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Antioxidation Mechanism Studies of Caffeic Acid: Identification of Antioxidation Products of Methyl Caffeate from Lipid Oxidation

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As a part of a research project on the elucidation of the chain-breaking antioxidation mechanism of natural phenols in food components, caffeic acid, a polyphenolic acid widely distributed in edible plants, was investigated. The identification and time course analysis of the antioxidation reaction products from methyl caffeate were carried out in the ethyl linoleate oxidation system. The antioxidation reaction produced a quinone derivative of methyl caffeate as an antioxidation product during the initial stage, which was identified by ^{13}C NMR. The quinone, however, was not the final product, and a further reaction occurred to produce several new peroxides. The isolation and structure determination of the peroxides revealed that they had tricyclic structures, which consisted of ethyl linoleate, methyl caffeate, and molecular oxygen. On the basis of the formation pathway of these products, an antioxidation reaction mechanism of methyl caffeate, including the redox reaction of the caffeate and Diels−Alder reaction of the produced peroxides, was proposed.

KEYWORDS: Antioxidation mechanism; caffeic acid; methyl caffeate; *o*-quinone; Diels−Alder reaction

INTRODUCTION

Phenolic acids have received much attention as powerful antioxidants to protect against the oxidative deterioration of food components, such as polyunsaturated fatty acids (*1*). Recently, some phenolic acids were found to be absorbed by the human body through an active transport system in the intestine (*2*), and then they act as antioxidants to prevent oxidative stress. Caffeic acid is one of the most potently antioxidative phenolic acids (*3*). It has *o*-diphenol and hydroxycinnamoyl structures, both of which are responsible for its potent activity (*4, 5*). The antioxidation mechanism of polyphenol is briefly understood as a quinone formation from the dihydroxybenzene structure, because dihydroxylbenzene is much more oxidizable than biological materials (*6*). However, detailed insight about the chain-breaking antioxidation, which polyphenols may demonstrate, clarified that the antioxidation reaction is divided into two stages (*7*):

1, radical trapping stage

$$S{-}OO\bullet + AH \rightarrow S{-}OOH + A\bullet \quad (1)$$

2, radical termination stage

$$A\bullet + X\bullet \rightarrow \text{nonradical materials} \quad (2)$$

S is the substance for oxidation, S−OO• is the peroxyl radical of S, AH is the antioxidant, A• is the antioxidant radical, and X• is another radical species including the same species as the A radical. Although the first stage is a reversible process, the second stage is irreversible and must produce stable radical termination products. Structural information about these nonradical products would afford important contributions to the detailed antioxidation reaction mechanism (*8–12*). It should be noted that most of the caffeic acid in edible plants exists as esters of other plant constituents (*13*). For example, chlorogenic acid in various fruits, coffee beans, and potatoes and rosmarinic acid in the Labiatae herbs are famous antioxidative caffeic esters (*14–17*). Therefore, methyl caffeate (**1**) (**Figure 1**) was employed as the simplest caffeic ester, and the antioxidation reaction mechanism of the methyl caffeate was investigated.

MATERIALS AND METHODS

Chemicals and Instruments. Methyl caffeate was synthesized from commercially available caffeic acid (Nacalai Tesque, Kyoto, Japan). 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) was obtained from Wako Pure Chemicals (Osaka, Japan). Ethyl linoleate was obtained from Kanto Chemicals (Tokyo, Japan) and used after purification by silica gel 60 (Merck, Darmstadt, Germany) chromatography developed with 2.5% ethyl acetate in hexane. All solvents and other reagents were

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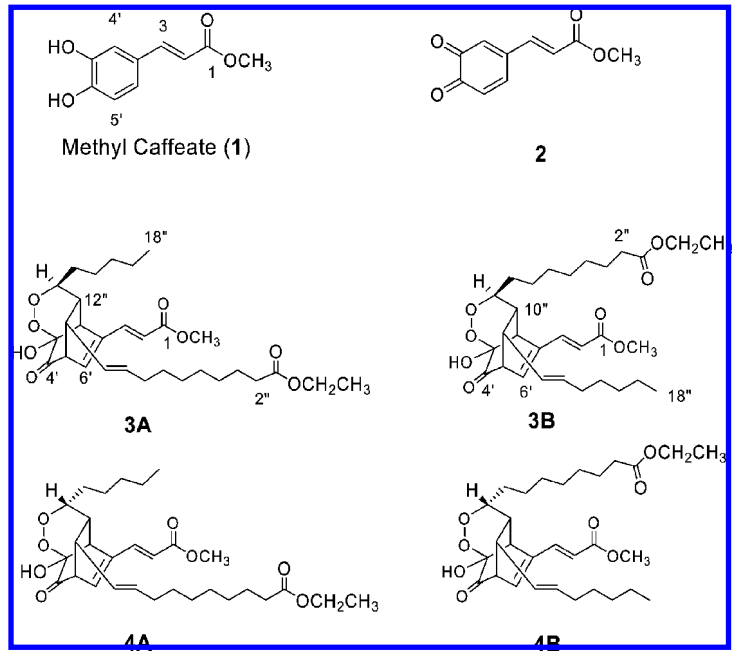

Figure 1. Chemical structures of methyl caffeate (**1**) and antioxidation reaction products **2**, **3A**, **3B**, **4A**, and **4B**. (Tentative position numbering is given on the basis of the numbering system of the starting caffeate and linoleate.)

