JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Procyanidins on the Color Stability of Oenin Solutions

CÉLINE MALIEN-AUBERT,[†,‡] OLIVIER DANGLES,[‡] AND MARIE JOSÈPHE AMIOT*[,‡]

Chr-Hansen, 92 avenue des Baronnes, 34730 Prades le Lez, France, and Sécurité et Qualité des Aliments d'Origine Végétale, UMR INRA-UAPV, Site Agroparc, 84914 Avignon Cedex 9, France

The aim of the present work was to specify the influence of the polymerization degree on the color stability of anthocyanins using model solutions under higher thermal conditions simulating rapid food aging. Results showed that an increase in polymeric degree improves the color stability of oenin. Solutions containing a catechin tetramer, purified from brown rice, displayed a remarkable stability. Flavanols as monomers, (+)-catechin and (−)-epicatechin, appeared to decrease stability with the formation of a xanthylium salt leading to yellowish solutions. For the dimers, procyanidin B2 and B3, different behaviors on red color stability have been observed corresponding to their different susceptibility to cleavage upon heating. In the presence of the trimeric procyanidin C2, the red color appeared more stable. However, the HPLC chromatograms showed a decrease in the amplitude of the peaks of oenin and procyanidin C2. Concomitantly, a new peak appeared with a maximal absorption in the red region. This newly formed pigment probably came from the condensation of oenin and procyanidin C2.

KEYWORDS: Oenin; anthocyanins; flavanols; procyanidins; condensed tannins; color stability; copigmentation; stacking; condensation; xanthylium; new pigment

INTRODUCTION

The use of naturally occurring pigments extracted from various edible plants is gaining importance in the food colorants industry. In particular, grape marc constitutes the main, relatively inexpensive, industrial source of anthocyanin-based colorants (*1*−*3*) that have various food applications, especially for coloring beverages. The main drawback in the use of anthocyanins as food colorants is their relatively low thermal and photochemical stability. We have recently studied some mechanisms improving the stability of various anthocyanin-rich extracts (*4*). The first protective mechanism is intramolecular copigmentation (*5*), which was shown in colorants rich in acylated anthocyanins, such as those extracted from purple carrot, red radish, and red cabbage (*4*). The second mechanism that prevails in colorants devoid of acylated anthocyanin (grape marc, elderberry, blackcurrant, and chokeberry) is intermolecular copigmentation, as previously described (*6*, *7*). Among grape marc colorants, those rich in flavonols display remarkable stability. Indeed, because of their planar polyphenolic nucleus, flavonols are excellent copigments that can tightly stack onto anthocyanins, thereby protecting the red flavylium chromophore from water addition at C2, an early step toward discoloration (*8*). In addition, flavonols are efficient UV screens that can protect the bound pigment from photooxidative degradation (*9*). In contrast, a high flavanol (catechins) content in grape extracts was related to low color stability and the corresponding colorants quickly turned yellowish in drink models (*4*). Catechins and procyanidins have been reported to couple with anthocyanin, either directly (*10*, *11*) or via acetaldehyde-derived ethyl bridges (*12*, *13*), the latter mechanism taking place in red wine and in aqueous alcoholic model solutions.

Because grape marc is rich in monomeric and oligomeric flavanols, the aim of the present study was to investigate the color evolution of anthocyanins in the presence of flavanols by using model solutions containing malvidin 3-glucoside (oenin), the main grape anthocyanin, and isolated flavanols with different degrees of polymerization (**Figure 1**). The chemical mechanisms are discussed in relation to the protection or degradation of anthocyanins.

MATERIALS AND METHODS

Plant Material and Chemicals. Malvidin 3-glucoside (Extrasynthèse, France) was used as the pigment. Copigments were (a) commercial standards: epicatechin and catechin (Sigma), or procyanidin B2 (Extrasynthèse); or (b) isolated compounds: proanthocyanidins B3 and C2 from barley (*14*), procyanidin B2 from apple (Red Chief variety) (*15*), or trimeric and tetrameric proanthocyanidins from brown rice (Griotto, France).

Extraction, Purification, and Identification of Flavanols from Different Plant Sources. The procedure described by Goupy et al. (*14*) was used for the extraction and purification of the different procyanidins from barley, apple, and brown rice. Procyanidins were purified by fractionation using a column (300 × 26 mm) filled with

* To whom correspondence should be addressed. Tel: (33) 4 32 72 24 98. Fax: (33) 4 32 72 24 92. E-mail: amiot@avignon.inra.fr.
† Chr-Hansen.
‡ UMR INRA-UAPV.

