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# Oxidation of caffeic acid in a wine-like medium: Production of dihydroxybenzaldehyde and its subsequent reactions with (+)-catechin

Marcus Lutter, Andrew C. Clark *, Paul D. Prenzler, Geoffrey R. Scollary

*National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga 2678, Australia*

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**Abstract**

Dihydroxybenzaldehyde was generated from the iron-mediated oxidation of caffeic acid in a model white wine containing the non-flavonoid, caffeic acid. Higher concentrations of this aldehyde were obtained with increased iron concentration. The reaction of dihydroxybenzaldehyde with (+)-catechin, a flavanol compound, resulted in the production of both colourless and yellow/red-coloured compounds. Based on LC–MS studies, the colourless compounds were identified as isomeric forms of 3,4-dihydroxybenzenemethine-bridged (+)-catechin dimers. The coloured compounds were found to be a result of dihydroxybenzaldehyde reacting with either one or two (+)-catechin units. Compared to the reaction between (+)-catechin and glyoxylic acid, the reaction of dihydroxybenzaldehyde with (+)-catechin had a considerably slower rate of (+)-catechin degradation and also less colour formation. These results suggest that dihydroxybenzaldehyde has the ability to form coloured compounds in a reaction mechanism that is relevant to the enhanced colour development apparent during the oxidation of white or red wine.


*Keywords:* White wine; Aldehyde; Caffeic acid; Dihydroxybenzaldehyde; Oxidation; Iron; Browning; Catechin

## 1. Introduction

The oxidative colouration of white wine is known to involve the phenolic compounds within the wine, specifically the flavanol compounds (Simpson, 1982; Sioumis, Kallithraka, Markris, & Kefalas, 2006). Although the non-flavonoid content of white wine has not been linked to increases in the yellow (420 nm) absorbances during the oxidation of white wine (Simpson, 1982; Sioumis et al., 2006), it has recently been shown that the oxidative colouration of white wine is more consistent with absorbance increases at both yellow (420 nm) and red (500 nm) wavelengths (Skouroumounis et al., 2005). Furthermore, it has been demonstrated that caffeic acid, a non-flavonoid hydroxycinnamic acid, can promote increased red colouration during the oxidation of a model system that contained both iron and a flavanol (George, Clark, Prenzler, & Scollary, 2006). These results suggest that some oxidation product of the caffeic acid may be a precursor of polymerisation reactions of flavanols.

The oxidation of caffeic acid (**1**), and its esterified form, has been examined by various enzymatic (Cheynier & Moutounet, 1992; Cheynier & Ricardo Da Silva, 1991; Singleton, Salgues, Zaya, & Trousdale, 1985) and non-enzymatic processes. The non-enzymatic processes have included oxidation induced by periodate, nitrite, Fenton chemistry, electrochemistry and also autoxidation (Antolovich et al., 2004; Cilliers & Singleton, 1989; Deiana et al., 2003; Fulcrand, Cheminat, Brouillard, & Cheynier, 1994; Napolitano & d'Ischia, 2002; Tazaki, Taguchi, Hayashida,

*Abbreviations*: LC-DAD, high performance liquid chromatography with photodiode array detector; LC–MS, liquid chromatography with mass spectrometry; UV, ultraviolet; VIS, visible.

* Corresponding author. Tel.: +61 2 6933 4181; fax: +61 2 6933 4068.
*E-mail address:* aclark@csu.edu.au (A.C. Clark).

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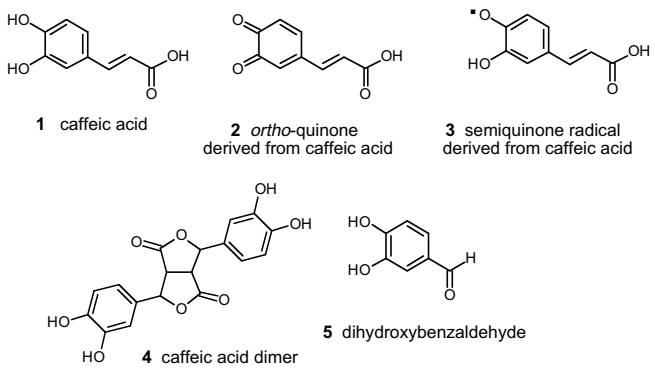
& Nabeta, 2001), the majority of which were performed under conditions not particularly relevant to wine systems.




