Interactions between Anthocyanins and Aroma Substances in a Model System. Effect on the Flavor of Grape-Derived Beverages

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Evaluation of the sensory quality of wine or grape-derived beverages led us to study the interactions between flavors and anthocyanins, the colored family of polyphenols. The flavylium cation–ligand complexation, resulting in copigmentation (rise in pigment visible absorption with a concomitant bathochromic shift), was investigated using visible absorption spectroscopy. Sole volatile phenols were found to markedly interact with malvidin-3,5-*O*-diglucoside. With series of guaiacyl-derived aroma substances, acyl-substituted ligands proved to be better copigments than alkyl-substituted ones. Association constants and 1:1 complex stoichiometry were further determined for several substrates. Decreasing binding to malvin was observed for acetosyringone, syringaldehyde, acetovanillone, vanillin, 3,5-dimethoxyphenol, and 4-ethylguaiacol. Addition of 10% ethanol lowered by one-third the association constants for malvin-ligand couples and for malvidin-3-*O*-glucoside with acetosyringone and syringaldehyde. The main driving force was ascribed to hydrophobicity, although this study evidenced an influence of the ligand substitution pattern on copigmentation.

Keywords: *Anthocyanin; aroma substance; visible absorption spectroscopy; association constant; copigmentation*

INTRODUCTION

As part of grape and wine polyphenols, anthocyanins deserve a special attention owing to their role in the pigmentation process. A 2-phenylbenzopyrylium cation structure variously hydroxylated or methoxylated leads to the different anthocyanins responsible for red to blue hues. Glycosylation of the 3- or 5-hydroxyl groups as well as acylation of the residual sugars with *p*-caffeoyl or *p*-coumaroyl groups bring about diversity among natural grape anthocyanins. Another pathway to color variations results from anthocyanin association with natural or artificial substrates giving rise to molecular complexes. This phenomenon has been observed with various classes of ligands such as flavonoids and natural polyphenols (Asen et al., 1972; Mistry et al., 1991; Mazza and Brouillard, 1990), purine and pyrimidine derivatives (Brouillard et al., 1991), and metals (Dangles et al., 1994a).

Except in strongly acidic aqueous solution, the flavylium cation stability is affected by various pH-dependent transformations. Nucleophilic addition of water on carbon 2 leads to a colorless hemiacetal in equilibrium with ring-opened chalcone forms. Deprotonation produces the quinonoidal bases. Noncovalent interactions between a ligand and the flavylium nucleus can prevent the hydration process, thus restoring color. This process, termed copigmentation, plays an important role in weakly acidic or neutral media extending from plant-colored cells to fruit-derived beverages.

Although originally related to grape composition, wine anthocyanins were found to evolve during wine aging.

Acid-catalyzed polymerization with procyanidins will give rise to more stable oligomeric pigments (Haslam and Lilley, 1988). This condensation is accelerated in the presence of ethanal with the formation of CH_3CH-bridged tannin-anthocyanin polymers (Dallas et al., 1996; Es-Safi et al., 1999).

Wine sensory quality is related to the balance between nonvolatile constituents in the medium and aroma substances, which bring a unique character to the product. Flavor partitioning between vapor and liquid phases was previously shown to be influenced by polysaccharides (Dufour and Bayonove, 1999a) and polyphenols other than anthocyanins (Dufour and Bayonove, 1999b). The present study will be devoted to the interactions between anthocyanin models malvidin-3,5-*O*-diglucoside (malvin) or malvidin-3-*O*-glucoside (oenin) and various wine aroma substances. Sole volatile phenolic compounds displayed a substantial affinity for malvin and were further investigated as copigments. Association constants and complex stoichiometries were determined using visible absorption spectroscopy (Brouillard et al., 1989). Influence of ethanol on copigmentation thermodynamic parameters was also examined.