10.1021/jf011392b CCC: $22.00
Published on Web 04/20/2002

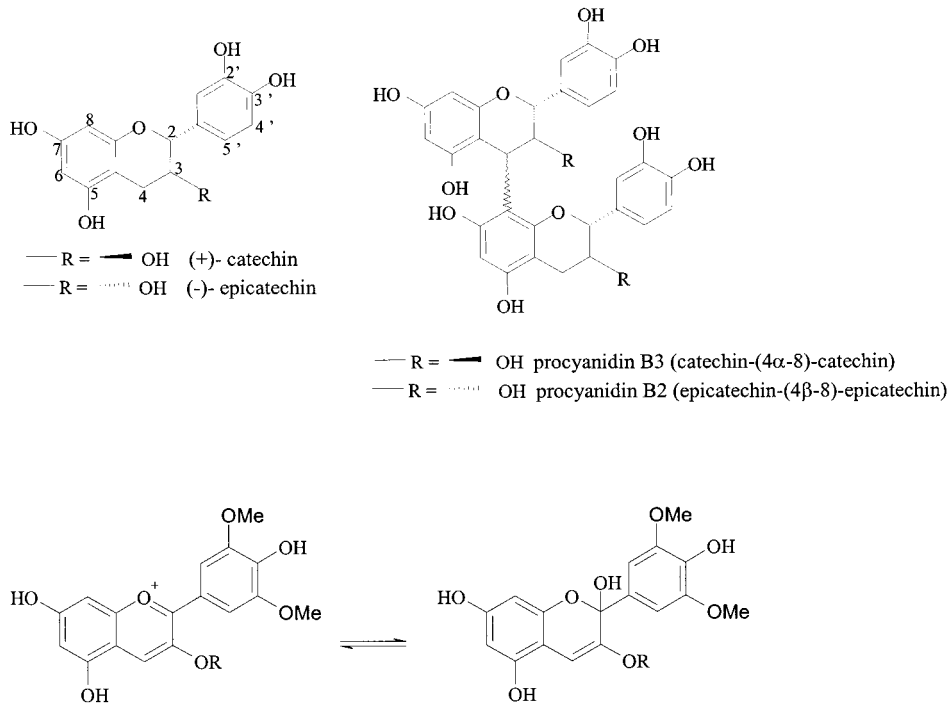

Figure 1. Structures of oenin (flavylium and hemiacetal forms) and flavanol monomers and dimers.

Fractogel Toyopearl HW-40(s), particle size 0.025−0.040 mm (TOSOH, Merck), and eluted with methanol as already described by de Freitas et al. (*16*). Flow rates were optimized according to the different plant extracts: 36 mL/h for barley, 42 mL/h for brown rice, and 30 mL/h for apple. Fractions (the volume of each was 7 mL for brown rice and apple and 6 mL for barley) were collected, and detection was performed at 280 nm. Flavanols were visualized after each fraction spotted on a cellulose plate was sprayed with 1% vanillin in 12 N HCl. The different fractions from barley, rice, and apple were analyzed by RPHPLC/diode array detection (DAD) with a Hewlett-Packard 1050, using a 5-μm Alltima C18 150 × 4.6 mm column with a 5-μm Alltima C18 guard column (Alltech, France). The solvent system used was a gradient of A (2.5% CH_3COOH), B (8% CH_3COOH), and C (acetonitrile). The best separation was obtained with the following gradient: at 0 min, 95% A/5% B; at 20 min, 80%A/10% B; at 50 min, 70%A/30% B; at 60 min, 100% B; at 100 min, 50% B and 50% C; at 110 min 100% C until 120 min. The solvent flow rate was 0.8 mL/min, and separation was performed at 35 °C. The volume injected was 10 μL. Flavanols were assayed by external standard calibration at 280 nm and expressed in mg/L equivalent of (+)-catechin. Thioacidolyses were performed for fractions from apple and brown rice according to the method described by Rigaud et al. (*17*) which allows terminal units to be distinguished as flavan-3-ols and the extension units to be distinguished as benzylthioethers.

Preparation of Model Solutions and Thermal Degradation. Equimolar solutions of malvidin 3-glucoside (oenin) (0.1 mM) with or without copigment (monomeric or oligomeric catechins) were prepared in a McIlvaine buffer at pH 3. The mixtures (three repetitions) were put in glass vials (1.5 mL), which were closed with a screw cap and placed into a hot air steam cabinet at 50 °C. Each tube was used for only one spectral measurement so as to minimize contact with oxygen. The first samples (t_0) were taken 1 h after the preparation of the solution. Synthetic solutions were also analyzed by HPLC/DAD. Determination of the phenolic composition was performed using an HPLC linked to a DAD (Hewlett-Packard 1100). Phenolic compounds were eluted from a 5-μm Lichrosorb RP18 250 × 4.6 mm column (Alltech, France) thermostated at 35 °C. Formic acid/water (10:90) (A) and formic acid/water/acetonitrile (10:60:30) (B) were used as eluents. A linear gradient from 20% B to 85% B (flow rate 0.8 mL/min) was established over 55 min. Quantification was performed by external calibration at 520 nm for oenin, at 280 nm for flavanols (monomers and oligomers), and at 450 nm for brown products. LC/MS analysis was carried out on the solution containing malvidin 3-glucoside and flavanols at the end of the assay. The chromatographic conditions were identical to those used for the HPLC analysis, except for the concentration of formic acid, which was 1%. The MS apparatus coupled to the chromatographic system (Hewlett-Packard 1050 with DAD detector) was a Micromass LYNX API equipped with an electrospray source ion. The MS detector was programmed to obtain fullscan between 150 and 1500 amu in the positive-ion mode. Ion spray voltage was set at 5 kV, and orifice voltage was set at 30 or 40 V. The column was connected with the ES (electrospray) interface via a capillary and a split to regulate the flow into the ES source. An UV−Vis spectrum was recorded on-line from 250 to 600 nm.

