Spectrophotometric Study of Anthocyan Copigmentation Reactions. 4. Malvin and Apigenin 7-Glucoside

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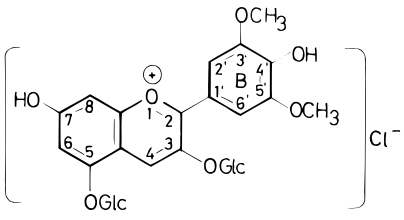
The existence of a copigmentation reaction was established between malvin chloride (malvidin 3,5-diglucoside) and apigenin 7-glucoside (5,4′-dihydroxyflavone 7-glucoside), and optimal conditions for its taking place were defined. Dependencies of the process on parameters such as the pH value of the solution, molecular concentration, and temperature were also determined. The kinetic and thermodynamic parameters obtained ($K = 137$ M^{-1}, $\Delta G° = -12.2$ kJ/mol, $\Delta H = -37.4$ kJ/mol, $\Delta S = -84.6$ J/K mol) were correlated with the structure of the flavone used.

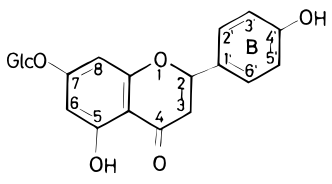
Keywords: *Copigmentation; apigenin 7-glucoside; UV−vis spectra; kinetic and thermodynamic parameters*

INTRODUCTION

As recognized, the process of copigmentation represents an important factor of anthocyan chromophore stabilization in the systems *in vivo* (Asen et al., 1970). Given that flavones from the class of flavonoid compounds belong to a group of good copigmentation molecules, we have continued to investigate the copigmentation process between malvin and apigenin 7-glucoside (see structures below). The structure of this flavone is characterized by a lower number of hydroxy groups in the molecule and the presence of a sugar molecule in position 7, which is unusual in these compounds. This is what led us to choose the present molecule, so that the effect of the lowered number of hydroxy groups could be investigated, having at the same time the sugar molecule in position 7, which is reported to be significant in the copigmentation reaction (Chen and Hrazdina, 1981).



MALVIN CHLORIDE
(MALVIDIN 3,5-DIGLUCOSIDE)



APIGENIN-7-GLUCOSIDE
(5,4'-DIHYDROXYFLAVONE-7-GLUCOSIDE)

EXPERIMENTAL PROCEDURES

Experimental procedures are the same as those in the first paper of this series (Baranac et al., 1997a).

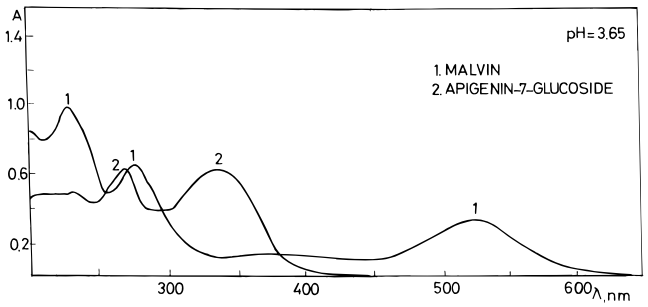


Figure 1. Absorption spectra of malvin ($c = 3.0 \times 10^{-4}$ M) and apigenin 7-glucoside ($c = 3.0 \times 10^{-5}$ M) in a pH 3.65 buffer solution.

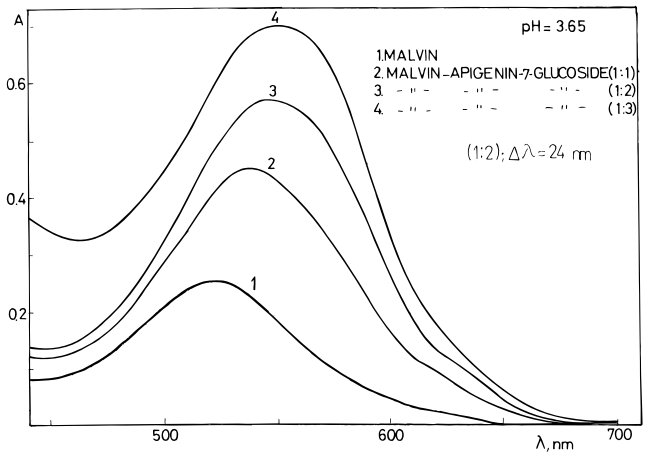


Figure 2. Absorption spectra of malvin ($c = 3.0 \times 10^{-4}$ M) and copigment malvin−apigenin 7-glucoside, mole ratios 1:1, 1:2, and 1:3.

RESULTS

The spectrum of the cation form of malvin at pH 3.65 is shown in Figure 1, together with an apigenin 7-glucoside spectrum. The choice of solutions of the specified pH value is explained elsewhere (Baranac et al., 1997a).