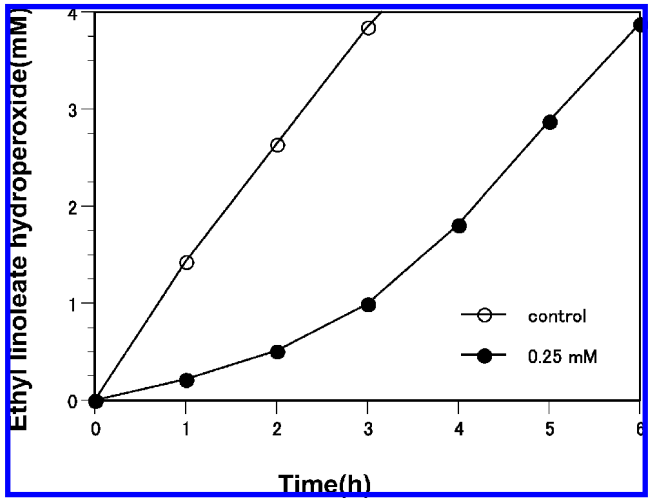

Figure 2. Antioxidant activity of methyl caffeate against AMVN-induced ethyl linoleate oxidation. (Concentration of reactants: 0.25 mM of methyl caffeate, 50 mM of ethyl linoleate, and 15 mM of AMVN.)

obtained from Nacalai Tesque. The 1D NMR spectra were measured with an EX-400 spectrometer (JEOL, Tokyo, Japan). The 2D NMR spectra were measured with a Unity Plus 500 spectrometer (Varian, Palo Alto, CA) using the manufacturer-supplied pulse sequences [^{1}H, ^{13}C, correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and total correlation spectroscopy (TOCSY)]. Variable-temperature NMR spectra were obtained by an ESP-400 spectrometer (JEOL). The mass spectra were measured with an LCT Premier ESI-TOFMS spectrometer (Waters Japan, Tokyo, Japan) in the positive ESI mode. A PU-980 high-pressure gradient system (Jasco, Tokyo, Japan) equipped with a photodiode array detector (SPD-M10AVP, Shimadzu, Kyoto, Japan) was used for the analytical HPLC. A PU-980 pump equipped with a UV-975 detector (Jasco) was used for the analysis of lipid hydroperoxides. An LC-6AD recycle system (Shimadzu) equipped with a UV-970 detector (JASCO) was used for preparative HPLC.

HPLC Analysis of Antioxidation Products from Methyl Caffeate and Linoleate Hydroperoxide in the Oxidation System. To 35 μL of ethyl linoleate in a 10 mL screw-capped tube were successively added 48 μL of a 10 mM methyl caffeate solution in CH_3CN, 100 μL of 0.3 M AMVN in CH_3CN, and 1.8 mL of CH_3CN. The control tube was also prepared in a similar manner with the addition of 48 μL of CH_3CN instead of the methyl caffeate solution. Both solutions were well stirred and then incubated at 37 °C in air for 6 h with shaking (100 times/min) by a water bath shaker. A 20 μL aliquot was removed from the solution at 1 h intervals and diluted with 380 μL of methanol. Ten microliters of the diluted solution was injected into the HPLC to analyze the ethyl linoleate hydroperoxides using a 150 × 4.6 mm i.d. ODS-A column (YMC, Tokyo, Japan) eluted with CH_3CN/H_2O (9:1, v/v) at 1.0 mL/min with detection at 234 nm. At the same intervals, an additional 10 μL aliquot was removed from the reaction mixture and injected into the HPLC to analyze the reaction products from the methyl caffeate using a 150 × 4.6 mm i.d. Cosmosil 5SL-II column (Nacalai Tesque) eluted with *n*-hexane (solvent A) and 1% AcOH in ethyl acetate (solvent B), using linear gradient from 15% of solvent B to 50% of solvent B for 25 min at 1.0 mL/min flow rate with detection at 280 nm.

Preparation and Identification of a Quinone Derivative of Methyl Caffeate (2). Methyl caffeate (10 mg) and DPPH (41 mg) were dissolved in acetone-d_6 (0.6 mL). After mixing well, the ^{13}C NMR of the solution was measured. The ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.0 (quinone CO), 180.6 (quinone CO), 166.4 (ester CO), 52.3 (CH_3O), and the other signals could not be assigned due to interference of the signals of diphenylpicrylhydrazine. A small part of the solution was withdrawn, diluted with CH_3CN, and injected into the HPLC under the analytical conditions mentioned above to identify the production of peak 2 [UV (EtOAc/hexane) λ_{max} 380, 295 nm].

