Characterization of the Products of Nonenzymic Autoxidative Phenolic Reactions in a Caffeic Acid Model System

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Products of nonenzymic autoxidation of caffeic acid were analyzed and isolated by analytical and preparative HPLC with diode array detection. By ^{1}H and ^{13}C nuclear magnetic resonance spectrometry, UV-visible spectrophotometry, and fast atom bombardment mass spectrometry, these oxidation products are shown to be specific (structures given) dimers and trimers of caffeic acid formed by reactions involving the side chain of at least one of the caffeic acid units. Compounds analogous to natural lignans and neolignans result, with dioxane, furan, or cyclohexene bridges between the caffeic units. The large effect of pH on the production rate indicates the involvement of phenolate anions in the formation of these products.

INTRODUCTION

The oxidative browning of polyphenols in food systems is well-known since this usually undesirable reaction results in a loss of nutritional and aesthetic value in food products from plants (Hurrel et al., 1984). This loss or its prevention costs the agricultural and food industries many millions of dollars each year. A loss in available food and nutritional value is especially serious in underprivileged countries. Two types of phenolic browning reactions are involved. Enzymic oxidation is the more important reaction in fresh fruits and juices and early in food processing, when polyphenol oxidase is present (Coseteng and Lee, 1987; Matheis, 1987). In processed foods with the enzyme removed or inactivated, substantial nonenzymic autoxidation can still take place. It can cause much damage in the presence of oxygen within only a few hours at pH 8.0 or after prolonged storage under acidic conditions even at low temperatures (Cilliers and Singleton, 1989). Autoxidation can also be catalyzed by metal ions naturally present or picked up from processing equipment and can even mimic enzyme action (Pandell, 1983).

The oxidative coupling of phenols is well documented with *o*- and *p*-dihydroxyphenols (Cha et al., 1986; Cilliers, 1989; Ghosh and Misra, 1987; Singleton, 1987). It involves the formation of a reactive electrophilic quinone intermediate that can readily undergo attack by nucleophiles. The presence of nucleophiles, e.g., thiols like glutathione, leads to the formation of addition products on different positions of the benzene ring. The 2-position of the benzene ring in caffeic acid is the most electrophilic, and nucleophilic addition occurs preferentially here (Cheynier et al., 1986). Upon further oxidation of this addition product by laccase to form its quinone, a second addition occurs in the 5-position (Salgues et al., 1986). We thus expected to find oxidation products that involved mainly ether and carbon to carbon linkages involving the benzene ring (Lundquist and Kristersson, 1985).

An extension of our earlier results (Cilliers and Singleton, 1989), this research is aimed at understanding the chemical reactions involved in autoxidation of a specific phenol common to foods. Browning occurs in the early stages of oxidation and correlates with the oxidative disappearance of caffeic acid. This early browning suggests that its formation is not dependent on the formation of large molecules with extended conjugation but rather the formation of small products, i.e., dimers and trimers, in which enough conjugation is present to extend into the visible region (Cilliers and Singleton, 1989). Combined with the earlier paper, this research provides information on the mechanism of the nonenzymic oxidative reactions and the specific oxidation products that form. Minimizing or preventing the effects of these oxidation reactions depends on such new understanding. Since caffeic acid derivatives (caftaric and chlorogenic acids) are found in relatively high concentrations as normal metabolites in many plants and plant-derived foods, we focused on caffeic acid as a model. These compounds are all susceptible to both enzymic and nonenzymic oxidation in the presence of oxygen. (Cheynier and Van Hulst, 1988; Singleton, 1987).

MATERIALS AND METHODS

Preparation of Oxidation Products. Caffeic acid (10.0 g) from Fluka (Ronkonkoma, NY) was added to 100 mL of water and the pH adjusted to 8.5 with solid KOH to dissolve the phenol and to increase the rate of oxidation. Oxygen was bubbled through while the mixture was stirred for 6 h at room temperature on a magnetic stirrer. After the oxidation the pH was adjusted to pH 5 with concentrated HCl and concentrated by evaporation of the water at 40 °C under reduced pressure (~95 kPa) with a rotary evaporator, the oxidation products were redissolved in 20 mL of 50% methanol and the solution was filtered through a 0.45-μm filter.

