Phytochemistry, 1963, Vol. 2, pp. 47 to 60. Pergamon Press Ltd. Printed in England

PREPARATION OF ANTHOCYANIDINS AND THEIR GLYCOSIDES FROM RELATED FLAVONOIDS

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(*Received* 9 *July* 1962)

Abstract—Improvements in the method of reductive acetylation of flavonols provide pure samples of anthocyanidins, the examples studied being cyanidin and its *O*-pentamethyl and *O*-5,7,3′,4′-tetramethyl derivatives. The method can also be used for the conversion of flavonol glycosides into anthocyanins; the preparation of cyanidin and pelargonidin-3-rhamnoglucosides are described. 3-Hydroxyflavanones, e.g. taxifolin, aromadendrin and dihydrorobinetin undergo isomeric change into ψ-base acetates of anthocyanidins on heating at 150–155° with acetic anhydride solution and a base catalyst and the ψ-base acetates undergo conversion into anthocyanidins in very good yields. The mechanism of this transformation is made clear by the isolation of intermediate stages in the case of taxifolin tetramethyl ether. This result suggests the possibility of a direct route for the biogenesis of 3-hydroxy anthocyanidins from the 3-hydroxy flavanones. But this change has not so far been successfully carried out with flavanones not having the 3-hydroxyl.

Flavones, e.g. apigenin and its trimethyl ether, yield on reductive acetylation, a mixture of products, in which flavenes seem to be predominant. The intermediates undergo conversion into apigeninidin chloride and its *O*-trimethyl derivative in good yields. 2′-Hydroxy-4,4′,6′-trimethoxy dibenzoyl methane on reduction with borohydride gives the diol which undergoes conversion in acids satisfactorily to apigeninidin trimethyl ether. Substituted flavan-4-ols, prepared by reduction of a flavanone, give the corresponding anthocyanidins (yield *ca.* 10 per cent) on heating in alcoholic hydrochloric acid.

THE conversion of related flavonoids (flavonols, flavones and their dihydro compounds) into anthocyanidins and anthocyanins is of considerable interest, because of the possibility of convenient methods of preparation of the latter for use in biological work and also of the possibility of throwing light on the biogenesis of anthocyanins. In earlier communications[1-3] have been outlined the results of our exploratory studies on the preparation of anthocyanidins from more readily available flavonoids. A fuller and connected account of this work is given in the present paper.

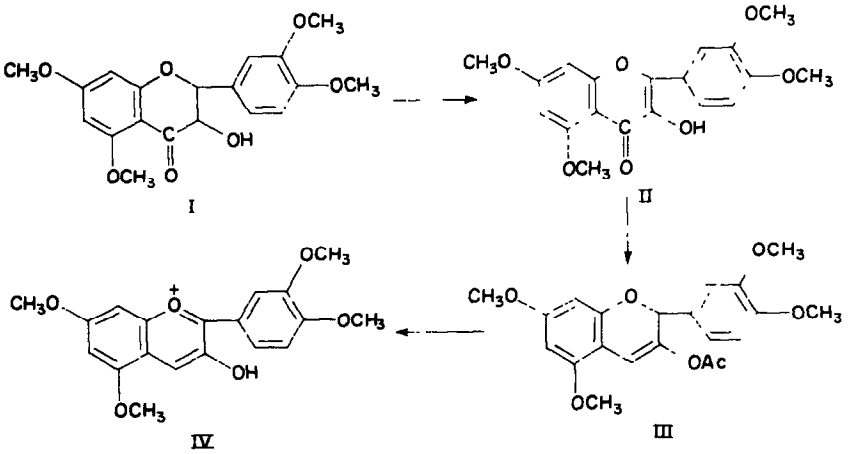
From flavonols by reductive acetylation

Many flavonols are readily available as natural products and they are also conveniently synthesised. Their conversion into the corresponding flavylium salts is best effected under the conditions reported by King and White.[4] During a study of leucoanthocyanidins it was shown that the reductive acetylation ($NaOAc\text{-}Ac_2O+Zn$) products of flavonols agree closely with the corresponding flav-3-ene-3-ol acetates which undergo conversion into the anthocyanidins when boiled with hydrochloric acid in alcohol. The anthocyanidin chlorides thus obtained are substantially pure but show lower absorption maxima of the order of 15–20 mμ in the visible. For analytical studies, paper chromatography can be used to effect the necessary purification, but for preparative purposes pure samples can be obtained by extraction of impurities with ethyl acetate or ether.

In an earlier publication,[1] reductive acetylation of quercetin pentamethyl ether was reported as giving 3,5,7,3′,4′-pentamethoxyflav-3-ene. We have examined this reaction a further number of times and find that the product is in general a mixture, and although the flavene is the major entity, the i.r. spectrum showed a fairly strong acetate band at 5·8 μ.

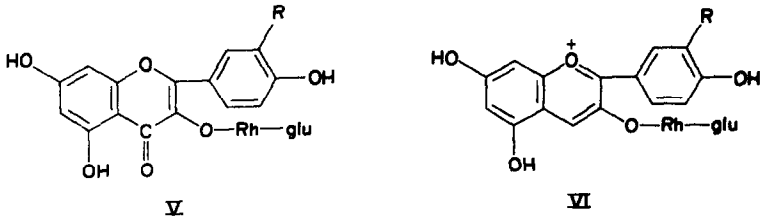
[1] K. R. LAUMAS and T. R. SESHADRI, *Proc. Ind. Acad. Sci.* **49**, 47 (1959).
[2] H. G. KRISHNAMURTY, T. R. SESHADRI and B. VENKATARAMANI, *J. Sci. Ind. Res.* **19B**, 115 (1960).
[3] H. G. KRISHNAMURTY and T. R. SESHADRI, *Curr. Sci.* **30**, 287 (1961).
[4] H. G. C. KING and T. WHITE, *J. Chem. Soc.*, 3701 (1957).

