Incidence of Molecular Structure in Oxidation of Grape Seed Procyanidins

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The kinetics of decomposition of the following flavan-3-ol derivatives isolated from grape seeds under oxidative conditions by airing and using metal ion catalysis (iron and copper) are determined: (+)-catechin and (−)-epicatechin; seven natural procyanidin dimers, B1 [(−)-epicatechin-(4→8)-(+)-catechin], B2 [(−)-epicatechin-(4→8)-(−)-epicatechin], B3 [(+)-catechin-(4→8)-(+)-catechin], B4 [(+)-catechin-(4→8)-(−)-epicatechin], B6 [(+)-catechin-(4→6)-(+)-catechin], B7 [(−)-epicatechin-(4→6)-(+)-catechin], and B8 [(−)-epicatechin-(4→6)-(−)-epicatechin]; trimers, C1 [(−)-epicatechin-(4→8)-(−)-epicatechin-(4→8)-(−)-epicatechin], (+)-catechin-(4→8)-(+)-catechin-(4→8)−(−)-epicatechin, and (+)-catechin-(4→8)-(−)-epicatechin-(4→6)-(+)-catechin, monogallate esters of B2 and B4 and digallate of B2, which were isolated from grape seeds. Kinetic decomposition comparisons were monitored by HPLC. The following order was found for oxidative decomposition for procyanidin dimers: B3 ≈ B4 > B7 ≈ B6 > B1 ≈ B2 > B8. In the conditions of this study, the gallate ester of (−)-epicatechin is more unstable than (−)-epicatechin; inversely, kinetic decompositions of dimeric procyanidins B2 and B4 are much more important than those of their gallate esters. Molecular mechanics (MM2*) and ^{1}H NMR studies of dimeric 3-O-gallate structures show a $\pi-\pi$ stacking arrangement between the aromatic gallate and catechol rings, absent in analogous dimeric procyanidins, which reduces the total surface accessible to oxidizing agents.

INTRODUCTION

Flavan-3-ol derivatives occur mainly as structure subunits of condensed tannins in plants (Weinges et al., 1968; Thompson et al., 1972; Haslam, 1979) and are important natural antioxidants in food and living cells. Grape polyphenol oxidation may be enzymatically catalyzed in the must or nonenzymatically produced during wine processing and aging. Enzymatic oxidation involves participation of grape polyphenol oxidase, which leads to browning in white musts and wines. For these cases, studies by Cheynier and co-workers, have shown that oxidation of hydroxycinnamic acids to respective *o*-quinones is the first step of phenolic compound oxidation (Cheynier et al., 1988, 1990; Cheynier and Ricardo da Silva, 1991). On the other hand, polyphenolic compounds scavenge active oxygen species, such as superoxide anion radicals and lipid peroxy radicals, and are supposed to give *o*-quinones, which are extremely reactive (Wildenradt and Singleton, 1974; Uchida et al., 1987; Singleton, 1987). Of particular interest in wines and teas are procyanidin compounds, which include (+)-catechin (**1**) and (−)-epicatechin (**2**) linked through C(4)−C(6) or C(4)−C(8) interflavanoid bonds, as well as various gallate esters (Czochanska et al., 1979; Nonaka et al., 1983; Boukharta et al., 1988; Ricardo da Silva et al., 1991a,b).

Although there have been many studies on their free radical scavenging reactions (Uchida et al., 1987; Ariga et al., 1988; Ariga and Hamano, 1990; Ricardo da Silva et al., 1991a,b), details of their antioxidant mechanisms and the relationship with procyanidin structures are not fully understood. Polyphenol oxidation in complex solutions, such as wine and tea, involves a simple step-by-step reaction not only with oxygen radical species (autoxidation) but also with other unknown oxidizing substrates involving different mechanisms (Singleton, 1987; Cheynier and Ricardo da Silva, 1991; Porter et al., 1986a,b; Cheynier et al., 1989).

In this work we investigated the influence of different structural factors, such as catechin structure units [(+)-catechin and (−)-epicatechin], interflavanoid bond linkage, gallic acid esterification, and degree of polymerization, in the kinetics of procyanidin decompositions in a model wine solution. This study was made under natural oxidative conditions (oxygen air-enriched solutions) at pH 3.2 in the presence of transition metal ion catalysts (iron and copper) (Porter et al., 1986a,b).

