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Structural Identification of New Curcumin Dimers and Their Contribution to the Antioxidant Mechanism of Curcumin

TOSHIYA MASUDA,*,† YUKIKO TOI,† HIROMI BANDO,† TOMOMI MAEKAWA,‡ YOSHIO TAKEDA,† AND HIDEMASA YAMAGUCHI‡

Faculty of Integrated Arts and Sciences, University of Tokushima, Tokushima 770-8502, Japan, and Graduate School of Human Life Science, Osaka City University, Osaka 558-8585, Japan

As a part of the research project on the elucidation of the chain-breaking antioxidant mechanism of natural phenolics against the oxidation of food components, curcumin, a main turmeric pigment, was investigated. A relatively high concentration of curcumin gave three dimers as radical termination products in addition to the coupling products with curcumin and the lipid hydroperoxide. The structural analysis of these dimers and quantitative analysis of their production rates revealed that radical−radical termination mainly occurred at the 2-position of curcumin. The contribution of the pathway for production of these dimers to the antioxidant mechanism of curcumin was estimated from the concentration-dependent data of the antioxidant activity and formation rates of these termination products. The A−A termination (dimer formation) was estimated to contribute at least about 40% of the entire antioxidant process against ethyl linoleate oxidation.

KEYWORDS: Curcumin; chain-breaking antioxidant; antioxidant mechanism; A−A termination; AOOS termination

INTRODUCTION

Curcumin is one of the most widely used natural food colorants, which is obtainable from the spice, turmeric (dry rhizomes of *Curcuma longa*), as the main yellow pigment (*1*). Curcumin is well-known to exhibit various biological activities (*2*), and most of them are mediated by the potent antioxidant activity of itself or related metabolites (*3*, *4*). Curcumin has a unique conjugated structure including two methoxylated phenols and the enol form of a β-diketone (structure **2** in **Figure 1**). Many phenols bearing the curcumin structure (curcuminoid) were found in plants of the Zingiberaceae family, which are used for spices or traditional medicine in Asia (*5*−*10*). Curcumin also shows potent antioxidant activity against the oxidation of food components by its radical chain-breaking ability (*11*, *12*). Generally, the antioxidation process of the chain-breaking antioxidant is divided into the following two stages: (i) radical trapping stage

$$\text{S-OO}\cdot + \text{AH} \leftrightarrows \text{SOOH} + \text{A}\cdot$$

(ii) radical termination stage

$$\text{A}\cdot \rightarrow \text{nonradical materials such as A−A, AOOS, and Aox}$$

(S, oxidation substrate; AH, antioxidant; A, antioxidant radical; A−A, dimer of A; AOOS, substrate antioxidant peroxide; Aox, oxidized A).

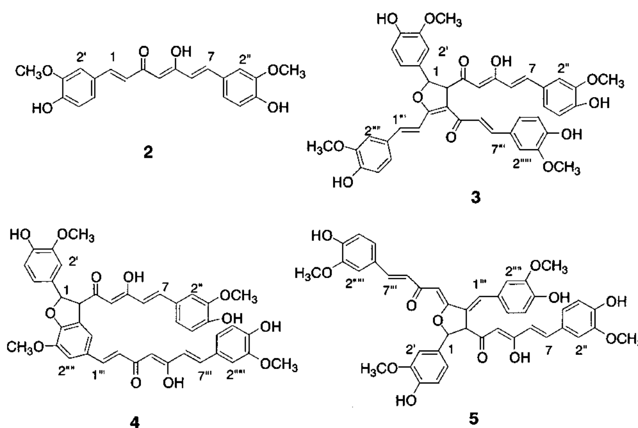

Figure 1. Chemical structures of isolated dimers (**3**–**5**) and curcumin (**2**).

Although the first stage is a reversible process, the second is irreversible and must produce stable radical termination compounds. The chemical structure of such a termination compound would afford important information in order to elucidate the antioxidant mechanism of the chain-breaking antioxidant. The antioxidant mechanism of curcumin has already attracted much attention, and several physiochemical studies were recently reported (*13*−*17*); however, it is still not yet well-understood because of the unique structure. During our studies of the antioxidant mechanism based on the chemical structures of the

* To whom correspondence should be addressed. Fax: +81-88-656-7244. E-mail: masuda@ias.tokushima-u.ac.jp.
† University of Tokushima.
‡ Osaka City University.