MATERIALS AND METHODS

Materials. All aroma compounds, sodium hydrogenphosphate, and citric acid were from Aldrich Chemical Co. (Milwaukee, WI) except 4-ethylguaiacol, eugenol, acetovanillone, and 2,6-dimethoxyphenol, which were from Interchim (Montluçon, France). Vanillol was obtained by $NaBH_4$ reduction of vanillin and its purity (>95%) checked by ^1H NMR analysis. HPLC-grade malvin and oenin chlorides (>95%) were from Extrasynthèse (Genay, France). The buffer solution was prepared from 0.1 M citric acid and 0.2 M sodium hydrogenphosphate (7:3, v/v) and adjusted to pH 3.5 or 3.7.

Visible Absorption Spectroscopy. Solutions of aroma substances (3, 6, 12, 24, and 48 mM) in buffer and 20% ethanol/

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buffer (v/v) were prepared and dissolution was achieved by sonication when required. A volume of 0.6 mM malvin chloride in buffer was added with an equal volume of the flavor solution and equilibrated for 2 h at 20 ± 1 °C. The UV−vis absorption spectra were recorded with a Uvikon 930 spectrometer (Kontron Instruments) in an 1-cm path length quartz cell. The ratio between molar extinction coefficients $[\epsilon_{AH(CP)}{}^+/\epsilon_{AH}{}^+]$ of fully complexed $[AH(CP)^+]$ and free anthocyanins (AH^+) was tentatively obtained by measuring absorbances at 525 nm of 1.5×10^{-5} M malvin chloride in 0.5 N HCl (pH 0.5) and 10% EtOH/0.5 N HCl (v/v) solutions in the presence and absence of a large excess of copigment (40 or 80 equiv). Visible absorbance of naturally colored aroma solutions was measured for concentrations used in the study and subtracted from malvin absorbance. A similar procedure was followed for oenin with an initial oenin concentration of 0.2 mM and 3, 6, 12, 24, and 36 mM syringaldehyde and acetosyringone solutions. Equilibration was conducted at 25 ± 1 °C.

Determination of Association Constants (K_a) and Stoichiometry Coefficients (n). At pH 3.5, malvin flavylium cation (AH^+) is mainly in equilibrium with its hemiacetal and *cis*-chalcone forms. *trans*-Chalcone and quinonoidal bases are minor species and are omitted for data treatment. In copigmentation, the variation of the absorbance A has been shown to be related to the initial copigment concentration $[CP]_0$ according to eq 1

$$\frac{(A - A_0)}{A_0} = K_a r [CP]_0^n \quad (1)$$

where A_0 is the absorbance without copigment, K_a the association constant, n the stoichiometry coefficient, and r the ratio between molar extinction coefficients $[\epsilon_{AH(CP)}{}^+/\epsilon_{AH}{}^+]$ of fully complexed $[AH(CP)^+]$ and free anthocyanins (AH^+) (Brouillard et al., 1989).

K_a and n were obtained by linear regression of $\ln[(A - A_0)/A_0]$ vs $\ln([CP]_0)$ within the software Excel (Microsoft Inc.). The stoichiometry coefficient is deduced from the slope and $\ln(K_a r)$ from the intercept. Although the copigment/anthocyanin ratio was set as large as allowed by solubility, no bathochromic or hypochromic effect was detected at pH <1 owing to complexation rates lower than 30%. Measured r values for malvin and oenin were found in the range 0.98−1.01 and r was thus kept at 1 throughout the studies. Thus, association constants are reported uncorrected for differences in molar extinction coefficients between free and fully complexed malvin.

Temperature Variation Studies. A volume of 1 mL of 0.2 mM oenin in a pH 3.7 buffer mixed with 1 mL of 12 mM syringaldehyde or acetosyringone in buffer or 20% EtOH/buffer was placed in a magnetically stirred quartz cell (optical path length, 1 cm). The temperature in the cell was varied from 15 ± 0.1 to 40 ± 0.1 °C (steps of 5 °C) by means of a water-thermostated bath. Spectra were recorded on a Hewlett-Packard 8453 diode-array spectrometer. The following data treatment is based on the same anthocyanin equilibrium scheme as described above. According to eq 2

$$\ln[(A - A_0)/A_0] = \ln(r[CP]_0) + \Delta S°/R - \Delta H°/(RT) \quad (2)$$

$\ln[(A - A_0)/A_0]$ vs the reciprocal of temperature is a linear plot where enthalpy $(\Delta H°)$ and entropy $(\Delta S°)$ changes can be, respectively, extracted from the slope and the intercept (Dangles and Brouillard, 1992). The ratio r was set at 1.

