-proton multiplet at 2·70–2·80 τ (B-ring)[*]. Two one-proton singlets at 4·09 and 3·47 τ were assigned to the allylic proton at the 2 position and the vinyl proton at the 4 position, respectively. No allylic coupling

[*] T. J. Batterham and R. J. Highet, *Austral. J. Chem.* 17, 428 (1964).

between the C_2 and C_4 protons was observed, even in the 100 M/s spectrum, indicating that the B-ring occupies an axial position in the flav-3-ene, since in this configuration σ–π overlap between the C_2—H bond and the C_3—C_4 double bond would be minimal.[10] None of the isomeric flav-2-ene (I, R = H) reported by Bergot and Jurd[4] was detected.

The second cyanidin precursor was eluted from the column with benzene–acetone (85:15 v/v) and crystallized with difficulty from methanol–acetone as white cubes, m.p. 206–207°d, (λ_{max} 268 (infl) and 284 (infl) mμ, log ϵ = 3·90 and 3·86). Elemental analysis showed this compound to be a hexaacetate and therefore should be either the 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene (I, R = OAc) or the isomeric flav-3-ene (IV, R = OAc). The NMR spectrum bore obvious similarities to that of the pentaacetoxyflav-3-ene (IV, R = H), with the exception of the additional acetate group singlet and a one-proton singlet at 5·74 τ. The latter could not be an aromatic ring proton and must, therefore, be due to the proton at the 2 or 4 position of the heterocyclic ring. Since the proton at the 2 position in the hexaacetoxyflav-3-ene (IV, R = OAc) would be expected to have a chemical shift close to the value of 4·09 τ found for this proton in the pentaacetoxyflav-3-ene (IV, R = H), the absorption at 5·74 τ can only be attributed to a proton in the 4 position. Thus the second cyanidin precursor must have the 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene structure (I, R = OAc).

While the UV spectra of the two polyacetoxyflavenes is not clearly definitive for the structural assignments, they are, at least, consistent for such assignments. It has been shown that, despite close similarity in chromophores, the UV spectral characteristics of flav-2-enes and flav-3-enes are distinctly different.[11] The flavenes used to demonstrate these spectral differences did not have structures directly comparable to the polyacetoxyflavenes isolated in our study. However, the general spectral characteristics reported are in agreement with spectra expected of compounds having the structural assignments given the polyacetoxyflavenes.

The behavior of the two cyanidin precursors on hydrolysis provides further evidence in support of the structural assignments, as well as explaining the anomalous results previously obtained on hydrolysis of the crude reduction product.[5] Both the pentaacetoxyflav-3-ene (IV, R = H) and the hexaacetoxyflav-2-ene (I, R = OAc), when hydrolyzed by methanolic sodium hydroxide gave the deep blue color of the pseudo-base which then yielded cyanidin on acidification. When the hydrolysis was performed in a nitrogen atmosphere however, the flav-3-ene gave a purple colored solution which turned colorless on acidification, indicating that an oxidation step is required for the formation of cyanidin from this compound. Hydrolysis of the flav-2-ene under the same conditions proceeded normally to give cyanidin as expected, since in both of these compounds, the heterocyclic ring is at the same oxidation state.

Paper chromatographic examination of the hydrolysis products of the two flavenes, obtained by either dilute acid or base followed by acid hydrolysis, showed that while the major product was cyanidin, other red anthocyanidin-like compounds having R_f values greater than cyanidin were also present (Table 2). Elution of these compounds from the paper and re-hydrolysis gave more cyanidin, indicating that these minor constituents of the hydrolysis product were partially acetylated derivatives

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[10] D. J. Collins, J. J. Hobbs and S. Sternhell, *Austral. J. Chem.* **16**, 1030 (1963).

[11] L. Jurd and A. C. Waiss, Jr., *Tetrahedron* **21**, 1471 (1965).

of cyanidin. Bergot and Jurd[4] had also observed these compounds on acid hydrolysis of the crude reduction product, though they suggested that these compounds could be traces of the quercetylene chloride (III) reported by Nierenstein.[5]