MATERIALS AND METHODS

Preparation of Grape Seed Catechin and Procyanidin Extracts. Grape seeds (*Vitis vinifera*) were extracted with 50% aqueous ethanol according to the procedure described in Darné and Madero (1979). The extract (360 mg) was subjected to chromatography over Fractogel TSK HW-40(s) (350 × 25 mm i.d.) using methanol as eluant at 0.8 mL/min to give four fractions containing various oligomeric procyanidins: fraction I (39.6 mg), fraction II (82.8 mg), fraction III

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(72.0 mg), and fraction IV (22.0 mg). Each fraction was freeze-dried after removal of the solvent by a rotatory evaporator under reduced pressure at 30 °C.

Kinetics of Oxidative Decomposition. *(a) Wine Model Systems.* The fraction residues were dissolved (0.5 g/L) in 12% aqueous ethanol model wine (5 g/L tartaric acid) at pH 3.2 with addition of a small amount of Cu(I) (1 mg/L) and Fe(II) (10 mg/L) and then analyzed by HPLC. To study the influence of cyanidin (anthocyanidin) in time–rate decomposition of oligomeric procyanidin, the residue from fraction II (0.5 g/L) was dissolved in the 12% aqueous ethanol wine model solution with addition of cyanidin (0.5 g/L). These solutions were sealed and stored at room temperature for several weeks. Every week, they were enriched with air oxygen through energetic shaking (oxidative medium) before HPLC analysis (Figure 1).

(b) White and Red Wines. The white and red wines were enriched with oligomeric procyanidins from fraction II (0.5 g/L), and their oxidative decomposition was performed by airing in the same conditions described above.

HPLC Analysis. Two Beckman Ultrasphere (C$_{18}$) ODS (250 × 4.6 mm i.d.) columns disposed in line and protected with a guard column packed with the same packing were used for all analyses. The chromatograms were monitored at 280 nm using a UV detector. The elution system consisted of two solvents: (A) 2.5% HOAc in H$_2$O; (B) 80% CH$_3$CN in solvent A. The following gradients were used: elution starting with 7% B in A isocratic for 5 min; 7–20% B in A, 5–90 min; 20–100% B in A, 90–95 min; 100% B, 95–100 min (isocratic); followed by washing and reconditioning of the column. The analysis was carried out at ambient temperature at 1 mL min^{-1}.

Semipreparative HPLC. Preparative chromatography was performed with the same chromatographic conditions and detection system employed for analytical HPLC. Compounds were collected manually at the time of their elution.

Identification of Procyanidins. Flavan-3-ol derivative structures were available from liquid secondary ion mass spectrometry (LSIMS), analytical HPLC by comparison with authentic standards, partial degradation with toluene-α-thiol (Rigaud et al., 1991), and enzymatic hydrolysis. The procyanidin dimers B1–B8 (**3**–**9**) and trimer C1 (**10**) used as standards were synthesized following the methods in Michaud et al. (1973) and Geissman and Yoshimura (1966).

(a) LSIMS Analysis. A few milligrams of sample was dissolved in the minimum quantity of anhydrous methanol required and dissolved in a matrix of glycerol. The LSIMS spectra were recorded using a VG Autospec EQ mass spectrometer, equipped with a Cs$^+$ gun in negative mode (beam energy = 35 keV). Calibration was performed with cesium iodide salt (200–3500 Da).

(b) Degradation with Toluene-α-thiol. The flavan-3-ol derivatives, contained in fractions I–IV, eluted from the HPLC columns, were collected in a vial, and the solvent was evaporated to dryness under vacuum at 30 °C. After addition of 45 μL of toluene-α-thiol, 5% (w/v) in EtOH, the solution was sealed in a vial and heated at 100 °C for 1 h. The solvent was then evaporated, and 30 μL of Raney nickel was added under H$_2$. The vial was sealed and heated at 50 °C for 1 h and then injected onto the HPLC system to analyze the reaction products.

(c) Enzymatic Hydrolysis. The flavan-3-ol derivatives, eluted from the HPLC columns, were collected, and the solvent was evaporated to dryness under vacuum at 30 °C. The residue was diluted with 1 mL of a 0.2 M acetate buffer (pH 4.5) and incubated with enzyme (1 mg mL^{-1}) (phenol heterosidase proceeding from *Aureobasidium pullulans*) at 30 °C for 2 h. After extraction with ethyl acetate (1 mL), the extract was injected onto the HPLC system.