RESULTS AND DISCUSSION

Only anthocyanidin monoglycosides have been identified in grapes from genus *Vitis vinifera* whereas both mono- and diglycosides occur in American *Vitis* species (e.g., *Vitis riparia*) (Ribereau-Gayon, 1982). Malvidin-3-*O*-glucoside, with a 3,5-dimethoxy-4-hydroxy-substituted B-ring, usually appears as the principal constitu-
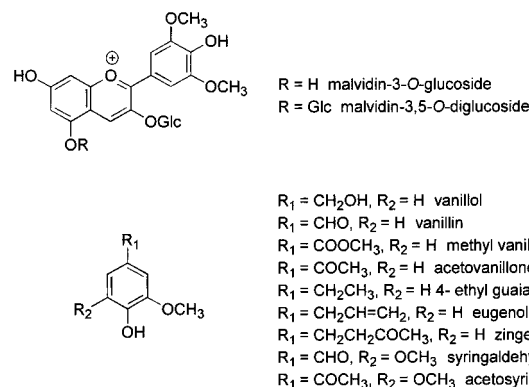

Figure 1. Structures for malvidin-3-*O*-glucoside, malvidin-3,5-*O*-diglucoside, and various phenolic aroma substances

Table 1. Hyperchromic Effects $[(A - A_0)/A_0]$ at 525 nm and Bathochromic Shifts $(\Delta\lambda_{max})$ for Copigmentation between Malvin (0.3 mM) and Various Guaiacyl- and Syringyl-Derived Phenols (6 mM) in pH 3.5 Citric Acid−Phosphate Buffer and 10% EtOH/Buffer at 293 K

ligand	buffer		10% EtOH/buffer	
	$(A - A_0)/A_0$	$\Delta\lambda_{max}$	$(A - A_0)/A_0$	$\Delta\lambda_{max}$
acetosyringone	185	11	100	7
syringaldehyde	135	10	76	6
acetovanillone	72	6	55	3
methyl vanillate	63	4	43	3
vanillin	48	4	26	3
zingerone	21	1	11	1
eugenol	21	1	15	0
4-ethylguaiacol	17	1	12	1
vanillol	16	1	12	1
4-ethylphenol	8	2		

ent among grape naturally occurring anthocyanidin monoglycosides (Figure 1). At pH 3.5, malvin, the corresponding 3,5-diglucoside, which is essentially in the hemiacetal form ($pK_{hydration} = 1.75$ at 25 °C), proves to be a better pigment than malvidin-3-*O*-glucoside ($pK_{hydration} = 2.7$ at 25 °C) for probing interactions with flavors at wine pHs (Dangles and Elhajji, 1994b). All ligands described as copigments in the literature possess an aromatic structure. Our results with various unsaturated cyclic aroma substances are consistent with this observation. Indeed, *trans-p*-menth-6-ene-2,8-diol, (+)-*p*-menth-1-en-9-ol, β-ionone, and maltol had no effect on malvin color. Other ligands such as furaneol, α-angelilactone, and 4-vinylphenol induced a discoloration of the malvin solution presumably through nucleophilic addition of these chemically reactive species onto the electron-deficient pyrylium ring as observed between 4-vinylphenol and oenin (Fulcrand et al., 1996). Aromatic compounds, benzaldehyde, furfural, and 2-isobutyl-3-methoxypyrazine proved to be inefficient copigments, whereas all phenol-derived flavors led to copigmentation effects of variable strength.