Enzymatic oxidation of caffeic acid in grape-juice and wine is normally more relevant after the crushing of grapes and before the fermentation of the must. Cheynier, Basire, and Rigaud (1989), Cheynier and Moutounet (1992), Cheynier and Ricardo Da Silva (1991) and Cheynier and Van Hulst (1988) have reported extensive kinetic and spectroscopic studies on the enzymatic oxidation of caffeic acid, and its predominant ester, caftaric acid, in grape-juice model systems, with the presence and absence of flavanols, tannins and/or protein. The studies were generally conducted at a pH relevant to juice and wine, typically pH 3.6. The enzymatic oxidations primarily result in the oxidation of the hydroxycinnamic acids into their corresponding *ortho*-quinone (**2**), followed by non-enzymatic reactions of the *ortho*-quinone, a reactive oxidising agent and electrophile. The hydroxycinnamic acid-derived *ortho*-quinone can oxidise flavanols, such as (+)-catechin or procyanidins (Cheynier et al., 1989; Cheynier & Ricardo Da Silva, 1991), to their subsequent *ortho*-quinones that can then undergo further reactions with flavanols to generate polymeric phenolic compounds (Guyot, Vercauteren, & Cheynier, 1996). Another well studied reaction of the hydroxycinnamic acid-derived *ortho*-quinone is its addition reaction with glutathionone (Cheynier & Van Hulst, 1988) to generate a colourless species known as the grape reaction product (GRP), identified at appreciable concentrations in white wine (Singleton et al., 1985). Brown pigments were reported during the enzymatic oxidation of caffeic acid in a model wine but the pigments were not identified (Cheynier & Moutounet, 1992). It is often suggested that the brown pigments may have been due to the oxidative polymerisation reactions induced by the *ortho*-quinone oxidation product (Cheynier & Moutounet, 1992; Singleton, 1987).

The main wine-related non-enzymatic studies on caffeic acid were performed by Cilliers and Singleton (1989, 1991), although no ethanol was present in any of the caffeic acid samples. Initial autoxidation studies were performed in the pH range 4.0–8.0, under oxygen gas and over the temperature range 5–35 °C (Cilliers & Singleton, 1989). It was suggested by Cilliers and Singleton (1989) that the major caffeic acid oxidation products were formed at all pH values while the relative concentrations of the oxidation products were different. However, later work showed additional products when the oxidation of caffeic acid was performed at higher initial concentrations of caffeic acid as well as at pH 8.5 (Cilliers & Singleton, 1991). Unlike enzymatic oxidation, it was suggested that an initial oxidation product of caffeic acid was a semi-quinone radical (**3**) that led to further polymerisation or degradation reactions. Some of the main products identified were dimers (**4**) and trimers of caffeic acid (Cilliers & Singleton, 1991) and similar polymers have been identified from the oxidation of caffeic acid by periodate (Fulcrand et al., 1994; Tazaki et al., 2001). Increases in brown products were observed during the autoxidation of caffeic acid, over the pH range 4.0–8.0, but no pigmented products were identified (Cilliers & Singleton, 1989, 1991).

The production of caffeic acid dimers and dihydroxybenzaldehyde (**5**) from caffeic acid during its oxidation with hydrogen peroxide and iron, that is, via Fenton chemistry, has been reported (Antolovich et al., 2004). Although this study was not performed under wine-like conditions, wine flavanols are sufficiently nucleophilic to react with aldehydes in the acidic conditions of wine (Fulcrand, Cheynier, Oszmianski, & Moutounet, 1997; Laurie & Waterhouse, 2006; Timberlake & Bridle, 1976) and polymerised phenolic pigments have been generated from such reactions (Es-Safi et al., 1999b). Therefore, dihydroxybenzaldehyde is a potential precursor for polymerisation reactions in wine. The mode of non-enzymatic oxidation of caffeic acid was found to be critical for the production of dihydroxybenzaldehyde in the study by Antolovich et al. (2004). For example, dihydroxybenzaldehyde was not detected from the oxidation of caffeic acid by periodate oxidation but was detected when the oxidation was performed with Fenton reagents.

As white wine phenolic compounds, such as caffeic acid, are some of the most easily-oxidisable species in white wine (Singleton, 1987), the oxidative production of dihydroxybenzaldehyde may be an important contributor to the polymerisation and/or pigmentation of white wine phenolic compounds. This study was conducted to assess the production of dihydroxybenzaldehyde from caffeic acid and iron under wine-like conditions and then to assess the reaction of this compound with (+)-catechin. The impact of such reactions on the colour of the model wine system was also to be examined.

## 2. Materials and methods

### 2.1. Reagents and apparatus

All glassware and plastic ware were soaked for at least 16 h in 10% nitric acid (BDH, AnalaR) and then rinsed with copious amounts of Grade 1 water (ISO 3696). Solutions and dilutions were prepared using Grade 1 water.

