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## On the red colour of raspberry (*Rubus idaeus*)

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### Abstract

Anthocyanins are the ubiquitous water-soluble pigments that are found in flowers and fruits, and are responsible for their impressive red and blue colours. Many examples of the strategies used by nature to obtain colour in plants are based on co-pigmentation, a phenomenon that stabilises and intensifies colours at pH values where the anthocyanin pigment alone is colourless. Several examples of these strategies were described in the last few years, in particular the structures responsible for colour in *Commelina communis* and *Ipomoea tricolor*. Here we show an alternative to co-pigmentation, used by nature in the colouring of raspberry (*Rubus idaeus*). This strategy does not involve co-pigmentation. © 2000 Elsevier Science Ltd. All rights reserved.

The colours of flowers and fruits have attracted the attention of man throughout history: the flowers as a fundamental source of artistic inspiration and beauty and fruits as an important part of the human diet. Anthocyanins are the ubiquitous water-soluble pigments that are found in flowers and fruits, and are responsible for their impressive red and blue colours.<sup>1–3</sup> Several references on the use of these compounds as colour pigments in decoration, namely to substitute for inorganic pigments when these are not available, or in ancient illustrations to give special lighting effects, have been reported. We have found descriptions of this type of use that range from the Roman Empire, by the famous architect Vitruvius,<sup>4</sup> to the paintings of the Maya civilisation,<sup>5</sup> passing through the recipes for illuminations described by Cennino Cennini in the 15th century.<sup>6</sup>

Due to the pioneering work of R. Willstätter,<sup>7,8</sup> Nobel prize in chemistry (1915), much information has been accumulated regarding the role played by anthocyanins and the colours they produce.<sup>9–13</sup> Isolation of the most common anthocyanins has shown that they follow a general pattern which is summarised in Fig. 1 for malvidin 3,5-diglucoside.<sup>13</sup>

The red flavylium cation is the dominant species in very acidic solutions; with increasing pH a series of more or less reversible chemical reactions can occur: (i) proton transfer leading to the blue quinoidal base, (ii) hydration of the flavylium cation giving rise to the colourless hemiacetal, (iii) tautomerization

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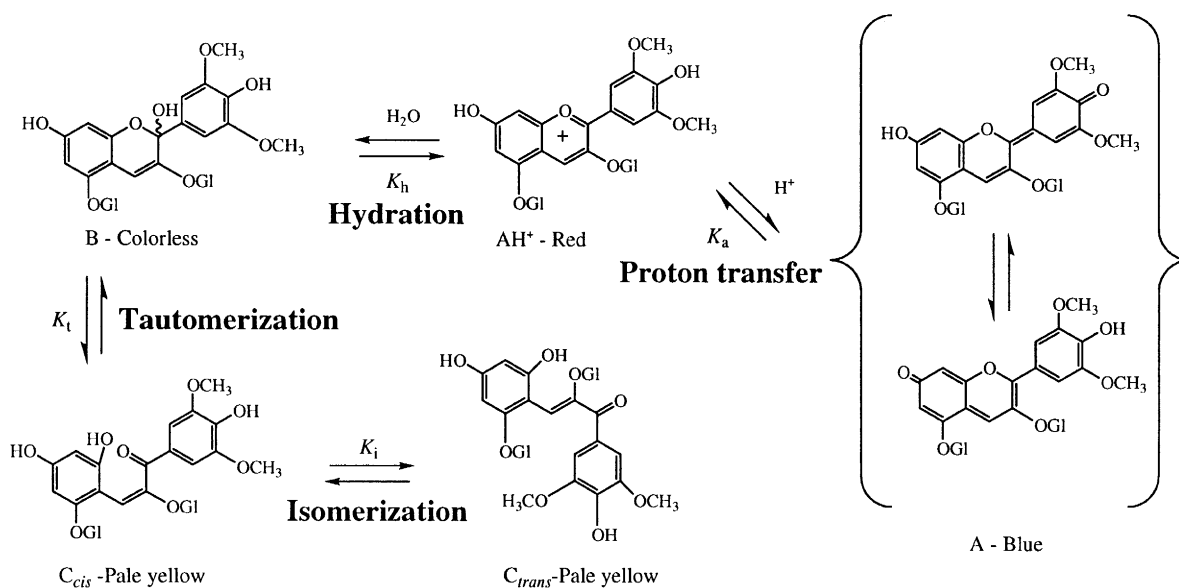


Fig. 1. Structural transformations of malvidin 3,5-diglucoside in aqueous solution

responsible for ring opening, to give the pale yellow *cis*-chalcone form, and finally, (iv) *cis*–*trans* isomerization to form the pale yellow *trans*-chalcone.