(d) NMR Measurements. The full peracetate of dimer B2 3″-*O*-gallate (**15**) was dissolved in CDCl$_3$, and the NMR spectra were recorded on a Bruker AMX 500 spectrometer at the temperature of 303 K (TMS as internal standard) in conditions described in Balas and Vercauteren (1994). ^{1}H and ^{13}C measurements were carried out at 500.13 and 125.77 MHz,

respectively. Acetylations of B2 3″-*O*-gallate was performed in Ac$_2$O/pyridine (1:1) at room temperature for 2 days in the dark. The purification was performed by preparative TLC (10 × 10 cm plates; silica gel 60 PF$_{254}$ 0.25 mm, from Merck) using EtOAc/CH$_2$Cl$_2$ (2/5) as eluant: ^{1}H NMR (500.13 MHz, CDCl$_3$) δ 3.0–3.1 (H-4aF and H-4bF, superposed), 4.44 (d, J = 2.4, H-4C), 4.73 (br s, $W_{1/2}$ = 2.1 Hz, H-2F), 5.60 (d, J = 1.6 Hz, H-2C), 5.30 (m, H-3F), 5.26 (dd, J = 1.6, 2.4 Hz, H-3C), 6.14 (d, J = 2.2 Hz, H-6A), 6.67 (s, H-6D), 6.26 (d, J = 2.2 Hz, H-8A), 7.40 (d, J = 1.8 Hz, H-2′B), 7.18 (H-2′E, superposed), 7.19 (d, J = 8.3 Hz, H-5′B), 7.07 (d, J = 8.3, H-5′E), 7.21 (dd, J = 1.8, 8.3 Hz, H-6′B), 6.97 (H-6′E, superposed), 1.6–2.5 (30 H, 10 × CH$_3$CO), 7.69 (s, H-gallate ring); ^{13}C NMR (125.77 MHz, CDCl$_3$) 34.8 (C-4C), 27.2 (C-4F), 71.8 (C-3C), 69.6 (C-3F), 74.6 (C-2C), 77.9 (C-2F), 108.2 (C-8A), 117.5 (C-8D), 109.5 (C-6A), 111.3 (C-6D), 112.4 (C-4aA), 112.2 (C-4aD), 122–143 (12 C, B and E catechol rings), 148.7 (C-5A), 149.7 or 149.9 (C-5D or C-7A), 148.7 (C-7D), 155.9 (C-8aA), 154.8 (C-8aD).

Molecular Modeling. Calculations were performed on an SGI Indigo platform running Macromodel version 5.0 (Columbia University, New York) or Insight II and Discover version 2.3.5 (Biosym Technologies). Conformational minima were found using the modified MM2* (1987 parameters) force field, as previously described (De Freitas, 1995). Build structures were minimized to a final RMS gradient ≤0.005 kJ Å^{-1} mol^{-1} via the truncated Newton conjugate gradient (TNCG) method (1000 cycles). Coupling constant calculations were performed using the method of Haasnoot et al. (1980), as implemented in Macromodel.