Preparative Separation. For semipreparative HPLC separation a Waters 810 system was used (Waters, Milford, MA) with two Model 6000A pumps equipped with extended flow pump-heads, each capable of handling 20 mL of solvent/min. A Lambda-Max Model 481 detector equipped with a 0.21-cm flow cell was used at either 280 nm under analytical conditions or 360 nm under preparative conditions. For developing the proper separation conditions, a Dynamax C_{18} (Rainin Instrument Co. Inc., Woburn, MA) 25 cm × 0.46 cm i.d. scout column with 8 μm packing material was used. The injection volume was 25 μL with a flow rate of 1.6 mL/min and detection at 280 nm. For preparative separation inexpensive mobile phases consisting of 0.1% formic acid as mobile phase A and 80% methanol (certified ACS grade Fisher Scientific, Fair Lawn, NJ) containing 0.1% formic acid as mobile phase B were used. Scaling up was done with a Dynamax Macro C_{18} column (Rainin) 2.14 cm i.d. × 25 cm with a 2.14 cm i.d. × 5 cm guard column, also packed with the same 8 μm packing material. Starting with 30% mobile phase B, a linear gradient was run to 75% B in 35 min. After the column was washed with 100% B and equilibrated with 30% B

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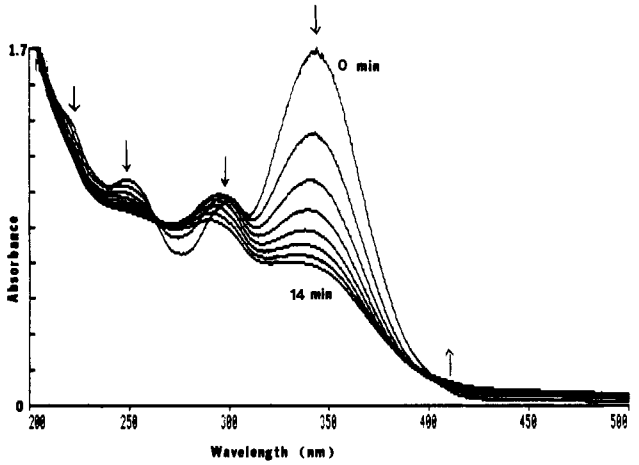

Figure 1. Oxidation of caffeic acid (1 mM) at pH 10.0 and room temperature. Stirring was done in the cuvette with no oxygen added. Spectra were taken at 2-min intervals. The first 14 min (7 spectra) are overlaid and increases and decreases at the different wavelengths indicated with arrows.

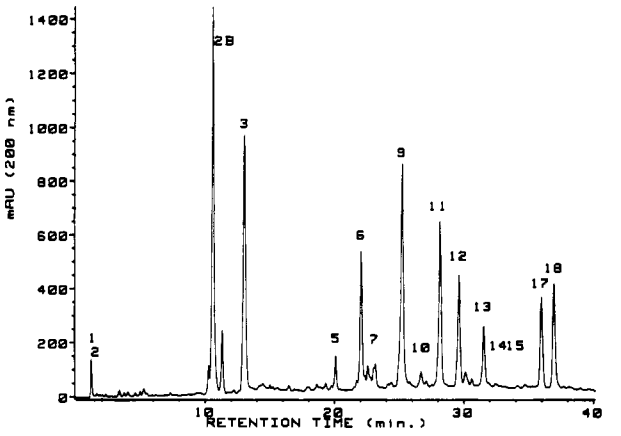

Figure 2. Oxidation of caffeic acid (0.56 M) at pH 8.5 and room temperature after 6 h in 100% oxygen. Analysis of a 20-μL sample of the oxidation product mixture to be used for preparative HPLC was done by C_{18} reversed-phase HPLC with mobile phase A, ammonium phosphate (0.05 M; pH 2.6) containing 0.001 M triethylamine, and mobile phase B, 80% acetonitrile in mobile phase A. Detection was at 200 nm. A linear gradient was applied from 0 to 40% mobile phase B in 30 min. Peak 3 is caffeic acid. The rest of the peaks are oxidation products.

for 10 min, the next sample was injected. A sample could be chromatographed every hour. Detection was done at 280 nm at a flow rate of 12 mL/min. The sample volume injected under these initial conditions was 500 μL. Fraction collection was done with an Isco (Lincoln, NE) programmable Foxy fraction collector connected to a Model 2150 peak separator from the same company. In order not to swamp the detector upon scaling up, detection was done at 360 nm, which is out of the optimum spectral range of these compounds. Up to 5 mL of sample could be manually injected by the sample loop on the Dynamax column. Once the system was optimized, fraction collection was based on peaks detected at 360 nm and separated by the peak separator with recycled collection of identical substance peaks.

Fractions obtained from the preparative system were then chromatographed on an analytical Hewlett-Packard (Santa Clara, CA) HPLC system as described by Cilliers and Singleton (1989) to verify the purity of each fraction. The diode array detector was also used to assess the purity of individual peaks by overlaying spectra. In this paper only spectra that proved to be pure with these criteria are presented. All fractions were refrigerated and kept in the dark. The matching fractions were pooled and concentrated under reduced pressure by rotary evaporator. Care was taken not to exceed 35 °C. Final drying was done in a vacuum oven (−92 kPa) at 35 °C for 24 h.