Potassium hydrogen tartrate (>99%) and L(+)-tartaric acid (>99.5%) were obtained from Sigma. (+)-Catechin monohydrate (Sigma, 98%), caffeic acid (Sigma, 99%), glyoxylic acid monohydrate (Aldrich, 98%) and dihydroxybenzaldehyde (Aldrich, 97%) were used without further purification. Iron(II) sulfate heptahydrate was obtained from AJAX.

Absorbance measurements and spectra were recorded on a μQuant Universal Microplate Spectrophotometer (Biotek Instruments) with the software KC4 v3.0 (Biotek Instruments). The absorbance measurements were recorded at 440 nm and spectra recorded from 200 to 600 nm.

LC-DAD experiments were conducted on a Waters 2690 Separation Module run by Millenium[32] software and connected to a Waters 2996 photodiode array detector. The column used was a reverse phase Wakosil C18RS column of particle size 5 μm and 250 × 2 mm with a guard column of the same type. The LC-DAD analyses were carried out as described previously (Clark & Scollary, 2002).

LC–MS experiments were conducted on a SpectraSYSTEM LC run by Xcalibar software with a P4000 sample pump, UV6000LP UV detector and a Finnigan AQA quadrapole MS with an electrospray source. The same column, solvent conditions and flow rate were used as for LC-DAD experiments. The sample injection was 20 μl. The LC–MS experiments were carried out in the positive ion mode, with an ion spray voltage of +3 kV and orifice voltage of +10 V, and in the negative ion mode, with an ion spray voltage of −3 kV and orifice voltage of −30 V. In experiments for investigating fragmentation products, the orifice voltages were increased to +80 V and −80 V in the positive and negative ion modes, respectively. Simultaneous wavelength detection at 278 nm and 440 nm was performed.

### 2.2. Reactions

The wine-like solution was prepared by adding 0.011 M potassium hydrogen tartrate and 0.008 M tartaric acid to aqueous ethanol (12% v/v, 2 l) and stirring overnight at room temperature. The pH of the wine-like solution was 3.2 ± 0.1. The phenolic compounds and iron(II) were added to this solution immediately prior to the commencement of an experiment.

The experiment involving the monitoring of caffeic acid oxidation products involved the preparation of 2 l of 200 mg/l (1.1 mM) caffeic acid. This was then divided into 9 x150 ml portions in 250 ml Schott bottles with screw top lids. Using a stock solution of 1 g/l iron(II), three of these 150 ml samples were adjusted to 1.5 mg/l iron(II), while another three samples were adjusted to 20 mg/l iron(II). The final three samples had 0 mg/l of added iron(II). All the samples were held in darkness at 45 °C and the sample bottles were only opened on measurement days.

The reaction of (+)-catechin with dihydroxybenzaldehyde was conducted with 1420 mg/l (10 mM) dihydroxybenzaldehyde and 150 mg/l (0.5 mM) (+)-catechin, a molar ratio of ~20:1. This reaction was performed in the wine-like solution and samples were stored in darkness at 45 °C and the sample bottles were only opened on measurement days.

## 3. Results and discussion

### 3.1. Formation of dihydroxybenzaldehyde from caffeic acid under wine-like conditions

Caffeic acid was added to the wine-like solution and, from this solution, three samples were prepared with 0, 1.5 and 20 mg/l iron(II). At the end of the experiment (14 days), dihydroxybenzaldehyde was identified in the samples (peak 1, Fig. 1) by co-elution with a standard of dihydroxybenzaldehyde, by its UV/VIS spectrum (Fig. 2) identical to a standard of dihydroxybenzaldehyde and also analysis by LC–MS. Peak 1 corresponded to a signal of 137 $m/z$ in the negative ion mode. This confirmed that dihydroxybenzaldehyde was generated from caffeic acid in wine-like conditions. The rates of production of dihydroxybenzaldehyde in the samples were 0.41 ± 0.03, 1.75 ± 0.09 and 4.7 ± 0.5 μM/day ($n = 3$, ±95% confidence limits) for the samples with 0, 1.5 and 20 mg/l of iron(II), respectively, highlighting the importance of iron in the production of this aldehyde. Intriguingly, dihydroxybenzaldehyde production was observed in the absence of added iron(II), although trace amounts of adventitious metal ions present in the sample were most likely sufficient to stimulate the oxidative

