Structural transformation reactions of anthocyanins

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Summary. Spectroscopic evidence shows that the flavylium cation (1) undergoes hydroxylation to form the carbinol base (2) and can also deprotonate to form the anhydro (quinonemethane) bases (3a and 3b). The anhydro bases hydrate to the carbinol base. The carbinol base above pH 6, or the ionized carbinol base (2a), undergoes ring fission to produce the cis-chalcone (4).

Investigations with synthetic flavylium salts \(^3-8\) showed that these compounds in aqueous media undergo structural transformation reactions. The mechanism in these reactions was suggested to be the deprotonation of the flavylium cation (1) to the anhydro (quinonemethane) base(s) (3a and 3b) and hydration of the anhydro base(s) in the 2-or 4-position to form the carbinol base. The formation of yellow chalcones (4) from the synthetic flavylium salts was reported to occur even at low pH values. Naturally occurring anthocyanins were not included in these experiments. Recent kinetic investigations by Brouillard et al.\(^{9,10}\) with malvidin 3-glucoside and 3,5-diglucoside suggested a similar behavior. A revised structural transformation mechanism proposed by the above authors excluded the hydration of the anhydro bases (3a and 3b) to form the colorless carbinol base (2) and claimed the formation of (2) by hydroxylation of the flavylium cation (1) exclusively. In this paper we present evidence for the existence of 8 structural transformation forms of naturally occurring anthocyanins in the pH range 1-7, and for the direct hydration of the anhydro bases. Hydroxylation of the flavylium cations from naturally occurring anthocyanins in the 4-position was not considered due to the deactivating effect of the 3-hydroxyl or glycosyl group. Previous studies showed that the major site for nucleophilic attack on the flavylium cation is at C-2.\(^{11}\)

Malvidin 3,5-diglucoside was isolated as described earlier.\(^{12-17}\) \(^{1}^H\)NMR (CD\(_3\)OD) \(\delta: 2.05 (s, 6H, 3,5-OMe), 3.00-3.90 (m, 12H, glucose protons), 5.15-5.50 (m, 2H, glucose C-1 protons), 7.14 (d, \(J=2.2\) Hz, H-6), 7.20 (d, \(J=2.2\) Hz, H-8), 8.06 (s, H-2', H-6'), 9.20 ppm (s, H-4).

Changes in the spectrum of malvidin 3,5-diglucoside solutions were investigated in the pH range 1-12.5. At pH 1 and below the pigment was present quantitatively in the red colored flavylium cation (1) form, exhibiting an absorption maximum at 517 nm. As the pH increased to 4.5, the

![Figure 1. Spectral changes of a malvidin 3,5-diglucoside solution in the pH range 1-4.](image1)

![Figure 2. Spectral changes of a malvidin 3,5-diglucoside solution in the pH range 5-7.5.](image2)
intensity of the red colored anthocyanin solution progressively decreased to nearly colorless, as the flavylum cation underwent nucleophilic attack by water to form the carbinol base (2) (fig. 1). Above pH 4.5, the purple color appeared and increased in intensity up to pH 6.5 (fig. 2). The visible spectrum of this solution exhibited absorption maxima at 532 and 558 nm, indicating the presence of 2 different chromophoric species. These are the 7-keto anhydro base (3a) \( \lambda_{max} 532 \text{ nm} \) and the 4'-keto anhydro base (3b) \( \lambda_{max} 558 \text{ nm} \) present in equilibrium in the solution (\( \lambda_{max} \) of the anhydro base from the 7-hydroxy-4'-methoxy flavylum chloride is at 484 nm, that of the 4'-hydroxy-7-methoxy flavylum chloride at 506 nm). Naturally occurring anthocyanins containing a 3-hydroxyl or glycosyl group apparently behave differently from synthetic flavylum salts because it was reported that 4',7-dihydroxy flavylum chloride produced the 7-keto anhydro base only.