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **4** (^{1}H, 500 MHz; ^{13}C, 125 MHz in Acetone-d_6)

position	^{13}C chemical shift	correlated ^{1}H in HMBC	^{1}H chemical shift[a]	correlated ^{1}H in NOESY
1	88.7	H-2′, H-6′	6.02 (d, 7.0)	H-2, H-2′, H-6′
2	61.3	H-1	4.48 (d, 7.0)	H-1, H-2′
3	197.7	H-1, H-2, H-4		
4	101.8		5.97 (s)	
5	184.3	H-6, H-7		
6	120.0	H-4, H-7	6.65 (d, 16.0)	H-2″, H-6″
7	141.5		7.55 (d, 16.0)	H-2″, H-6″
1′	129.1	H-1, H-2		
2′	110.7		7.06 (d, 2.0)	H-1, H-2, 3′-OCH_3
3′	148.7			
4′	147.8			
5′	115.8		6.81 (d, 8.0)	
6′	120.0		6.87 (dd, 8.0, 2.0)	H-1
1″	127.9	H-6		
2″	111.4[b]		7.28 (d, 2.0)	H-6, H-7, 3″-OCH_3
3″	148.7			
4″	150.0[c]			
5″	116.1		6.84 (d, 8.0)	
6″	123.8		7.13 (dd, 8.0, 2.0)	H-6, H-7
1‴	140.9		7.57 (d, 16.0)	H-2⁗
2‴	122.9		6.71 (d, 16.0)	H-2⁗, H-6⁗
3‴	183.5	H-1‴, H-2‴		
4‴	100.4		5.94 (s)	
5‴	178.6	H-6‴, H-7‴		
6‴	122.2		6.65 (d, 16.0)	H-6′′′′′
7‴	141.9		7.58 (d, 16.0)	H-2′′′′′, H-6′′′′′
1⁗	130.2	H-2‴		
2⁗	113.6	H-1‴	7.30 (brs)	H-1‴, H-2‴, 3⁗-OCH_3
3⁗	145.8			
4⁗	151.3	H-2		
5⁗	132.4	H-2		
6⁗	118.6	H-2	7.24 (brs)	H-2‴
1′′′′′	128.0	H-6‴, H-7‴		
2′′′′′	111.5[b]		7.30 (d, 2.0)	H-6‴, 3′′′′′-OCH_3
3′′′′′	148.7			
4′′′′′	150.2[c]			
5′′′′′	116.1		6.85 (d, 8.0)	
6′′′′′	124.1		7.14 (dd, 8.0, 2.0)	H-6‴
3′-OCH_3	56.2		3.80 (s)	H-2′
3″-OCH_3	56.2		3.87 (s)	H-2″
3⁗-OCH_3	56.4		3.91 (s)	H-2⁗
3′′′′′-OCH_3	56.2		3.87 (s)	H-2′′′′′

[a] Coupling pattern and coupling constants (J in Hertz) are in parentheses. [b,c] Assignment may be interchangeable.

radical termination products, we reported that curcumin reacted with the peroxyl radicals of linoleate to produce stable cyclic compounds by a unique pathway through the AOOS termination step (*18*). We also found that radical reaction of curcumin under nonlipidic conditions gave a dimer as an A−A termination compound in addition to several fragmented products (*19*). A question has arisen as to which termination pathway is important for the antioxidant mechanism of curcumin. To elucidate the contribution of the A−A and AOOS terminations toward the antioxidation mechanism, we reexamined the radical termination process by the quantitative analysis of the formation rates of the termination products, in addition to the intensive isolation and structure analysis of any new termination compounds.