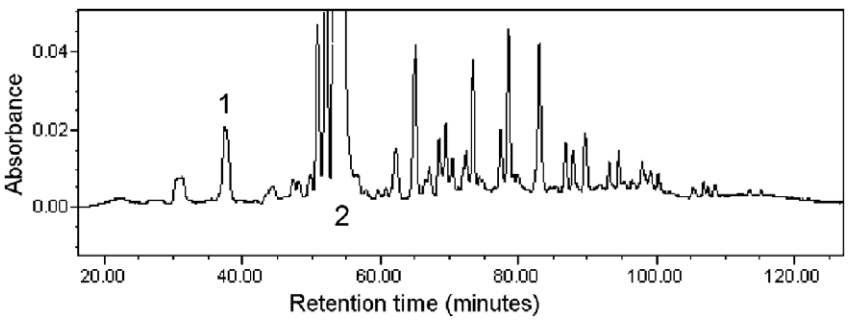



Fig. 1. The extracted chromatogram (280 nm) for the model white wine, containing 200 mg/l caffeic acid and 1.5 mg/l of iron, after 14 days of reaction. Peak 1 is due to dihydroxybenzaldehyde and peak 2 is due to caffeic acid.

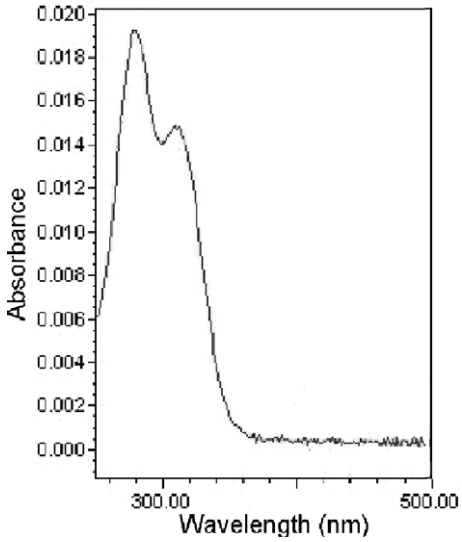



Fig. 2. The UV/VIS spectrum corresponding to peak 1 (Fig. 1).

process (Buettner et al., 1996; Clark, Prenzler, & Scollary, 2007).

Many other degradation products of caffeic acid were observed in the 280 nm chromatogram (Fig. 1). The majority of these degradation products eluted after dihydroxybenzaldehyde and caffeic acid on the reverse phase chromatography column, and hence were less polar than dihydroxybenzaldehyde and caffeic acid. The polymeric forms of caffeic acid were reported to elute after caffeic acid under similar reverse phase chromatography analysis conditions (Antolovich et al., 2004; Cilliers & Singleton, 1989). The samples with added iron(II) exhibited a fine black precipitate, formed during the experiment, and increased amounts were generated at higher metal ion concentrations. The ability of an oxidation product of caffeic acid to precipitate iron has been reported previously (Deiana et al., 2003).

### 3.2. Reaction of dihydroxybenzaldehyde with (+)-catechin

Given the ability of caffeic acid to generate the aldehyde, dihydroxybenzaldehyde, and the potential for the reaction between aldehydes and flavanols to generate pigments (Es-Safi, Cheynier, & Moutounet, 2003; Es-Safi et al., 1999b), the reaction between dihydroxybenzaldehyde and the flavanol, (+)-catechin, was investigated. Dihydroxybenzaldehyde and (+)-catechin were reacted in the wine-like solution. During the experiment, the sample colour changed from a faint yellow-colour (<0.02 absorbance units at 440 nm) to an intense yellow/red-colour (Fig. 3a and b). No colour change occurred upon reaction of caffeic acid with dihydroxybenzaldehyde (Fig. 3a), confirming the importance of the flavonoid ring structure of (+)-catechin in its reaction with the aldehyde.

Fig. 4a shows part of the 280 nm chromatogram for the (+)-catechin/dihydroxybenzaldehyde sample at the end of

