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# REACTIONS OF THE ANTHOCYANIDIN-3,5-DIGLUCOSIDES: FORMATION OF 3,5-DI-(*O*-β-D-GLUCOSYL)-7-HYDROXY COUMARIN

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**Abstract**—Anthocyanidin-3,5-diglucosides form 3,5-di-(*O*-β-D-glucosyl)-7-hydroxy coumarin at pH 3–7 in aqueous solutions. The coumarin derivative was characterized by its chromatographic and spectral properties and its aglucone was shown to be identical with 3,5,7-trihydroxy coumarin.

## INTRODUCTION

ANTHOCYANIN pigments in plant extracts and aqueous solutions decompose upon prolonged storage at room temperature or upon heating in the presence of oxygen to give reaction products of unknown structure. The decomposition is enhanced in the plant extracts in the presence of sugars,[1] amino acids,[2] ascorbic acid[3] and certain fungal enzymes,[4] however, these effects cannot be accounted for in model solutions.

Lukton *et al.*[5] suggested that the destruction of anthocyanins in aqueous solutions is directly proportional to the amount of pigment present in the pseudobase form. Since it was shown that flavylium salts[6–11] and anthocyanins[12] form highly coloured quinoidal anhydro bases in the pH range of 3–6, the pH of most plant extracts, it is expected that the structural form present at this pH are at least partially responsible for the irreversible colour loss of the pigments.

The present work describes the isolation and characterization of a coumarin glucoside, apparently formed as by product by structural transformation of the anthocyanins.

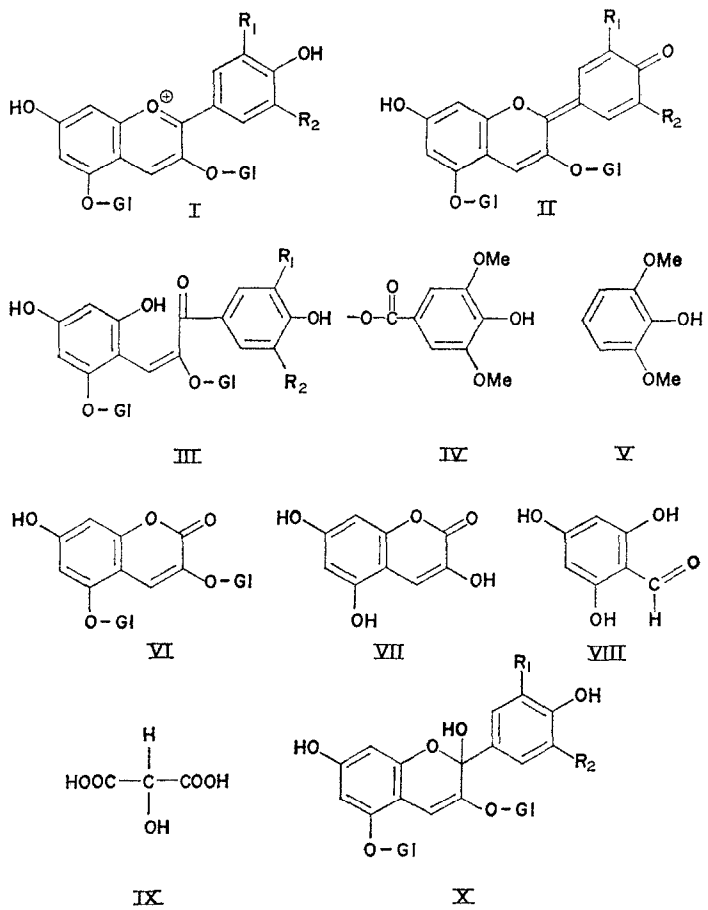
## RESULTS

The visible and u.v. spectrum of the anthocyanidin-3,5-diglucosides changes drastically at high temperatures and neutral pH. Malvidin-3,5-diglucoside (I. $R_1 = R_2 =$ OMe) converts quantitatively into its anhydrobase (II) upon dissolving in buffer solutions of pH 7 and shows an absorption maximum at 607 nm. During heating, the purplish-blue colour of