The reductive acetylation of quercetin-5,7,3',4'-tetramethyl ether (II) has therefore been studied with the hope of getting more information. The compound was prepared by the dehydrogenation of taxifolin-5,7,3',4'-tetramethyl ether (I) using a variety of dehydrogenating agents; (1) iodine in the presence of potassium acetate[5] (yield, 51 per cent), (2) alkaline ferricyanide (30 per cent), and (3) 2*N* sulphuric acid[6] (55 per cent). The reductive acetylation product of (II) had no definite melting point and the analytical values are in agreement with 5,7,3',4'-tetramethoxyflav-3-ene-3-ol acetate (III). It gave cyanidin 5,7,3',4'-tetramethyl ether (IV) identical with a sample prepared by the Robinson method of synthesis.[7]



From flavonol glycosides

The reductive acetylation method can also be applied for the preparation of anthocyanidin glycosides from the corresponding flavonol glycosides. In these cases certain special precautions have to be taken to prevent glycosidic hydrolysis. As typical examples, the conversion of rutin (quercetin-3-rhamnoglucoside, V, R = OH) into cyanidin-3-rhamnoglucoside (VI, R = OH) and of kaempferol-3-rhamnoglucoside (V, R = H) into pelargonidin-3-rhamnoglucoside (VI, R = H) are reported here. After the flavonol glycosides



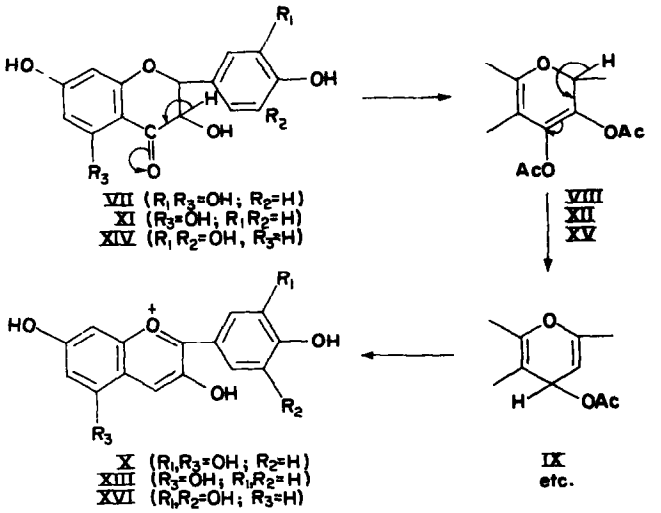
had been subjected to reductive acetylation, the reduced acetates were hydrolysed with cold aqueous potassium hydroxide in an inert atmosphere and converted into anthocyanins by acidification at a low temperature. The anthocyanins thus obtained agreed in their properties with natural samples of cyanidin-3-rhamnoglucoside[8,9] and pelargonidin-3-rhamnoglucoside[9] respectively. The yields are about 35 per cent.

[5] R. N. GOEL, V. B. MAHESH and T. R. SESHADRI, *Proc. Ind. Acad. Sci.* **47**, 184 (1958).
[6] J. PEW, *J. Am. Chem. Soc.* **70**, 3033 (1948).
[7] B. VENKATARAMANI and T. R. SESHADRI, *J. Sci. Ind. Res.* **19B**, 477 (1960).
[8] R. WILLSTATTER and E. H. ZOLLINGER, *Brit. Chem. Abs.* **1**, 45 (1917).
[9] J. B. HARBORNE, *Biochem. J.* **72**, 22 (1958).

From 3-hydroxyflavanones by isomeric change

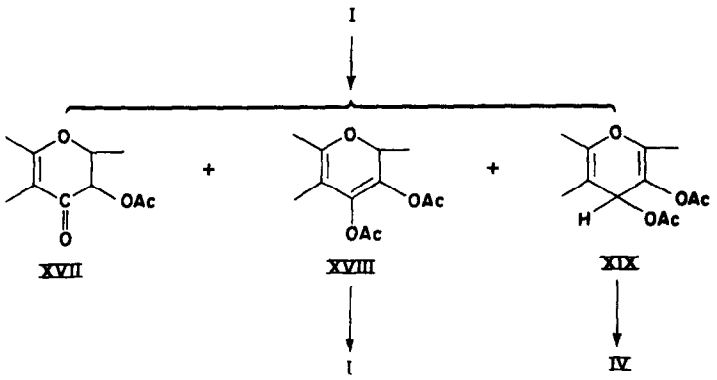
The earliest experiment reported to yield cyanidin[1] (X) from taxifolin (VII) by reductive acetylation could not be repeated successfully. In the course of our attempts to find out the cause of this discrepancy, it was found that 3-hydroxyflavanones (dihydroflavonols) undergo a novel facile isomeric change when boiled with acetic anhydride in the presence of anhydrous sodium acetate or pyridine as catalyst. Under these conditions taxifolin gave a new product, a hexa-acetate, to which the formula of cyanidin 4-ψ base hexa-acetate (IX) was assigned based on analytical data, infra-red spectrum and its reactions towards acids and alkalis (see below). The new product when boiled with alcoholic hydrochloric acid underwent deacetylation and yielded cyanidin chloride. This conversion was also effected by treatment with alkali in hydrogen or nitrogen atmosphere followed by acidification at room temperature. The yield of pure cyanidin chloride was over 55 per cent of the acetate taken. This high yield confirms that the material hydrolysed is really a pseudobase. The experiments could be repeated with aromadendrin (XI) and dihydrorobinetin (XIV) giving pelargonidin (XIII) and robinetinidin chlorides (XVI) through the ψ-base acetates (XII) and (XV) respectively.

The mechanism of this isomeric change has already been outlined[2] and is summarized in the formulae given below. Evidence in support of the suggestion that the reaction proceeds through taxifolin-enol acetate (VIII) is now provided by the experiments discussed below.



Originally it was stated that the above isomeric change seemed to be specific only for hydroxy dihydroflavonols and not their methyl ethers.[2] A detailed study of taxifolin tetramethyl ether (I) has necessitated revision of this inference. This tetramethyl ether when boiled with acetic anhydride and sodium acetate under the same conditions as used for taxifolin, gave a product which on careful examination, is found to be a mixture of three compounds, (i) *O*-tetramethyl-taxifolin 3-acetate (XVII), (ii) *O*-tetramethyl-taxifolin enol di-acetate (XVIII) and (iii) *O*-tetramethyl cyanidin-4-ψ-base di-acetate (XIX). The mixture could be separated by fractional crystallization from ethanol. The identity of the normal acetate was based on mixed melting point with a sample prepared by acetylation of

taxifolin tetramethyl ether with pyridine and acetic anhydride at room temperature. The characterization of the enol acetate was based on analytical data, i.r. spectrum (absence of ketonic $>C=O$ at 5·9 μ) and acid hydrolysis reforming taxifolin tetramethyl ether. The last fraction as the ψ-base was proved by conversion into cyanidin 5,7,3′,-4′-tetramethyl ether (IV). In our attempts to achieve the total conversion of taxifolin tetramethyl ether into the ψ-base acetate, we have found that anhydrous potassium acetate, serves the purpose far better, the change is complete and no enol acetate was present. In a separate experiment the enol-acetate was found to get completely converted into the ψ-base acetate by boiling with potassium acetate acetic anhydride mixture. In the case of taxifolin the isomeric change was complete with sodium acetate itself (time 1 hr) but the change was quicker with potassium acetate. The use of these two reagents with the tetramethyl ether has enabled the study of the intermediate stages in the new isomeric change.