TABLE 2. PAPER CHROMATOGRAPHY OF THE HYDROLYSIS PRODUCTS OF THE POLYACETOXYFLAVENES

Compound	R_f Values in solvent					Spray reagent				
	A	B	C	D	E	1	2	3	4	
Cyanidin chloride (Std)	0·51	0·15	0·30	0·17	—	R	R	Bl	—	
d-Catechin	0·71	0·63	--	0·67	0·71	N	O	O	YO	
1-Epicatechin	—	0·59	—	0·53	0·60	N	O	O	YO	
Acid hydrolysis products of 3,3′,4′,5,7-pentaacetoxyflav-3-ene										
spot 1	0·50	0·13	—	0·19	—	R	R	—	—	
spot 2	0·61	0·63	—	—	—	P	dP	—	—	
spot 3	0·71	0·65	—	0·67	0·70	N	O	—	YO	
Acid hydrolysis products of 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene										
spot 1	0·52	0·13	—	—	—	R	R	Bl	—	
Alkaline hydrolysis products of 3,3′,4′,5,7-pentaacetoxyflav-3-ene								—		
spot 1	0·51	0·14	0·29	—	—	R	R	Bl	—	
spot 2	0·80	0·40	0·66	—	—	R	R	Bl	—	Trace
Alkaline hydrolysis products of 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene										
spot 1	0·52	0·15	0·29	—	—	R	R	Bl	—	
spot 2	0·80	0·41	0·66	—	—	R	R	Bl	—	Trace
spot 3	0·88	0·77	—	—	—	R	R	Bl	—	Trace

Chromatography solvents:

A. Water, conc HCl, AcOH (30:3:10)
B. Water, conc HCl AcOH (80:3:17)
C. Water, conc HCl, formic acid (30:20:50)
D. 15% AcOH
E. Butanol:AcOH: Water (4:15) (Upper phase)

Spray reagents:

1. None
2. Bis-diazotized benzidine
3. 10% NaOHaq
4. Cinnamaldehyde-HCl Reagent

Legend: Bl = blue, R = red, O = orange, Y = yellow, P = purple, N = no color visible, d = dark

When chromatograms of the acid hydrolysis product of the pentaacetoxyflav-3-ene (IV, R = H) were sprayed with tetrazotized benzidine or cinnamaldehyde–hydrochloric acid reagent, another compound was detected, along with the expected cyanidin. This compound was shown to be catechin by chromatographic and chromogenic comparison with an authentic sample of *d*-catechin (Table 2). When the acid hydrolysis of the flav-3-ene (IV, R = H) was performed under a nitrogen atmosphere, the same color development and products were obtained as when this hydrolysis was performed in air. Thus the formation of cyanidin and catechin does not require the presence of oxygen and must involve a disproportionation reaction, whereas alkaline hydrolysis does require an aereal oxidation step. A disproportionation reaction of the 3-ketoflavan produced initially by hydrolysis would lead to the formation of cyanidin and catechin.[12] Such a disproportionation reaction probably involves

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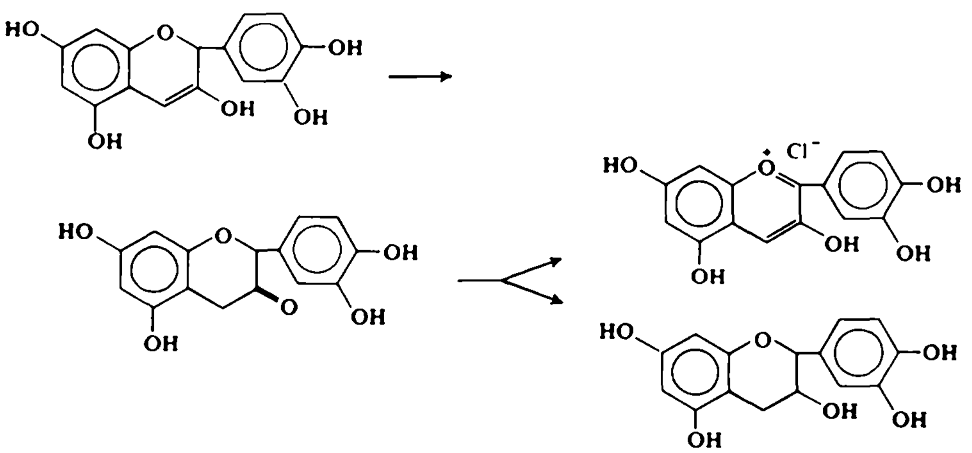
[12] N. Campbell, *Chemistry of Carbon Compounds* (Edited by E. H. Rodd) Vol. IVB; p. 868. Elsevier, N. York (1959).



a hydride abstraction step in acid medium, precedents of which have been described.[13]

The formation of a 1 : 1 mixture of cyanidin chloride and catechin could account for Nierenstein's[5] conclusion that quercetylene chloride (III) was formed, since the elemental analysis, particularly the chloride content, would be close to that of quercetylene chloride.