Copigmentation. The UV−vis spectra of equilibrated solutions of pigment and copigment at pH 3.5 (0.2 M citrate buffer, ionic strength adjusted to 0.5 M by NaCl) were recorded for different copigment concentrations with a Hewlett-Packard diode array spectrometer (HP 8452) fitted with a quartz cell (1 cm) equipped with a stirring magnet. A constant temperature in the cell ($T = 25.0$ (± 0.1) °C) was obtained using a Lauda water-thermostated bath.

RESULTS

Isolation and Analyses of Procyanidins from Barley, Apple, and Brown Rice Extracts. A purified phenolic extract from each plant material (barley, apple, and brown rice) was submitted to a preliminary analysis in order to collect the major fractions corresponding to different procyanidin structures (**Figure 2**). Thioacidolyses were performed on the purified phenolic extracts, and the degree of polymerization was confirmed by LC/MS. Oligomeric flavanols from apple were only procyanidins, and the monomeric units were mainly epicatechin as reported by Yanagida et al. (*18*) and Foo and Lu (*19*). Procyanidin B2 was identified by RPHPLC/DAD by comparison with standards. Two dimers, procyanidin B3 and prodelphinidin B3, and one trimer, procyanidin C2, were purified from barley (**Figure 2**). Structural elucidation was confirmed by comparison with the standards previously isolated by Goupy et al. (*14*). No structural identification of phenolic compounds from brown rice has been published so far. From the thioacidolyses of the purified fractions (**Figure 2**), a dimer, a trimer, and a

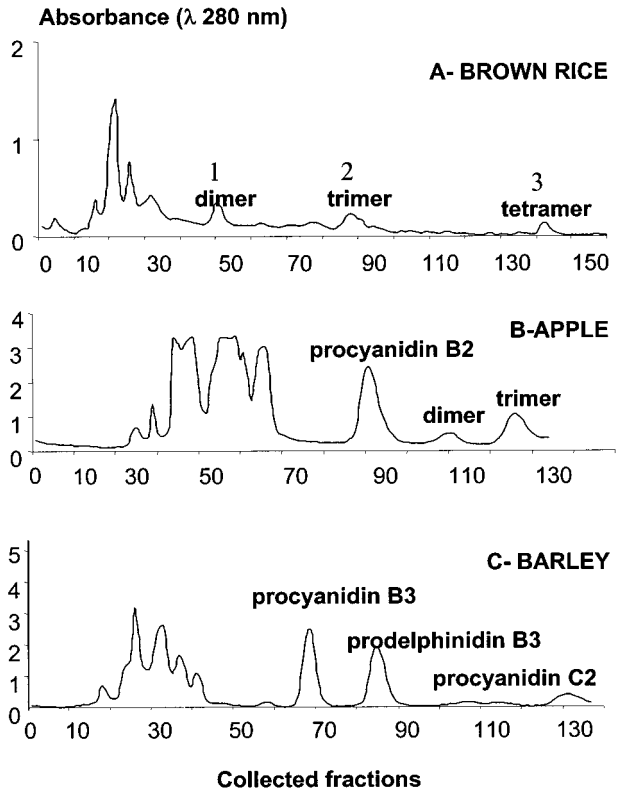

Figure 2. Separation of procyanidins from barley, brown rice, and apple extracts on Fractogel Toyopearl TSK 40 (eluent methanol).

Table 1. Results of Thioacidolysis and LC/MS of Fractions from Brown Rice Extract by Order of Elution

fraction	ratio catechin/ thiobenzylcatechin	type of oligomer	$[M - H]^-$ *m/z*
1	1.06	dimer	577
2	2.08	trimer	865
3	3.19	tetramer	1153

tetramer of catechin were identified. These results were confirmed by LC/MS, which gave the following $[M - H]^-$ peaks: *m*/*z* 577 for the dimer, *m*/*z* 865 for the trimer, and *m*/*z* 1153 for the tetramer (**Table 1**).

Copigmentation between Oenin and Flavanols. Addition of flavanols to a solution of oenin at pH 3.5 (flavanol/anthocyanin molar ratios: 10−30 for dimers, 10−100 for monomers) induced only weak changes in the visible absorption band of the pigment. The small bathochromic shift (<5 nm) and the increase in absorbance (ca. 10%) for a flavanol/oenin molar ratio of 30 suggest a relatively weak stacking interaction between the flavylium ion and the catechin nucleus. In the case of dimers B2 and B3, contamination by low concentrations of oxidation byproducts absorbing around 450 nm prevented a rigorous quantitative analysis. In the case of catechin, the copigmentation binding constant *K* could be evaluated from a general mathematical treatment (*20*) that takes into account the thermodynamics of water addition onto the flavylium ion ($pK_h = 2.7$) (*6*). Assuming a 1:1 stoichiometry for the complex and no complexation between the copigment and the colorless forms, the variations of visible absorbance A as a function of the total copigment concentration CP_t can be expressed as eq 1, where A_0 = visible absorbance in the absence of copigment, r = ratio of the molar absorption coefficient of the complex to that of the free flavylium ion, $a = 1/(1 + K_h 10^{pH})$) which can be easily converted into eq 2.