Spectrophotometry. The oxidation of caffeic acid was followed with a Perkin-Elmer (Norwalk, CT) diode array spectrophotometer. Caffeic acid (1 mM) was oxidized at pH 10.0 with stirring in the cuvette at room temperature. Spectra were taken at 2-min intervals for 30 min. Oxygen was not replenished.

Mass Spectrometry. A ZAB-HS-2F (VG Analytical, Wythenshawe, U.K.) with ionization FAB (Xe°, 8 keV, 1-mA beam current) and accelerating potential of 8 kV and resolution (M/M10% valley) of 1000 was used. The FAB matrix used was 2-nitrophenyl octyl ether (for TMS derivatives) and dithiothreitol/dithioerythritol (3:1 v/v) (for methyl esters and underivatized products). Methyl esters were made of the carboxylic acid groups present in oxidation product peaks designated 2b, 6, 9, and 18 by using BF_3 in methanol.

NMR. A Nicolet NT-360 spectrometer was used with ^1H frequency of 360.065 and ^{13}C frequency of 90.547. Proton spectra were accumulated with 16K data points over 8-kHz bandwidth and ^{13}C spectra with 16K data points over 25-kHz bandwidth with and without broadband proton noise decoupling.

RESULTS AND DISCUSSION

Caffeic acid oxidation was followed spectrophotometrically at pH 10.0. In Figure 1 spectra are overlayed to accentuate spectral changes. The decrease of absorption at wavelength 343 nm (bathochromic shift from 326 nm due to the high pH) indicates the involvement of the caffeic acid side chain in this reaction. However, since not all this absorption is lost during oxidation (even with all of the caffeic acid removed by oxidation), it has to be concluded that roughly 30% side chain ethylene conjugation still exists in this crude mixture of oxidized products. The increase in absorption at 420 nm indicates an increase in visible brown color. Absorption at wavelengths 249 and 296 nm decreased in this time period. The increase at 420 nm correlated well with the decreases at 343 (r = 0.982, n = 16, $P \leq 0.001$) and 249 nm (r = 0.993, n = 16, $P \leq 0.001$). These spectra provide only qualitative information since dissolved oxygen also became limiting soon after the pH was adjusted and this was not replenished except for normal diffusion.

These compounds were chromatographically separated to obtain quantitative structural, spectral, and physical information. The analytical chromatography has been discussed (Cilliers and Singleton, 1989). By preparative separation of these oxidation products, further characterization was possible.

A chromatogram of the oxidation products obtained for preparative chromatography after 6 h of oxidation at pH 8.5 is shown in Figure 2. The high concentration and excess of caffeic acid (560 mM) resulted in the production of all the oxidation products plus some additional ones which we had not encountered before at lower (10 mM) concentrations (Cilliers and Singleton, 1989). The latter included the formation of substantial concentrations of peaks 2b, 17, and 18, which were also isolated and analyzed. The HPLC peak numbering system is the same and data are comparable between these results and those previously published. Oxidation conditions were chosen for some caffeic acid (peak 3) to remain when the reaction was stopped by acidification to get the whole spectrum of oxidation products including initial, intermediate, and final products. Compounds that were isolated by preparative HPLC included peaks 2b, 6, 9, 11–13, 17, and 18 as shown in Figure 2.

After separation by HPLC, diode array detection gave UV–visible spectra from 200 to 400 nm. It was found that spectra of certain peaks were similar, suggesting similar chromophores. The overlayed spectra of caffeic acid and compounds 9, 11–13, 17, and 18 are shown in Figure 3. The similarity of these spectra indicates they might be

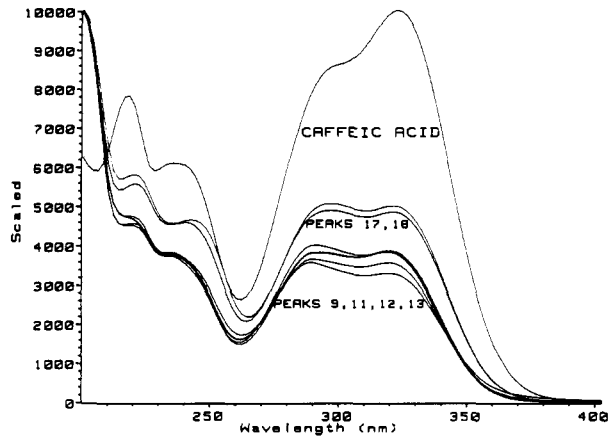

Figure 3. Normalized UV–visible diode array spectra obtained in mobile phase of caffeic acid, caffeicins A, B, C, and D (compounds corresponding to peaks 9, 11, 12, and 13 respectively), and peaks 17 and 18.