Isolation Procedure for the Coupling Products (3A, 3B, 4A, and 4B). To a 100 mL straight vial (40 mm, diameter) were added 480 μL of 40 mM methyl caffeate (CH_3CN solution), 400 μL of 0.3 M AMVN (CH_3CN solution), 560 μL of ethyl linoleate, and 6.6 mL of CH_3CN. The vial was incubated at 60 °C in the dark with shaking (100 times/min) for 2 h. The combined reaction solution from 80 vials was cooled at −30 °C to freeze the ethyl linoleate, and the produced supernatant was collected. The precipitate was dissolved again in CH_3CN (60 mL) and then cooled at the same temperature to collect the next supernatant. This procedure was repeated once more, and all supernatants were combined and evaporated in vacuo. The residue was subjected to column chromatography on BW-350 silica gel (Fuji Silysia Chemical, Kasugai, Japan) developed with hexane and then hexane/ethyl acetate (4:1) to remove the residual lipid and AMVN. The fraction, which contained the coupling products, was evaporated and next purified by preparative HPLC on a 250 × 10 mm i.d. Cosmosil 5SL-II column eluted with hexane/ethyl acetate (85:15) at 6 mL/min with detection at

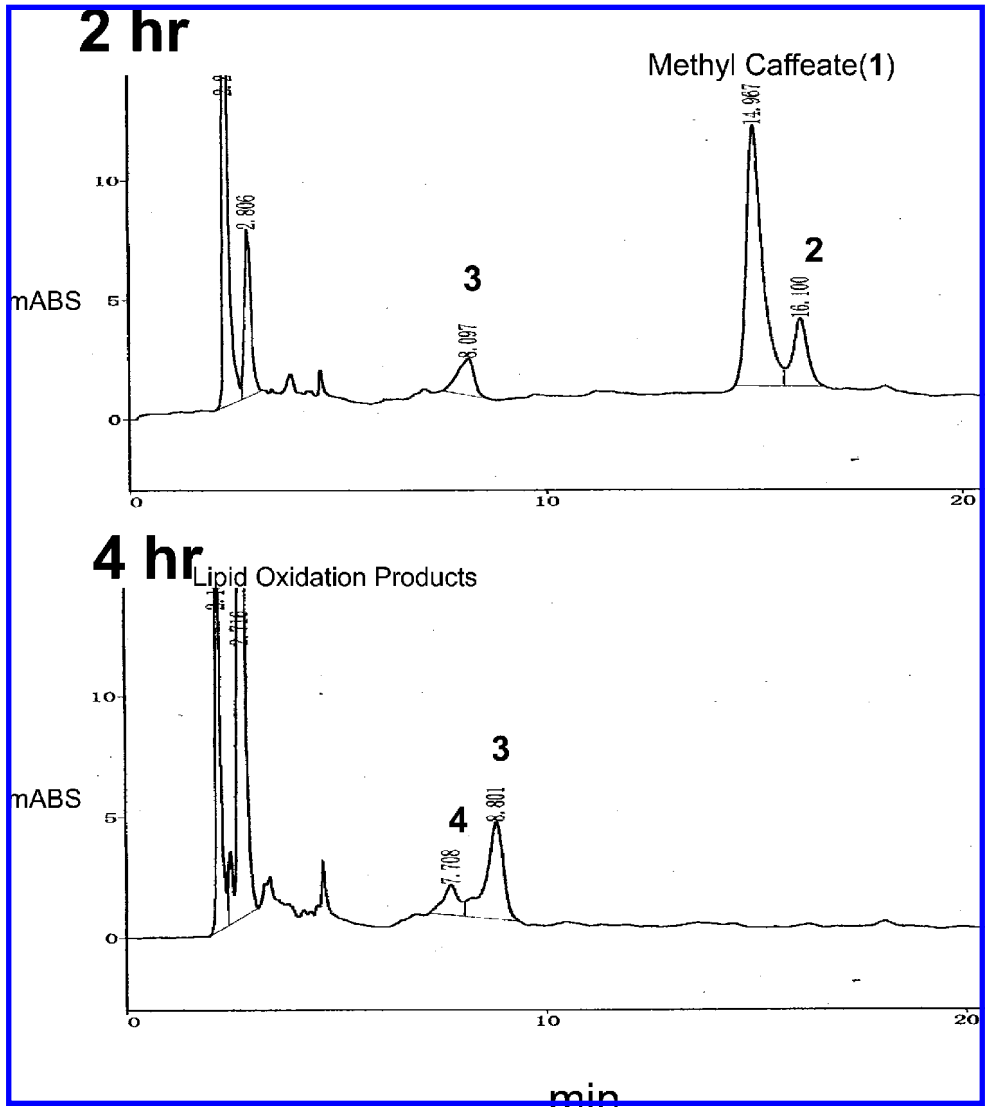

Figure 3. HPLC analytical data of antioxidation reaction mixture at 2 and 4 h.

280 nm to give peak compounds **3** (38 mg) and **4** (63 mg). The HPLC purification using a reversed phase column of peak 3 was carried out on a 250 × 20 mm i.d. Cosmosil 5C18-AR-II eluted with CH_3CN/H_2O (75:25) at 5.5 mL/min, with detection at 280 nm to give compounds **3A** and **3B** in 13.9 and 13.5 mg yields, respectively. Peak 4 was also purified by HPLC under the same conditions to give compounds **4A** and **4B** in 7.3 and 6.3 mg yields, respectively.