$$A = A_0 \frac{1 + rK\mathrm{CP_t}}{1 + aK\mathrm{CP_t}} \quad (1)$$

$$\frac{A_0}{A - A_0} = \frac{a}{r - a} + \frac{1}{(r - a)K} \frac{1}{\mathrm{CP_t}} \quad (2)$$

Hence, the plot of $A_0/(A - A_0)$ as a function of $1/CP_t$ was linear with an intercept/slope ratio of a*K* from which the *K* value is readily obtained. The *K* value of 89/M estimated for the oenin−catechin complexation confirms that catechin is a much weaker copigment than flavonols ($K > 10^3$/M) (*6*, *7*, *21*).

Influence of the Degree of Polymerization of Procyanidins on Color Stability. During storage of the model solutions at 50 °C and pH 3.0, a strong decrease in the visible absorbance took place for all model solutions (**Figure 3**). Spectrometric analyses during 240 h revealed for solutions containing catechin a strong decrease at 520 nm with a concomitant increase at 430 nm (**Figure 3**). For procyanidin C2, the decrease at 520 nm and the increase at 430 nm were less pronounced. The bleaching was significantly weaker in the presence of the trimer. HPLC analysis after 240 h consistently revealed that the consumption of oenin was lower in the C2-containing solutions (ca. 30%) than in the catechin-containing solutions and the control (no flavanol) (ca. 50%). Remarkably, the brown index (BI = *A*(430 nm)/*A*(520 nm)) increased in all model solutions, although at a lower rate when the polymerization degree of the flavanol increased (**Figure 3B**). The change in brown index was less for solutions containing the tetramer, which remained red, than for solutions supplemented with catechin, epicatechin, or B3, which turned yellow.

When the model solutions were analyzed by HPLC after storage at 50 °C for 240 h, two main peaks were detected (**Figure 4**). The first peak (P1) was characterized by two absorption maxima at 292 nm (major) and at 336 nm (minor). From its *m*/*z* value, it was tentatively identified as the coumarin formed upon removal of the B ring from oenin. The second peak (P2) was identified as syringic acid by comparison with a commercial standard. Its structure was confirmed by HPLC/MS. In the catechin-containing model solution, a new pigment, P3, with an absorption maximum at 438 nm and a *m*/*z* peak at 785 (electrospray, positive mode) was also detected (**Figure 4**, **Table 2**). Such characteristics strongly suggest that P3 is the glycosylated xanthylium ion resulting from the condensation of oenin with catechin followed by an intramolecular dehydration step to give the xanthylium chromophore. The *m*/*z* value (785) would correspond to the adduct between a sodium ion and the neutral conjugated base of the xanthylium ion. Fragment ions were detected at *m*/*z* 623 (adduct with a sodium ion) and 465, corresponding to the loss of the glucose moiety and the additional loss of the B ring of oenin, respectively. Another important fragment ion was detected at *m*/*z* 611, which corresponds to the product of a retro-Diels−Alder reaction taking place in the catechin unit in agreement with previous findings (*22*). The fragment at *m*/*z* 449, corresponding to the loss of the glucose moiety of the retro-Diels−Alder product, was also detected. A new pigment similar to P3 was also obtained with epicatechin, the formation of which was slower than with catechin. Despite the large consumption of oenin in the catechin-containing solutions, the concentration of catechin remained high (ca. 97% of its initial value). Hence, it can be estimated that the yield in xanthylium pigment (P3) was lower than 3% in the model solutions after 10 days at 50 °C. Despite its low concentration, P3 can be observed directly from the time-

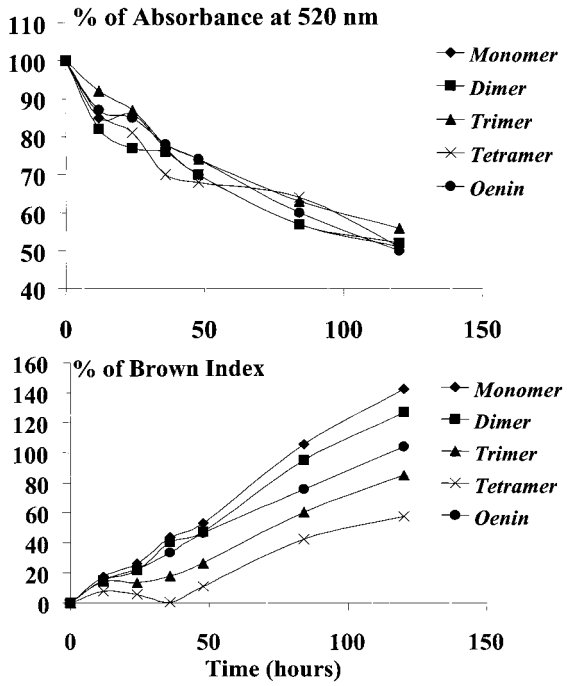

Figure 3. Time-dependent (0–240 h) changes in the visible absorbance at 520 nm (A) and brown index (BI), normalized to 100% at time zero, of model solutions containing oenin and procyanidins during storage (pH 3, 50 °C). Results are the mean of three replicates with a variation less than 1%.

dependent spectroscopic changes at 440 nm occurring in the solutions containing oenin and catechin (data not shown).