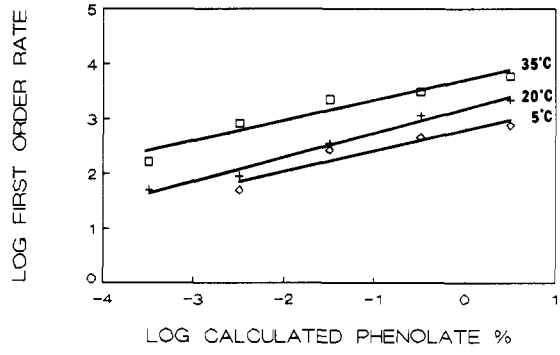

Figure 4. Effect of phenolate ion concentration on the first-order rate constant of caffeic acid oxidation at temperatures of 5, 20, and 35 °C in 100% oxygen.

structural analogues. Their differences (and similarities) with the caffeic acid spectrum indicate the possibility of modified caffeic acid oligomers. Since the spectra do not have a 40–80-nm bathochromic shift, it is believed they are not quinoid but are regenerated phenolic products. Some side chain conjugation still exists in the oxidized products to give the 290- and 326-nm peak maxima, although the 326-nm absorbance relative to that at 290 nm is decreased about half, indicating the involvement and loss of half of the side-chain conjugation during oxidation. An increase in absorbance can also be seen at 200 nm. A similar observation with respect to structural isomers was made with compounds **2b** and **4** (not shown). Their UV–visible spectra were so similar that we continued with compound **2b** to obtain structural information in the place of compound **4**, which we were unable to isolate in this experiment.

The rate of oxidation of caffeic acid is very pH dependent (Cilliers and Singleton, 1989). With a phenolic pK_a value of 9.5 for caffeic acid (based on the pK_a for catechol) the percent phenolate ions at the different pH values was calculated with the Henderson Hasselbalch equation (Lehninger, 1986). Without correction for differences due to temperature effects, a plot of log first-order rate constant for caffeic acid oxidation vs the log of the calculated phenolate ion concentration gave lines with correlation coefficients of 0.962, 0.992, and 0.950 for temperatures of 35, 20, and 5 °C, respectively. These linear relationships are shown in Figure 4 and indicate the involvement of phenolate ions in this oxidation. The phenolate is believed necessary to react directly by charge transfer with triplet oxygen to form a semiquinone which will then undergo further reaction. The oxygen triplet accepts the electron to become superoxide in this process. This avoids the spin-forbidden interaction of triplet oxygen with singlet phenol and does not require an activated form of oxygen.

Table I. ^{1}H NMR Data (in ppm) of Caffeicins A–D[a]

^{1}H no.	A	B	C	D	coupl[b]	const (J, Hz)
ring B						
2′	6.70	6.70	6.70	6.68	s	0
5′	6.70	6.70	6.70	6.68	s	0
6′	6.80	6.80	6.80	6.78	s	0
2	5.29	5.30	5.39*	5.43*	d	4, *3
3	5.10	5.08	5.10*	5.08*	d	4, *3
ring A						
8	7.30	7.33	7.29	7.38	d	1.8
6	7.21	7.21	7.22	7.22	dd	1.8 and 8.6
5	6.95	6.93	7.02	6.95	d	8.6
11	7.50	7.50	7.52	7.53	d	16
12	6.39	6.40	6.40	6.40	d	16

[a] All spectra were done in deuterated DMSO and are presented as two caffeic acid units A and B joined as a dimer. [b] s, singlet; d, doublet.

Investigation by ^{1}H NMR confirmed the UV–visible data that compounds **9** and **11–13** (obtained from the corresponding peaks indicated in the chromatogram) are structural analogues (isomers). We have shown them to be dimers and named them caffeicins A–D. These data are summarized in Table I. Small differences are observed for the chemical shifts of protons H-2 and H-3, indicating caffeicins A–D differ by steric effects. The presence of one *trans*-alkene (cinnamate) side chain is seen at 7.5 and 6.4 ppm as two doublets (J = 16 Hz), indicating the protons (H-11 and H-12) in the trans form. Loss of the second alkene side chain conjugation is indicated by the appearance of two doublets (H-2 and H-3) at 5.3 and 5.1 ppm. With J = 3 or 4 Hz, the positions of these two protons are indicated as cis in the fused ring. The couplings of all these side-chain protons were confirmed with decoupling experiments. The presence of two carboxylic acid groups was confirmed with ^{13}C NMR and FAB mass spectrometry by running these samples without derivatization and repeating it after the methyl esters were prepared. Mass spectrometric data show that compound 11 is a dimer with a molecular mass of 358. With FAB in the positive ion mode, the dimethyl ester $[M + H]^+$ peak at 387 was seen with fragments 355 $[M + H - CH_3OH]^+$, 323 $[M + H - 2CH_3OH]^+$, and 197 [M + H – caffeic acid methyl ester monomer]$^+$. The ^{1}H and ^{13}C NMR data for the caffeicins are given in Tables I and II, respectively. Assignments for all NMR data were made by calculation and the use of standard spectra given in Sadtler (some of these are 16962*C*, 16857*C*, 10407*C*, 4814*C*, 6806*C*, 2960*C*, 12801*M*, and 6930*M*). Values for similar compounds described in the literature were also compared, and these are mentioned in the discussion of each compound. It is thus possible that spectral lines in close proximity could be interchanged.