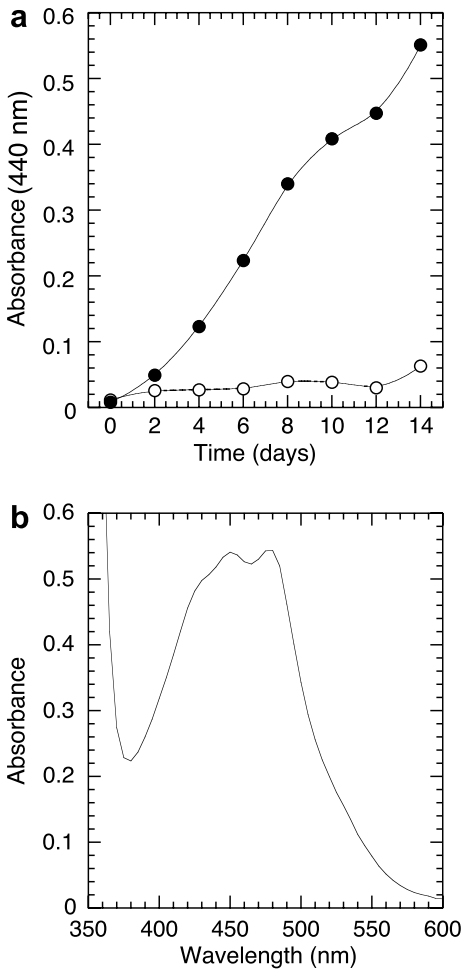



Fig. 3. The 440 nm absorbance of the model white wine containing dihydroxybenzaldehyde and either caffeic acid (○) or (+)-catechin (●) (a) and the UV/VIS spectrum of the model white wine containing dihydroxybenzaldehyde and (+)-catechin after 14 days of reaction (b).

the experiment. Apart from the peaks due to (+)-catechin and dihydroxybenzaldehyde (not shown; retention times <42 min), the peaks in Fig. 4a are the most intense peaks in the 280 nm chromatogram. Peaks 5 and 6 were due to contaminants in the standard of dihydroxybenzaldehyde, and their respective peak areas did not change throughout the experiment. After analysis by LC–MS, peaks 3 and 7 were found to coincide with $m/z$ values of 701 and 699 in the mass chromatograms of the positive and negative ion modes, respectively (Table 1). A further peak, peak 8 (Fig. 4b), was identified with the same $m/z$ values. The $m/z$ values for these ions correspond to the bridging of two (+)-catechin molecules by a dihydroxybenzenemethine unit (Fig. 5).

The extracted LC-DAD UV/VIS spectra, corresponding to peaks 3 and 7, had single maxima at 280 nm and no tailing into the visible region of the spectrum, similar to the spectrum for (+)-catechin. Hence, they differed to the spectrum of dihydroxybenzaldehyde (Fig. 2) due to the absence of any absorbance maximum at 310 nm. This latter absorbance maximum is due to the side chain aldehyde

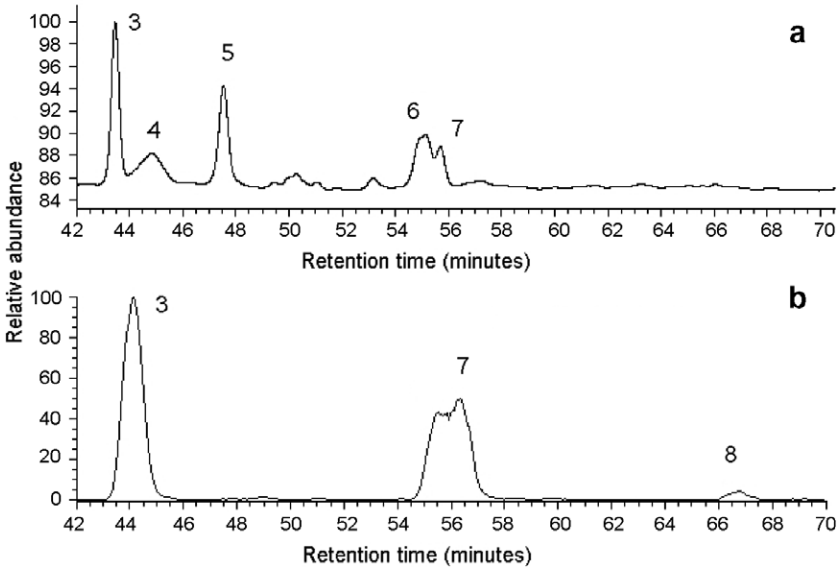
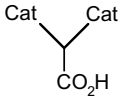



Fig. 4. The extracted chromatograms, 280 nm (a) and 701 *m/z* (positive ion mode) (b), for the model white wine containing both dihydroxybenzaldehyde and (+)-catechin after 14 days of reaction. The peaks in the UV and mass chromatograms are offset by approximately 0.8 min, due to the LC–MS hardware set up.