Malvin Interactions with Guaiacyl- and Syringyl-Derived Substances. Malvin copigmentation was estimated based on the hyperchromic effect $[(A - A_0)/A_0]$ at 525 nm and the bathochromic shift $(\Delta\lambda_{max})$, i.e., the shift of the maximum absorption wavelength toward longer wavelengths. In both pH 3.5 buffer and 10% ethanol/buffer, the largest copigmentation effects were recorded for syringyl-derived compounds, acetosyringone and syringaldehyde (Table 1). Hyperchromic effects ranging from 16 to 72% were obtained for the guaiacyl series pointing out an influence of the substituent in para to the phenol hydroxyl group. Indeed, alkyl-substituted guaiacols seem to present less affinity

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Table 2. Association Constants (K_a) with Malvin and Stoichiometry Coefficients (n) for Selected Phenolic Aroma Substances in pH 3.5 Citric Acid−Phosphate Buffer and 10% EtOH/Buffer at 293 K

	buffer		10% EtOH/buffer	
ligand	$K_a{}^b$ ± SD	n ± SD	$K_a{}^b$ ± SD	n ± SD
acetosyringone	348 ± 51	1.03 ± 0.03	219 ± 10	1.05 ± 0.01
syringaldehyde	208 ± 15	1.00 ± 0.01	137 ± 5	1.01 ± 0.01
acetovanillone	205 ± 5	1.09 ± 0.01	130 ± 5	1.07 ± 0.01
vanillin	72 ± 4	0.98 ± 0.01	52 ± 9	1.02 ± 0.03
4-ethylguaiacol	21 ± 5	0.94 ± 0.05	6.8 ± 0.3	0.79 ± 0.01
3,5-dimethoxy-phenol[a]	101 ± 9	1.04 ± 0.02	77 ± 7	1.07 ± 0.02

[a] Residual absorbance from ligand subtracted. [b] K_a values are uncorrected ($r = 1$; cf. Materials and Methods).

for the flavylium cation than acyl-substituted ones. The only simple phenol studied here, 4-ethylphenol, appears to be twice less effective than 4-ethylguaiacol. Furthermore, the association constant and the stoichiometry coefficient were determined for aryl aldehydes and aryl methyl ketones (Table 2). As expected, the magnitude of K_a is in good agreement with the corresponding hyperchromic effect. Association constants for acetosyringone and syringaldehyde, respectively, 348 and 208 M^{-1} in buffer, are greater than those for the corresponding guaiacyl-derived compounds acetovanillone (205 M^{-1}) and vanillin (72 M^{-1}). Within the two series, aryl aldehydes prove to be weaker copigments than the corresponding aryl methyl ketones. Besides, a 1:1 complex stoichiometry was established for all couples studied. The two glucose residues on malvin led to face discrimination for ligand access. Molecular modeling showed a highly stable conformation for malvin with both glycosyloxy residues located on the same side of the chromophore and nearly orthogonal to the benzopyrylium plane (Dangles and Elhajji, 1994b).

Acetosyringone and syringaldehyde exhibited similar copigmentation abilities as the more hydrophilic chlorogenic acid. Malvin-chlorogenic acid association constants evaluated by variation of the temperature at fixed species concentrations (Dangles and Brouillard, 1992) and variation of the sole copigment concentration (Brouillard et al., 1989) were found to be, respectively, 220 M^{-1} (pH 3.4; 298 K) and 390 M^{-1} (pH 3.65; 293 K). Small discrepancies appear to be mostly linked to evaluation methods outlining once more that temperature, ionic strength, and medium pH strongly influence copigmentation. Interactions between malvin and various polyphenols were also investigated by Mistry et al. (1991). Flavan-3-ol monomers and oligomers display weaker copigmentation effects than hydrolyzable tannins. Evaluation of the association constant for β-1,2,3,4,6-penta-*O*-galloyl-D-glucose, the best ligand in the series, gave, respectively, 987 M^{-1} (UV−vis, 295 K) and 508 M^{-1} (^{1}H NMR, 318 K) for a 1:1 complexation. Finally, these authors found that quercetin-3-β-D-galactoside, with hidden 2,4,6-trihydroxybenzoyl and caffeoyl substructural moieties, was the most potent copigment (K_a = 1686 M^{-1}, 295 K).