The MS data of compounds **2b** (caffeicin E), **6** (caffeicin F), caffeicin A, and compound 18 are summarized in Table III. The abundance of certain fragments corresponds with the structure of the dimer. This results in the absence or low abundance of some fragments of one dimer type compared to a larger amount in another. Some fragments were shared by all four different groups of dimers that were analyzed by MS.

Caffeicins A–D are deduced to be neolignans of the 2,3-dihydro-1,4-benzodioxin type (Chemical Abstracts Service nomenclature). Similar compounds occurring naturally have been isolated and characterized, although these specific compounds do not appear to have been reported. Examples of somewhat similar natural neolignans are

Table II. Comparison of ^{13}C Spectra of Caffeicins A and B (in ppm)[a]

C no.	A	B	signals[b]
13	171.6	170.0	s
7′	170.9	169.5	s
3′	146.2	145.2	s
10	145.4	144.9	d
9	145.8	144.7	s
11	145.4	144.6	s
4′	143.6	142.0	s
1′	129.4	128.1	s
6	128.7	127.3	s
7	124.3	123.0	d
6′	120.4	119.2	d
12	119.8	118.0	d
5	118.1	116.5	d
8	117.7	116.2	d
5′	117.2	115.8	d
2′	115.8	114.5	d
3	76.8	75.5	d
2	75.9	75.2	d

[a] Caffeicin A was analyzed on a 360-MHz instrument as described under Materials and Methods and caffeicin B on a 300-MHz General Electric NMR. [b] s, singlet; d, doublet.

Table III. FAB Mass Spectrometric Data[a]

[M + H]$^+$	E	F	A	18
521				6
387	20	47	52	4
355	10	11	23	
327	28	2	8	12
323	6	34	5	5
293	78	29	16	48
288			12	3
193	100	100	100	100
163	3	3	52	11
175	25	14	8	18
157	51	30		33
153	5	86	6	6
145	34	9	12	23
135			19	7
119	44	6	23	32

[a] Comparison of esterified caffeicins A, E, and F and compound 18. All numbers are expressed in terms of m/e 193, which is the base peak in the mass range 110–521.

Americanin A (Antus et al., 1986; Woo et al., 1978) and eusiderin (Cavalcante et al., 1985; Fernandes et al., 1980) and of coumarinolignoids in which the cinnamate side chain is lactonized are daphneticin (Lin-gen et al., 1983) aquillochin (Bhandari et al., 1982), propacin (Zoghbi et al., 1981) and cleomiscosin (Arnoldi et al., 1984). These naturally occurring compounds usually seem to have the protons in the dioxane ring in the trans form (^1H NMR; J = 8 Hz) with the exception of eusiderin C and D (Fernandes, 1980). Caffeicins A–D are in the cis form (J = 3 or 4 Hz). The stereospecific synthesis of 2,3-disubstituted 1,4-benzodioxins is described by Arnoldi et al. (1986), Proietti et al. (1981), and Su et al. (1977).

Several different dimers formed from caffeic acid oxidation in a model system. These reactions specifically involved the side chain. This is not uncommon and has been observed with the dimerization and polymerization of styrene (Gotoh et al., 1981). Caffeicins A (peak 9), B (peak 11), C (peak 12), and D (peak 13) have the four structures *rel*-(2*S*,3*S*)- and *rel*-(2*R*,3*R*)-6-(2-carboxyethenyl)-3-(3′,4′-dihydroxyphenyl)-2-carboxy-1,4-benzodioxin and *rel*-(2*S*,3*S*)- and *rel*-(2*R*,3*R*)-7-(2-carboxyethenyl)-3-(3′,4′-dihydroxyphenyl)-2-carboxy-1,4-benzodioxin as shown in Figure 5. The specific isomers involved have not been related to the specific HPLC peaks. On the basis of the coupling constants of 3 and 4 Hz for H-2 and H-3, they are formed in two sets in the cis form. The four different