Table 1
LC–MS analysis of the products from the reaction between (a) (+)-catechin and glyoxylic acid, and (b) (+)-catechin and dihydroxybenzaldehyde

| Reaction | Peak identity | Ionisation mode | Parent ion (*m/z*) | Fragmentation ion (*m/z*) – fragment identity |
|---|---|---|---|---|
| (a) Glyoxylic acid + catechin | Carboxymethine-linked (+)-catechin dimer | Positive | 637 | 485 – RDA<br>347 – loss of (+)-catechin portion<br>195 – loss of (+)-catechin portion and subsequent RDA |
| |  | Negative | 635 | 345 – loss of (+)-catechin portion<br>301 – loss of (+)-catechin and decarboxylation<br>289 – (+)-catechin |
| (b) DHB + catechin | Peaks 3, 7, 8[a] (Fig. 4b) Assigned as **6** (Fig. 5) and its isomers | Positive | 701 | 549 – RDA<br>411 – loss of (+)-catechin portion<br>259 – loss of (+)-catechin portion and subsequent RDA |
| | | Negative | 699 | 547 – RDA<br>409 – loss of (+)-catechin portion<br>289 – (+)-catechin<br>257 – loss of (+)-catechin portion and subsequent RDA |
| | Peak 9 (Fig. 7) | Positive | 967 and 427 | 545 – RDA of 967 ion<br>409, 275, 257 – not assigned |
| | | Negative | 965 and 425 | 177, 407 – not assigned |
| | Peak 10 (Fig. 7) | Positive | 409 | 257 – RDA |
| | | Negative | 407 | 255 – RDA |

DHB, dihydroxybenzene; RDA, retro-Diels Alder and Cat, catechin with linkage at either positions 6 or 8.
[a] Parent ions were detected for peak 8 but no fragmentation ions were detected.

functionality present in dihydroxybenzaldehyde and the absence of this maximum absorbance in the proposed (+)-catechin dimer is consistent with the non-existence of this aldehyde moiety in the proposed dimer.

From the LC–MS analyses of other aldehyde-derived flavonoid dimers (Laurie & Waterhouse, 2006; Pissarra, Mateus, Rivas-Gonzalo, Buelga, & de Freitas, 2003; Saucier, Guerra, Pianet, Laguerre, & Glories, 1997), the loss of a (+)-catechin portion and/or the retro-Diels Alder degradation of the flavonoid moiety of these dimmers, are characteristic fragmentation patterns (Fig. 6). For example, Table 1 shows this type of fragmentation from LC–MS analysis of the carboxymethine-linked (+)-catechin dimer, the dimer resulting from reaction between (+)-catechin and glyoxylic acid. This particular dimer has the added fragmentation pathway of decarboxylation due to the acid

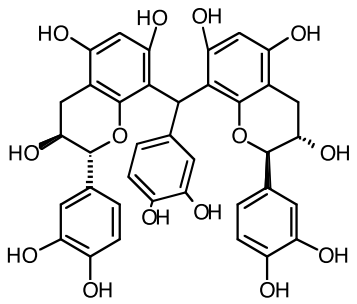



Fig. 5. Dihydroxybenzenemethine-linked (+)-catechin dimer.

group that it contains. Similarly, loss of 152 atomic mass units gives fragment ions of 549 and 547 *m*/*z* in the positive and negative ion modes, respectively, from the dihydroxybenzenemethine-linked (+)-catechin dimer. This loss may be attributed to the retro-Diels Alder fragmentation. Further, loss of 289 atomic mass units gives fragment ions of 411 and 409 *m*/*z* in the positive and negative ion modes, respectively, due to loss of a single catechin unit from the dimer. These results allow assignments of peaks 3 and 7 to isomers of the dihydroxybenzaldehyde-linked (+)-catechin dimer.

As the UV/VIS spectral and fragmentation data for peak 8 were of poor intensity, the identification of peak 8 (Fig. 4b) remains tentative. However, the formation of bridged-flavonoid dimers is expected to result in four connective-isomers, whereby the aldehyde-derived moiety connects two flavonoids at positions 8–8 (as shown in Fig. 5), 6–8, 8–6 or 6–6 on the flavonoid molecules. Related studies (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999a; Es-Safi, Guernevé, Cheynier, & Moutounet, 2000; Es-Safi, Guernevé, Cheynier, & Moutounet, 2002) have shown that production of the 8–8 isomer is favoured and it elutes first on a reverse phase chromatography column. The 6–8 and 8–6 connective-isomers are the next most abundant isomers and also the next isomers to be eluted, often being unresolved from each other. Finally, the 6–6 connective isomer is the least abundant isomer generated and it elutes with the longest retention time. This elution pattern and abundance suggests that peak 3 (Fig. 4) may be the 8–8 connective isomer, and peak 7 (Fig. 4) may be the 6–8 and 8–6 connective-isomers.

Two main groups of peaks (9 and 10 in Fig. 7) were observed in the 440 nm chromatogram of the dihydroxybenzaldehyde and (+)-catechin sample at the end of the experiment. The extracted spectra of these peaks (Fig. 8) were similar to the spectrum of the sample solution (Fig. 3b) and show absorbance spectra tailing to wavelengths corresponding to red colouration (>500 nm).