Analytical Data for the Coupling Products (3A, 3B, 4A, and 4B). ***3A***: ESI-TOFMS (*m*/*z*) $[M + H]^+$ calcd for $C_{30}H_{45}O_8$, 533.3114; found, 533.3088; IR (film) ν cm^{-1} 3379 (OH), 2928 (CH), 1718 (CO); ^1H NMR (500 MHz, $CDCl_3$) δ 6.15(d, J = 15.8 Hz, 1H, H-2), 7.40 (d, J = 15.8 Hz, 1H, H-3), 3.24 (dd, J = 3.3 and 2.0 Hz, 1H, H-2′), 3.37 (dd, J = 6.5 and 3.3 Hz, 1H, H-5′), 6.44 (dd, J = 6.5 and 2.0 Hz, 1H, H-6′), 2.28 (t, J = 7.5 Hz, 2H, H-2″), 1.61 (br q, J = 7.5 Hz, 2H, H-3″), 1.24−1.34 (complex, 14H, H-4″, H-5″, H-6″, H-7″, H-15″, H-16″, and H-17″), 1.95 (br q, J = 7.0 Hz, 2H, H-8″), 5.48 (dt, J = 15.0 and 7.0 Hz, 1H, H-9″), 5.22 (br dd, J = 15.0 and 9.0 Hz, 1H, H-10″), 2.90 (dt, J = 9.0 and 3.3 Hz, 1H, H-11″), 2.01 (dt, J = 3.3 and 1.0 Hz, 1H, H-12″), 4.37 (br t, J = 6.0 Hz, 1H, H-13″), 1.40 (complex, 1H, H-14″), 1.53 (m, 1H, H-14″), 0.88 (br t, J = 7.0 Hz, 3H, H-18″), 4.12 (q, J = 7.5 Hz, 2H, H-1‴), 1.25 (t, J = 7.5 Hz, 3H, H-2‴), 3.78 (s, 3H, 1-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$ δ 167.2 (C-1), 118.5 (C-2), 141.0 (C-3 or C-1′), 141.1 (C-3 or C-1′), 42.8 (C-2′), 92.7 (C-3′), 204.6 (C-4′), 54.6 (C-5′), 132.3 (C-6′), 173.9 (C-1″), 34.3 (C-2″), 24.9 (C-3″), 29.1, 29.1, 29.0, 28.9 (C-4″, C-5″, C-6″, and C-7″), 32.3 (C-8″), 132.6 (C-9″), 130.4 (C-10″), 40.5 (C-11″), 41.4 (C-12″), 85.1 (C-13″), 29.8 (C-14″), 25.4 (C-15″), 31.6 (C-16″), 22.4 (C-17″), 14.0 (C-18″), 60.2 (C-1‴), 14.3 (C-1‴), 51.9 (1-OCH_3).

3B: ESI-TOFMS (*m*/*z*) $[M + H]^+$ calcd for $C_{30}H_{45}O_8$, 533.3114; found, 533.3103; IR (film) ν cm^{-1} 3368 (OH), 2928 (CH), 1719 (CO); ^1H NMR (500 MHz, $CDCl_3$) δ 6.15 (d, J = 16.0 Hz, 1H, H-2), 7.39 (d, J = 16.0 Hz, 1H, H-3), 3.24 (dd, J = 3.1 and 2.0 Hz, 1H, H-2′), 3.38 (dd, J = 6.7 and 3.1 Hz, 1H, H-5′), 6.44 (dd, J = 6.7 and 2.0 Hz, 1H, H-6′), 2.28 (t, J = 7.5 Hz, 2H, H-2″), 1.60 (br q, J = 6.9 Hz, 2H, H-3″), 1.26−1.35 (complex, 10H, H-4″, H-5″, H-6″, H-16″, and H-17″), 1.40 (complex, 2H, H-7″), 1.54 (m, 2H, H-8″), 4.36 (br t, J = 6.3 Hz, 1H, H-9″), 2.00 (dt, J = 3.1 and 1.0 Hz, 1H, H-10″), 2.90 (dt, J = 8.3 and 3.1 Hz, 1H, H-11″), 5.22 (br dd, J=15.0 and 8.3 Hz, 1H, H-12″), 5.48 (dt, J = 15.0 and 7.0 Hz, H-13″), 1.95 (br q, J = 7.0 Hz, 1H, H-14″), 1.40 (complex, 1H, H-14″), 1.20 (m, 2H, H-15″), 0.88 (br t, J = 6.9 Hz, 3H, H-18″), 4.12 (q, J = 6.9 Hz, 2H, H-1‴), 1.25 (t, J = 6.9 Hz, 3H, H-2‴), 3.77 (s, 3H, 1-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2 (C-1), 118.5 (C-2), 141.1 (C-3 or C-1′), 141.0 (C-1′ or C-3), 42.8 (C-2′), 92.7 (C-3′), 204.6 (C-4′), 54.6 (C-5′), 132.3 (C-6′), 173.9 (C-1″), 34.3 (C-2″), 24.9 (C-3″), 29.2, 29.0, 28.9, 28.9 (C-4″, C-5″, C-6″, and C-15″), 25.7 (C-7″), 29.9 (C-8″), 85.0 (C-9″), 41.3 (C-10″), 40.5 (C-11″), 130.3 (C-12″), 132.8 (C-13″), 32.3 (C-14″), 31.3 (C-16″), 22.4 (C-17″), 14.1 (C-18″), 60.2 (C-1‴), 14.2 (C-1‴), 51.9 (1-OCH_3).