RESULTS AND DISCUSSION

Identification of Catechins and Procyanidins. The **first fraction** studied contains (+)-catechin (**1**) and (−)-epicatechin (**2**) identified by coelution with authentic standards through analytical HPLC; the mass spectrum gave [M − H]$^-$ ion peaks at m/z 289. The **second fraction** LSI mass spectrum gave [M − H]$^-$ ion peaks m/z 441, 577, 729, and 865 corresponding to catechin gallates, dimers, dimer gallates, and trimers, respectively. Procyanidin dimers (**3**–**10**) and trimer C1 (**11**) were identified by cochromatography with authentic reference samples by analytical HPLC and also from partial degradation by thiolysis. The (−)-epicatechin *O*-gallate (**14**) was collected from the HPLC column, and its structure was elucidated by enzymatic hydrolysis with a phenol heterosidase possessing an esterase activity (isolated from *A. pullulans*); the (−)-epicatechin and gallic acid moiety structures released were identified by analytical HPLC by comparison with authentic standards. The B2 3″-*O*-gallate (**15**) structure was identified by NMR spectroscopy. The **third fraction** LSI mass spectrum gave [M − H]$^-$ ion peaks m/z 865 and 729 corresponding to trimers and dimer gallates, respectively. The procyanidin trimers (**12** and **13**) were isolated by HPLC from semipreparative scale, and their structures were elucidated by partial thiolysis with toluene-α-thiol followed by reduction with Raney nickel: the trimer (**12**) released the fragments (+)-catechin, (−)-epicatechin, dimer B3, and dimer B4; on the other hand, the trimer (**13**) released (+)-catechin, dimer B4, and dimer B7. All of the fragments were identified through analytical HPLC by comparison with authentic standards. To our knowledge, the structures of trimers (**12** and **13**) in grapes are suggested here for the first time. The B4 *O*-gallate structure was elucidated by enzymatic hydrolysis as described above for (−)-epicatechin *O*-gallate. The **fourth fraction** contains dimer digallates and tetramers; its LSI mass spectrum gave the corresponding [M − H]$^-$ ion peaks m/z 881 and 1153. The B2 digallate dimer was collected
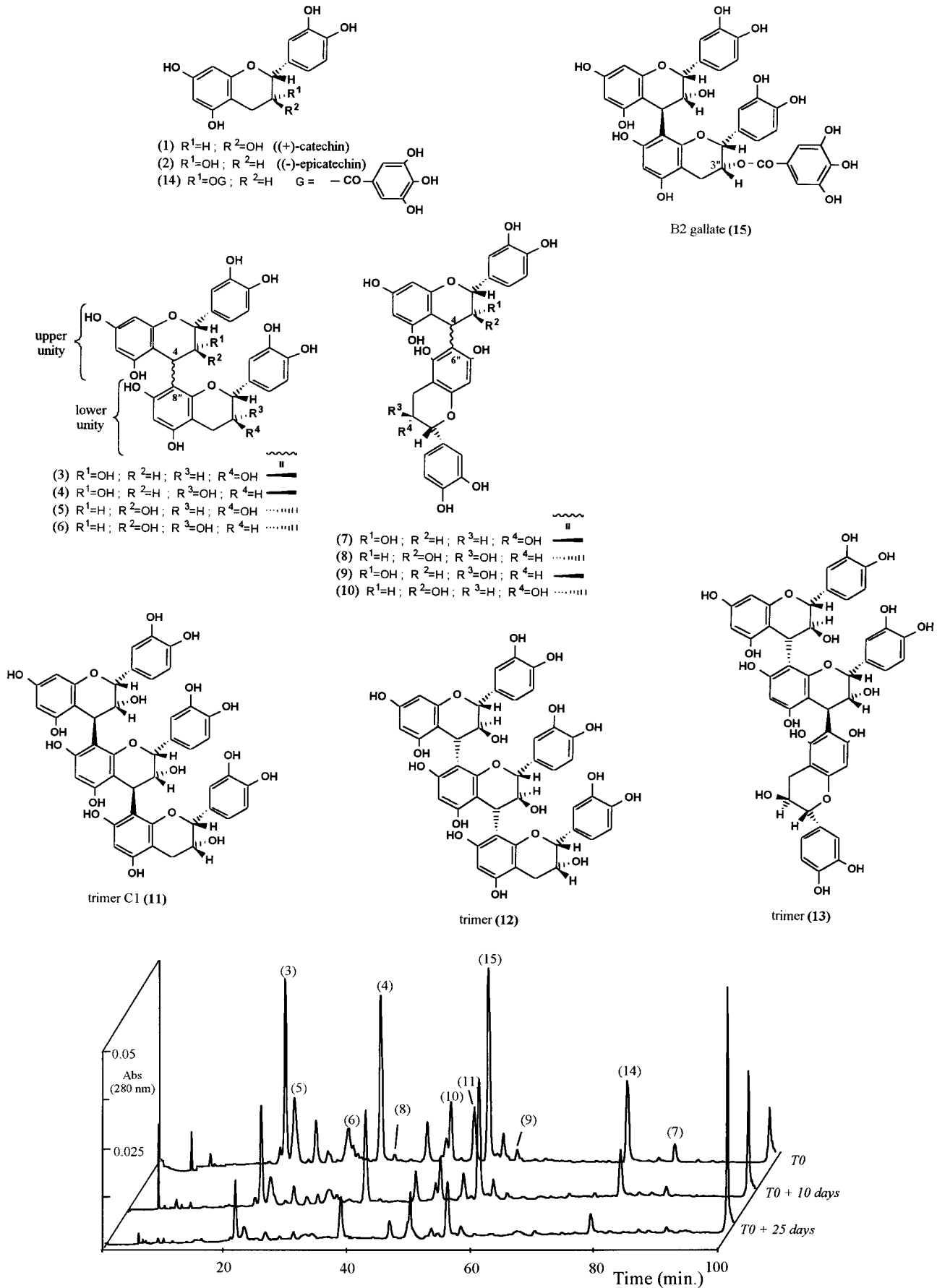
(1) R^1=H ; R^2=OH ((+)-catechin)
(2) R^1=OH ; R^2=H ((-)-epicatechin)
(14) R^1=OG ; R^2=H   G = —CO—

B2 gallate **(15)**

upper unity

lower unity

(3) R^1=OH ; R^2=H ; R^3=H ; R^4=OH
(4) R^1=OH ; R^2=H ; R^3=OH ; R^4=H
(5) R^1=H ; R^2=OH ; R^3=H ; R^4=OH
(6) R^1=H ; R^2=OH ; R^3=OH ; R^4=H