Biogenetic significance of the new transformation

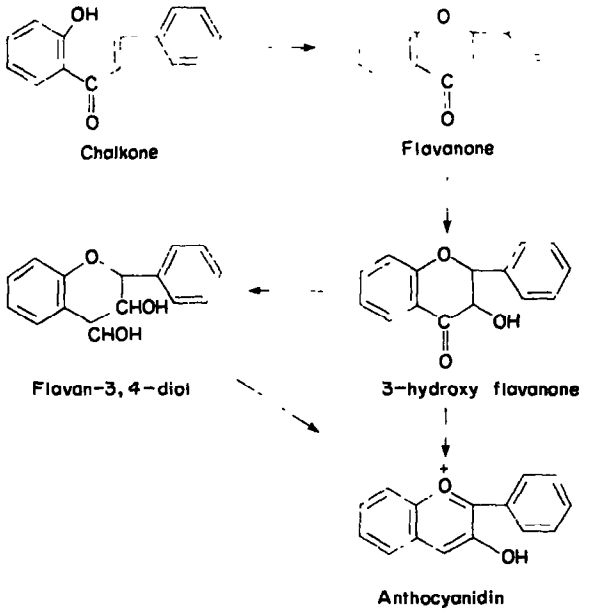
In an earlier publication [10a] the possible course of biogenesis of anthocyanidins based on flavan-3,4-diols or leucoanthocyanidins was discussed. The suggestions are embodied in the scheme presented below (see following Chart A). 3-Hydroxyflavanones which undergo easy reduction to flavan-diols, can be considered to be the precursors of leucoanthocyanidins which in their turn require an oxidation step to form anthocyanidins. It follows, therefore, that starting from a 3-hydroxyflavanone, a reduction and a reoxidation steps are necessary to reach anthocyanidins. The new transformation of 3-hydroxyflavanones just discussed suggest a more direct route. 3-Hydroxyanthocyanidins and 3-hydroxyflavanones are in the same oxidation level with respect to the C_3 bridge and the easy isomeric change of the latter into ψ-base (acetates) would represent the simplest mode of formation of anthocyanidins. For the laboratory method, heat and a base catalyst are needed and the transformation is smooth. In plants an efficient enzyme reaction could be visualized. That 3-hydroxyflavanones constitute important intermediates in the evolution of flavonoids has already been discussed.[10b] The present work adds further emphasis to this hypothesis.

It will be appropriate to mention here the recent results reported by Griesbach and Patschke.[11] They have found that 2′-glucoside of 2′,4′,6′,4-tetrahydroxy-(β^{14}C)-chalkone is incorporated into the synthesis of cyanidin in red cabbage and into cyanidin and quercetin in buckwheat. In these cases no randomization of the radioactivity is noted. They concluded

[10] T. R. SESHADRI, (a) *Proc. Bose. Res. Inst.* **22**, 139 (1958); (b) *Colloques Internationau Les Héterocycles Oxygénés, du centre National La Recherche Scientifique* (1957).
[11] H. GRIESBACH and L. PATSCHKE, *Z. Naturf.* **16B**, 645 (1961).

that the chalkone is most probably a common precursor in the biosynthesis of flavonols and anthocyanidins. These results provide support for the earlier ideas of flavonoid biogenesis, based on the inference that chalkones constitute the earliest step in the C_{15} skeleton and that 3-hydroxyflavanones are important stages.

CHART A



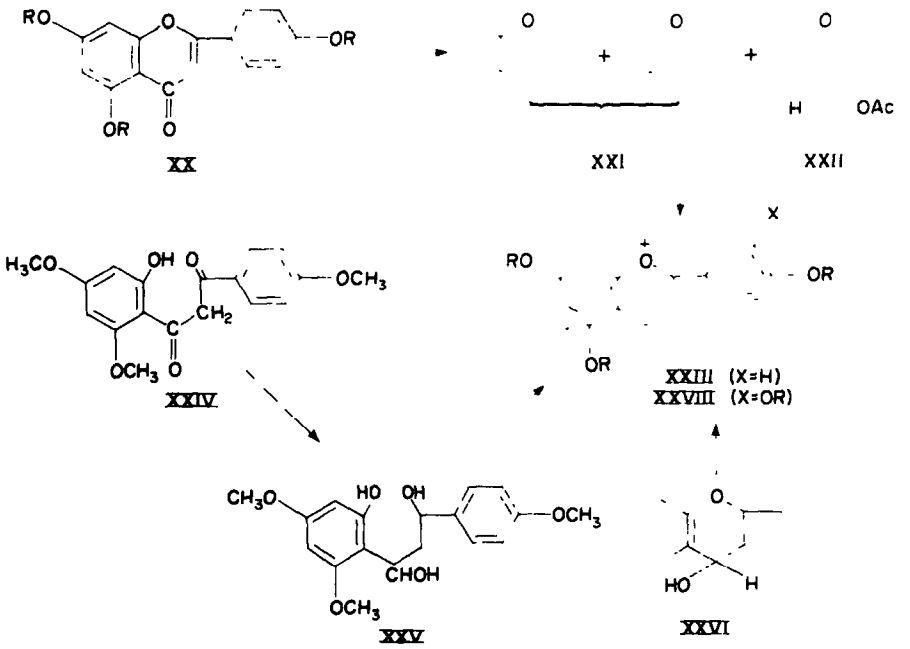
From flavones by reductive acetylation

Flavones behave on reductive acetylation in a manner quite similar to flavonols to yield flavylium salts of the apigeninidin type (XXIII). The method works well with hydroxy and methoxyflavones and gives good yields (40–50 per cent) of chromatographically and spectrophotometrically pure anthocyanidins. In a detailed study, apigenin (XX R = H) and apigenin trimethyl ether (XX, R = CH_3) were used and the reductive acetylation products seemed to be a difficultly separable mixture of flavenes (XXI) and flavan-4-ol acetate (XXII). This conclusion was based on the analytical values and absorption spectra. The high yield of flavylium chlorides obtained is probably due to the presence of a high percentage of flavenes in the mixture, which are capable of undergoing ready conversion owing to facile oxidation of the active CH_2(position 4) or CH(position 2) group.