The copigment formation is a fast process, manifested by a bathochromic and a hyperchromic shift of the absorption maximum of the malvin cation form ($\Delta\lambda = 24$ nm, $\Delta A = 0.340$), as presented in Figure 2. From Figure 2 it is also observable that the magnitude of the

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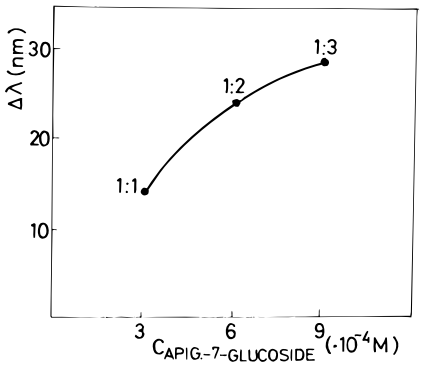

Figure 3. Shift of the absorption maximum position of a malvin ($c = 3.0 \times 10^{-4}$ M)−apigenin 7-glucoside solution as a function of apigenin 7-glucoside concentration.

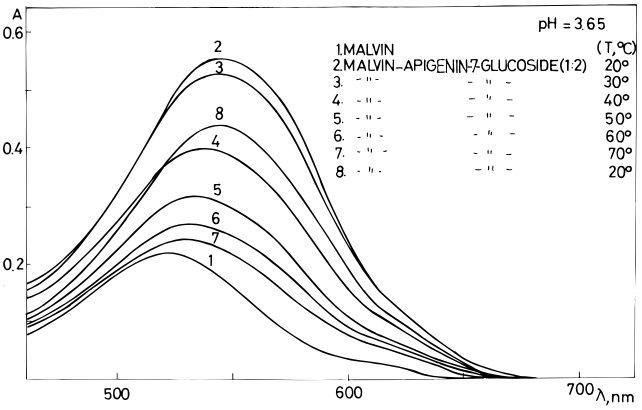

Figure 4. Change in absorption spectra of the copigment malvin ($c = 3.0 \times 10^{-4}$ M)−apigenin 7-glucoside with temperature (mole ratio 1:2).

changes depends on the apigenin 7-glucoside concentration, i.e., on the mole ratio of components [(1:1) curve 2, (1:2) curve 3, and (1:3) curve 4]. The optimal mole ratio at the pH chosen and malvin concentration utilized ($c = 3.0 \times 10^{-4}$ M) is 1:2, which is defined by the magnitude of the bathochromic shift. The 1:3 mole ratio solution could not be used due to precipitate formation. In Figure 3 the dependence of the copigment absorption band shift on the apigenin 7-glucoside concentration is given.

Our further investigations dealt with the effect of temperature on the copigmentation process. An increase in temperature results in the hypsochromic and hypochromic shifts (Figure 4, curves 2−7). At 70 °C the copigment band almost coincides with the band of the malvin cation form (Figure 4, curve 1). By lowering the temperature, the copigment regenerates to a certain extent (Figure 4, curve 8). The same behavior is noticed at mole ratio 1:1. The change in absorbance of the copigment absorption band for mole ratios 1:1 and 1:2, as a function of temperature, is given in Figure 5. An extrapolation to 100 °C suggests that the effect of copigmentation weakens as the temperature increases, regardless of the apigenin 7-glucoside concentration.

The stoichiometric ratio of the components, the equilibration constant, and the standard Gibbs free energy change were determined from the plot of the parameter $\ln[(A - A_o)/A_o]$ (A is absorbance of a copigment solution and A_o is absorbance of a pure malvin solution) versus the analytical concentration of apigenin 7-glucoside (Figure 6). The stoichiometric ratio of components is 1:1. The constant was determined to be $K = 137$ M^{-1} and the standard Gibbs free energy change $\Delta G° = -12.2$ kJ/mol. The plot of the above parameter versus reciprocal temperature (Figure 7) was used to determine the enthalpy change, $\Delta H = -37.4$ kJ/mol. The entropy change is negative, $\Delta S = -84.6$ J/K mol.

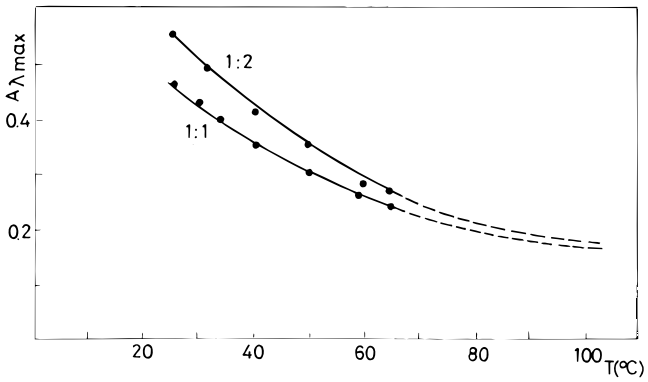

Figure 5. Change in absorption of the copigment as a function of temperature for different malvin ($c = 3.0 \times 10^{-4}$ M)−apigenin 7-glucoside mole ratios.