(7) R^1=OH ; R^2=H ; R^3=H ; R^4=OH
(8) R^1=H ; R^2=OH ; R^3=OH ; R^4=H
(9) R^1=OH ; R^2=H ; R^3=OH ; R^4=H
(10) R^1=H ; R^2=OH ; R^3=H ; R^4=OH

trimer C1 **(11)**

trimer **(12)**

trimer **(13)**

Figure 1. Analytical HPLC chromatograms of grape seed procyanidins in fraction II from Fractogel TSK at 280 nm for different times of oxidation (0, 10, and 25 days). Peaks: 3, dimer B1; 5, dimer B3; 6, dimer B4; 4, dimer B2; 8, dimer B6; 10, dimer B8; 11, trimer C1; 15, dimer B2 3″-O-gallate; 9, dimer B7; 14, (−)-epicatechin gallate; 7, dimer B5.

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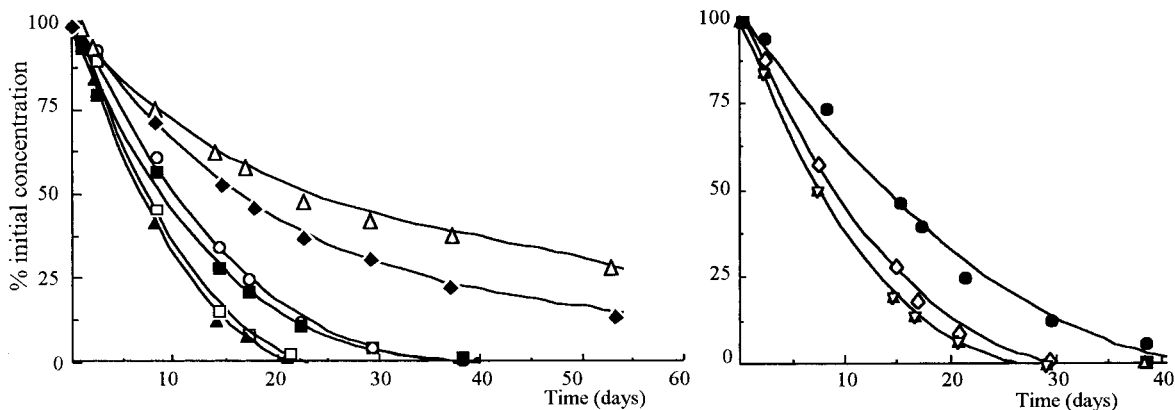*J. Agric. Food Chem.,* Vol. 46, No. 2, 1998 **379**

Figure 2. Time course of catechin and procyanidin dimer destruction (fractions I and II from Fractogetl TSK), in a 12% ethanol model wine system [pH 3.2; tartaric acid, 5.0 g/L; Fe(II) 10.0 mg/L; Cu(I), 1.0 mg/L], aired every week: (△) (+)-catechin; (◆) (−)-epicatechin; (○) procyanidin B1; (■) procyanidin B2; (□) procyanidin B4; (▲) procyanidin B3; (●) procyanidin B8; (◇) procyanidin B6; (☆) procyanidin B7.
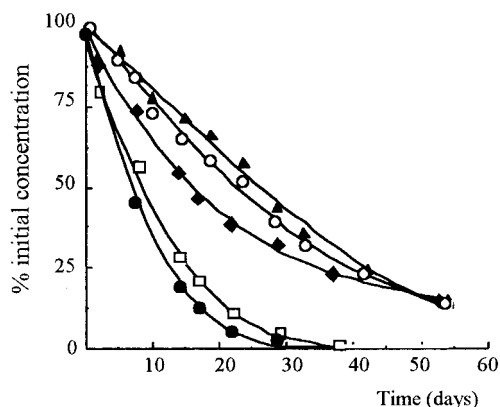
Figure 3. Influence of degree of polymerization in the time course of (◆) (−)-epicatechin, (▲) trimer 1, (○) trimer 2, (□) dimer B2, and (●) trimer C1 destruction.

Figure 4. Influence of gallic acid esterification in the time course of (−)-epicatechin destruction: (○) (−)-epicatechin; (●) (−)-epicatechin gallate.

Figure 5. Influence of gallic acid esterification in the time course of procyanidin dimer destruction: (○) B2 monogallate; (■) B4 monogallate; (△) B2 digallate; (●) B2; (□) B4.

from the HPLC column, and its structure was elucidated by enzymatic hydrolysis, from which we obtained dimer B2 and gallic acid.

Oxidative Decomposition. Figure 1 shows an important decrease of the compounds in time. Determination of the kinetic order of the oxidative decomposition of procyanidins was not possible because of the complex mechanisms involved. Therefore, we illustrate our results by plotting percent initial concentration versus time.