Interactions between aromatic ligands and the flavylium nucleus are thought to involve ring vertical stacking. The hydrophobic contribution is presumably the most important factor influencing copigmentation in aqueous media. Nevertheless, our results with the guaiacyl and syringyl series pointed out an influence of the ligand substitution pattern. Two factors appear to be involved. First, addition of methoxy groups on

Table 3. Hyperchromic Effects [$(A - A_0)/A_0$] at 525 nm and Bathochromic Shifts ($\Delta\lambda_{max}$) for Copigmentation between Malvin (0.3 mM) and Various Volatile Phenols (6 mM) in pH 3.5 Citric Acid−Phosphate Buffer and 10% EtOH/Buffer at 293 K

	buffer		10% EtOH/buffer	
phenol	$(A - A_0)/A_0$	$\Delta\lambda_{max}$	$(A - A_0)/A_0$	$\Delta\lambda_{max}$
3,5-dimethoxyphenol[a]	47	4	35	1
3,4-dimethoxyphenol	38	3	19	0
2,6-dimethoxyphenol	30	2	18	1
2,3-dimethoxyphenol	20	2	3	0
5-methoxyresorcinol	34	2	22	1
methoxyhydroquinone[a]	24	2	17	0
3-methoxycatechol	13	1	11	0
phloroglucinol			24	1
1,2,4-benzenetriol[a]			3	0
pyrogallol			12	1
gallic acid	61	5	40	2

[a] Residual absorbance from ligand subtracted.

positions 3 and 5 of the ligand strengthens the electronic density on the aromatic ring. The favorable influence of electron-donating groups on copigmentation has been evidenced for chlorogenic acid compared to 5-*O*-*p*-coumaroyl quinic acid devoid of the aromatic 3-hydroxy substituent (Mistry et al., 1991). Malvin-delocalized positive charge reflects an almost planar flavylium cation. Interactions between the electron-deficient pigment and electron-rich ligands could then be favored. Second, electronic delocalization of 4-hydroxybenzoyl species induces the formation of a permanent dipole moment. In this work, ligands presenting resonance structures, thus a larger dipole, were assessed as the best malvin copigments. This result can be interpreted in terms of development of a charge−dipole interaction.

Malvin Interactions with Volatile Phenols. Phenolic aroma substances also comprise simple volatile phenols. Among them, methoxyhydroquinone, 2,6-, 3,4-, and 3,5-dimethoxyphenols have been identified in grape while others were studied to get an insight into phenol−flavylium cation interactions. Copigmentation was then evaluated for series of benzenetriols, methoxydiphenols, and dimethoxyphenols in aqueous and 10% hydro alcoholic media and compared to standard gallic acid (Table 3). For similar substitution patterns, the series increasing ability to copigment malvin ranked as follows: benzenetriols, methoxydiphenols, dimethoxyphenols. Hydrophobicity remains apparently a key parameter in this complexation. Besides, 1,3,5-trisubstituted compounds such as 3,5-dimethoxyphenol and 5-methoxyresorcinol gave rise to the strongest interactions, suggesting a further complex stabilization through hydrogen bonding. Another explanation may be linked to intrinsic steric hindrance in other methoxyphenols, precluding planarity, thus, a close contact between partners.

Oenin Interactions with Syringyl-Derived Substances. Copigmentation visual effects with oenin are weaker owing to a lower hydration constant compared to malvin. Hence, the association with oenin was evaluated for the two best copigments, syringaldehyde and acetosyringone. For a ligand/oenin ratio of 30, the hyperchromic and bathochromic effects were found to be, respectively, 62% and 6 nm for acetosyringone and 59% and 6 nm for syringaldehyde in a pH 3.7 buffered medium. The stoichiometric coefficients, reported in Table 4, are close to unity reflecting a 1:1 complexation as with malvin. Again, acetosyringone proves to be a better copigment than syringaldehyde. Calculated as-

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Table 4. Influence of 10% Ethanol on Various Thermodynamic Parameters in the Copigmentation of 0.1 mM Oenin with Acetosyringone and Syringaldehyde in a pH 3.7 Citric Acid−Phosphate Buffer (298 K)

		method 1[a]		method 2[b]		
solvent	ligand	$K_a \pm SD$ (M^{-1})	$n \pm SD$	$\Delta H^\circ \pm SD$ (kJ/mol)	$\Delta S^\circ \pm SD$ (J/K/mol)	K_a calc (M^{-1})
buffer	acetosyringone	173 ± 23	0.95 ± 0.02	−18.5 ± 0.2	−17.5 ± 0.7	210
10% EtOH/buffer	acetosyringone	155 ± 3	1.00 ± 0.01	−26.5 ± 0.7	−48.0 ± 2.2	137
buffer	syringaldehyde	110 ± 22	0.93 ± 0.01	−18.5 ± 0.3	−19.2 ± 1.1	173
10% EtOH/buffer	syringaldehyde	77 ± 4	0.93 ± 0.01	−23.9 ± 0.6	−41.0 ± 2.2	113