MATERIALS AND METHODS

Chemicals and Instruments. Curcumin was purchased from Wako Chemicals (Osaka, Japan) and used after repeated recrystallization with ethyl acetate-ethanol. 2,2′-Azobis(isobutyronitrile) (AIBN) was obtained from Tokyo Kasei (Tokyo, Japan). Ethyl linoleate was obtained from Kanto Chemicals (Tokyo, Japan). The silica gel thin-layer chromatography (TLC) plates (silica gel 60 F_{254} PLC) were purchased from Merck (Darmstadt, Germany). All solvents and other reagents were obtained from Nacalai Tesque (Kyoto, Japan). The nuclear magnetic resonance (NMR) spectra were measured with a Unity Plus 500 spectrometer (Varian, Palo Alto, CA) or an EX-400 spectrometer (JEOL, Tokyo, Japan) using the manufacturer-supplied pulse sequences [^{1}H, ^{13}C, correlated spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC)]. The mass spectra were measured with an SX-102A spectrometer (JEOL) in the negative fast atom bombardment (FAB) mode. The IR spectra were recorded on a Shimadzu FTIR-8400 (Kyoto, Japan). An LC-10A low-pressure gradient system (Shimadzu) equipped with a photodiode array detector (Shimadzu, SPD-M10AVP) and a PU-980 pump equipped with a UV-975 detector (JASCO, Tokyo, Japan) were used for the analytical HPLC. An LC system consisting of two PU-980 pumps and a UV-970 detector (JASCO) was used for the preparative HPLC. A medium-pressure pump 91-M-8 (Chemco, Osaka, Japan) equipped with a UV-8011 detector (Tosoh, Tokyo, Japan) was used for the medium-pressure liquid chromatography (MPLC).

Antioxidation Reaction and Analysis of the Products. To 3.0 g of ethyl linoleate in a 50 mL screw-capped straight vial (40 mm diameter) were added the appropriate amount of curcumin in dimethyl sulfoxide (200 μL), CH_3CN (3 mL), and AIBN (600 mg). The solution was well-stirred and incubated at 40 °C. At 1 h intervals, a 100 μL aliquot of the reaction mixture was removed and then diluted with CH_3CN (200 or 700 μL). Ten microliters of the diluted solution was then injected into the gradient HPLC system and analyzed under the following conditions: column, Develosil CN-5, 250 mm × 4.6 mm i.d., 5 μm particle size (Nomura Chemicals, Seto, Japan) equipped with a 10 mm × 4.6 mm i.d. guard column; solvent system, *n*-hexane−CH_3CO_2H (100:2) (solvent A) and ethyl acetate−CH_3CO_2H (100:2)

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of **5** (^{1}H, 500 MHz; ^{13}C, 125 MHz in Acetone-d_6)

position	^{13}C chemical shift	correlated ^{1}H in HMBC	^{1}H chemical shift[a]	correlated ^{1}H in NOESY
1	88.5		5.91 (d, 2.0)	H-2′, H-6′
2	59.8	H-1	4.23 (t, 2.0)	H-4, H-2′, H-2′′′′
3	198.5			
4	99.0		5.91 (s)	H-2, H-6, H-7
5	180.0	H-4, H-6, H-7		
6	119.5	H-7	6.68 (d, 15.5)	H-4, H-2′′, H-6′′
7	142.3		7.58 (d, 15.5)	H-4, H-2′′, H-6′′
1′	132.7			
2′	110.2		7.06 (d, 2.0)	3′-OCH_3
3′	148.4			
4′	147.8			
5′	116.1		6.82 (d, 8.5)	
6′	119.1	H-1	6.88 (ddd, 8.5, 2.0, 0.5)	H-1
1′′	127.8[b]			
2′′	111.1		7.33 (d, 2.0)	H-6, H-7, 3′′-OCH_3
3′′	148.8			
4′′	150.3			
5′′	116.1		6.84 (d, 8.0)	
6′′	124.5	H-7	7.13 (dd, 8.0, 2.0)	H-6, H-7
1′′′	132.6	H-2	7.61 (d, 1.5)	H-4′′′, H-2′′′′, H-6′′′′
2′′′	129.4	H-2, H-1′′′, H-4′′′		
3′′′	167.1	H-1, H-1′′′, H-4′′′		
4′′′	97.0		6.11 (s)	H-1′′′, H-6′′′, H-7′′′
5′′′	186.9	H-4′′′, H-6′′′, H-7′′′		
6′′′	126.0		7.39 (d, 16.0)	H-4′′′, H-2′′′′′, H-6′′′′′
7′′′	140.9	H-6′′′	7.50 (d, 16.0)	H-4′′′, H-2′′′′′, H-6′′′′′
1′′′′	127.9[b]			
2′′′′	113.3	H-1′′′	7.20 (d, 2.0)	H-2, H-1′′′, 3′′′′-OCH_3
3′′′′	148.6			
4′′′′	149.3[c]			
5′′′′	116.2		6.86 (d, 8.5)	
6′′′′	125.4	H-1′′′	7.10 (dd, 8.5, 2.0)	H-1′′′
1′′′′′	128.5	H-6′′′		
2′′′′′	111.1		7.18 (d, 2.0)	H-6′′′, H-7′′′, 3′′′′′-OCH_3
3′′′′′	148.5			
4′′′′′	149.5[c]			
5′′′′′	116.1		6.83 (d, 8.0)	
6′′′′′	123.4	H-7′′′	7.09 (dd, 8.0, 2.0)	H-6′′′, H-7′′′
3′-OCH_3	56.2		3.77 (s)	H-2′
3′′-OCH_3	56.2		3.86 (s)	H-2′′
3′′′′-OCH_3	56.2		3.84 (s)	H-2′′′′
3′′′′′-OCH_3	56.2		3.80 (s)	H-2′′′′′