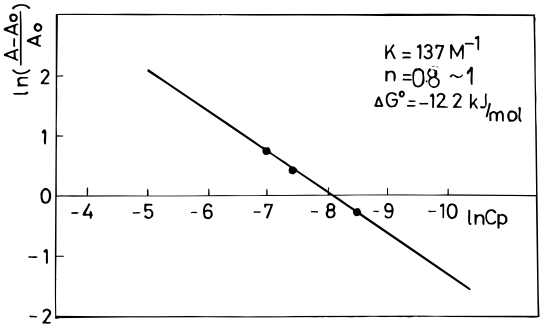

Figure 6. Plot of $\ln[(A - A_o)/A_o]$ as a function of logarithm of apigenin 7-glucoside concentration.

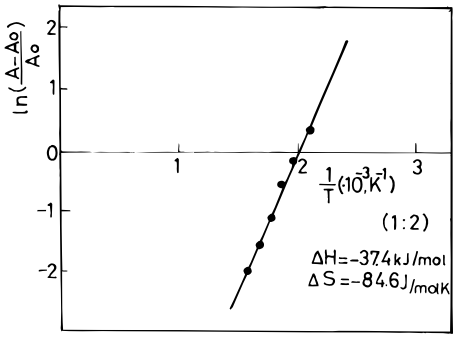

Figure 7. Plot of $\ln[(A - A_o)/A_o]$ for malvin ($c = 3.0 \times 10^{-4}$ M)−apigenin 7-glucoside solution as a function of reciprocal temperature (mole ratio 1:2).

DISCUSSION AND CONCLUSIONS

The apigenin 7-glucoside molecule used differs by structure from the previously investigated molecules. Only one-half the number of hydroxy groups, with only one in the B ring, and one sugar molecule in position 7 affect the characteristics of the copigment formed. The proof of the copigmentation reaction with apigenin 7-glucoside was the magnitude of the bathochromic and hyperchromic effects on the absorption maximum of the malvin cation form ($\Delta\lambda = 24$ nm, $\Delta A = 0.340$) in the pH 3.65 buffer solution, at the optimal mole ratio 1:2. This relatively large bathochromic shift, characteristic to the malvin−rutin system as well (Baranac et al., 1996), can be attributed to the apigenin 7-glucoside structure, most probably to the sugar molecule in position 7. According to our measurements, another

flavone, apigenin, which does not have the sugar molecule in position 7, does not show a bathochromic shift on the absorption maximum of the malvin cation form. We presume that an existent sugar molecule in position 7 causes, in the copigmentation reaction, a greater delocalization of the π electrons in the copigment formed and lowering of the electronic transition energy, i.e., increasing $\Delta\lambda$ (Goto et al., 1979).

The stoichiometric ratio of components was determined to be 1:1. The equilibrium constant was found to be $K = 137\ M^{-1}$. It is the lowest, compared with the other systems investigated, i.e., ca. 30 times lower than in the case of rutin (Baranac et al., 1996), which has four hydroxy groups and two sugar molecules. The magnitude and sign of the Gibbs free energy change, $\Delta G° = -12.2$ kJ/mol, indicates that the copigmentation is, in this case too, spontaneous, but the position of the equilibrium is shifted toward the starting reactants, compared with the malvin−quercetin and malvin−morin systems (Baranac et al., 1997a,b). This supports the assumption that the copigment is formed via hydrogen bonding and that the lower number of hydroxy groups in the molecule causes their instability. On the other hand, the value obtained for the equilibrium constant indicates that the copigment is formed to a lesser extent than in the previously investigated systems (Baranac et al., 1996, 1997a,b).

Temperature increase results in copigment decomposition (Figure 4, curves 2−7) and its decrease to partial regeneration (Figure 4, curve 8). The magnitude of enthalpy change, $\Delta H = -37.4$ kJ/mol, indicates a great affinity of the reacting components. The negative sign of the entropy change, $\Delta S = -84.6$ J/K mol, is the proof of a greater ordering of the system.

In accordance with the acceptable assumptions of other authors (Chen and Hrazdina, 1981), that the structure of the interacting molecules affects the course and outcome of the copigmentation reactions, we can also attribute the characteristics of the copigment formed to the structure of apigenin 7-glucoside, i.e., to a sugar molecule in position 7. We can finally conclude that this paper is another of our attempts to gain more information, albeit *in vitro*, on the essence of the copigmentation process, taking into account the effects of the nature, number, and position of the substituents.

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Received for review August 12, 1996. Revised manuscript received February 3, 1997. Accepted February 13, 1997.[⊗]

JF960612W

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.