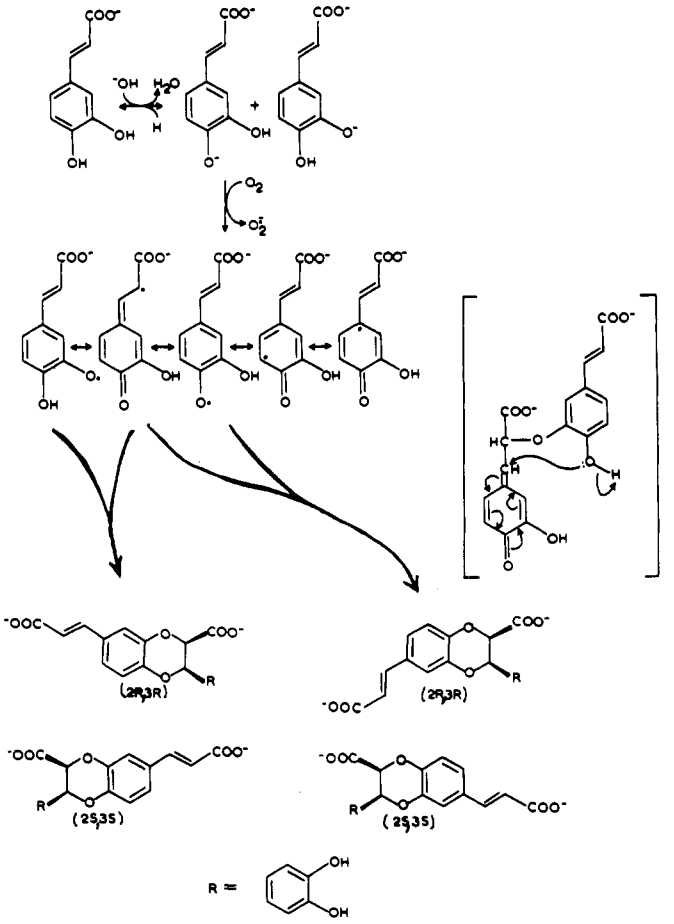

Figure 5. Postulated mechanism for the formation of caffeicins A–D. After phenolate ion formation takes place, reaction with oxygen produces the semiquinones which can couple to form the different structural isomers.

isomers are thus accounted for by formation from combining with the side chain of either first the 3- or the 4-hydroxyl group which would give the 6- and 7-(2-propenoic acid) isomers. Combination could then occur from either the bottom (2*S*,3*S*) or the top (2*R*,3*R*) of the side chain to give the remaining two isomers. The cis form is preferred since the bulky groups are all in the equatorial position with the dioxane ring in the boat form.

Compounds 17 and 18 (corresponding to peaks 17 and 18; Figure 2) are postulated as monodecarboxylated trimers (Cilliers, 1989) involving three caffeic acid units. ^1H NMR spectral similarities between these two compounds suggest they are structural analogues. In addition, ^1H NMR indicates the addition of a caffeic acid unit to a caffeicin (A, B, C, or D) with loss of a carboxylic acid group. The presence of only two carboxylic acid functions is confirmed with ^{13}C NMR and mass spectrometry (molecular ion of the methyl ester is 521; Table III). UV–visible spectra (Figure 3) agree with these findings, particularly regarding the loss of one conjugated side chain as linking occurs, thus raising the 280-nm absorbance relative to that at 320 nm.

Compound 6 (caffeicin F) is a further substituted benzofuranoid neolignan [Figure 6(middle)] of the 2,3-dihydrobenzofuran type (Chemical Abstracts Service nomenclature). Examples of natural benzofuranoid compounds that have been isolated and identified are chrysophyllins A and B (Ferreira et al., 1982) and some other unusual ones from *Licaria chrysophylla* (Lopes et al., 1986). NMR data for caffeicin F are as follows: ^1H NMR 7.50 (d, J = 16.2 Hz, Ar CH=), 7.13 (s, 1 Ar H, H-4 or H-6), 7.03 (s,

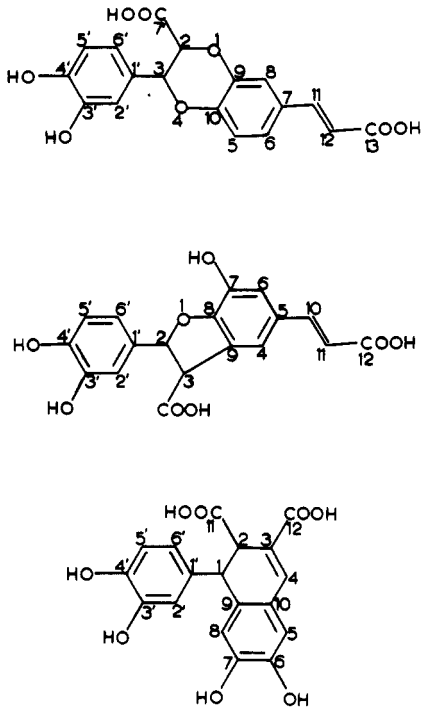

Figure 6. Structures of (top) caffeicins A–D as 2,3-dihydro-1,4-benzodioxan-type neolignans (two of these isomers have the 2-propenoic acid side chain in the 6-position and the other two in the 7-position); (middle) caffeicin F as a substituted 2,3-dihydrobenzofuran-type neolignan; and (bottom) caffeicin E as a substituted 1,2-dihydronaphthalene-type compound.