From diketones by borohydride reduction

As a simplification in steps, the use of 2-hydroxydibenzoyl methanes (XXIV), related to flavones, has also been examined for the preparation of flavylium salts. These diketones are easily available by the Baker-Venkataraman transformation of 2-aroyloxyacetophenones. 2'-Hydroxy-4,4',6'-trimethoxy dibenzoyl methane (XXIV) underwent complete reduction with sodium borohydride to give the diol (XXV). This diol is very sensitive to strong acids and gives a deep carmine colour even in the cold. It slowly undergoes change into apigeninidin 5,7,4'-*O*-trimethyl ether (XXIII, R = CH_3) and some polymeric products.

By carrying out this reaction with a mixture of glacial acetic acid and concentrated hydrochloric acid, the flavylium chloride has been isolated in satisfactory yields (20–30 per cent). The flavylium ferrichloride was directly obtained by performing the above conversion in the presence of anhydrous ferric chloride. The method seems to be of general use.



From flavanones by borohydride reduction

Flavanones are in the same state of oxidation as flavylium salts (without 3-hydroxyl group), and hence the former might be expected to give the latter by an intramolecular rearrangement similar to 3-hydroxyflavanones. But experiments carried out so far to achieve this have been unsuccessful. No definite reasons could be given. It may be that appropriate conditions have still to be found out. Flavanones undergo easy and quantitative reduction with lithium aluminium hydride or sodium borohydride to give flavan-4-ols; the use of the second reagent is more convenient. These flavan-4-ols which are analogous to leucoanthocyanidins are converted into the corresponding flavylium chlorides when treated with hydrochloric acid in alcoholic solution. This process is attended by loss of water to give a flavene followed by oxidation (aerial). By the usual method of partition between isoamyl alcohol and dilute hydrochloric acid, the pure anthocyanidin can be obtained in an overall yield of 15 per cent. As representative examples, apigeninidin (gesneridin, XXIII) has been prepared from the corresponding flavan-4-ol (XXVI) and its identity proved by comparison with authentic sample prepared by the method of Robinson.[12] Similarly, apigeninidin trimethyl ether (XXIII, R = CH_3), luteolinidin[13,14] (XXVIII, R = H) and its *O*-tetramethyl ether[13] (XXVIII, R = CH_3) have also been prepared. The properties of these samples agree fully with those reported in the literature.

[12] R. ROBINSON and A. TODD, *J. Chem. Soc.*, 809 (1934).
[13] D. D. PRATT and R. ROBINSON, *J. Chem. Soc.* 1935 (1925).
[14] J. B. HARBORNE, *Chem. and Ind.* 229 (1959).

EXPERIMENTAL

Melting points are uncorrected. Ethanolic solutions are used for u.v. absorption measurements. Only essential i.r. bands are recorded here.

General procedure for the reductive acetylation of flavonols and flavones

The compound (2 parts) was dissolved in acetic anhydride (20 parts), freshly fused sodium acetate (1 part) was added followed by pure zinc dust (2 parts): further additions of zinc was made after 1 hr of refluxing. The heating was continued for 1 hr more. The mixture was filtered hot, the residue washed with warm glacial acetic acid and the combined filtrate poured over crushed ice. After keeping the mixture overnight the solid product was filtered, washed with water, dried and recrystallized.

(i) *Quercetin pentamethyl ether*

This gave quantitative yield of the reduction product which crystallized as pale yellow rectangular prisms from a mixture of ethyl acetate-light petroleum (60–80°) m.p. 80–90° (Found: C, 65·5; H, 6·4. $C_{20}H_{22}O_6$) (flav-3-ene) requires C, 67·0; H, 6·1; $C_{22}H_{24}O_8$(flavan-4-ol acetate) requires C, 63·5; H, 5·8%); λ_{max} 279 mμ ($E^{1\%}_{1\,cm}$ 310); i.r. ($CHCl_3$): 5.75 μ.

(ii) *Quercetin-5,7,3′,4′-tetramethyl ether: Preparation of quercetin 5,7,3′,4′-tetramethyl ether (II)*

The sample required for the present work has been prepared by the oxidation of taxifolin tetramethyl ether[15] by the following methods.

(a) *Iodine Oxidation.* The reaction was carried out as described by Goel *et al.*[5] The flavonol crystallized from ethanol as pale yellow needles, m.p. 194–195°. Yield (51 per cent.) Mixed melting point with a sample prepared by methylation of rutin followed by hydrolysis was undepressed.

(b) *Aerial oxidation in presence of* 2N *sulphuric acid.* A suspension of taxifolin tetramethyl ether (0·2 g) in 2*N* sulphuric acid (50 ml) was heated on a steam bath for 15 hr. The methyl ether went into solution and a yellow substance separated. After 30 hr the precipitate was filtered, dried and crystallized from ethanol yielding pale yellow needles, m.p. and mixed m.p. 194–195° (0·11 g; 55 per cent).

(c) *Alkaline potassium ferricyanide.* To a boiling solution of taxifolin tetramethyl ether (1 g) in ethanol (100 ml) was added a warm solution of potassium ferricyanide (1·8 g) and KOH (0·3 g) in water (15 ml). The mixture was left overnight and then diluted with water. Extraction with ethyl acetate and removal of the solvent gave a residue which crystallized from ethanol yielding quercetin 5,7,3′,4′-tetramethyl ether, melting point and mixed melting point 194–195° (0·3 g; 30%).

Reductive acetylation. The tetramethyl ether (0·1 g) afforded a buff coloured solid, which crystallized from dilute ethanol, as aggregates of prisms, m.p. 85–95°, (0·1 g) (Found: C, 65·1; H, 5·7; $COCH_3$, 9·9. $C_{21}H_{22}O_7$ requires C, 65·2; H, 5·7; $COCH_3$, 11·4%). λ_{max} 280 mμ (log ε 3·88); min 266 mμ (log ε 3·83); i.r. (KBr): 5·70 μ.

(iii) *Apigenin*

Apigenin or its triacetate (0·5 g) on reductive acetylation gave orange yellow prisms (0·4 g) melting between 150–170° (Found: C, 64·1; 64·4; H, 4·7; 4·7. $C_{21}H_{18}O_7$ (flavene)

[15] H. L. HERGERT, P. COAD and A. V. LOGAN, *J. Org. Chem.* **21**, 304 (1956).

requires: C, 65·9; H, 4·7; $C_{23}H_{22}O_9$ (flavan-4-ol acetate) requires: C, 62·4; H, 5·0%); λ_{max} 380 mμ ($E^{1\%}_{1\,cm}$ 77·6).