[a] K_a and n from $\ln[(A - A_0)/A_0]$ vs $\ln$(ligand concentration) plots. K_a values are uncorrected ($r = 1$; cf. Materials and Methods). [b] ΔH° and ΔS° obtained from $\ln[(A - A_0)/A_0]$ vs the reciprocal of temperature plots, K_a calculated [$K_a = \exp(-\Delta G^\circ/RT)$]. K_a and ΔS° values are uncorrected ($r = 1$; cf. Materials and Methods).

sociation constants appear, however, weaker than those reported for malvin. In fact, the mathematical treatment used, namely method 1, underestimates association constants and stoichiometric coefficients. Authors Dangles and Elhajji (1994b) refined this treatment by integrating the mass-law equation for the hydration equilibrium. Validity of their treatment extends to the oenin copigmentation case where the difference between pH and anthocyanin p$K_{hydration}$ is small. However, for medium comparison, the latter data treatment proved to be particularly heavy requiring an accurate determination of pH and p$K_{hydration}$. Indeed, a rapid evaluation of these two parameters in a pH 3.7 buffer showed that adding 10% ethanol lowered p$K_{hydration}$ and increased pH, both by ca. 0.1 unit. In the case of 1:1 complexation and athermal hydration process, various thermodynamic parameters can be obtained from temperature variation of a copigmented solution (method 2; Dangles and Brouillard, 1992). With enthalpy and entropy changes in hand, the association constant is then easily derived (eq 2). Interestingly, oenin hydration was found slightly temperature dependent in a pH 3.7 buffer while nearly athermal in 10% ethanol/buffer. In the latter solvent system, a malvin-type treatment as described in eq 2 is then well-adapted. Although the association constants calculated with method 2 are still lower than for malvin (Table 4), the addition of 10% ethanol reduces by one-third these values as usually observed for malvin-ligand complexation. Thus, method 2 provides a convenient way for ligand and medium comparisons in oenin copigmentation (Figure 2). Dangles and Elhajji (1994b) reported close association constants for malvidin-3-glucoside (187 M^{-1}) and malvin (200 M^{-1}) with chlorogenic acid in weakly acidic solutions. This suggests that ligand binding to malvidin-3,5-diglucoside may not be very different from binding to malvidin-3-glucoside which is the major anthocyanin in the genus *V. vinifera*.

Effect of Ethanol. Copigmentation has never been so spectacular than in water. A lowering in the magnitude of the hyperchromic effect is produced by addition of protic and aprotic cosolvents to water (Brouillard et al., 1991). Despite hydroxylated cosolvents appear the less damaging, they disrupt interactions through perturbation of the three-dimensional network of the hydrogen-bonded water molecules. Actually, a large influence of the water organization on intermolecular interactions is characteristic of a hydrophobic driving force. Other contributions such as hydrogen bonding and charge−dipole interaction do not predominate although they should be considered to increase complex stability. Our results are in strong agreement with this effect. Indeed, $(A - A_0)/A_0$ values were nearly halved in the presence of 10% ethanol (v/v). As mentioned earlier, association constants were reduced by one-third, although complex stoichiometry remained unvaried re-
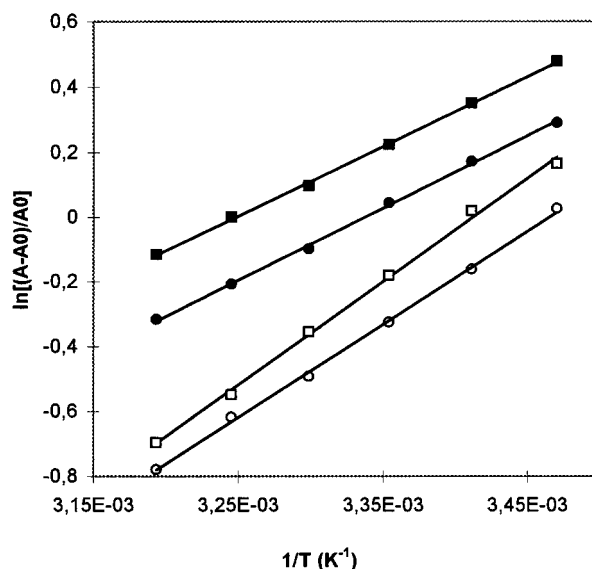