(a) Influence of Catechin Structure Units and Interflavanoid Bond Linked. Our results show that (−)-epicatechin (**2**) is more oxidizable than (+)-catechin (**1**) and both are degraded at a slower rate than the dimer procyanidins (**3−10**) (Figure 2). These results may be explained by the major capacity of dimers to trap oxygen radical species comparatively to the monomers (autoxidation). However, in our conditions, polyphenol transformations involve probably a simple reaction with oxygen radical species and also with other unknown oxidizing substrates. The time−rate decomposition profile of procyanidin dimers shows an important effect of the C(4)−C(6) or C(4)−C(8) interflavanoid bond and of the structural monomeric unity (Figure 2). Under our experimental conditions, the kinetics of the oxidative degradation of the procyanidin dimers B3 (**5**) and B4 (**6**) containing the interflavanoid bond C(4)−C(8) are identical and greater than those of the procyanidin dimers B1 (**3**) and B2 (**4**), suggesting that the oxidability depends essentially on the nature of the upper struc-
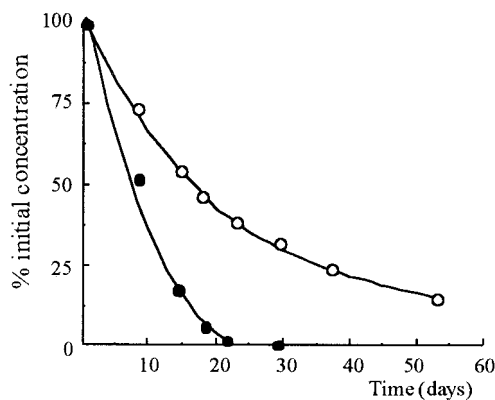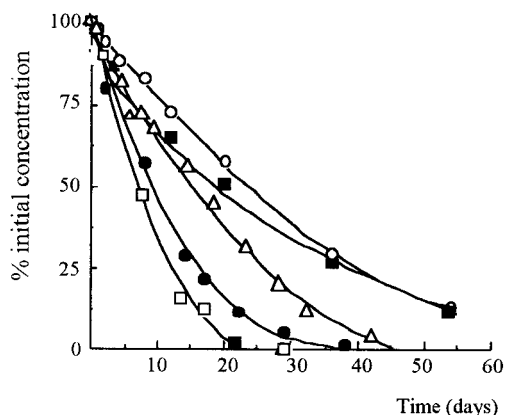
tural monomeric unity, whatever the inferior unity is; the oxidability is greater for the (+)-catechin unity. Inversely, for the procyanidin dimers containing the C(4)−C(6) interflavanoid bond (**8−10**), we noticed that the kinetics of the oxidative degradations depend essentially on the nature of the lower structural monomeric unity, whatever the upper unity is. So, kinetics of degradations of dimers B6 (**8**) and B7 (**9**) are slightly identical and faster than those of B8 (**10**). In conclusion, these results show that B1 and B2 were degraded at a slower rate than B3 and B4 and at faster rate than B6 and B7. Procyanidin B8 is the more stable dimer.
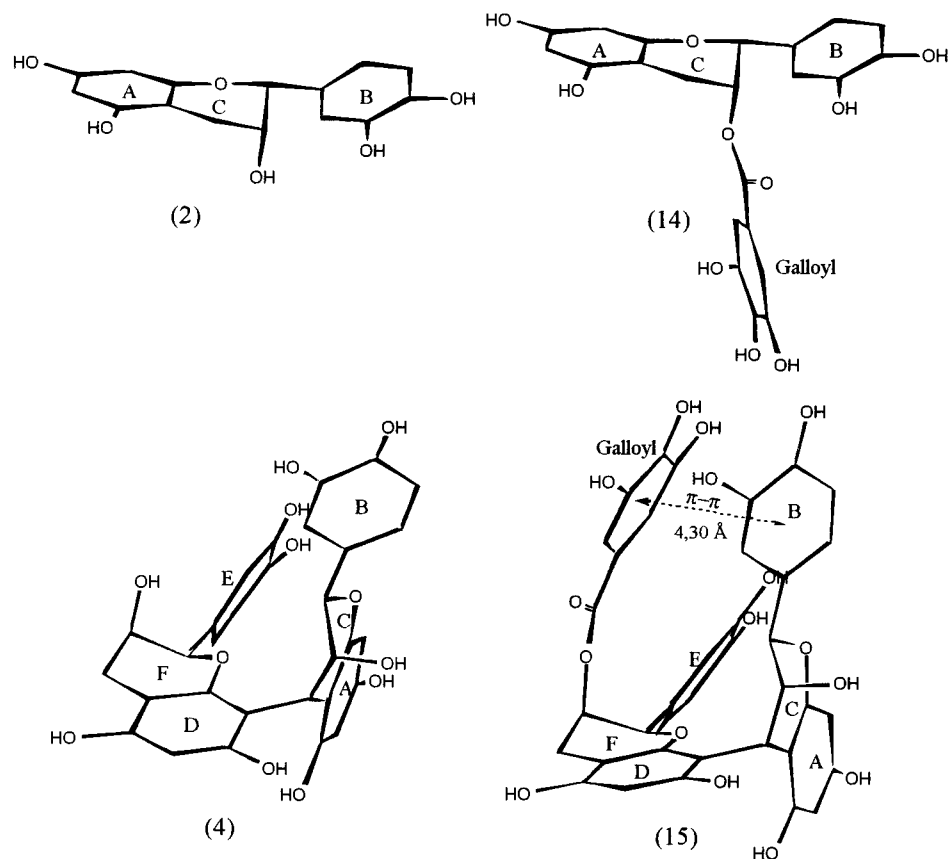
Figure 6. Representations of the preferred conformations of (−)-epicatechin (**2**), dimer B2 (**4**), and their analogous gallates (**13** and **14**), determined using Allinger's MM2* force field parameters.