The products isolated and identified from this reduction reaction can best be explained by consideration of the reaction as a stepwise process. Reductive acetylation of the carbonyl group must occur initially to give the hexaacetoxyflav-2-ene (I, R = OAc), followed by reduction of the olefinic double bond to give 3,3′,4,4′,5,7-hexaacetoxyflavan. Spontaneous loss of the elements of acetic acid between C_3 and C_4 would give the pentaacetoxyflav-3-ene (IV, R = H). Loss of the element of acetic acid between the C_2 and C_3 positions is also possible, if the stereochemistry at these positions is suitable. However, this would yield 3′,4,4′,5,7-pentaacetoxyflav-2-ene (I, R = H), which would not be a cyanidin precursor. Such a reaction sequence is contrary to that proposed by Laumas and Seshadri,[6] who suggest that the pyrone double bond is reduced first, by analogy with the reduction of chalcone to dihydrochalcone. Further reduction of the pentaacetoxyflav-3-ene can be envisaged which may account for some of the other reduction products detected by chromatography, but again, none of these compounds would be cyanidin precursors. Complete definition of the steps involved in the reductive acetylation of quercetin requires the identification of those products formed which are not cyanidin precursors.

EXPERIMENTAL

M.ps are uncorrected and were determined with a Fischer–Johns m.p. apparatus. Elemental analyses were performed by Drs. Pascher and Pascher, Bonn, West Germany. IR spectra were determined with a Beckman Model IR-5 spectrometer and UV spectra with a Beckman Model DB spectrophotometer. NMR spectra were determined with a Varian Model A-60 spectrometer at 60 M/c using TMS.

Reductive acetylation of quercetin. Quercetin (2·0 g) was dissolved in Ac_2O (75 ml). Anhyd AcONa (1·0 g) and technical grade (90%) Zn dust (2·0 g) were added and the soln was refluxed for 1 hr, treated with more Zn dust (2·0 g), and refluxed for an additional hr. The warm soln was filtered and the filtrate poured into water (500 ml). The Zn residues were washed with warm glacial AcOH (50 ml)

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[13] N. C. Deno, H. J. Peterson and G. S. Saines, *Chem. Rev.* **60**, 7 (1960).

and the filtered washings were added to the aqueous soln. After cooling in a refrigerator (5°) for approximately 3 hr, this soln was filtered and a dark orange-red solid was collected and washed repeatedly with water. The solid was dried in a vacuum desiccator over NaOH for approximately 48 hr, total yield of the crude reaction product, 2·74 g.

TLC of the crude reaction product. The crude reaction mixture was examined by TLC on Silica Gel G. Following development, the chromatograms were air dried, examined under UV light, and treated with spray reagents selected to indicate the presence of any anthocyanidin precursors in the reaction product. The results of the TLC analysis of the crude reaction mixture are presented in Table 1.

Isolation and identification of anthocyanidin precursors found in the reaction mixture. The crude reduction product (1 g) was chromatographed on a column of silicic acid (Bio-Rad A, 200–325 mesh) in benzene–acetone (85:15). The separation was monitored by periodically collecting a very small sample of the eluate (3 or 4 drops) and treating this with a drop of 10% methanolic NaOH. The presence of either of the anthocyanidin precursors was indicated by the formation of a blue soln which turned red on acidification. In this manner the fraction corresponding to spot seven (Table 1) was collected. The solvent was removed under vacuum with a rotary evaporator yielding a crude product (0·33 g) which was recrystallized twice from MeOH to give 3,3′,4′,5,7-pentaacetoxyflav-3-ene as colorless needles m.p. 138–139°. The yield of the crystalline product was 0·22 g (18·3%). $[\alpha]_D^{29}$ = 0·0 (*c*, 0·81; acetone). IR absorption (nujol): 1758 cm^{-1} (acetyl CO groups). UV absorption (95% EtOH): λ_{max} 273 and 302 mμ; log ε = 3·97 and 3·76. NMR spectrum ($CDCl_3$): multiplet, 2·70–2·80 τ; 3 protons (B ring aromatic protons); doublet pair, calculated chemical shifts 3·51 τ and 3·42 τ; J = 2·2 c/s; 2 protons (A ring aromatic protons); singlet, 3·53 τ; 1 proton (aliphatic proton at 2-position); singlet, 4·09 τ; 1 proton (aliphatic proton at 4-position); singlets, 7·70, 7·74, 7·78, and 7·98 τ; 3,6,3, and 3 protons respectively (Me protons of acetate groups). (Found: C, 60·30; H, 4·41; MeCO, 43·2. $C_{25}H_{22}O_{11}$ requires: 60·24; H, 4·45; MeCO, 43·18%.)