4A: ESI-TOFMS (*m*/*z*) $[M + H]^+$ calcd for $C_{30}H_{45}O_8$, 533.3114; found, 533.3165; IR (film) ν cm^{-1} 3356 (OH), 2928 (CH), 1732 (CO); ^1H NMR (500 MHz, $CDCl_3$) δ 6.16 (d, J = 15.8 Hz, 1H, H-2), 7.38 (d, J = 15.8 Hz, 1H, H-3), 3.45 (br t, J = 2.5 Hz, 1H, H-2′), 3.42 (dd, J = 6.5 and 2.5 Hz, 1H, H-5′), 6.47 (dd, J = 6.5 and 1.5 Hz, 1H, H-6′), 2.28 (t, J = 7.5 Hz, 2H, H-2″), 1.61 (q, J = 7.5 Hz, 2H, H-3″), 1.24−1.38 (complex, 16H, H-4″, H-5″, H-6″, H-7″, H-14″, H-15″,

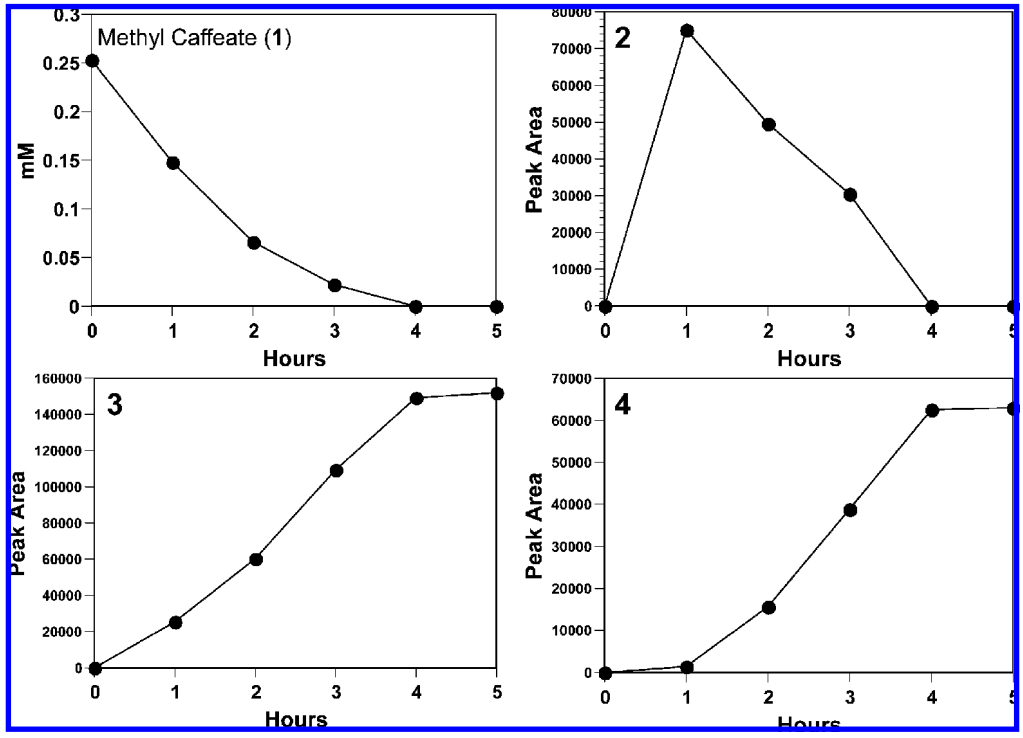

Figure 4. Time course analytical data of methyl caffeate and peaks 2−4 from the antioxidation reaction for 5 h.

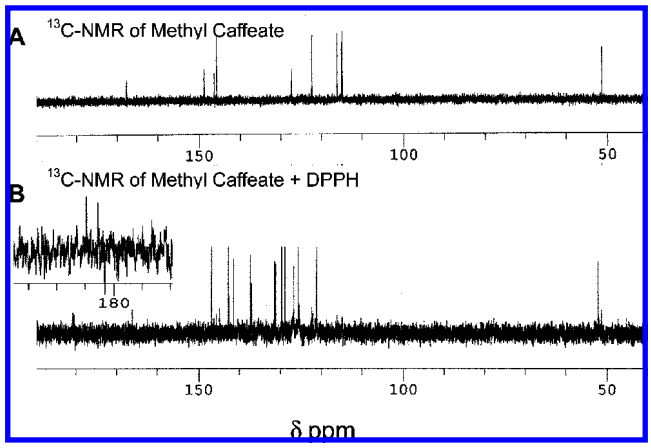

Figure 5. ^{13}C NMR data of (**A**) methyl caffeate and (**B**) DPPH reaction with methyl caffeate.