During storage at 50 °C, HPLC analyses of the model solutions containing B2 and B3, respectively, revealed the presence of epicatechin and catechin due to the cleavage of the interflavan bond. In addition, a brown precipitate appeared in the course of the reaction with B3. In the presence of C2, the red color of the model solutions appeared more stable (**Figure 3**). However, HPLC analysis revealed the gradual consumption of oenin and C2 with the simultaneous build-up of a new pigment, P4, with absorption maximum at 534 nm (**Figure 5**, **Table 2**). By contrast, no new pigment could be detected in the solutions containing oenin and the tetrameric procyanidin, even though both components were also consumed. HPLC/MS analysis after 120 h of storage showed a peak at *m*/*z* 1357 for P4, corresponding to the direct condensation between oenin and C2. Fragment ions with *m*/*z* values of 1339 and 1177 were also detected which correspond to loss of a water molecule and loss of the glucose moiety, respectively. After 240 h of storage, P4 could no longer be detected.

Table 2. Identification of New Products Formed by HPLC/DAD and LC/MS in Synthetic Model Solutions during Storage at 50 °C for 240 h

	t_r (HPLC)	abs. (nm)	molecular weight	fragments E (+) *m*/*z*	identification
P1	12.1	292	356		coumarin structure
P2	14.7	275	198		syringic acid
P3	16.5	438	785	785; 623, 611, 465, 449	xanthylium salt
P4	15.1	534	1357	1339; 1177; 867; 495	new red pigment

DISCUSSION

Colorless polyphenols are well-known to enhance the color of anthocyanin solutions by preferentially interacting (π-stacking) with the planar chromophore of the flavylium form (*6*, *7*). This phenomenon protects the flavylium nucleus from the nucleophilic attack of water molecules at C2 that leads to the colorless forms, hemiacetals and chalcones. Our results suggest that copigmentation of anthocyanins by flavanols is rather weak, in agreement with previous investigations (*12*). Even though catechin and dimers B2 and B3 act as weak copigments slightly enhancing the red color of oenin solutions, the samples rapidly turn yellow upon heating. Under the same conditions, the trimer (C2) and tetramer more efficiently protect the red color (**Figure 3**). HPLC analysis showed the formation of two degradation products of oenin, P1 and P2 (**Figures 4**

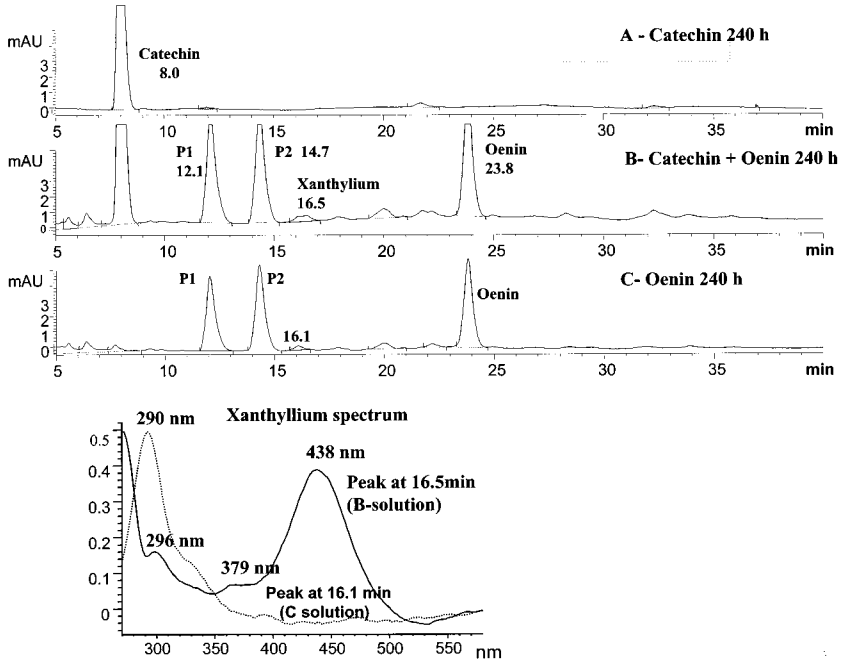

Figure 4. HPLC chromatogram and UV–visible spectrum showing the formation of a new xanthylium pigment in model solutions of oenin (200 μM) and catechin (200 μM) after storage (pH 3, 50 °C) for 240 h.

