Spectrophotometric Study of Anthocyan Copigmentation Reactions. 3. Malvin and the Nonglycosidized Flavone Morin

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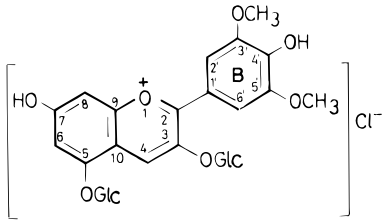
The process of copigmentation between malvin chloride (malvidin 3,5-diglucoside) and morin hydrate (3,5,7,2′4′-pentahydroxyflavone) was studied. The UV−vis absorption spectra were used to define the optimum conditions for the formation of copigment, as well as their characteristics. It was established that the copigmentation process does not depend only on the number of hydroxy groups but also on their mutual position. The copigment formation is spectrally manifested in a bathochromic and hyperchromic shift of the longest wavelength absorption band. The copigment formed is defined by kinetic and thermodynamic parameters.

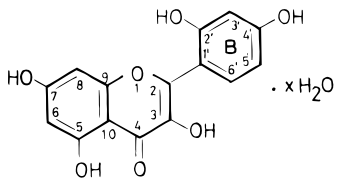
Keywords: *Copigmentation; morin; UV−vis spectra; kinetic and thermodynamic parameters*

INTRODUCTION

Since in our previous work (Baranac et al., 1996, 1997) we established the effect of structure on the characteristics of copigments formed, we continued the investigations in that context in the present paper. We chose the malvin−morin system (see structures below), i.e., the reaction of copigmentation with another nonglycosidized flavone, that differs from the previous one, quercetin, in the hydroxy group position within the B ring (meta in morin and ortho in quercetin). In order to gain more information on the essence of the process, we have tried to define the kinetic and thermodynamic parameters, as characteristics of the copigments formed.



MALVIN CHLORIDE
(MALVIDIN -3,5-DIGLUCOSIDE)



MORIN HYDRATE
(3,5,7, 2',4' - PENTAHYDROXY FLAVONE)

EXPERIMENTAL PROCEDURES

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

RESULTS

In Figure 1 absorption spectra of malvin and morin, in the pH 3.65 buffer solution, are presented.

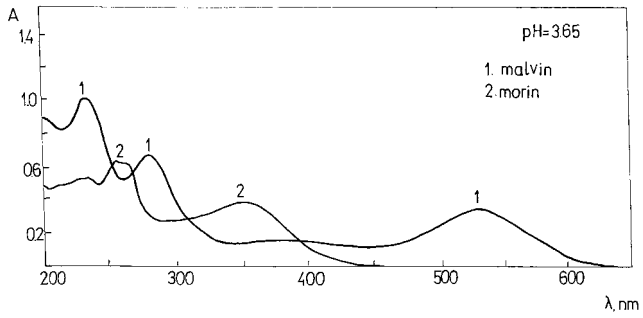


Figure 1. Absorption spectra of malvin ($c = 3.0 \times 10^{-4}$ M) and morin ($c = 3.0 \times 10^{-5}$ M) in a pH 3.65 buffer solution.

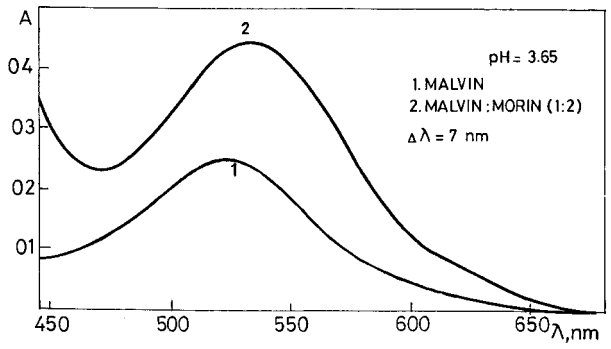


Figure 2. Absorption spectra of malvin ($c = 3.0 \times 10^{-4}$ M) and copigment malvin−morin, mole ratio 1:2.

Our first investigations dealt with defining the conditions under which the copigmentation reaction takes place between morin and malvin, which is shown is Figure 2. The formed copigment was expressed by a bathochromic and a hyperchromic shift of the absorption maximum of the pure malvin at $\lambda = 525-532$ nm ($\Delta\lambda = 7$ nm, $\Delta A = 0.200$). The copigmentation process of malvin and morin was observed through the morin concentration effect on the position and intensity of the malvin cation form absorption. With this aim, the solutions made by mixing malvin and morin in different mole ratios were recorded (Figure 3, 1:1, 1:2, 1:3) in the pH 3.65 buffer. It is clear from Figure 3 that, in the concentration range used, the optimum mole ratio is 1:2 (malvin:morin), while the ratio 1:3 could only be observed in the first few minutes after making the solution, which was followed by precipitation. A wider