1 Ar H, H-4 or H-6), 6.75 (s, 1 Ar H, H-2′), 6.73 (d, 1 Ar H, overlap H-5′), 6.68 (dd, J = 8.1 and 1.8 Hz, 1 Ar H, ortho, meta, H-6′), 6.20 (d, J = 16.2 Hz, =CH), 5.82 (d, J = 7.2 Hz, H-2), 4.40 (d, J = 7.2 Hz, H-3); ^{13}C NMR 174.9 (s, C-7′ or C-12), 172.0 (s, C-7′ or C-12), 149.5 (s, C-8), 147.1 (s, C-10), 145.2 (s, C-3′), 145.0 (s, C-4′), 140.9 (d, C-7), 132.8 (s, C-5), 129.1 (s, C-1′), 126.7 (s, C-9), 119.0 (d, C-6′), 118.9 (d, C-4), 117.5 (d, C-11), 117.2 (d, C-5′), 115.9 (d, C-2′), 115.3 (d, C-6), 87.7 (d, C-2), 55.8 (d, C-3).

Compound **2b** (caffeicin E) is a substituted 1,2-dihydronaphthalene-type [shown in Figure 6(bottom)] compound (Chemical Abstracts Service nomenclature). Some natural compounds of this type are also found, e.g., magnoshinin (Kadota et al., 1987). NMR data for caffeicin E are as follows: ^{1}H NMR 7.5 (s, =CH, H-4), 6.8 (s, Ar, H-5), 6.6 (d, J = 8.1 Hz, ortho coupling Ar, H-5′), 6.5 (s, Ar, H-8), 6.3 (d, J = 8.1 Hz, Ar, H-2′), 6.25 (dd, J = 8.1 and 1.8 Hz, ortho, meta coupling, Ar, H-6′), 4.3 (d, J = 1.8 Hz, H-1), 3.7 (d, J = 1.8 Hz, H-2), 3.2 (s, OCH_3); ^{13}C NMR 178.2 (s, COOH), 171.2 (s, COOH), 147.8 (s, C-3′), 144.8 (s, C-4′), 144.0 (s, C-6), 143.7 (s, C-7), 140.7 (d, C-4), 135.7 (s, C-3), 131.7 (s, C-1′), 124.8 (s, C-9), 122.8 (s, C-10), 121.0 (d, C-6′), 118.0 (d, C-5′), 117.2 (d, C-5), 117.0 (d, C-8), 116.6 (d, C-2′), 49.9 (q, OCH_3), 48.7 (d, C-2), 46.3 (d, C-1).

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LITERATURE CITED

Antus, S.; Seligmann, O.; Wagner, H. *Liebigs Ann. Chem.* **1986**, 647–654.

Arnoldi, A.; Arnone, A.; Merlini, L. Synthesis of the natural coumarinolignoids propacin and cleomiscosin A and B. An empirical spectroscopic method to distinguish regioisomers of natural benzodioxane lignoids. *Heterocycles* **1984**, *22*, 1537–1544.

Arnoldi, A.; Camarda, L.; Merlini, L. Synthesis and sweet taste of some 2-phenylbenzodioxanes. *J. Agric. Food Chem.* **1986**, *34*, 339–344.

Bhandari, P.; Pant, P.; Rastogi, R. P. Aquillochin, a coumarinolignan from *Aquilaria agallocha*. *Phytochemistry* **1982**, *21*, 2147–2149.

Cavalcante, S. H.; Yoshida, M.; Gottlieb, O. R. Neolignans from *Virola carinata* fruit. *Phytochemistry* **1985**, *24*, 1051–1055.

Cha, J. A.; Berry, K. B.; Lim, P. K. Aerobic coupling of aqueous phenols catalyzed by binuclear copper: ring substituent effect and the kinetics of the coupling of *o*-methylphenol. *AIChE J.* **1986**, *32*, 477–485.

Cheynier, V. F.; Van Hulst, M. J. W. Oxidation of *trans*-caftaric acid and 2-S-glutathionylcaftaric acid in model solutions. *J. Agric. Food Chem.* **1988**, *36*, 10–15.

Cheynier, V. F.; Trousdale, E. K.; Singleton, V. L.; Salgues, M. J.; Wylde, R. Characterization of 2-S-glutathionylcaftaric acid and its hydrolysis in relation to grape wines. *J. Agric. Food Chem.* **1986**, *34*, 217–221.