(iv) *Apigenin trimethyl ether. Preparation of apigenin trimethyl ether* (*XX*, $R = CH_3$)

This was earlier prepared by Kostanecki[16] by his well-known method, but it is more conveniently prepared by Baker-Venkatarman method as follows: (1) *2-anisoyl ester of phloracetophenone dimethyl ether* m.p. 98–99°, was prepared in almost quantitative yield by heating (100°) for 1 hr a mixture of phloracetophenone 4 : 6-dimethyl ether and anisoyl-chloride in the presence of pyridine, (2) *Isomerization to the dibenzoyl methane*: The above ester (3 g) in anhydrous pyridine (20 ml) was treated with powdered and dry potassium hydroxide (3 g) and the mixture vigorously shaken, with occasional warming (water bath, 60°) for 30 min. After 90 min crushed ice was added the mixture acidified with acetic acid and allowed to stand overnight. The solid was collected, washed and dried. It crystallized from alcohol as lemon yellow needles, m.p. 137–138° (2·5 g) (3) *Cyclization*: The foregoing diketone (1 g) in glacial acetic acid (15 ml) containing 5 drops of concentrated sulphuric acid was heated under reflux for 2 hr. Removal of acetic acid under reduced pressure and dilution with ice-cold water, precipitated a pale yellow solid (0·8 g). The product crystallized from ethanol as colourless needles, m.p. 157–158° (lit[16] 157–158°).

Reductive acetylation. The product was obtained as a yellow solid, which separated from alcohol as small aggregates of cubes m.p. 188–194°, sintering at 180° (Found: C, 70·8, 70·3; H, 6·1; 5·8. $C_{20}H_{18}O_4$ (flavan-4-ol acetate) requires: C, 67·0; H, 6·2; $C_{18}H_{22}O_6$ (flavene) requires C, 72·4; H, 6·0%) λ_{max} 275 mμ ($E^{1\%}_{1\,cm}$ 194); i.r. ($CHCl_3$): 5·68 μ.

Acetylation of 3-hydroxy flavanones. 3-Hydroxyflavanones yield the normal acetates on acetylation with acetic anhydride and pyridine at room temperature.[15]

Conversion of taxifolin into cyanidin ψ-base hexa-acetate (*IX*). (*a*) Taxifolin or its penta-acetate (0·5 g), acetic anhydride (10 ml) and pyridine (5 ml) or freshly fused sodium acetate (0·5 g) were heated under reflux at 145–150° for 1 hr. Within 15 min the mixture turned red and remained as a deep-red solution. It was then poured into crushed ice and allowed to stand till the excess of acetic anhydride had decomposed. An orange-red amorphous solid was obtained. It was dissolved in the minimum amount of glacial acetic acid, filtered and reprecipitated by pouring into water. Purification by the usual methods of fractional crystallization and also by chromatography failed to give any fraction with a sharp melting point. It separated from hot methanolic solution on cooling as pale brown aggregates of prisms, m.p. 130–135° (Found: C, 58·1; H, 5·0; CH_3CO, 49·1. $C_{27}H_{24}O_{13}$ requires C, 58·2; H, 4·4; CH_3CO, 46·4%). There is no characteristic maximum in u.v.; i.r. ($CHCl_3$): 5·70 μ.

(*b*) Acetylation of taxifolin (0·5 g) with acetic anhydride (10 ml) and fused sodium acetate (0·5 g) at 100° for 2 hr gave mainly taxifolin penta-acetate (0·56 g) along with a small amount (0·08 to 0·18 g) of coloured (reddish) by-product which could be easily converted into cyanidin chloride with hot alcoholic hydrochloric acid and thus it is of the same nature as the cyanidin-ψ-base acetate.

Acetylation of taxifolin tetramethyl ether

(a) *Cold acetylation* (*normal acetate* (*XVII*)). A clear solution of taxifolin tetramethyl ether (0·2 g) in acetic anhydride (3 ml) and pyridine (5 drops) was kept for 48 hr at room temperature. The solution was diluted with water, when a colourless solid separated. It

[16] S. T. VON KOSTANECKI and J. TAMBOR, *Ber. deut. chem. Ges* 33, 1991 (1900).

crystallized from ethanol, as colourless needles, (0·2 g) m.p. 179–180° (lit.[15] 171–172°) (Found: C, 62·6; H, 6·0. Calc. for $C_{21}H_{22}O_8$: C, 62·6; H, 5·5%); i.r. (KBr): 5·70 and 5·90 μ.

(b) *Boiling acetic anhydride and sodium acetate (mixture of normal (XVII)), enol (XVIII) and ψ-base acetates (XIX).* Taxifolin tetramethyl ether (0·5 g), acetic anhydride (10 ml) and fused sodium acetate (0·5 g) were refluxed for 1 hr at 150–160°. The mixture was poured into crushed ice. The pale yellow precipitate thus obtained was reprecipitated from acetic acid and subjected to fractional crystallization from ethanol.

The first fraction was obtained as colourless needles (0·2 g) m.p. 134–135° (Found: C, 62·6; H, 5·9. $C_{23}H_{24}O_9$ requires C, 62·2; H, 5·4%); λ_{max} 251 mμ (log ε 4·02) and 344 mμ (log ε 4·32) i.r. ($CHCl_3$): 5·65, and in (KBr): 5·70 μ. In all its properties it agreed with the enol-acetate. It developed no colour on boiling with alcoholic hydrochloric acid, but underwent hydrolysis to the original taxifolin derivative. The above enol acetate (0·1 g) in ethanolic hydrochloric acid (20 ml; 10% v/v) was boiled under reflux for 2 hr. The solvent was removed completely under reduced pressure. The product crystallized from ethanol as colourless needles (0·06 g), m.p. 171–172°, undepressed by taxifolin tetramethyl ether.

The second fraction was obtained as colourless needles (0·2 g), m.p. 179–180°. It was identified as taxifolin tetramethyl ether acetate by mixed m.p.

The third fraction was obtained as pale brown amorphous powder. By boiling with alcoholic hydrochloric acid this could be converted into cyanidin 5,7,3′,4′-tetramethyl ether and hence agrees with the ψ-base acetate given below.

(c) *Boiling acetic anhydride and potassium acetate (cyanidin tetramethyl ether ψ-base acetate (XIX)).* Taxifolin tetramethyl ether or its acetate (0·2 g), freshly fused potassium acetate (0·2 g) and acetic anhydride (5 ml) were refluxed for 1 hr at 150–160°. The dark-red solution was poured into crushed ice and the orange-brown solid was filtered, dissolved in acetic acid and reprecipitated with water. Repeated crystallization from methanol afforded pale brown amorphous solid, m.p. 140–150° (decomp.). Chromatography on cellulose gave a pale brown solid which crystallized from benzene-light petroleum, as short rectangular prisms and needles, m.p. 185–190° (decomp.) with previous sintering at 170° (Found: C, 63·3; H, 5·3. $C_{23}H_{24}O_9$ requires C, 62·2; H, 5·4%). There is no characteristic maximum in u.v.; i.r. (KBr): 5·70 μ.