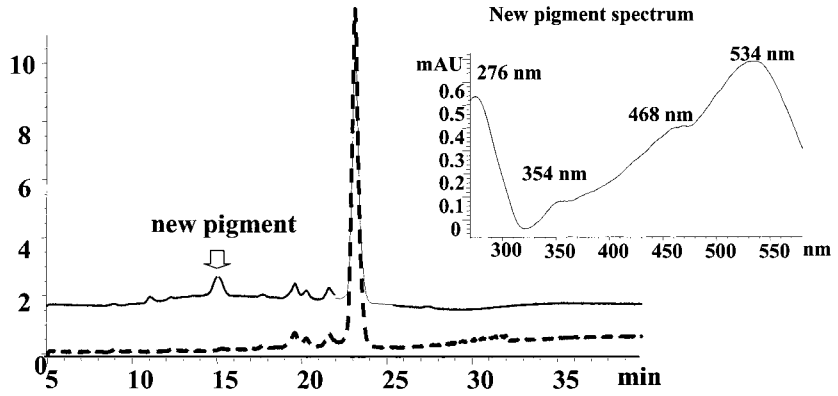

Figure 5. HPLC chromatogram and UV–visible spectrum showing the formation of a new flavylium pigment in model solutions of oenin (200 μM) and trimer C2 (200 μM) after storage (pH 3, 50 °C) for 240 h.

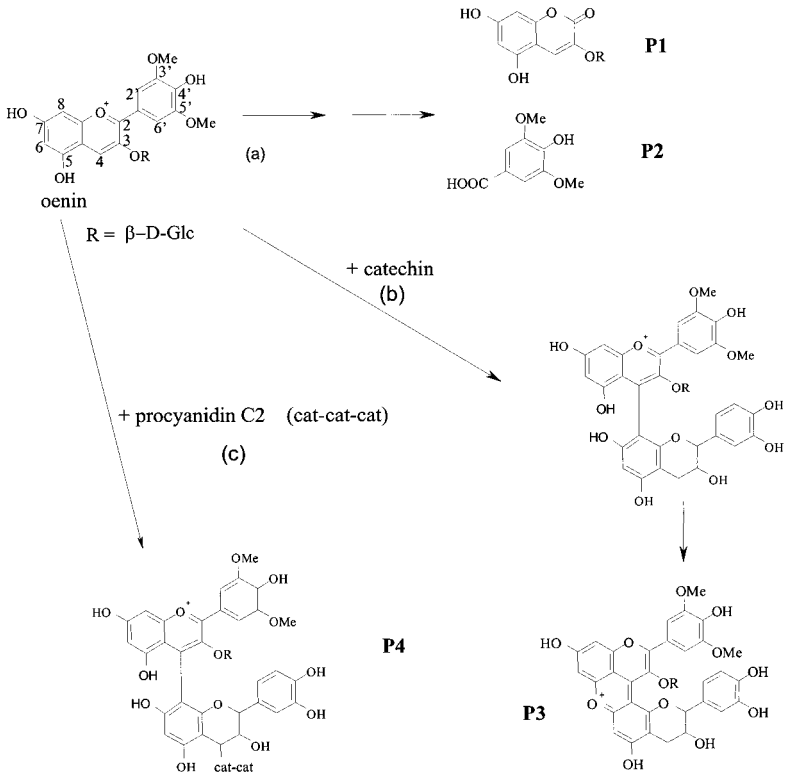

Figure 6. Proposed structures for the degradation products of oenin (P1, P2) and for the new xanthylium (P3) and flavylium (P4) pigments formed upon reaction between oenin and catechin and C2, respectively.

and **6**), in agreement with previous work (*23*). HPLC analysis of solutions containing oenin and C2 showed the gradual disappearance of both components with the simultaneous buildup of a new pigment with absorption maximum at 534 nm (**Figure 5**). This new pigment probably forms upon attack of one of the nucleophilic C_6 or C_8 atoms of procyanidin C2 at the electrophilic C_4 position of the flavylium nucleus of oenin, with subsequent air oxidation of the colorless flavene intermediate to regenerate a flavylium chromophore (**Figure 6**). Such a mechanism (*24*) is consistent with the well-known ability of carbon-centered nucleophiles to attack anthocyanins at C_4 (*11*, *12*, *25*−*27*). However, no new pigment could be detected in solutions containing oenin and the tetrameric procyanidin. Upon heating, covalent coupling between catechin and oenin can also occur in accordance with the above-mentioned mechanism. In this case, however, the new flavylium ion undergoes an additional step of intramolecular dehydration leading to a yellow xanthylium ion (**Figure 6**), as previously described by Escribano-Bailon et al. (*12*). Such condensation reactions probably take place in red wine and participate in the evolution of the color in competition with acetaldehyde-mediated anthocyanin−flavanol coupling reactions (*13*, *14*, *28*−*34*). In agreement with the mechanism proposed, experiments carried out in the presence of oxygen (air bubbling) led to higher xanthylium concentrations than those conducted under an inert atmosphere of N_2 (data not shown). However, the presence of oxygen may also favor the subsequent degradation of the xanthylium pigment (*10*, *31*).