Further elution gave the fraction corresponding to spot one (Table 1). The solvent was removed under vacuum with a rotary evaporator to yield the crude product (0·11 g). This substance was dissolved in a minimum amount of acetone and the soln was diluted to approximately 15 ml with MeOH and a trace of water. This soln was placed in a refrigerator. After 5 days the mother liquor was decanted leaving 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene adhering to the bottom and sides of the recrystallization vessel as small clear cubes, m.p. 206–207° (dec), yield of the crystalline product was 0·08 g (6·5%). $[\alpha]_D^{22}$ = 0·0 (*c*, 0·04; acetone). IR absorption (nujol): 1760 cm^{-1} (acetyl CO groups). UV absorption (95% EtOH): λ_{inn} 232, 265, 282 mμ; log ε = 4·46, 3·94 and 3·89 respectively. NMR spectrum ($CDCl_3$): multiplet, 2·67–2·83 τ; 3 protons (B ring aromatic protons); doublet pair, calculated chemical shifts 3·16 τ and 3·20 τ; J = 2·5 c/s; 2 protons (A ring aromatic protons); singlet, 5·73 τ; 1 proton (aliphatic proton at 4-position); singlets, 7·62, 7·71, 7·74, and 8·24 τ; 3,6,6, and 3 protons respectively (Me protons of acetate groups) (Found: C, 58·52; H, 4·23. $C_{27}H_{24}O_{13}$ requires: C, 58·30; H, 4·31%.)

Acid hydrolysis of polyacetoxyflavenes. Small samples (10·0 mg) of 3,3′,4′,5,7-pentaacetoxyflav-3-ene and 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene were dissolved in solutions of 10% (w/w) HCl in anhyd MeOH (10–15 ml) and refluxed for 20 to 25 min. The resulting red solns were taken to dryness with a rotary evaporator and the products were dissolved on small volumes of 0·1% HCl in 95% EtOH. These solns were chromatographed on Whatman No. 1 Chromatographic Paper in five solvent systems (Table 2). Finally, each of the solns was diluted with water (50 ml) and extracted with AcOEt (2 × 25 ml), these extracts being discarded, followed by n-butanol (3 × 25 ml). The n-butanol extracts from each work-up were combined and taken to dryness with a rotary evaporator. The products were then dissolved in 0·1% HCl in 95% EtOH for UV analysis.

UV absorption (0·1% HCl in 95% EtOH): Extracted hydrolysis product of 3,3′,4′,5,7-pentaacetoxyflav-3-ene, λ_{max} 279 and 551 mμ. Extracted hydrolysis product of 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene, λ_{max} 279 and 551 mμ.

Alkaline hydrolysis of polyacetoxyflavenes. For qualitative analysis small samples (2–3 mg) of 3,3′,4′,5,7-pentaacetoxyflav-3-ene and 3,3′,4,4′,5,7-hexaacetoxyflav-2-ene were dissolved in minimum amounts of acetone and chloroform respectively. These solns were diluted to 2 to 3 ml by the addition of 2% NaOHaq in MeOH (1/2, v/v; pH 12·0) and allowed to stand at room temp for approximately 1 min before acidification with 10% methanolic HCl (w/w). Both solns became deep blue in color when treated with alkali and bright red on acidification. The resulting solns were chromatographed on Whatman No. 1 Chromatographic Paper in 3 solvent systems and the chromatograms were examined with a number of chromogenic spray reagents (Table 2).

For UV analyses 10 mg of each of the two cyanidin precursors were hydrolyzed as described above. The acidified solns were diluted with water (50 ml) and extracted with AcOEt (2 × 25 ml) and n-butanol (3 × 25 ml). The combined butanol extracts from each work-up were taken to dryness with a rotary evaporator and the products were dissolved in 0·1% HCl in 95% EtOH.

UV absorption (0·1% HCl in 95% EtOH): Extracted hydrolysis product of 3,3',4',5,7-pentaacetoxyflav-3-ene, λ_{max} 279 and 551 mμ. Extracted hydrolysis product of 3,3',4,4',5,7-hexaacetoxyflav-2-ene, λ_{max} 279 and 551 mμ.