S0021-8561(96)00606-1 CCC: $14.00

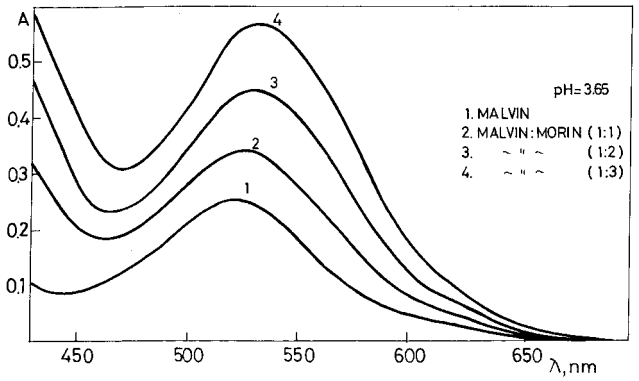


Figure 3. Absorption spectra of malvin ($c = 3.0 \times 10^{-4}$ M) and copigment malvin−morin, mole ratios 1:1, 1:2, and 1:3.

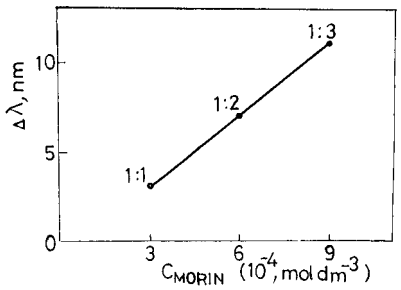


Figure 4. Shift of the absorption maximum position of a malvin ($c = 3.0 \times 10^{-4}$ M)−morin solution as a function of morin concentration.

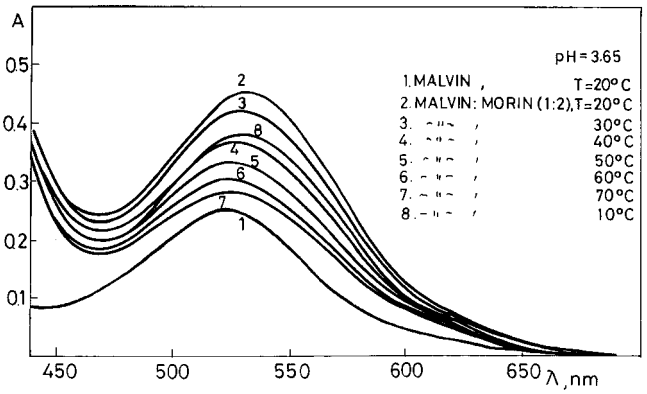


Figure 5. Change in absorption spectra of the copigment malvin ($c = 3.0 \times 10^{-4}$ M)−morin (mole ratio 1:2) with temperature.

study showed that the optimum mole ratio of the components changed at different pH values and that the copigmentation reaction is dependent on the morin concentration in the system, as shown in Figure 4.

Besides the copigment concentration, temperature has a great effect on the copigmentation process. In Figure 5 the effect of temperature on the copigment formed, at the optimum mole ratio 1:2, is presented. As in the systems investigated so far (Baranac et al., 1996, 1997), a temperature increase to 70 °C results in hypsochromic and hypochromic shifts of the absorption maximum of the copigment formed. At 70 °C (Figure 5, curve 7) the absorption maximum almost coincides with the absorpton maximum of pure malvin (Figure 5, curve 1). Decreasing the temperature to 10° C (Figure 5, curve 8) regenerates the copigment. Such behavior was also observed at mole ratio 1:1. The absorbance change, at the absorption maxima wavelengths, as a function of temperature (Figure 6) shows that at around 100 °C (the extrapolated parts of the

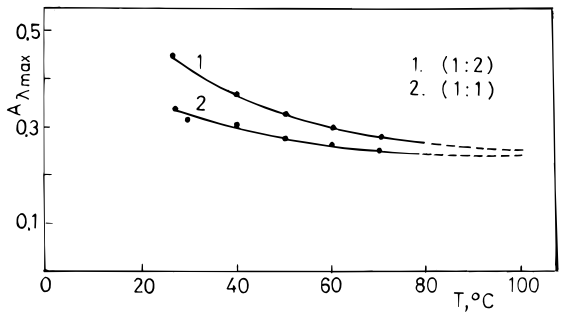


Figure 6. Change in absorption of the copigment as a funcion of temperature for different malvin ($c = 3.0 \times 10^{-4}$ M)−morin mole ratios.

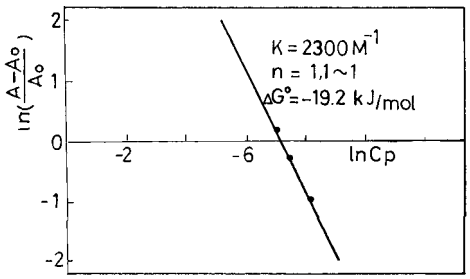


Figure 7. Plot of ln $[(A - A_o)/A_o]$ as a function of logarithm of morin concentration.