A useful way to illustrate the pH domain of the red and blue colours is to represent the pH dependence of the mole fraction distribution of the species (Fig. 2a), when the system has reached thermodynamic equilibrium. In Fig. 2 the key parameter is  $pK_{ap}$ <sup>14</sup> defined as the pH value for which the mole fraction distribution of the red flavylium cation is reduced by one half.

Anthocyanins are located inside vacuoles, where the pH can assume a large range of values, depending on the species of the plant considered.<sup>15</sup> However, as can be visualised in the band at the top of Fig. 2a, the colouring power of malvidin 3,5-diglucoside is very low even for the most favourable pH values that can appear in the vacuoles of certain plants. There is no doubt that malvidin 3,5-diglucoside, for example at pH ca. 3.1, does not exhibit any substantial colouring power, neither red nor blue. For the formation of a blue colour some interaction between the blue quinoidal base and other compounds is required, Fig. 2b. This phenomenon is called co-pigmentation and can involve other natural compounds, and metals.<sup>8,9,16–22</sup> The mole fraction distribution of the several malvidin 3,5-diglucoside forms, obtained upon complexation, can be simulated for a simplified model system consisting of a single 1:1 equilibrium with association constant  $K'_{cp}$  involving the species A and the co-pigment.<sup>22</sup> The results are shown in Fig. 2b, and indicate that a blue colour can now be obtained at moderately acidic pH values.

A red flower or fruit in which the pH of the vacuoles is larger than 3.0 and the colorant is malvidin 3,5-diglucoside ( $pK_{ap}=1.7$ ), or cyanidin 3,5-diglucoside ( $pK_{ap}=1.85$ ) for example, immediately suggests the existence of co-pigmentation with a flavylium cation. This is the case for the red buds of *Ageratum*, described sixty years ago by G. Robinson,<sup>12</sup> which contain acylated cyanidin diglucoside at pH ca. 6. In this and other similar cases, the red adduct must be stabilised in comparison with free  $AH^+$  in order to shift the  $pK_{ap}$  of the red species to high pH values. The resulting mole fraction distribution can also be easily simulated as shown in Fig. 2c, where the enlarging of the pH domain of the red species is evident.

Many examples of the strategies used by nature to obtain colour in plants are based on co-pigmentation. Several examples of intermolecular, intramolecular or self association co-pigmentation have been reported,<sup>16–20</sup> in particular the fascinating structures described by Goto, Kondo and collaborators.<sup>18–21</sup>

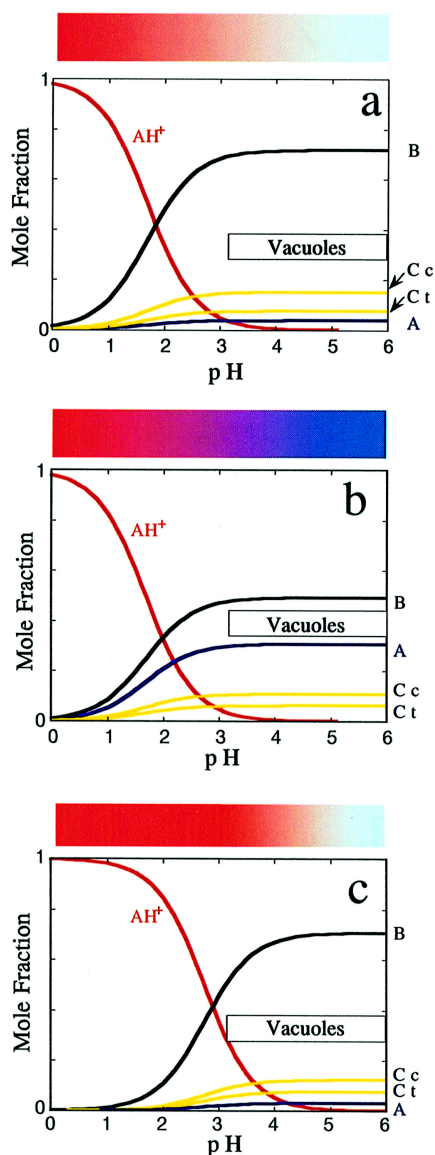


Fig. 2. Mole fraction distribution of the several species of malvidin 3,5-diglucoside: (a) free ( $pK_{ap}=1.7$ ); (b) simulation in the case of co-pigmentation with A for a co-pigment concentration of 0.1 M and an association constant,  $K'_{cp}=100\text{ M}^{-1}$ ; (c) the same as in B but when exclusive co-pigmentation with  $AH^+$  occurs,  $K_{cp}=100\text{ M}^{-1}$ . The band at the top simulates the colour that is obtained at different pH values. For details about the physical meaning of  $pK_{ap}$ ,  $K_{cp}$  and  $K'_{cp}$  see Ref. 22.