Conversion of enol acetate (XVIII) into ψ-base acetate (XIX)

The enol acetate when refluxed with acetic anhydride (5 ml) and pyridine (2 ml) was recovered unchanged. But by refluxing for 1 hr (0·1 g) with freshly fused potassium acetate (0·2 g) and acetic anhydride (5 ml) an orange-red solution was produced. It was poured on crushed ice and the brown solid was filtered and dried. It could be easily converted into cyanidin 5,7,3′,4′-tetramethyl ether on boiling with hot alcoholic hydrochloric acid. Its i.r. spectrum is identical with that of cyanidin 5,7,3′,4′-tetramethyl ether ψ-base acetate described earlier.

Conversion of aromadendrin (XI) into pelargonidin-ψ-base penta-acetate (XII)

A mixture of aromadendrin[17] (0·2 g), fused sodium acetate (0·2 g) and acetic anhydride (5 ml) was heated under reflux for 1 hr. It became deep orange-red, within 15 min and remained so afterwards. The mixture was poured over crushed ice and left overnight. The product was obtained as amorphous powder melting between 135–150° (0·31 g). (Found

[17] A. K. Ganguly and T. R. Seshadri, *J. Chem. Soc.* 2787 (1961).

in a sample dried at room temperature: C, 60·8; H, 4·6. $C_{25}H_{22}O_{11}$ requires C, 61·0; H, 5·0%).

Conversion of dihydrorobinetin (XIV) into robinetinidin ψ-base hexa acetate (XV)

A mixture of dihydrorobinetin (0·1 g), freshly fused sodium acetate (0·2 g) and acetic anhydride (2 ml) was heated at 150–155° under reflux for 1 hr. The product was isolated as in the earlier cases. It was a pale red amorphous solid with indefinite melting point (0·14 g) (Found: C, 59·1, H, 4·7. $C_{27}H_{24}O_{13}$ requires C, 58·2; H, 4·5%).

General procedure for the reduction of flavanones with sodium borohydride

The flavanone (1 mole) was dissolved in excess of methanol and the solution cooled in ice-water. Sodium borohydride (1 mole) was added in one lot and the reaction mixture was allowed to stand at room temperature for 24 hr. The solution was then just acidified with glacial acetic acid (a few drops) and the solvent distilled under reduced pressure. The residue was dried in a vacuum desiccator over KOH, macerated with a small amount of water, filtered or decanted, dried again and crystallized. The yields are quantitative.

(i) *5,7,4'-Trimethoxyflavan-(β)-4-ol (XXVI).* 5,7,4'-Trimethoxyflavanone (1g) in methanol was reduced with sodium borohydride (0·14 g). The flavan-4-ol crystallized readily from methanol or benzene as colourless stout needles, m.p. 158° (Found: C, 67·8; H, 6·7. Calc. for $C_{18}H_{20}O_5$: C, 68·3; H, 6·4%). λ_{max} 272 mμ (log ε 3·4); min 255 mμ (log ε 2·23); i.r. ($CHCl_3$): 2·7 μ. Its alcoholic solution gives an intense red-pink colour (λ_{max} 545 mμ) when treated with concentrated hydrochloric acid. Geissman and Clinton[18] recorded m.p. 159–159·5° for 5,7,4'-trimethoxyflavan-4-ol obtained from 5,7,4'-trimethoxyflavanone by reduction using (1) sodium amalgam and (2) hydrogen in the presence of Adam's catalyst. The product was designated as 5,7,4'-trimethoxyflavan (β)-4-ol. The identity of the present sample with that obtained by catalytic hydrogenation was established by mixed m.p.

(ii) *5,7,3',4'-Tetramethoxyflavan-(β)-4-ol.* The tetramethoxyflavanone (0·5 g) in methanol was reduced with sodium borohydride. Crystallization of the product from ethanol yielded, very pale cream coloured prisms, m.p. 116–117° (Found: C, 66·3; H, 6·4. $C_{19}H_{22}O_6$ requires: C, 65·9; H, 6·4%). λ_{max} 278 mμ (log ε 3·69); min 256 mμ (log ε 3·46); i.r. ($CHCl_3$): 2·7 μ. The flavan-4-ol gives a permanganate colour with concentrated hydrochloric acid in alcohol.

(iii) *Flavan β-4-ol.* Similarly prepared from flavanone, crystallized as colourless woolly needles, m.p. 147–148°, (lit.[18,19] 147–148°).

(iv) *7-Methoxyflavan-(β)-4-ol.* The flavan (β)-4-ol was obtained as colourless fine needles, m.p. 103° (Found: C, 74·9; H, 6·3. Calc. for $C_{16}H_{16}O_3$: C, 75·0; H, 6·4%); i.r. ($CHCl_3$): 2·7 μ.

Preparation of anthocyanidins

General procedure for the conversion of reduction and isomerization products into the anthocyanidins

A solution of the compound in 10% (v/v) alcoholic hydrochloric acid (d. 1·16) was refluxed for 2 hr. About three-fourths of the alcohol was removed under reduced pressure and the residue diluted with water till a slight but permanent turbidity appeared. The

[18] T. A. GEISSMAN and R. O. CLINTON, *J. Am. Chem. Soc.* **68**, 705 (1946).
[19] R. BOGNÁR, M. RÁKOSI, H. FLETCHER, D. KEHOE, E. M. PHILBIN and T. S. WHEELER, *Tetrahedron* **18**, 135 (1962).

solution was cooled and filtered from any precipitated solid (phlobaphene), this material being rejected. The filtrate (*F*) was twice extracted with ethyl acetate, the extract being rejected. The aqueous solution was extracted with small quantities of butyl alcohol. The butyl alcohol extract was evaporated under reduced pressure. During evaporation, small quantities of methanol saturated with hydrogen chloride gas, was added at intervals, so as to keep the solution acidic. The residue was macerated with concentrated hydrochloric acid. A crystalline mass was usually formed. Further purification was done by dissolution in methanolic hydrochloric acid and reprecipitation with dry ether.

For the purpose of identification the following further procedure was adopted. The aqueous solution (*F*) was extracted repeatedly with small quantities of isoamyl alcohol and the anthocyanidin chloride was transferred into acqueous dilute hydrochloric acid (1%) by the addition of a large volume of petroleum and by shaking. The acid solution was extracted once with ether and several times with benzene to remove traces of amyl alcohol. The amyl alcohol free acid solution was evaporated at room temperature, in a vacuum desiccator over KOH.