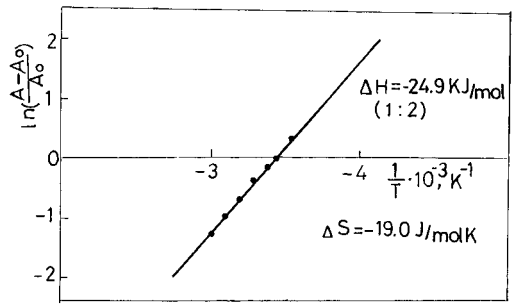


Figure 8. Plot of ln $[(A - A_o)/A_o]$ for a malvin ($c = 3.0 \times 10^{-4}$ M)−morin solution (mole ratio 1:2) as a function of reciprocal temperature.

curves) the effect of copigmentation weakens, regardless of the morin concentration in the system.

The stoichiometric ratio of the components in the copigment, the equilibrium constant, and the change in the standard Gibbs free energy of the copigment formed were determined from the plot of the logarithm of the main experimental parameter $\ln[(A - A_o)/A_o]$ (A is absorbance of the copigment solution and A_o is absorbance of the pure malvin solution) versus the logarithm of morin concentration (Figure 7). The stoichiometric ratio of the components is 1:1. The obtained equilibrium constant is $K = 2300$ M^{-1}. The change in standard Gibbs free energy is $\Delta G = -19.2$ kJ/mol. From the plot of $\ln[(A - A_o)/A_o]$ as a function of the reciprocal temperature (Figure 8), we determined the change of the system enthalpy to be $\Delta H = -24.9$ kJ/mol. The entropy change is $\Delta S = -19.0$ J/K mol.

DISCUSSION AND CONCLUSIONS

As our investigations employed UV−vis absorption spectra, the principal proof of copigment formation was the amount of the bathochromic and hyperchromic shifts, which are also characteristic to other systems (Williams and Hrazdina, 1979; Goto, 1987), of the absorption maximum of the malvin cation form with which morin reacts. This showed that *in vitro* a colored

form of anthocyan in solution is stabilized by the process of copigmentation, at a pH value close to the natural medium (Harborne, 1976; Timberlake and Bridle, 1975). The bathochromic shift in the case of morin is lower ($\Delta\lambda$ = 7 nm) than the corresponding one with quercetin ($\Delta\lambda$ = 12 nm) (Baranac et al., 1997).

The optimum mole ratio of the components in the copigmentation process is 1:2, in the concentration range used. The stoichiometric ratio of the copigment components malvin−morin is 1:1, i.e., the same as in the previously studied malvin−rutin (Baranac et al., 1996) and malvin−quercetin (Baranac et al., 1997) systems.

The equilibrium constant of $K = 2300\ M^{-1}$, as a thermodynamic parameter, points to a relatively increased stability of the copigment formed, compared with the copigment malvin−quercetin ($K = 650\ M^{-1}$) (Baranac et al., 1997). The large difference in equilibrium constants is probably the consequence of the effect of hydroxy groups in the B ring, which are in the meta position in morin and the ortho position in quercetin. An even higher value of the equilibrium constant, $K = 3300\ M^{-1}$, for the malvin−rutin system (Baranac et al., 1996) undoubtedly confirms that the copigmentation reaction is a consequence of the effect of structure of the interacting molecules (Chen and Hrazdina, 1981). The greater stability of the copigment malvin−morin is also confirmed by the value of the standard Gibbs free energy change, $\Delta G° = -19.2$ kJ/mol. This value is higher than the corresponding one for the copigment malvin−quercetin ($\Delta G° = -16.1$ kJ/mol) (Baranac et al., 1996, 1997).

The effect of temperature was observed at all mole ratios investigated. An increase in temperature, at the optimum mole ratio 1:2 (Figure 5), results in the decomposition of the copigment formed, while a decrease in temperature almost quantitatively regenerates it (Figure 5, curve 8). Such temperature dependence points to the thermodynamic character of the whole copigmentation process.

The value of enthalpy change is $\Delta H = -24.9$ kJ/mol. The entropy change is $\Delta S = -19.0$ J/K mol, and it favors the copigment formation, as it indicates a greater degree of order of the system, compared with the free reactants. The difference in the enthalpy change values between this and other systems (Baranac et al., 1996, 1997) indicates different affinities of the reacting components. Comparing the obtained characteristics of morin with quercetin, earlier investigated (Baranac et al., 1997), it can be concluded that with morin, where the affinity is lower, a more stable copigment is formed than in the case of quercetin, where the situation is inverted.

The mechanism itself and the structure of the copigment formed have not been elucidated to date, but we, as well as other authors (Osawa, 1982; Williams and Hrazdina, 1979; Brouillard et al., 1989), assume that hydrogen bonding takes place. This is supported by the equilibrium constant determined for the malvin−rutin system ($K = 3300\ M^{-1}$) (Baranac et al., 1996), which contains two sugar molecules and probably has a greater ability for hydrogen bonding.

Since the copigmentation process, dealt more with in our previous paper (Baranac et al., 1996), represents an important factor of stabilization of anthocyan molecules, which are present in many products for human use, it is clear that investigating this new system contributes to a better understanding and points to the influence not only of the nature of present substituents in the molecule investigated but also of their position.

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

JF9606060

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