When analysed by LC–MS, with minimal fragmentation conditions, the peak labelled 9 (Fig. 7) gave *m*/*z* signals at both 695 and 425 in the negative ion mode, and also *m*/*z* signals at both 697 and 427 in the positive ion mode. As no adduct formation could explain the difference between these mass units, this suggested that peak 9 was most likely due to the co-elution of at least two different species. A compound with *m*/*z* of 695 (in the negative ion mode) can be achieved by intramolecular rearrangements of the dihydroxybenzene-linked (+)-catechin dimer (699 *m*/*z*, negative ion mode), with the loss of four protons. Similar intramolecular rearrangements have been observed for

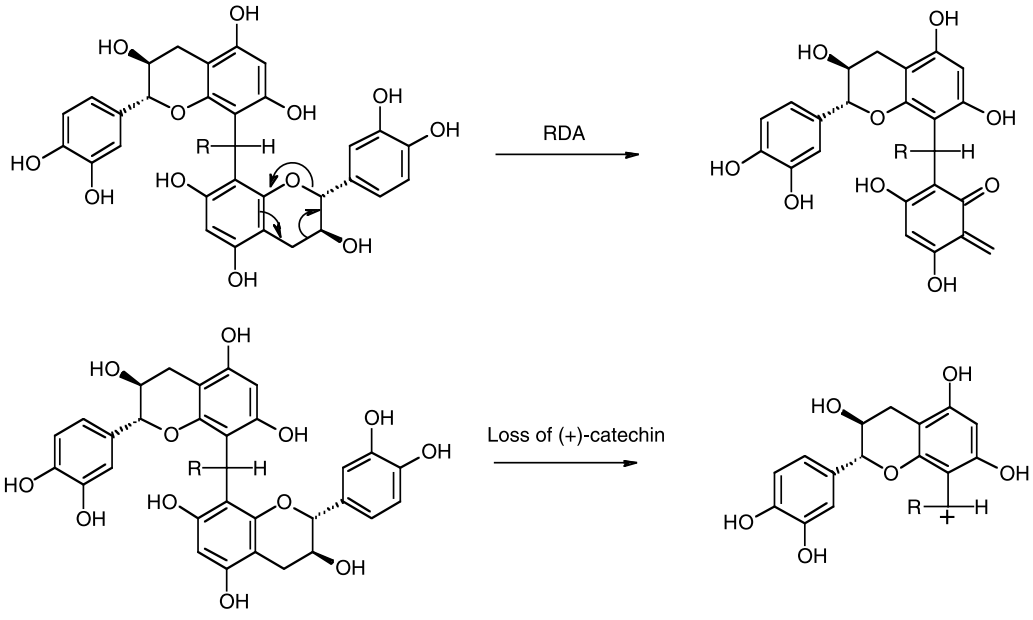



Fig. 6. The common fragmentation pattern of aldehyde-bridged flavonoid dimers (Laurie & Waterhouse, 2006; Pissarra et al., 2003; Saucier et al., 1997). RDA, retro-Diels alder degradation. When R = dihydroxybenzene, detection of the RDA fragment was at 549 and 547 in the positive and negative ion modes, respectively, and detection of the catechin-cleaved fragment was at 411 and 409 in the positive and negative ion modes, respectively.

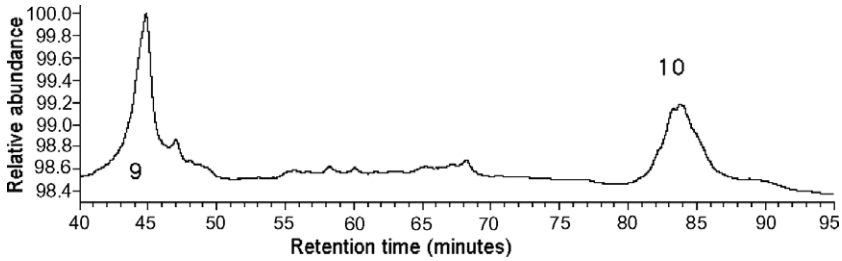



Fig. 7. The extracted chromatogram (440 nm) for the model white containing both dihydroxybenzaldehyde and (+)-catechin after 14 days of reaction.