In this communication we wish to report a simple alternative to co-pigmentation, used by nature in the colouring of raspberry (*Rubus idaeus*). This fruit presents a very intense red colour, and easily gives rise to an abundant juice containing the colorant. HPLC of the juice without any manipulation, in order to avoid hydrolysis of some anthocyanin species containing acylated sugars, was performed. The results indicate the existence of four anthocyanins (Fig. 3), cyanidin 3-glucosylrutinoside, cyanidin 3-sophoroside, cyanidin 3-rutinoside, and cyanidin 3-glucoside, in agreement with a previous study carried out by Harborne and Hall, for a range of raspberries.<sup>23</sup> Further confirmation of the exclusive existence of species containing sugars at position 3 was obtained by heating the juice, in the presence of  $HCl\sim 6\text{ M}$ ,

at 60°C for 45 min to promote sugar hydrolysis. Only cyanidin 3-glucoside and cyanidin were detected, upon HPLC analysis.

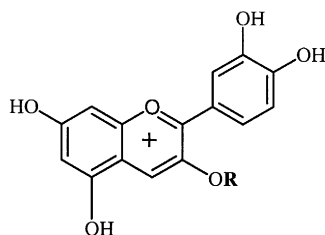


Fig. 3. Structures for the 3-monoside, 3-biosides and 3-trioside of cyanidin found in *Rubus idaeus*: cyanidin 3-glucose, **R**=-Glc; cyanidin 3-sophoroside, **R**=-Glc-Glc; cyanidin 3-rutinoside, **R**=-Glc-Rha; cyanidin 3-glucosylrutinoside, **R**=-Glc(-Glc)-Rha. The abbreviations used in the structures are: Glc=glucose; Rha=rhamnose. Cyanidin, **R**=H.

The existence of cyanidin exhibiting 3-substituted sugars suggests a simple strategy to obtain colour. Comparing the mole fraction distribution of malvidin 3,5-diglucoside ( $pK_{ap}=1.7$ ) or cyanidin 3,5-diglucoside ( $pK_{ap}=1.85$ ) with cyanidin 3-glucoside ( $pK_{ap}=2.8$ ), clearly shows that, for this last compound, the domain of the red species is enlarged by ca. 1 pH unit. We also observed that the extract is strongly buffered, mainly with citric acid/citrate, at pH=3.1. At this pH the mole fraction distribution of cyanidin 3,5-diglucoside is only ca. 0.05, while for cyanidin 3-glucoside it is 0.33. Moreover, the titration curve of the crude juice is coincident, within experimental error, with the curve of cyanidin 3-glucoside in the presence of 0.1 M citrate. This result seems to indicate that no co-pigmentation takes place, otherwise the pH domain of the red colour would be shifted to higher pH values as has been discussed in Fig. 2c. These results also lead to the conclusion that ca. 33% of the maximum colouring power of the dye is used by the plant to obtain its colour.

The second aspect of this strategy is the large concentration of the colorant. We have found ca. 2.4 mg of anthocyanin (expressed in cyanidin 3-glucoside) per gram of fresh fruit. In a certain way, nature uses this variable to compensate for the fact that is not using the total colouring power of the anthocyanin.

In conclusion, by means of 3-glycosides and without having recourse to co-pigmentation, nature is able to produce an intense red colour at acidic pH values that can be easily found in the vacuoles of many fruits.

Several studies have pointed out the anti-oxidant properties of anthocyanins and their positive role in our diet.<sup>24</sup> In the past anthocyanins were more accessible, in the form of wild fruits and berries. Presently, with the increasing dependence on processed food our diet has become deficient in anthocyanins. Raspberries, due to the strategy adopted to produce colour, possess a large amount of these compounds, and thus can play a therapeutic role in the prevention of chronic diseases related to processes resulting from oxidative stress, namely in the reduction of coronary heart disease and atherosclerosis.<sup>25,26</sup>

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