[a] Coupling pattern and coupling constants (J in Hertz) are in parentheses. [b],[c] Assignment may be interchangeable.

(solvent B); elution mode, linear gradient from 14 to 71% of solvent B for 20 min and then isocratic of 100% of solvent B for 5 min; flow rate, 2 mL/min; column temperature, 25 °C. The area of each observed peak was calculated using Shimadzu M-10A data analyzing software. The concentration of each peak compound was calculated by each calibration curve, which was obtained using the corresponding isolated compound in this or previous studies (*19*) except peak **6**.

Analysis of Hydroperoxide of Ethyl Linoleate. At 4 h, 10 μL of the antioxidation reaction mixture was removed from the above-mentioned vial and diluted with 5 mL of methanol. Ten microliters of the solution was injected into the analytical HPLC system and analyzed under the following conditions: column, YMC ODS-A, 150 mm × 4.6 mm i.d. (YMC, Kyoto, Japan); solvent, CH_3CN-H_2O (9:1); flow rate, 1 mL/min; detection, 240 nm. The area of the hydroperoxide peak was calculated using a Shimadzu CR-6A integrator. The hydroperoxide concentration was calculated using a calibration curve, which was obtained from pure ethyl linoleate hydroperoxide.

Isolation Procedure of Compounds 3−5. The screw-capped reaction vial (40 mm diameter × 60 mm), which contained curcumin (18 mg) in dimethyl sulfoxide (70 μL), CH_3CN (3.9 mL), and AIBN (360 mg), was filled with oxygen gas. The vial was then incubated for 18 h at 37 °C in an air chamber (CI-310, Advantec, Tokyo, Japan). The reaction mixture was combined from the 50 vials, and the solution was concentrated to ca. 100 mL. The concentrate was stored at −20 °C for 5 h, and the produced precipitate (AIBN) was filtered off. The filtrate was concentrated again to 50 mL and then stored at −20 °C overnight. The produced precipitate was filtered again. The obtained filtrate was stored at −20 °C until purification. The stock solution (2.5 mL) was evaporated and injected into the MPLC for purification under the following conditions: column, Develosil LOP CN, 24 cm × 3 cm i.d., 30 μm particle size (Nomura Chemicals); solvent, a 60:40 mixture of hexane containing CH_3CO_2H (0.2%)−ethyl acetate for 20 min and then a 50:50 mixture of these solvents; flow rate, 10 mL/min; detection, 300 nm. The eluent from 20 to 57 min, which was eluted after curcumin, was collected. This procedure was repeated five times. Thus, obtained eluents were combined and evaporated to give 0.7 g of the residue. One hundred milligrams of the residue was then precisely separated by preparative HPLC under the following conditions: column, Develosil CN-5, 250 mm × 8.0 mm i.d. (Nomura Chemicals); solvent, 65:35 mixture of hexane containing CH_3CO_2H (1%) and ethyl acetate containing CH_3CO_2H (1%); flow rate, 6 mL/min. Each eluent corresponding to the peaks at 7.4, 8.7, and 12.0 min was separately collected. After seven applications, each eluent of all the applications was combined and evaporated to give the three fractions containing compounds **3**−**5**, respectively. Fraction 3 was finally purified by silica gel TLC (5% methanol in CH_2Cl_2) to give compound **3** (3 mg; isolation yield (%), 0.33). Fractions 4 and 5 were also purified by silica gel TLC under the same conditions to give compound **4** (18 mg; isolation yield (%), 2.0) and compound **5** (28 mg; isolation yield (%), 3.1), respectively.