[1] I. J. TINSLEY and A. H. BOCKIAN, *Food Res.* **25**, 161 (1960).
[2] I. J. TINSLEY and A. H. BOCKIAN, *Food Res.* **24**, 410 (1959).
[3] E. SONDHEIMER and Z. KERTESZ, *Food Res.* **18**, 475 (1953).
[4] N. T. HUANG, *J. Agri. Food Chem.* **3**, 141 (1955).
[5] A. LUKTON, C. O. CHICHESTER and G. MACKINNEY, *Food Technol.* **10**, 427 (1956).
[6] L. JURD, *J. Org. Chem.* **28**, 987 (1963).
[7] L. JURD and T. A. GEISSMAN, *J. Org. Chem.* **28**, 2394 (1963).
[8] C. F. TIMBERLAKE and P. BRIDLE, *Chem. & Ind.* 1520 (1965).
[9] C. F. TIMBERLAKE and P. BRIDLE, *J. Sci. Food Agri.* **18**, 473 (1967).
[10] K. A. HARPER and B. V. CHANDLER, *Australian J. Chem.* **20**, 731 (1967).
[11] K. A. HARPER and B. V. CHANDLER, *Australian J. Chem.* **20**, 745 (1967).
[12] C. F. TIMBERLAKE and P. BRIDLE, *Nature* **212**, 158 (1966).

the pigment solution fades, becoming green after 10 min, yellow green after 1 hr and finally yellow after 2 hr, while the absorbance of the solution in the u.v. region at 278 nm decreased and developed into a $\lambda$ min. with the appearance of two new peaks (shoulders) at 260 and 248 nm (Fig. 1). The spectral curves in Fig. 1 have two well defined isosbestic points at 295 and 545 nm, showing that the formation of the new compounds is directly proportional with the decomposition of the anhydrobase (II).

Upon acidification (to pH 1·0) the yellow solution developed a light pink colour. The spectrum of the acidified solution produced a slight increase in the 260–280 nm region, the shoulder at 370 nm disappeared and a new peak was observed at 520 nm, apparently due to the conversion of the chalcone (III) to the flavylium salt form (I), in accord with Jurd's observation on flavylium salts.[6,7] The other absorption maxima (248, 260 and 330 nm), however, were not affected.




TLC on cellulose showed the presence of a major compound, ($R_f$'s in solvent 1: 0·28; solvent 2: 0·51) found for all five anthocyanins investigated, e.g. cyanidin-, peonidin-, delphinidin-, petunidin and malvidin-3,5-diglucoside), 0·52 (not identified), 0·94 (mixture of

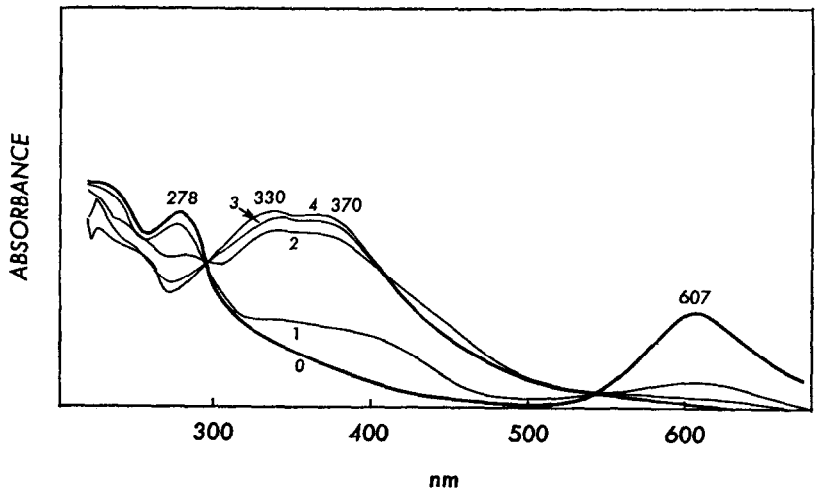



FIG. 1. SPECTRAL CHANGES OF MALVIDIN-3,5-DIGLUCOSIDE AT 90° AND pH 7·0. After 0 time (0); 10 min (1); 30 min (2); 60 min (3); 120 min (4).

syringic acid (IV) and 2,6-dimethoxy phenol (V),* and a brownish spot, which remained at the origin.

The u.v. fluorescent compound was isolated (2 mg) from malvidin-3,5-diglucoside (200 mg) as white microcrystalline powder. It showed no distinct melting point, became brown at 240° and decomposed at 273°. The compound is difficultly soluble in MeOH and EtOH but goes easily into solution in aqueous media. Its u.v. spectrum (1 mg/100 ml MeOH) showed a $\lambda_{max}$ at 329 nm, ($\epsilon = 8000$), a $\lambda_{min}$ at 274 nm and shoulders at 248 and 260 nm. The addition of MeONa produced a significant bathochromic shift ($\Delta\lambda$ 49 nm) to $\lambda_{max}$ 378 nm, ($\epsilon = 9250$), $\lambda_{min}$ 290 nm and shoulders at 276, 251 and 238 nm, as shown in Fig. 2.