H-16″, and H-17″), 1.95 (br q, J = 7.0 Hz, 1H, H-8″), 5.49 (dt, J = 15.0 and 7.0 Hz, 1H, H-9″), 5.16 (dd, J = 15.0 and 8.5 Hz, 1H, H-10″), 2.89 (br d, J = 8.5 Hz, 1H, H-11″), 1.83 (br s, 1H, H-12″), 0.90 (t, J = 6.5 Hz, 3H, H-18″), 4.12 (q, J = 7.0 Hz, 2H, H-1‴), 1.25 (t, J = 7.0 Hz, 3H, H-2‴), 3.79 (s, 3H, 1-OCH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.1 (C-1), 118.7 (C-2), 141.0 (C-3 or C-1′), 140.9 (C-1′ or C-3), 37.1 (C-2′), 94.3 (C-3′), 54.9 (C-5′), 132.6 (C-6′), 173.9 (C-1″), 34.3 (C-2″), 24.9 (C-3″), 29.2, 29.1, 29.0, 28.8 (C-4″, C-5″, C-6″, and C-7″), 32.3 (C-8″),132.8 (C-9″), 130.2 (C-10″), 47.7 (C-11″), 42.8 (C-12″), 86.0 (C-13″), 30.2 (C-14″), 25.3 (C-15″), 31.5 (C-16″), 22.5 (C-17″), 14.0 (C-18″), 60.2 (C-1‴), 14.3 (C-2‴), 51.9 (1-OCH$_3$).

4B: ESI-TOFMS (m/z) [M + H]$^+$ calcd for $C_{30}H_{45}O_8$, 533.3114; found, 533.3145; IR (film) ν cm^{-1} 3363 (OH), 2927 (CH), 1734 (CO); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.15 (d, J = 15.8 Hz, 1H, H-2), 7.38 (d, J = 15.8 Hz, 1H, H-3), 3.44 (br t, J = 2.5 Hz, 1H, H-2′), 3.42 (dd, J = 6.5 and 2.5 Hz, 1H, H-5′), 6.46 (dd, J = 6.5 and 2.5 Hz, 1H, H-6′), 2.29 (t, J = 7.5 Hz, 2H, H-2″), 1.62 (br t, J = 7.0 Hz, 2H, H-3″), 1.21−1.31 (complex, 12H, H-4″, H-5″, H-6″, H-15″, H-16″, and H-17″), 1.32 (m, 2H, H-7″), 1.53 (m, 2H, H-8″), 1.83 (br s, 1H, H-10″), 2.89 (br d, J = 8.8 Hz, 1H, H-11″), 5.16 (dd, J = 15.0 and 8.8 Hz, 1H, H-12″), 5.49 (dt, J = 15.0 and 7.0 Hz, 1H, H-13″), 1.95 (q, J = 7.0 Hz, 2H, H-14″), 0.88 (t, J = 6.9 Hz, 3H, H-18″), 4.12 (q, J = 7.0 Hz, 2H, H-1‴), 1.25 (t, J = 6.9 Hz, 2H, H-2‴), 3.78 (s, 3H, 1-OCH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.1 (C-1), 118.7 (C-2), 141.9 (C-3 or C-1′), 140.0 (C-1′ or C-3), 37.2 (C-2′), 94.0 (C-3′), 54.9 (C-5′), 132.7 (C-6′), 173.9 (C-1″), 34.3 (C-2″), 24.9 (C-3″), 29.2, 29.1, 29.0, 28.8 (C-4″, C-5″, C-6″, and C-15″), 25.5 (C-7″), 30.3 (C-8″), 85.8 (C-9″), 42.8 (C-10″), 47.7 (C-11″), 130.0 (C-12″), 132.9 (C-13″), 32.3 (C-14″), 31.3 (C-16″), 22.5 (C-17″), 14.1 (C-18″), 60.2 (C-1‴), 14.3 (C-2‴), 51.9 (1-OCH$_3$).

RESULTS AND DISCUSSION

Antioxidation Reaction of Methyl Caffeate. The antioxidant reaction conditions were designed by using freshly purified ethyl linoleate (50 mM) as the oxidation substrate and AMVN (15 mM) as the radical oxidation initiator. The antioxidant activity of 0.25 mM methyl caffeate is shown in **Figure 2**. Methyl caffeate shows a very strong antioxidant activty for the initial 2 h; then the activity potency gradually decreased, and from 4 h, no activity was observed. **Figure 3** shows the HPLC analysis of the reaction mixture at 2 and 4 h. For the 2 h data, new peaks at retention times of 16 min (peak 2) and 8 min (peak 3) were observed besides the peak of methyl caffeate (**1**) at 15 min. On the other hand, the methyl caffeate peak and peak 2 disappeared and peak 4 was observed in the 4 h data. A time course analysis of peaks 2−4 and the methyl caffeate peak (**1**) was carried out, and these data are shown in **Figure 4**. The concentration of methyl caffeate continuously decreased during the 4 h antioxidation period. Peaks 3 and 4 continuously increased during this period; however, peak 2 increased for the initial 1 h and then decreased up to 4 h, suggesting that the product corresponding to peak 2 was an intermediate for the other antioxidation products and the peak 3 and 4 compounds were stable antioxidation reaction products.