Figure 2. Variation of $\ln[(A - A_0)/A_0]$ as a function of the reciprocal of temperature for copigmentation between oenin (0.1 mM) and ligands (6 mM). (■) Acetosyringone and (●) syringaldehyde in a pH 3.7 buffer; (□) acetosyringone and (○) syringaldehyde in 10% EtOH/buffer.

flecting no major conformational change in anthocyanins (Tables 2 and 4). Copigmentation between oenin and the two ligands studied corresponds to an exothermic process. The negative enthalpy change mainly results from synergistic effects between a lower energy state of the complex in the gas phase and a favorable desolvation of this latter. An unfavorable ordering of both complex in the gas phase and solvent during desolvation account for a negative entropy change. Addition of ethanol had a larger effect on entropy than on enthalpy change (Table 4). Dangles and Brouillard (1992) suggested that the disruption of the water network was weaker than expected, owing to a preferential rearrangement of the cosolvent around the complex. In fact, a small bathochromic effect (2−4 nm) was registered for free anthocyanins upon addition of ethanol pointing out a reduction of the polarity around the flavylium nucleus. Last, the variation extent of enthalpy and entropy changes are consistent with results obtained upon addition of ethanol on malvin copigmentation with catechin and chlorogenic acid (Dangles and Brouillard, 1992).

Impact on Sensory Quality and Color of Grape-Derived Beverages. Volatile phenols are mainly produced by biochemical degradation of phenolic acids in grape juice. Aging of alcoholic beverages in oak barrels contributes to the level in volatile phenols through extraction of various tannins, lignin, and lignin-derived substances (Etievant, 1981) and their subsequent chemi-

cal and enzymic modifications. Concentrations in vanillin and syringaldehyde, the two main aromatic aldehydes, increased with time for red wines aged in oak barrels (Puech, 1987). With contents reaching $1-2$ mg/L, vanillin may contribute to red wine aroma. Indeed, odor threshold values reported in the literature appear to be ranging between 65 and 500 μg/L for a model wine and $320-500$ μg/L for a red wine (Boidron et al., 1988; Guth, 1997; Puech, 1987). Although the syringaldehyde concentration in barrel-aged wines is usually higher than 1 mg/L, its sensory impact is probably null owing to a high odor threshold (>50 mg/L). Other important sensory contributors are eugenol, 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, and 4-vinylguaiacol with an overall wine level greater than 1 mg/L (Etievant, 1981; Chatonnet et al., 1992).

With the association constants in hand, one can evaluate the impact of anthocyanins on the sensory quality of grape-derived beverages. For instance, in a model hydro alcoholic solution containing 10 mg of vanillin equiv/L of phenolic flavor substances and 1 g of malvin equiv/L of anthocyanins (upper levels found in red wines), calculations using K_a 72 M^{-1} lead to complexation rates of 7% for vanillin and 0.3% for the anthocyanin. In absence of ethanol, the respective complexation rates are found to be 10 and 0.4%. With the same level in a better copigment such as syringaldehyde ($K_a = 208$ M^{-1}), the complexation rate reaches 24% for the flavor and 0.9% for malvin. Again ethanol decreases the rates to, respectively, 17 and 0.6%. As a general trend, the strength of the complexation has no impact on color owing to the highly dilute concentrations in flavor. However, a 2-fold increase in the anthocyanin concentration or in the association constant value will nearly double the complexation rate of the phenolic volatile substances. Hence, the sensory quality of grape-derived beverages may be weakly influenced by high levels in anthocyanin. Presence of copigments such as flavonoids and tannins will, however, depress this effect.

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