(b) Influence of Esterification with Gallic Acid. Esterification of (−)-epicatechin with gallic acid at the C(3) hydroxyl function increases its oxidability (Figure 4). Inversely, degradation of procyanidin dimers B2 (**4**) and B4 (**6**) is systematically faster than that of their gallic esters (Figure 5). These results are not in agreement with the major ability of gallic esters to scavenge oxygen radical species (autoxidation) (Cheynier and Ricardo da Silva, 1991; Ricardo da Silva et al., 1991a,b), which indicates that, in our oxidative condition, free oxygen has no direct action in gallic ester transformation.

To attempt an interpretation of our results, a study of molecular aspects was performed using molecular mechanics (MM2*) (Fronczek et al., 1985; Porter et al., 1986a,b; Viswanadhan et al., 1987; Balas et al., 1995). Using Allinger's MM2 force field parameters (Allinger, 1977), we have determined the possible conformer structures (within a 15 kJ/mol energy range window) for each procyanidin dimer and their corresponding gallates. The $^3J_{2,3}$, $^3J_{3,4}$ (^{1}H NMR) proton−proton coupling constants of their pyranic rings were calculated using the empirical method of Altona and co-workers (Altona et al., 1980) and compared to the experimental ones. Vicinal coupling constants give information on the dihedral angle between residual H2, H3, and H4 protons. From comparisons of these coupling constants with those measured in solution (chloroform) for procyanidin dimers and B2 3″-*O*-gallate peracetates, we estimated the conformations we expect to be the most probable in solution (this approximation is based on the fact that ^{1}H NMR J proton−proton coupling constants described for natural dimers B3 and B4 measured in methanol are quite identical with those of their peracetates measured in chloroform, which suggests that in

this particular case the pyranic ring geometry of procyanidins is not greatly affected by acetylation of the hydroxyl functions). It was concluded that these preferred conformations have systematically both catechol rings (B and E) in a pseudoequatorial position and the two pyranic rings (C and F) adopt a half-chair conformation. Interestingly, it was noticed that these conformations corresponded to the least energy conformer only for the C(4)−C(8) bonded dimers (B1−B4). The preferred conformation for B2 3″-*O*-gallate (**15**) (corresponding in this case to the most stable one) shows an intramolecular π−π stacking arrangement between the aromatic gallate and catechol moieties, the distance between the centers of the aromatic rings being, respectively, 4.30, 4.15, and 4.71 Å for B2, B4, and B6. It is interesting to note that this "cage" structure is not present in (−)-epicatechin gallate (**14**) (Figure 6).

While it is not possible to extrapolate the behavior of all gallate dimers in solution, MM2* calculations point to the importance of π−π arrangements which reduce the total surface of gallate ester exposed to water and protect the interior moiety from the oxidizing agents. This effect is probably the property responsible for the lower oxidability of gallate esters relative to the procyanidin dimers.

To quantify this visual aspect, we have computed for each compound the van der Waals surface (S_{VDW}) of the preferred conformation and its relative solvent-accessible surface (S_{solv}) (a sphere of 1.4-Å radius was used to mimic water molecules), which must be normally superior to S_{VDW} (Table 1). A weak increase of S_{solv} relative to S_{VDW} (percent increase) means that a more or less important part of the molecule surface is not accessible for the solvent owing to a compact structure.