Identification of Peak 2 Compound. To identify the produced peak compounds, we attempted the isolation of each peak compound from the reaction mixture. Unfortunately, the isolation of the peak 2 compound failed due to its instability. The photodiode array detection of peak 2 gave a bathochromic shifted UV spectrum (λ_{max} 380 nm), which indicated that **2** was

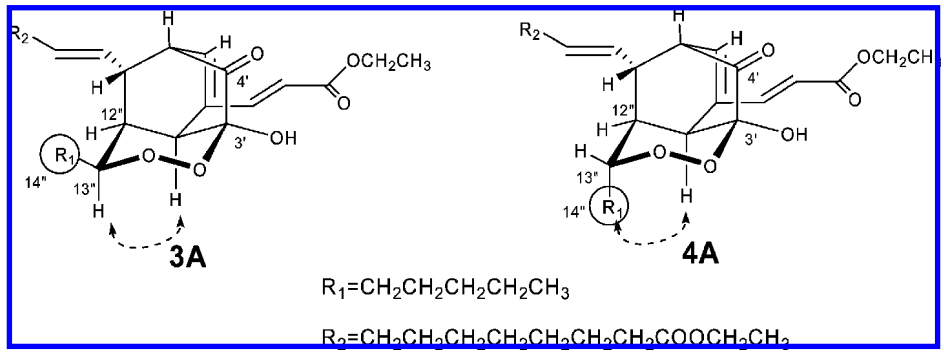

Figure 6. Stereostructures around dioxacyclohexane ring of **3A** and **3B**. (Dashed arrows indicate NOE correlations observed in NOESY.)

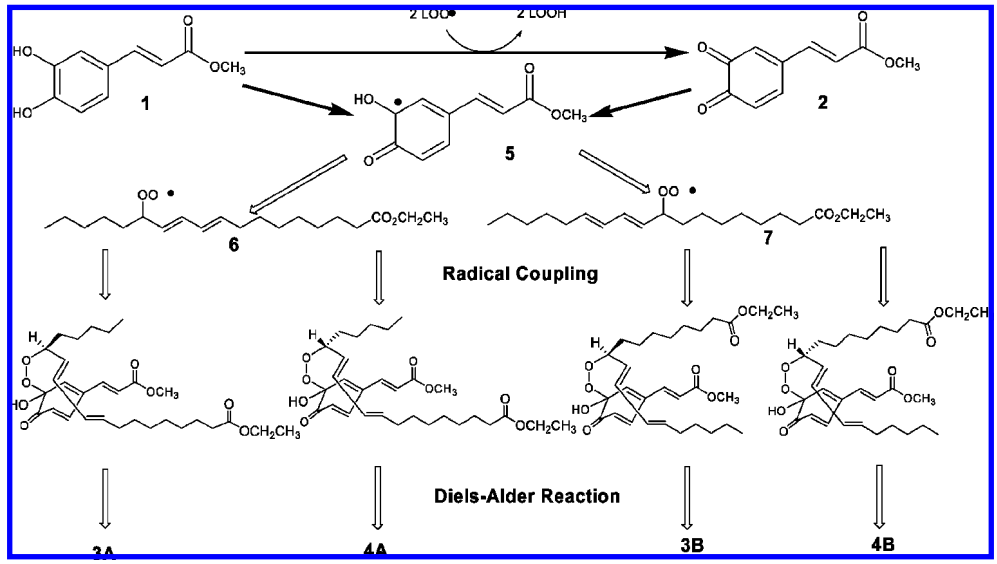

Figure 7. Scheme for the proposed mechanism of antioxidation reaction of methyl caffeate in ethyl linoleate.

a quinone derivative of methyl caffeate. Sawai and co-workers (*18, 19*) reported the identification of the quinone derivatives of several polyphenols, which were produced by DPPH oxidation, using ^{13}C NMR without purification. The ^{13}C NMR spectrum of the DPPH-treated methyl caffeate, which was obtained by Sawai's procedure, revealed two quinone carbonyl signals at 181.0 and 180.6 ppm (**Figure 5**). This DPPH oxidation product was identical to the peak 2 compound by HPLC analysis. Therefore, the peak 2 compound should be the *o*-quinone of methyl caffeate (**2**) as depicted in **Figure 1**.

Identification of Peak Compounds 3 and 4. To clarify the peak 3 and 4 compounds, we isolated them and determined their chemical structures. Peaks 3 and 4 were not observed in the reaction without the lipid; therefore, these compounds might be coupling products with ethyl linoleate, not dimeric products previously observed during the oxidation of caffeic acid (*20, 21*). The antioxidation reaction of methyl caffeate in the presence of a large amount of ethyl linoleate was carried out, and the peak 3 and 4 compounds were isolated from the reaction mixture. The final purification of peaks 3 and 4 using reversed phase HPLC afforded compounds **3A** and **3B** from peak 3 and compounds **4A** and **4B** from peak 4.