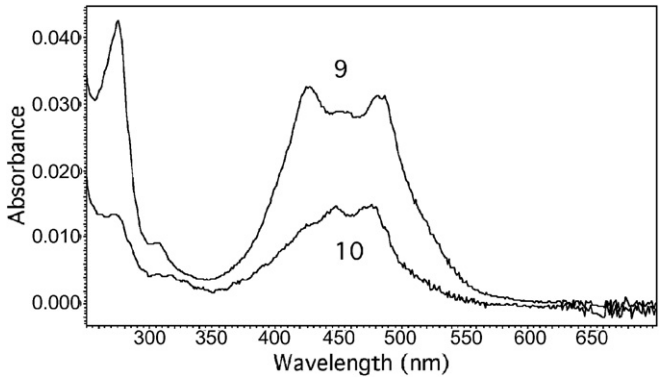



Fig. 8. The extracted spectra corresponding to peaks 9 and 10 (Fig. 7).

colourless (+)-catechin dimmers, to provide coloured compounds (Guyot et al., 1996). The ion at 425 *m*/*z* (negative ion mode) can result from the reaction of dihydroxybenzaldehyde (140 amu) and one (+)-catechin unit (290 amu) with subsequent intramolecular rearrangement and loss of four protons. Variations of the chromatographic conditions were conducted in an attempt to resolve peak 9 into separate components, however, these experiments proved unsuccessful.

The second group of peaks, labelled as 10 in Fig. 7, had a single *m*/*z* signal at 407 in the negative ion mode, and 409 in the positive ion mode. These data are consistent with the reaction of dihydroxybenzaldehyde with (+)-catechin in a manner similar to that reported for sinapaldehyde and (+)-catechin (de Freitas, Sousa, Silva, Santos-Buelga, & Mateus, 2004), the latter system providing a brick-red ($\lambda_{max} = 500$ nm) catechin-pyrylium-derived pigment. Fig. 9 shows an example of one isomeric form of a product that would be generated from the reaction of dihydroxybenzaldehyde and (+)-catechin in a similar manner as

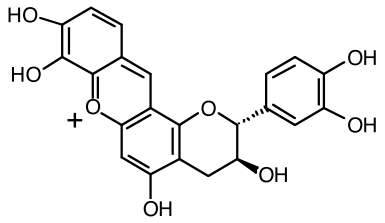



Fig. 9. The suggested structure for a compound responsible for peak 10 in Fig. 7.

reported for sinapaldehyde and (+)-catechin. Although only a single fragmentation peak was detected (Table 1), generated via a retro-Diels Alder degradation, this is consistent with the mode of fragmentation observed for the sinapaldehyde-derived pigment (Sousa, Mateus, Perez-Alonso, Santos-Buelga, & de Freitas, 2005).

The production of the dihydroxybenzaldehyde-derived pigments (Fig. 7) was relatively slow compared to the pigments generated from the reaction of (+)-catechin with glyoxylic acid. For example, to provide a similar level of 440 nm absorbance intensity in a wine-like solution of (+)-catechin (0.5 mM), the concentration of dihydroxybenzaldehyde (10 mM) needed to be 40-fold that of glyoxylic acid (0.25 mM). In part, this was because the main product generated from the dihyroxybenzaldehyde and (+)-catechin reaction was a colourless product while glyoxylic acid and (+)-catechin readily formed a yellow xanthylium cation (Es-Safi et al., 1999b). Also, the reaction of dihydroxybenzaldehyde with (+)-catechin was much slower than the reaction of glyoxylic acid with (+)-catechin. After reaction at 14 days at 45 °C, the rate of (+)-catechin loss was $2.2 \times 10^{-5}$ M/day and $1.2 \times 10^{-5}$ M/day for the 10 mM dihydroxybenzaldehyde and 0.25 mM glyoxylic acid samples, respectively. In other words, the 40-fold excess of dihydroxybenzaldehyde compared to glyoxylic acid only increased the rate of (+)-catechin loss by less than a factor of 2.

Previous studies have shown that, during the oxidation of phenolic compounds in wine or wine-like solutions, aldehydes are produced that stem from the coupled oxidation of non-phenolic components, such as ethanol, glycerol or tartaric acid (Fulcrand et al., 1997; Laurie & Waterhouse, 2006; Timberlake & Bridle, 1976). This current study shows that the oxidation of a wine-like solution led to the production of an aldehyde, dihydroxybenzaldehyde, which stems directly from a phenolic compound, caffeic acid. This work also identifies a dihydroxybenzenemethine-linked (+)-catechin dimer formed from (+)-catechin and dihydroxybenzaldehyde and shows the presence of several yellow/red pigments, also formed simultaneously. The production of these pigments is consistent with the observation by a recent study (George et al., 2006) that the presence of caffeic acid in a model wine system, also containing (+)-catechin and iron, led to the eventual production of a red tint in the samples.

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