Analytical Data for Compounds 3−5. Compound **3**: FABMS (negative) (*m/z*): $[M - H]^-$ 733. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.78 (1H, d, $J = 16$ Hz), 7.54 (1H, d, $J = 16$ Hz), 7.49 (1H, d, $J = 16$ Hz), 7.40 (1H, d, $J = 16$ Hz), 7.30 (1H, d, $J = 2$ Hz), 7.26 (1H, d, J

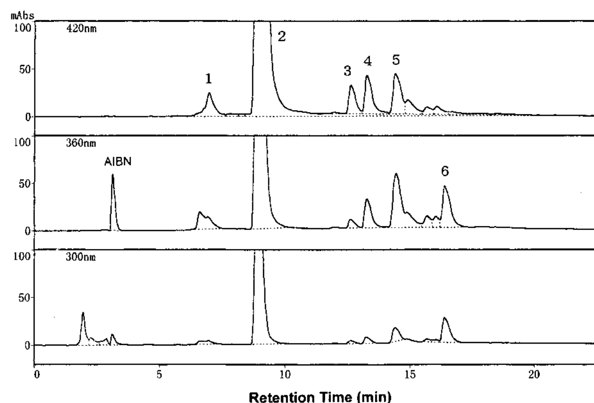

Figure 2. HPLC analytical data of antioxidation products from curcumin. Reaction conditions: curcumin, 13 mM; ethyl linoleate, 1.5 M; AIBN, 0.56 M; temperature, 40 °C; reaction time, 4 h. Analytical conditions: column, Develosil CN-5 (250 mm × 4.6 mm i.d.); solvent system, *n*-hexane–CH_3CO_2H (100:2) (solvent A) and ethyl acetate–CH_3CO_2H (100:2) (solvent B); elution mode, linear gradient from 14 to 71% of solvent B for 20 min and then isocratic of 100% of solvent B for 5 min; flow rate, 2 mL/min; column temperature, 25 °C.

= 2 Hz), 7.22 (1H, d, J = 2 Hz), 7.15 (1H, dd, J = 8 and 2 Hz), 7.11 (2H, dd, J = 8 and 2 Hz), 7.08 (1H, d, J = 2 Hz), 7.00 (1H, d, J = 16 Hz), 6.92 (1H, dd, J = 8 and 2 Hz), 6.87 (1H, d, J = 8 Hz), 6.86 (1H, d, J = 8 Hz), 6.83 (1H, d, J = 8 Hz), 6.80 (1H, d, J = 8 Hz), 6.65 (1H, d, J = 16 Hz), 5.98 (1H, s), 5.70 (1H, d, J = 6 Hz), 4.42 (1H, d, J = 6 Hz), 3.88 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 3.79 (3H, s).

Compound 4. HR−FABMS (negative) (*m/z*): $[M - H]^-$ calcd for $C_{42}H_{37}O_{22}$, 733.2285; found, 733.2249. IR (dry film): ν_{max} 3416, 1626, 1574, 1514, 1269 cm^{-1}. ^1H and ^{13}C NMR, see **Table 1**.