Some specific behaviors of oligomeric procyanidins were observed in our model solutions. The influence of dimers B3 (catechin-4α,8-catechin) and B2 (epicatechin-4β,8-epicatechin) on color stability suggests that a combination of factors is at work. In particular, the thermal degradation of the oligomeric procyanidins (cleavage of the interflavan linkage) must be taken into account. For instance, our observation that catechin, epicatechin, and B3 altered the color of oenin solutions to the same extent after 240 h of storage at 50 °C is consistent with

the formation of large concentrations of catechin in the B3-containing solutions. Moreover, the better stability of the B2-containing solutions is in agreement with the fact that B2 is less prone to thermal degradation than B3 (*35*, *36*), although epicatechin was easily detected in the B2-containing solutions upon heating. Hence, flavanol monomers, either introduced as such or formed in the course of interflavan bond cleavage in dimers, seem relatively effective at coupling with anthocyanins to yield yellow xanthylium pigments. The situation may be different with C2 and the tetramer, as no monomer seems to form in significant concentrations when the latter procyanidins are heated in the presence of oenin. However, both were readily consumed, with more than 60% loss after 10 days at 50 °C, an observation suggesting that rearrangement of flavanol units within oligomers may readily take place. With C2, a new flavylium ion is formed that does not undergo dehydration in the corresponding xanthylium ion (not detected). In our model solutions, no colorless flavene intermediate or bicyclic adduct formally resulting from addition of an OH group of the catechin A ring to the flavene carbon−carbon double bond (*11*, *25*) could be detected. However, such compounds are best evidenced from solutions kept under inert atmosphere. Nor could we detect any presence of adduct resulting from the coupling between oenin and the benzylic carbocations formed by cleavage of interflavan bonds in C2 (*11*).

Anthocyanin−flavanol copigmentation exerts a dual, and eventually paradoxical, influence on color intensity: first, it causes a gain in red color by increasing the concentration of flavylium ion, at the expense of the colorless forms (hemiacetal, chalcones). However, copigmentation also favors anthocyanin−flavanol covalent coupling that may ultimately lead to a loss of red color. Indeed, unlike the colorless forms, the flavylium ion displays an electrophilic C4 center for coupling with a flavanol molecule. Moreover, copigmentation brings the anthocyanin and flavanol molecules into a molecular contact that may well favor subsequent covalent coupling. The loss of red color is particularly important with flavanol monomers because the coupling products readily evolve toward yellow xanthylium pigments. The situation may be different with oligomeric flavanols (trimers and higher oligomers) that may couple with the flavylium ion without suppressing the flavylium chromophore (no further condensation into a xanthylium chromophore), thereby preserving the red color for a longer period. It may be speculated that the inertia brought about by the large oligomeric flavanol moiety about the flavylium−flavanol linkage can freeze the red pigment in a twisted conformation which cannot be dehydrated to give the flat xanthylium nucleus.

ACKNOWLEDGMENT

We thank Ralph Beisson for his help in proofreading the manuscript.