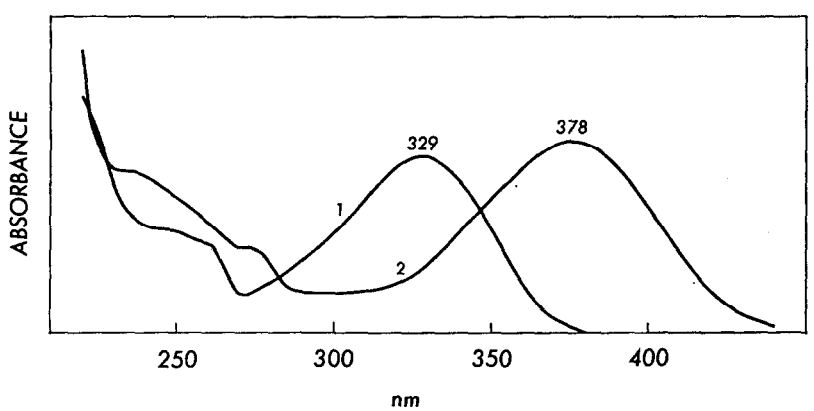



FIG. 2. SPECTRAL CHARACTERISTICS OF 3,5-DI(*O*-β-D-GLUCOSYL)-7-HYDROXY COUMARIN IN MeOH (1) AND IN MeO-Na (2).

* TLC on precoated silica gel plates using solvent (5) showed the presence of syringic acid ($R_f$: 0·44) and 2,6-dimethoxyphenol ($R_f$: 0·29), both visible as blue spots after spraying with Gibbs reagent.

Partial hydrolysis of the compound and subsequent chromatography of the hydrolysate (cellulose TLC solvent 1, organic phase) showed the presence of three compounds, the original product ($R_f$: 0·28) and two new compounds with $R_f$ 0·58 and 0·78 respectively (presumably the monoglucoside and aglucon), both compounds emitting strong blue fluorescence in u.v. light, as does the original compound. The fact that the compound ($R_f$ 0·28) was formed from five anthocyanidin-3,5-diglucosides which differ only in the substitution in the B-ring, its chromatographic behavior as a diglucoside and spectral characteristics identified it as the 3,5-di-(*O*-β-D-glucosyl)-7-hydroxy coumarin (VI).

The structure of IV was confirmed by the successive synthesis of its aglucon, the 3,5,7-trihydroxy coumarin (VII) from phloroglucin aldehyde (VIII) and tartronic acid (IX), using the modified Knoevenagel condensation.[13] Synthetic (VII) showed in the mass spectrometer