Identification of the anthocyanidins

Colour reactions using 1% hydrochloric acid solutions, circular paper chromatography and absorption spectra have been used. For absorption spectra 0·1% alcoholic hydrochloric acid solutions were used. The following solvents were used for chromotography (25°C): (A) phenol saturated with water, (B) butanol–acetic acid–water (4 : 1 : 5 v/v) (upper phase); (C) lower phase; (D) water–acetic acid–conc. hydrochloric acid (10 : 30 : 3 v/v); (E) 2*N*-hydrochloric acid–acetic acid (7 : 3 v/v).

Cyanidin chloride (X) from ψ-base acetate (IX)

(a) *By acid hydrolysis.* The cyanidin ψ-base hexa-acetate (0·4 g) on acid hydrolysis yielded cyanidin chloride (yield 57 per cent). R_f values: 0·70 (A); 0·83 (B); 0·55 (C); 0·55 (D); λ_{max}, 542 mμ.

(b) *By alkaline hydrolysis.* The cyanidin-ψ-base hexa-acetate (0·1 g) was kept overnight at 20-25° in 2% aqueous ethanolic (1 : 1) solution of potassium hydroxide (15 ml), boiled previously to expel air and then saturated with nitrogen. A deep green solution was immediately formed and this changed to bright red on acidification with cold dilute hydrochloric acid. The acid solution was diluted with water (20 ml) and extracted twice with ethylacetate (20 ml each time). The colouring matter was isolated as given in the general procedure. Its identity with cyanidin chloride was established by colour tests, chromatography and light absorption (λ_{max}, 545 mμ).

Cyanidin tetramethyl ether (IV)

(a) *From reductive acetylation product of quercetin-5,7,3′,4′-tetramethyl ether.* It was obtained, on acid deacetylation, in an yield of 45-50 per cent. R_f values: 0·95 (D); 0·77 (E); λ_{max} 525 mμ, same as an authentic sample.[7]

(b) *From the ψ-base acetate (XIX) obtained from taxifolin tetramethyl ether.* By similar hydrolysis cyanidin tetramethyl ether was obtained in an yield of 50–55 per cent.

Cyanidin pentamethyl ether

This was obtained in an yield of 40 per cent from the reductive acetylation product of quercetin pentamethyl ether on acid hydrolysis. R_f Value 0·86 (E); λ_{max} 520 mμ.

Pelargonidin chloride (XIII)

Pelargonidin-ψ-base penta-acetate, obtained from aromadendrin, on acid hydrolysis, gave pelargonidin chloride, yield 62 per cent of the acetate taken. R_f values 0·90 (A), 1.00 (B), 0·54 (C); λ_{max} 530 mμ.

Robinetinidin chloride (XVI)

The robinetinidin ψ-base hexa-acetate on acid hydrolysis, yielded robinetinidin chloride in an yield of 58 per cent. With amyl alcohol and sodium acetate it gave a violet colour which changed to greenish blue on addition of a drop of ferric chloride. It is completely destroyed in the oxidation test. R_f value 0·58 (D); λ_{max} 529 mμ (lit.[20] 532 mμ).

Apigeninidin chloride (XXIII, R = H)

(a) *From flavan-(β)-4-ol*: 5,7,4′-trihydroxyflavan-β-4-ol was refluxed with alcoholic hydrochloric acid. The deep red violet colour (λ_{max} 550 mμ) produced in the beginning changed to deep orange-red in the course of about 40 min. The anthocyanidin chloride was isolated by the general procedure in an yield of 15 per cent. R_f values: 0·80 (D), 0·57 (C); λ_{max} 484 mμ ($E^{1\%}_{1\,cm}$ 1371). (b) *From the reductive acetylation product of apigenin.* It gave on acid hydrolysis apigeninidin chloride in an yield of 45–50 per cent. The above two samples agree fully in their colour reactions, R_f values and absorption spectrum with an authentic sample.[12]

Apigeninidin trimethylether (XXIII, R = CH_3)

(a) *From the flavan-4-ol (XXVI).* 5,7,4′-Trimethoxyflavan-(β)-4-ol in alcoholic hydrochloric acid was refluxed for 1·5 hr. The intense carmine colour (λ_{max} 545 mμ) produced in the cold changed to reddish orange. From the resulting solution apigeninidin trimethyl ether was isolated in an yield of 15 per cent; m.p. 159–162° (decomp.) R_f: 0·70 (C); λ_{max} 475 mμ ($E^{1\%}_{1\,cm}$ 1231). King and Clark-Lewis[21] recorded melting point 159–160°, and λ_{max} 476 mμ. (b) *From the reductive acetylation product of apigenin trimethyl ether.* This on acid hydrolysis yielded apigeninidin trimethyl ether in an yield of 51 per cent. It agreed in melting point, chromatography and absorption maximum with the sample described under (a).

Luteolinidin chloride (XXVIII, R = H). Eriodictyol[6] (0·1 g) was reduced with borohydride (0·05 g) as usual and the reduction product was directly refluxed with alcoholic hydrochloric acid (10%) for 1·5 hr. An intense purple colour was produced in the cold. From the resulting solution, the anthocyanidin was isolated by the partition method. It was obtained as an orange-red crystalline mass not melting below 290°. The colour reactions agreed with those reported by Leon and Robinson.[22] λ_{max} 495 mμ (lit.[14] 495 mμ).

Luteolinidin tetramethyl ether (XXVIII, R = CH_3). 5,7,3′,4′-tetramethoxyflavan-(β)-4-ol on conversion gave luteolinidin tetramethyl ether in an yield of 20 per cent, m.p. 160–162° (decomp.); λ_{max} 492 mμ (Gramshaw *et al.*,[23] recorded m.p. 161-162° (decomp.), and λ_{max} 494 mμ).

Reduction of 2-hydroxy-4,6,4′-trimethoxy dibenzoyl methane (XXIX) to the diol (XXV)

The dibenzoyl methane (0·5 g) in methanol (100 ml) was treated with sodium borohydride (0·2 g) and after leaving the solution overnight at room temperature (20–22°), it

[20] K. FREUDENBERG and D. J. ROUX, *Nature. Wiss.* **41**, 450 (1954).
[21] F. E. KING and J. W. CLARK-LEWIS, *J. Chem. Soc.* 1345 (1955).
[22] A. LEON and R. ROBINSON, *J. Chem. Soc.* 2732 (1931).
[23] J. W. GRAMSHAW, A. W. JOHNSON and T. J. KING, *J. Chem. Soc.* 4040 (1958).

was worked up as usual. Crystallization of the product from benzene-light petroleum (40–60°) yielded a colourless crystalline solid, m.p. 134–135° (decomp.) i.r. spectrum ($CHCl_3$) showed no carbonyl absorption band.