LITERATURE CITED

(1) Francis, F. J. Food Colorants: Anthocyanins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 273−312.
(2) Timberlake, C. F.; Henry B. S. Plant pigments as natural food colours. *Endeavour New Series* **1986**, *10*, 31−36.
(3) Mazza, G. Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 341−371.
(4) Malien-Aubert, C.; Dangles, O.; Amiot, M. J. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. *J. Agric. Food Chem.* **2001**, *49*, 170−176.
(5) Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect. *Phytochemistry* **1993**, *34*, 119−124.
(6) Brouillard, R.; Mazza, G.; Saad, Z.; Albrecht-Gary, A. M.; Cheminat, A. The copigmentation reaction of anthocyanins: a microprobe for the structural study of aqueous solutions. *J. Am. Chem. Soc.* **1989**, *111*, 2604−2610.
(7) Dangles, O.; Brouillard, R. Polyphenol interactions. The copigmentation case: thermodynamic data from temperature variation and relaxation kinetic. Medium effect. *Can. J. Chem.* **1992**, *70*, 2174−2189.
(8) Furtado, P.; Figueiredo, P.; Neves, H. C.; Pina, F. Photochemical and thermal degradation of anthocyanidins. *J. Photochem. Photobiol. A* **1993**, *78*, 113−118.
(9) Sweeny, J. G.; Wilkinson, M. M.; Iacobucci, G. A. Effect of flavonoid sulfonates on the bleaching of anthocyanins in acid solution. *J. Agric. Food Chem.* **1981**, *29*, 563−567.
(10) Santos-Buelga, C.; Bravo-Haro, S.; Rivas-Gonzalo, J. C. Interactions between catechin and malvidin-3-monoglucoside. *Z.-Lebensm.-Unters. Forsch.* **1995**, *201*, 269−274.
(11) Remy, S.; Fulcrand, H., Labarbe, B., Cheynier, V.; Moutounet, M. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *J. Sci. Food Agric.* **2000**, *80*, 745−751.
(12) Escribano-Bailon, M. T.; Dangles, O.; Brouillard, R. Coupling reactions between flavylium ions and catechin. *Phytochemistry* **1996**, *41*, 1583−1592.
(13) Es-Safi, N.-E.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-*O*-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47*, 2096−2102.
(14) Goupy, P.; Hugues, M.; Boivin, P.; Amiot, M. J. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extract, and of isolated phenolic compounds. *J. Sci. Food. Agric.* **1999**, *79*, 1625−1634.
(15) Guyot, S.; Marnet, N.; Drilleau, J. F. Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. *J. Agric. Food Chem.* **2001**, *49*, 14−20.
(16) de Freitas, V. A. P.; Glories, Y.; Laguerre, M. Incidence of molecular structure in oxidation of grape seed procyanidins. *J. Agric. Food Chem.* **1998**, *46*, 376−382.
(17) Rigaud, J.; Perez-Ilzarbe, J.; Ricardo Da Silva, J. M.; Cheynier, V. Micro method for the identification of proanthocyanidin using thiolysis monitored by high-performance liquid chromatography. *J. Chromatogr.* **1991**, *540*, 401−405.
(18) Yanagida, A.; Kanda, T.; Shoji, T.; Ohnishi-Kameyama, M.; Nagata, T. Fractionation of apple procyanidins by size-exclusion chromatography. *J. Chromatogr. A* **1999**, *855*, 181−190.
(19) Foo, L. Y.; Lu, Y. Isolation and identification of procyanidins in apple pomace. *Food Chem.* **1999**, *64*, 511−518.
(20) Dangles, O.; El Hajji, H. Synthesis of 3-methoxy- and 3-(β-D-glucopyranosyloxyl) flavylium ions. Influence of the flavylium substitution pattern on the reactivity of anthocyanins in aqueous solutions. *Helv. Chim. Acta* **1994**, *77*, 1595−1610.
(21) Alluis, B.; Dangles, O. Quercetin glycosides and sulfates: chemical synthesis, complexation, and antioxidant properties. *Helv. Chim. Acta* **2001**, *84*, 1133−1156.
(22) Mirabel, M.; Saucier, C.; Guerra, C.; Glories, Y. Copigmentation in model wine solutions: Occurrence and relation to wine aging. *Am. J. Enol. Vitic.* **1999**, *50*, 211−218.
(23) Markakis, P. Stability of anthocyanins in foods. In *Anthocyanins as Food Colors*; Markakis, P. Ed.; Academic Press: New York, 1982; pp 163−180.
(24) Liao, H.; Cai, Y.; Haslam, E. Polyphenol interactions. Anthocyanins: Co-pigmentation and color changes in red wines. *J. Sci. Food Agric.* **1992**, *59*, 299−305.
(25) Bishop, P. D.; Nagel, W. C. Characterization of the condensation product of malvidin 3,5-diglucoside and catechin. *J. Agric. Food Chem.* **1984**, *32*, 1022−1026.
(26) Fulcrand, H.; Cameiras dos Santos, P. J.; Sarni-Manchado, P.; Cheynier, V.; Fabre-Bonvin, J. Structure of new anthocyanin-derived wine pigment. *J. Chem. Soc., Perkin Trans. 1* **1996**, 735−739.

(27) Romero, C.; Bakker, J. Interactions between grape anthocyanins and pyruvic acid, with effect of pH and acid concentration on anthocyanin composition and color in model solutions. *J. Agric. Food Chem.* **1999**, *47*, 3130−3139

(28) Baranowski, E. S.; Nagel, C. W. Kinetic of malvidin-3-glucoside condensation in wine model system. *J. Food Sci.* **1983**, *48*, 419−421 and 429.

(29) Bakker, J.; Picinelli, A.; Bridle, P. Model wine solutions: colour and composition changes during aging. *Vitis* **1993**, *32*, 111−118.

(30) Rivas-Gonzalo, J. C.; Bravo-Haro, S.; Santos-Buelga, C. Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde. *J. Agric. Food Chem.* **1995**, *43*, 1444−1449.

(31) Dallas, C.; Manuel Ricardo-da-Silva, J.; Laureano, O. Interactions of oligomeric procyanidins in model wine solutions containing malvidin-3-glucoside and acetaldehyde. *J. Sci. Food Agric.* **1996**, *70*, 493−500.

(32) Saucier, C.; Little, D.; Glories, Y. First evidence of acetaldehyde-flavanol condensation products in red wine. *Am. J. Enol. Vitic.* **1997**, *48*, 370−373.

(33) Saucier, C.; Guerra, C.; Pianet, I.; Laguerre, M.; Glories, Y. (+)-Catechin-acetaldehyde condensation products in relation to wine aging. *Phytochemistry* **1997**, *46*, 229−234.

(34) Es-Safi, N.-E.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Competition between (+)-catechin and (−)-epicatechin in acetaldehyde-induced polymerization of flavanols. *J. Agric. Food Chem.* **1999**, *47*, 2088−2095.

(35) Francia-Aricha, E. M.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Effect of malvidin-3-monoglucoside on the browning of monomeric and dimeric flavanols. *Z.-Lebensm.-Unters. Forsch.* **1998**, *207*, 223−228.

(36) de Freitas, V. A. P.; Glories, Y.; Laguerre, M. Incidence of molecular structure in oxidation of grape seed procyanidins. *J. Agric. Food Chem.* **1998**, *46*, 376−382.

Received for review October 19, 2001. Revised manuscript received February 19, 2002. Accepted February 19, 2002. This work was financially supported by the Association Nationale de la Recherche Technique in France and Chr-Hansen-SEFCAL.

JF011392B