TABLE 1. MASS SPECTRAL DATA OF 3,5,7-TRIHYDROXY COUMARIN

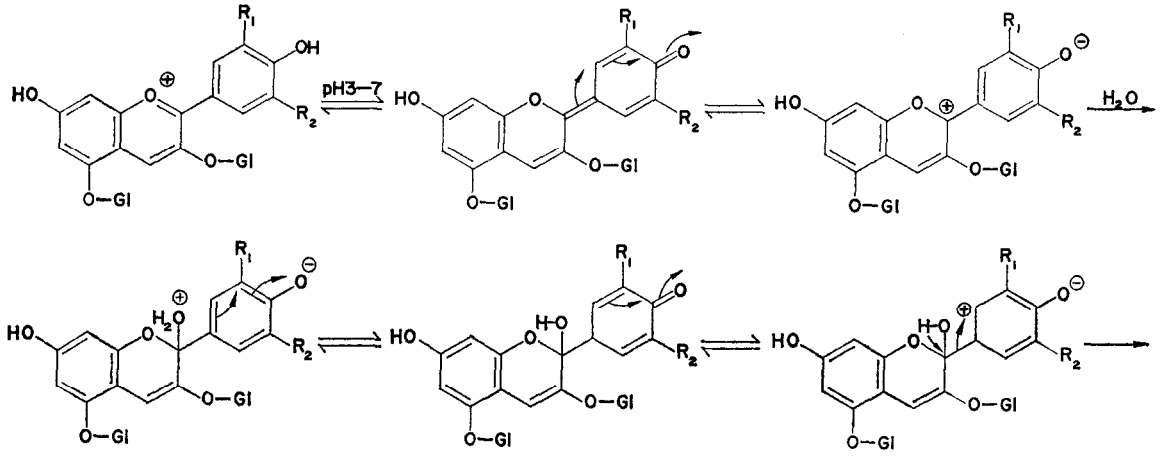
| *m/e* | % Relative abundance | *m/e* | % Relative abundance |
|---|---|---|---|
| 36 | 1·76 | 80 | 1·76 |
| 37 | 5·26 | 81 | 8·78 |
| 38 | 9·11 | 82 | 29·12 |
| 39 | 20·20 | 83 | 10·52 |
| 41 | 11·40 | 84 | 1·76 |
| 42 | 10·52 | 87·68 m | |
| 43 | 7·02 | 91 | 1·76 |
| 44 | 3·86 | 92 | 5·27 |
| 45 | 3·52 | 93 | 2·28 |
| 46 | 5·27 | 94 | 2·81 |
| 49 | 2·64 | 95 | 4·39 |
| 50 | 10·52 | 96 | 2·81 |
| 51 | 14·05 | 97 | 14·38 |
| 52 | 7·02 | 98 | 1·76 |
| 53 | 28·05 | 108 | 2·12 |
| 54 | 7·90 | 109 | 6·15 |
| 55 | 20·19 | 110 | 42·10 |
| 56 | 2·45 | 111 | 5·27 |
| 57 | 2·81 | 112 | 0·88 |
| 60 | 2·11 | 114·71 m | |
| 61 | 3·16 | 119 | 2·10 |
| 62 | 5·27 | 120 | 2·20 |
| 63 | 12·58 | 121 | 4·40 |
| 64 | 5·62 | 122 | 0·87 |
| 65 | 6·49 | 123 | 2·20 |
| 66 | 7·19 | 137 | 7·90 |
| 67 | 3·86 | 138 | 29·8 |
| 68 | 4·38 | 139 | 3·50 |
| 69 | 63·20 | 142·09 m | |
| 70 | 3·86 | 165 | 10·50 |
| 71 | 2·98 | 166 | 38·60 |
| 73 | 1·76 | 167 | 4·40 |
| 74 | 3·51 | 168 | 0·88 |
| 75 | 2·63 | 177 | 1·40 |
| 76 | 1·76 | 186 | 1·75 |
| 77 | 4·39 | 193 (M − 1) | 5·25 |
| 78 | 2·11 | 194 (M) | 100·00 |
| 79 | 6·68 | 195 (M + 1) | 11·40 |
| | | 196 (M + 2) | 2·10 |

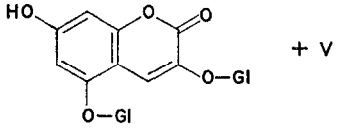
[13] P. N. KURIEN, K. C. PANDYA and V. R. SURANGE, *J. Indian Chem. Soc.* **11**, 823 (1934).

the typical fragmentation of coumarins hydroxylated in the 3-position: m* at 142·09 (M − CO), 114·71 (M − 2CO) as shown in Table 1. The NMR spectrum of the trimethyl silylated (VII) (in $CCl_4$, TMS as internal reference) revealed the presence of a vinyl proton (4H, s, $\delta = 7{\cdot}00$ ppm) and two aromatic protons (8H, d, $\delta = 6{\cdot}34$ ppm; $J = 2{\cdot}0$ c/s and 6H, d, $\delta = 6{\cdot}09$ ppm; $J = 2{\cdot}2$ c/s), thus identifying the synthetic compound unequivocally as VII.

## DISCUSSION

The investigated anthocyanins (cyanidin-, peonidin-, delphinidin-, petunidin-, and malvidin-3,5-diglucosides) convert quantitatively into their anhydrobases (or ionized anhydrobases) upon dissolving in buffer solutions of pH 7. The purplish-blue colour caused by the anhydro base ($\lambda_{max}$ 607 nm) fades and finally becomes yellow ($\lambda_{max}$ 370 nm) as a result of the transformation of the anhydrobase (II) to the chalcone (III). This transformation requires, approximately 4 days at room temp., but is reached at 90° after only 2 hr. The simultaneous development of the absorption band at 340 nm and the well defined isosbestic point at 295 nm are strong indications that the formation of (VI) is directly proportional to the decomposition of (II). This is supported by the fact that the prolonged heating of the solution after the disappearance of the blue colour did not result in significant increase of the absorption in the 340 nm region of the spectrum. Since the coumarin glucoside was formed as well in the presence of oxygen as in a nitrogen atmosphere, but could not be detected by the oxidation of malvidin-3,5-diglucoside with $H_2O_2$[14] in the reaction mixture, it is assumed that oxidation is not involved in the reaction. The formation of (VI) is probably caused by structural transformations of the anhydro base as proposed by the following reaction mechanism:




+ V

[14] G. HRAZDINA, unpublished data.

Since the formation of the coumarin glucoside VI requires the presence of the anhydro-base and VI could not be detected in solutions with pH under 3, it is assumed, that the structural transformations of the anthocyanins in the pH range 1–3 involve only the flavylium salt form (I) and the pseudobase (X).

## EXPERIMENTAL

*Isolation of 3,5-di-(-O-β-D-Glucosyl)-7-hydroxy Coumarin (VI)*

200 mg Malvin Ch-ide was dissolved in 50 ml $H_2O$ and 50 ml McIlvaine buffer at pH 7·0 were added. The resulting dark purple pigment solution was heated at 90° for 4 hr, cooled and evaporated to *ca.* 20 ml. VI was isolated using chromatography and rechromatography on 500 μ cellulose TLC with solvent 1, organic phase. The compound was eluted from the cellulose with $H_2O$, evaporated to *ca.* 1 ml and stored in the refrigerator, whereupon VI precipitated as white powdery material (Yield: 2 mg), $\lambda_{max}$ in MeOH: 329, 260 (shoulder), 248 nm (shoulder), $\epsilon = 8000$, $\lambda_{max}$ in NaOMe: 378, 276 (shoulder), 251 (shoulder) 238 nm (shoulder), $\epsilon = 9250$. The compound has no true melting point, becomes brown at 240°, and decomposes at 273–5°.

*Hydrolysis of (VI)*

1·0 Mg VI was refluxed in 10 ml 1N HCl for 40 min and the hydrolysate extracted with ethyl acetate (3 × 10 ml). The ethyl acetate extract was dried ($Na_2SO_4$), evaporated to dryness, and the aglucon dissolved in 100 ml MeOH for spectral measurements.

*Synthesis of 3,5,7-Trihydroxycoumarin (VII)*

1·9 g phloroglucinaldehyde (VIII) and 1·2 g tartronic acid (IX) was refluxed with 1·0 ml pyridine at 71° for 24 hr. The resulting dark brown syrup was dissolved in $H_2O$, whereupon an orange-brown precipitate formed. The precipitate was filtered off, the filtrate containing (VII) was evaporated to dryness, dissolved in ethyl acetate, and purified on a silica gel column (2 × 20 cm) with ethyl acetate as eluent. The effluent containing VII was evaporated to dryness, the oily residue dissolved in hot $H_2O$ and allowed to stand at room temp. over night, whereupon VII crystallized as orange prisms. Yield: 75·4 mg (3% of theory $\lambda_{max}$ in MeOH: 329, 264 (shoulder), 255 nm (shoulder), $\epsilon = 12{,}000$, $\lambda_{max}$ in MeONa: 340, 265 (shoulder), 255 nm (shoulder), $\epsilon = 4800$. (Found: C, 55·69; H, 3·08. Calc. for $C_9H_6O_5$: C, 55·68; H, 3·11.)

*Thin Layer Chromatography*

For the isolation and identification of the reported compounds, the following solvent systems were used:

Cellulose TLC: 1. BAW (4:1:5); 2. 5% AcoH; 3. Benzene–MeOH (1:1).
Silica gel: 4. MeOH–Benzene (1:2); 5. MeOH–Benzene (12:40).

The u.v. spectra were recorded in a Beckman DK spectrophotometer using 1 cm silica cells, the mass spectrum with a Hitachi–Perkin Elmer RMU-6 mass spectrometer equipped with solid-insertion chamber.

3,5,7-trihydroxycoumarin (VII) (10 mg) was silylated according to the method of Mabry *et al.*[15] and the NMR spectrum recorded with a Varian HA-100D spectrometer.

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[15] T. J. MABRY, J. KAGAN and H. ROESLER, *The University of Texas Publication No.* 6418 (1964).