The reduced diketone is slightly soluble in water, very easily in all the common organic solvents but insoluble in light petroleum. It developed reddish brown colour on the surface on exposure to light and air. Concentrated hydrochloric acid added to an alcoholic solution of the diol gave a purple-red colour.

Formation of apigeninidin trimethyl ether

(i) *With alcoholic hydrochloric acid.* A solution of the reduced diketone (*ca.* 20 mg) in alcoholic hydrochloric acid (10 ml, 10%) was refluxed for 1·5 hr. The anthocyanidin chloride was isolated, according to the procedure described already, as a solution in hydrochloric acid. Examination of this solution spectrophotometrically (λ_{max} 472 mμ) and chromatographically showed the presence of apigeninidin trimethyl ether.

(ii) *With glacial acetic acid and concentrated hydrochloric acid.* A mixture of the diol (0·1 g) in acetic acid (4 ml) and concentrated hydrochloric acid (1 ml) was kept at room temperature for 48 hr. Dilution with dry ether deposited an orange solid, m.p. 155–160°. This material was once washed with dry ethylacetate and recrystallized from alcoholic hydrochloric acid, m.p. 158–160° (decomp.); yield 25–30 mg. It was identical with apigeninidin trimethyl ether.

Formation of apigeninidin trimethyl ether ferric chloride

A mixture of the diol (0·1 g), anhydrous ferric chloride (0·1 g), glacial acetic acid (5 ml) and concentrated hydrochloric acid (1 ml) was kept overnight at room temperature. The reddish brown precipitate which separated from the clear solution was filtered and crystallized twice from glacial acetic acid. The flavylium ferric chloride sintered at 180° and melted with decomposition at 185–187°. Pratt, Robinson and Robinson[24] and Gramshaw *et al.*[23] record the same melting point.

Preparation of anthocyanins

Cyanidin-3-rhamnoglucoside (VI, R = OH)

Rutin (0·2 g) on reductive acetylation gave an orange-red product which crystallized from aqueous acetic acid, pale yellow aggregates of prisms, m.p. 125–140° (0·35 g) (Found: C, 55·3; H, 5·6; $C_{47}H_{52}O_{25}$ (flav-3-ene) requires: C, 55·5; H, 5·1%).

It (0·3 g) was suspended in alcoholic potassium hydroxide solution (20 ml, 10%) and kept at room temperature (25°) for 1·5 hr, while a slow stream of pure hydrogen was bubbled through the mixture. The solid went into solution within 15 min and the solution developed a dark green colour. It was acidified with ice-cold hydrochloric acid to make it approximately 2%, extracted three times with ethyl acetate (10 ml, portions) and then taken to dryness at room temperature in a vacuum desiccator over potash. The residue was extracted with 0·5% absolute methanolic hydrochloric acid, filtered and the pigment precipitated by the addition of a large excess of dry ether. The dark red crystalline solid was dissolved in 1% aqueous hydrochloric acid containing a few drops of methanol. The acid concentration was slowly increased to 5% by the addition of calculated volume of 10% hydrochloric acid and the solution left in the ice chest for crystallization. The anthocyanin was obtained in the form of dark red prisms. It did not melt below 200°.

[24] D. D. Pratt, G. M. Robinson and R. Robinson, *J. Chem. Soc.* 1975 (1927).

It is readily soluble in methanol, ethanol and moderately in cold water but readily in warm water and in 1% aqueous hydrochloric acid giving a red solution, the colour fades on exposure to light. Aqueous sodium carbonate gives a red-violet colour and sodium hydroxide a blue colour changing to green. Aqueous ferric chloride gives a blue colour which changes to stable blue-violet on dilution with water (3-hydroxyl is not free). There was very little extraction of the pigment by isoamyl alcohol, but on saturating the solution with sodium chloride, the pigment was completely extracted. The identity of the present sample with a natural sample (from *Antirrhinum* magenta flowers) was proved by co-chromatography and absorption spectra. (Table 1.)

TABLE 1. COMPARISON* OF NATURAL AND SYNTHETIC ANTHOCYANINS

Compound	Source	R_f value in† 1	2	3	4	λ_{max} MeOH : HCl	$\frac{E_{440}}{E_{max}}$
Cyanidin-3-*rhamnoglucoside*	From *Antirrhinum*	0·31	0·22	0·16	0·39	282, 523 mμ	23%
	Synthetic	0·32	0·21	0·15	0·39	283, 526 mμ	23%
Pelargonidin-3-*rhamnoglucoside*	From *Antirrhinum* pink flowers	0·31	0·33	0·22	0·46	278, 508 mμ	40%
	Synthetic	0·33	0·33	0·20	0·45	278, 508 mμ	42%

* Supplied by Dr J. B. Harborne.
† Solvents: 1, solvent B; 2, BuOH-2N HCl 1 : v/v; 3, 1% HCl; 4, Acetic acid : conc. HCl : H_2O : 15 : 3 : 82.

Pelargonidin-3-(*rhamnoglucoside*)[25] (*VI*, $R = H$)

Kaempferol-3-rhamnoglucoside on reductive acetylation gave an yellow product which crystallized from aqueous acetic acid as pale yellow amorphous powder, m.p. 130–140° with previous sintering at 115° (Found: C, 55·7; H, 5·4; $C_{45}H_{50}O_{23}$ (flav-3-ene) requires: C, 56·4; H, 5·2%). It was converted into the pelargonidin-3-rhamnoglucoside in the manner described above. The anthocyanin was obtained as orange red prisms, m.p. above 200°.

It is readily soluble in methanol, ethanol and moderately in cold water but readily in warm water and in 1% aqueous hydrochloric acid giving an orange-red solution. This solution gives a violet colour with sodium carbonate and a red-violet colour with sodium acetate. There was very little extraction of the pigment by isoamylalcohol, but on saturating the solution with sodium chloride, the pigment was completely extracted. The identity of the present sample with a natural sample (from *Antirrhinum* pink flowers) was proved by chromatography and co-chromatography and absoption spectra. (Table 1.)

Acknowledgements—The authors are thankful to Dr. J. B. Harbone for the comparison of cyanidin and pelargonidin-3-rhamnoglucosides with the natural samples, and to Dr. V. V. S. Murti for a sample of kaempferol-3-rhamnoglucoside.

[25] V. V. S. MURTI, P. V. RAMAN, T. R. SESHADRI and R. S. THAKUR, *J. Sci. Ind. Res.*, **21B**, 80 (1962).