Compounds **3A** and **3B** were isolated as colorless oils. Their molecular formulas were estimated to both be $C_{30}H_{44}O_8$ from the ESI-MS results. The molecular formula indicated that **3A** and **3B** were oxidative coupling products consisting of methyl caffeate, ethyl linoleate, and molecular oxygen. In the ^1H NMR of **3A** and **3B**, both signal sets due to the original methyl caffeate and ethyl linoleate were observed; however, several signals assignable to the aromatic part of methyl caffeate and the olefinic part of ethyl linoleate had disappeared, indicating that the double bond of ethyl linoleate reacted with the aromatic part of methyl caffeate. These NMR data were very similar to those of the tricyclic coupling product of methyl ferulate and ethyl linoleate peroxide, which was previously reported as an antioxidation product from methyl ferulate (*12*). The structures of **3A** and **3B** were confirmed by the 2D NMR techniques (COSY, NOESY, HMQC, and HMBC). The distinction between the structures of **3A** and **3B** was carried out by TOCSY correlations (H-13″−H-18″ and H-2″−H-8″ in **3A**, H-2″−H-9″ and H-18″−H-14″ in **3B**) to determine the structures **3A** and **3B** as depicted in **Figure 1**.

Compound **4A** was also isolated as a colorless oil, and its molecular formula was determined to be $C_{30}H_{44}O_8$ from the ESI-MS. All of the spectroscopic data were very similar to those of **3A**, indicating that **4A** was one of the stereoisomers of **3A**. Very interestingly, a carbon signal (C-4′) and a proton signal (H-13″) disappeared and four carbon signals (C-3′, C-12″, C-13″, and C-14″) were broadened in the NMR spectra. We presumed that various conformational isomers existed in **4A** and exchange between the conformers hid and broadened the NMR signals. The NMR measurement of **4A** at elevated temperature failed because **4A** decomposed at 80 °C; however, in the low-temperature experiment (−50 °C), several conformers of **4A** were observed in its ^1H NMR and the carbonyl signal at the 4′-position of one of the conformers was observed at 205.5 ppm in its ^{13}C NMR. The NOE correlations (H-2′−H-14″), which were observed in the NOESY of **4A**, indicated that a bridgehead proton (H-2′) and an alkyl group at the 13″-position should have the same orientation as illustrated on the right side of

Figure 6. As shown in **Figure 6**, the axial alkyl group at the 13″-position labilized the chair conformation of the dioxacyclohexane ring and generated several conformers that interfered with the NMR signal assignment of **4A**. Compound **4B** has the same molecular formula as **4A** and spectroscopic data very similar to those of **4A**. Only the TOCSY data were different from those of **4A**. In the TOCSY spectra of **4A** and **4B**, correlations were observed from H-2″ to H-8″ and H-9″ in **4A** and from H-2″ to H-8″ and H-10″ in **4B**. These data indicated that **4A** and **4B** have the structures **4A** and **4B** as depicted in **Figure 1**.

Proposed Antioxidation Reaction Mechanism of Methyl Caffeate in Linoleate. Potent antioxidatively active polyphenols, which bear the ortho- or para-substituted diphenol structure, have been believed to afford their quinone derivatives as the antioxidation products because the diphenol structure is easily oxidizable to a quinone. In this investigation, we found that the quinone is not the final antioxidation product of methyl caffeate. From our elucidation of the chemical structures of the five compounds including the *o*-quinone of methyl caffeate, we propose the antioxidation reaction mechanism of methyl caffeate in ethyl linoleate as illustrated in **Figure 7**. As shown in **Figure 7**, methyl caffeate traps two radicals at both the phenolic groups to produce the *o*-quinone (**2**). Tazaki et al. (*22*) revealed that the *o*-quinone of caffeic acid underwent a redox reaction with caffeic acid. Therefore, the *o*-quinone (**2**) is unstable in the presence of methyl caffeate to produce a semiquinone radical (**5**) in the next step. The radical **5** reacts with two types of peroxyl radicals of the ethyl linoleate (**6** and **7**) at the 3′-position, affording four coupling products through a peroxyl linkage. This coupling is the important radical-scavenging step of the antioxidation of the methyl caffeate. The coupling products are not very stable because the aromatic stability of the original benzene ring is disrupted by the coupling reaction. Thus, the subsequent Diels−Alder reaction occurs in the coupling products. In our previous antioxidation mechanism studies, curcumin and methyl ferulate also provided this type of stabilized cyclic peroxide (*10, 12*) in the presence of a large amount of linoleate. These Diels−Alder products of peroxides would be common stable antioxidation products of the antioxidant bearing a hydroxycinnmamoyl structure.

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Supporting Information Available: Four tables of correlation data of COSY, NOESY, HMBC, and TOCSY of products **3A**, **3B**, **4A**, and **4B**. This material is available free of charge via the Internet at http://pubs.acs.org.

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