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Table 1. Comparison of van der Waals Surface (S_{VDW}) for the Least Energy Conformation of Procyanidin Dimers and Gallate Esters, as well as the Relative Surface Accessible by Water Size Molecules (S_{solv})

procyanidin	S_{VDW} (Å^2)	S_{solv} (Å^2)	% increase[a]
B2	540	731	35.4
B4	534	734	37.5
B6	526	688	30.8
B2 gallate	661	780	18.0
B4 gallate	661	820	24.1
B6 gallate	660	849	28.6

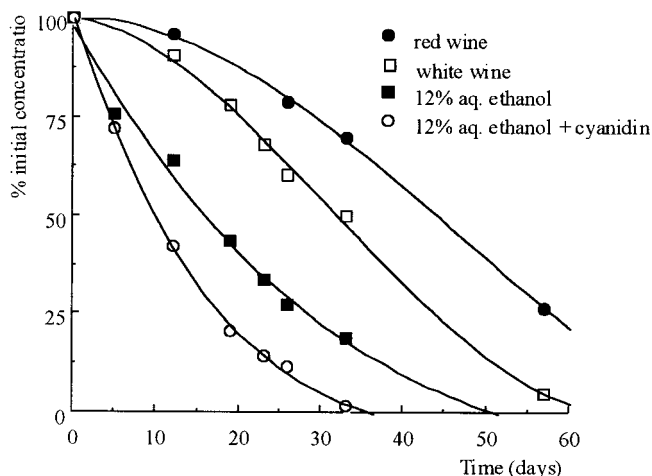[a] Percent increase = $(S_{solv} - S_{VDW})/S_{VDW}$.

Figure 7. Time course of total procyanidin dimer destruction in a 12% ethanol model wine system [pH 3.2; tartaric acid, 5.0 g/L; Cu(I), 1.0 mg/L; Fe(II), 10.0 mg/L] with and without cyanidin as well as in white and red wines.

In fact, the relative increase for the gallate esters was found low relative to the analogous dimer procyanidins because of the $\pi-\pi$ arrangements which are absent in the dimer structures.

(c) Influence of Degree of Galloylation. For procyanidin dimer B2 (**4**), the oxidability is not proportional to the number of gallic acid units. In effect, our results show that the decomposition of dimer B2 digallate is faster than that of B2 monogallate, suggesting that the effect of $\pi-\pi$ arrangements is probably less significant in this case. Nevertheless, the dimer B2 digallate is more stable than its analogue dimer B2 (**4**).

(d) Influence of Degree of Polymerization. Degradation rates of (−)-epicatechin (**2**), procyanidin dimer B2 (**4**), and trimer C1 (**11**) increase with the degree of polymerization (Figure 3). Although the sites accessible to oxidation increase proportionally to the number of flavan-3-ol units, we observe that the rise in oxidability is not proportional to this.

Inversely, the trimers (+)-catechin-(4a→8)-(+)-catechin-(4a→8)-(−)-epicatechin (**12**) and (+)-catechin-(4a→8)-(−)-epicatechin-(4b→6)-(+)-catechin (**13**) are less oxidizable than catechins (**1** and **2**) (Figure 3). This may also mean that the conformations of procyanidin trimers which include the (+)-catechin and (−)-epicatechin units are probably more complex and compact, thus protecting the structures from the oxidizing agents.

(e) Oxidative Decomposition of Total of Procyanidin Dimer Directly in Wines and in Wine Model Systems. Figure 7 show the time−rate decomposition profile of the total procyanidin dimers in wine models with and without cyanidin as well as in white and red wines, all with addition 0.5 g/L of oligomeric procyanidins proceeding from the second fraction.

The procyanidins in wines are degraded at a slower rate compared to the wine models systems. This result suggests that the wines contain other compounds even more oxidizable which protect the procyanidins from oxidation. These unknown substrates seem to have a protective effect more important in red wines than in white wines. Oppositely, cyanidin, which contributes to the color of red wines, plays a pro-oxidant role relative to dimer procyanidins. It appears probable that additional compounds resulting from hydrolytic degradation of the cyanidin are implied in the procyanidin dimer degradation. Several authors suggested that anthocyanins quickly complex with procyanidins and are incorporated into the condensed tannin structures to give polymeric phenolic pigments (Somers, 1971; Singleton and Trousdale, 1992), which is generally believed to be due to an acid-catalyzed condensation of procyanidins and anthocyanin.

This study shows the important relationship between grape seed procyanidin structures and their oxidizing properties. They contribute differently to the oxidability of foods and beverages. On the other hand, under natural conditions of oxidation, esterification by gallic acid stabilizes the procyanidins, despite the major ability of gallate ester to scavenge oxygen radical species.

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