Cilliers, J. J. L. Nonenzymic autoxidative browning reactions of natural polyphenolic compounds with reference to foods and beverages. Ph.D. dissertation, University of California, Davis, 1989.

Cilliers, J. J. L.; Singleton, V. L. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. *J. Agric. Food Chem.* **1989**, *37*, 890–896.

Coseteng, M. Y.; Lee, C. Y. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci.* **1987**, *52*, 985–989.

Fernandes, J. B.; Ribeiro, M. N. D.; Gottlieb, O. R.; Gottlieb, H. E. Eusiderins and 1,3-diarylpropanes from Virola species. *Phytochemistry* **1980**, *19*, 1523–1525.

Ferreira, Z. S.; Roque, N. C.; Gottlieb, O. R.; Gottlieb, H. E. An unusual porosin type neolignan from *Licaria chrysophylla*. *Phytochemistry* **1982**, *21*, 2756–2758.

Ghosh, U.; Misra, T. N. Spectroscopic studies of photochemical reactions in organic solids: photodimerization of *o*-hydroxycinnamic acid. *Indian J. Phys.* **1987**, *61B*, 530–538.

Gotoh, T.; Yamamoto, M.; Nishijima, Y. Radiation-induced cationic dimerization and polymerization of styrene in methylene chloride solution. I. *J. Polym. Sci., Polym. Chem. Ed.* **1981**, *19*, 1047–1060.

Hurrell, R. F.; Finot, P. F. Nutritional consequences of the reactions between proteins and oxidized polyphenolic acids. *Adv. Exp. Med. Biol.* **1984**, *177*, 423–435.

Kadota, S.; Tsubono, K.; Makino, K.; Takeshita, M.; Kikuchi, T. Convenient synthesis of magnoshinin, an anti-inflammatory neolignan. *Tetrahedron Lett.* **1987**, *28*, 2857–2860.

Lehninger, A. L. *Principles of biochemistry*, 4th printing; Worth Publishers: New York, 1986; p 83.

Lin-gen, Z.; Seligmann, O.; Wagner, H. Daphneticin, a coumarinolignoid from *Daphne tangutica*. *Phytochemistry* **1983**, *22*, 617–619.

Lopes, M. N.; Da Silva, M. S.; Barbosa, J. M.; Ferreira, Z. S.; Yoshida, M.; Gottlieb, O. R. Unusual benzofuranoid neolignans from *Licaria chrysopylla*. *Phytochemistry* **1986**, *25*, 2609–2612.

Lundquist, K.; Kristersson, P. Exhaustive laccase-catalysed oxidation of a lignin model compound (vanillyl glycol) produces methanol and polymeric quinoid products. *Biochem. J.* **1985**, *229*, 277–279.

Matheis, G. Polyphenol oxidase and enzymic browning of potatoes (*Solanum tuberosum*).II. Enzymic browning and potato constituents. *Chem. Mikrobiol. Technol. Lebensm.* **1987**, *11*, 33–41.

Pandell, A. J. Mechanism of the Fe(III)-catalyzed peracetic acid oxidation of catechol. A biomimetic reaction for pyrocatechase. *J. Org. Chem.* **1983**, *48*, 3908–3912.

Proietti, G.; Corsano, S.; Castagnino, E. Stereospecific synthesis of 2,3-disubstituted 1,4-benzodioxans. *J. Heterocycl. Chem.* **1981**, *18*, 415–416.

Sadtler. *Standard NMR, Carbon-13 NMR Spectra*; Sadtler Research Laboratories: Philadelphia, PA, 1966–1984.

Salgues, M.; Cheynier, V.; Gunata, Z.; Wylde, R. Oxidation of grape juice 2-S-glutathionyl caffeoyl tartaric acid by *Botrytis cinerea* laccase and characterization of a new substance: 2,5-di-S-glutathionyl caffeoyl tartaric acid. *J. Food Sci.* **1986**, *51*, 1191–1194.

Singleton, V. L. Occurrence and reactions of hydroxycinnamoyl tartrates, the major phenols of white grape juices and wines. Presented at the 193rd National Meeting of the Div. Agric. Food American Chemical Society, Denver, CO, April 1987; Division of Agriculture and Food Chemistry, Abstract 43.

Su, T.; Schulz, J.; Hansel, R. *Chem. Ber.* **1977**, *110*, 3867–3873.

Woo, W. S.; Kang, S. S.; Wagner, H.; Chari, V. M. *Tetrahedron Lett.* **1978**, *35*, 3239–3242.

Zoghbi, M. D. B.; Roque, N. F.; Gottlieb, O. R. Propacin, a coumarinolignoid from *Protium opacum*. *Phytochemistry* **1981**, *20*, 180.

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