In addition to the 2 above absorption maxima a new \( \lambda_{max} \) at 610 nm appeared when the pH of the solution was increased to 6 and above, as shown in figure 2. This band is caused by the presence of the ionized anhydro base (5). Since there is only 1 absorption maximum, it is obvious that the same ionized anhydro base is obtained from either of the 2 anhydro base structures (3a and 3b).

At neutral pH the anhydro bases were quantitatively ionized and the color of the anthocyanin solution appeared blue. In addition to the absorption maximum at 610 nm, a new peak appeared at 398 nm, caused by the formation of the yellow chalcone (4). In solutions with increasing alkalinity the absorbance at 610 nm progressively decreased as the chalcone was formed. The chalcone is also formed when the anthocyanin solution is stored at alkaline pH's, as seen in figure 3.

At pH 12 the color of the anthocyanin solution is yellow \( (\lambda_{max} 398 \text{ nm}) \) and the presence of the ionized anhydro base (5) could not be detected. In addition to the absorption peak at 398 nm a shoulder at 430 nm was also observed in the spectrum, deriving from the ionized chalcone (4a).

Malvidin 3-glucoside18 behaved similarly. Due to the higher \( pK \) values of anthocyanin 3-glucosides, the relative amount of the structural transformation forms was slightly different from those of malvidin 3,5-diglucoside. The color of the malvidin 3-glucoside solution was more intense at pH values of 3–4.5 than that of the 3,5-diglucoside, caused by the presence of higher amounts of the flavylum ion in the solution. The formation of the ionized anhydro bases occurred at higher pH's. Complete ionization of the anhydro bases occurred at pH 8.5. At pH 12 the color of the malvidin 3-gluco side solution appeared green, due to the presence of both the ionized anhydro bases and the yellow chalcone. At this pH no ionization of the chalcone could be detected.

When a solution of malvidin 3,5-diglucoside with the pH of 1.0 was placed in a buffer solution with the pH of 4.0, the initial red color of the resulting solution slowly faded to colorless. This indicated the conversion of the flavylum cation (1) to the carbinol base (2). When a pigment solution with the pH of 6.0, containing the anhydro bases (3a and 3b) was added to the same buffer solution with the pH of 4.0, the original purple color also faded slowly, producing the same carbinol base (2) by hydration. During the course of the reaction the formation of the flavylum cation (1) could not be detected in the spectrum.

When a malvidin 3,5-diglucoside solution with the pH of 1.0 was added to a buffer solution with the pH of 6.5, the original deep red solution immediately turned purple and then slowly faded to colorless. This indicated a deprotonation of the flavylum cation (1) to the anhydro bases (3a and 3b) in a fast reaction and hydration of the anhydro bases (3a and 3b) to the colorless carbinol base (2) in a 2nd, slow reaction. In this reaction a small peak at 398 nm also appeared in the spectrum, indicating the formation of the chalcone (4). The chalcone does not seem to appear below this pH in detectable amounts (fig. 3). Addition of malvidin 3,5-diglucoside solution with the pH of 1.0 to a pH 7.5 buffer solution resulted in an immediate appearance of blue color, caused by the formation of the ionized anhydro base (5). The blue color of the solution slowly changed to greenish-yellow \( (\lambda_{max} 398 \text{ nm}) \) as the chalcone was formed.

These above experiments indicate an equilibrium between the flavylum cation (1) and carbinol base (2) and also between the anhydro bases (3a and 3b) and the carbinol base.

From our data and observations the structural transformation of anthocyanins appears to take place as follows:

Structural transformation forms from naturally occurring anthocyanins other than the flavylum cation (1) eluded isolation and chemical characterization. However, investigations with simple flavylum salts such as 3-methyl 4'-hydroxy and 3-ethyl 4'-hydroxy flavylum chloride support the above mechanism. Both these compounds form readily the carbinol bases in aqueous solutions and the corresponding 2-methoxyflav-3-enes in aqueous methanolic solutions and permit their isolation and characterization. The equilibrium between anhydro base and the carbinol...