Compound 5. HR−FABMS (negative) (*m/z*): $[M - H]^-$ calcd for $C_{42}H_{37}O_{22}$, 733.2285; found, 733.2278. IR (dry film): ν_{max} 3377, 1632, 1582, 1520, 1283 cm^{-1}. ^1H and ^{13}C NMR, see **Table 2**.

RESULTS AND DISCUSSION

Quantitative HPLC Analysis of Radical Reaction Products of Curcumin. In our previous publication (*18*), we reported the formation of the coupling products of curcumin and ethyl linoleate hydroperoxide in the antioxidation reaction for a low concentration (ca. 1.2 mM) of curcumin in a large amount of ethyl linoleate (ca. 1.5 M). However, the antioxidant activity of this concentration of curcumin is not very strong and the further addition of curcumin to the reaction enhanced the activity. An analysis of the reaction products at the higher concentration of curcumin was carried out in this investigation. **Figure 2** shows the HPLC analytical data of the antioxidation reaction in about a 10-fold higher concentration of curcumin (13 mM) as compared to our previous investigation. In the HPLC data, four outstanding peaks, **1** and **3**−**5**, were observed during the 420 nm detection along with the curcumin peak **2** and an additional peak **6**, also observed along with AIBN and curcumin peaks during the 360 nm detection. A comparison of the retention time of each peak with that of the coupling compounds from curcumin and the lipid hydroperoxide, which has already been isolated in our previous studies (*18*), revealed that peak **1** was identical to the peak of the coupling compounds. The time−course and quantitative analyses of peaks **1**−**6** were carried out using various concentrations of curcumin; these data are shown in **Figure 3**. The concentration of curcumin continuously decreased during the antioxidation reaction, and the curcumin at the lowest concentration (1.3 mM) was almost completely consumed after 3 h as shown in **Figure 3B**. Although only the reaction at the lowest concentration of curcumin showed

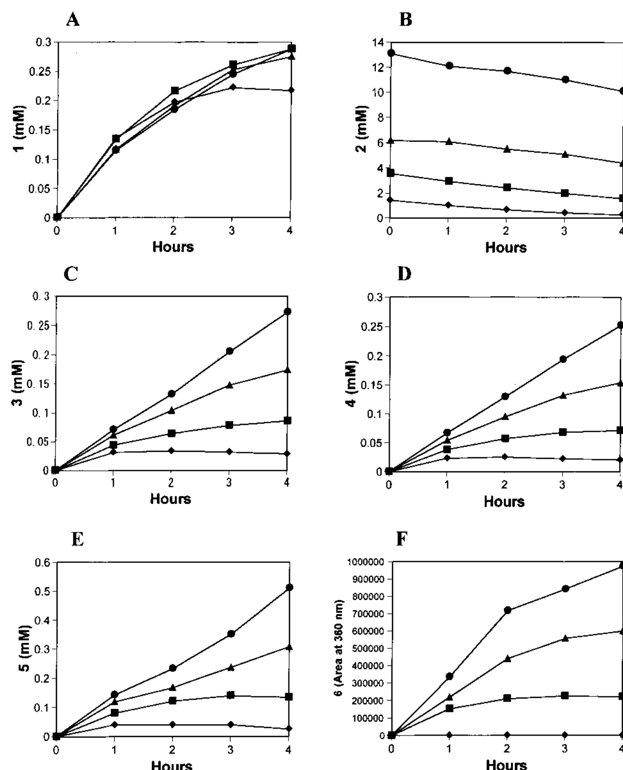

Figure 3. Time–course analytical data for peak compounds **1**–**6**. Concentration of starting curcumin: ●, 13 mM; ▲, 6.2 mM; ■, 3.1 mM; ♦, 1.3 mM.

a decrease after 3 h, the intensity of peak **1**, which contained a mixture of six curcumin−lipid hydroperoxide coupling products (*18*), increased by a similar rate at the four different curcumin concentrations (**Figure 3A**). The compounds **3**−**6** also accumulated during the reaction; however, the production rates were different between the concentration conditions of curcumin. The initial concentration of curcumin affected the production rates of these four compounds **3**−**6**. The clear tendency was observed that the higher concentration of curcumin gave higher amounts of these compounds as shown in **Figures 3C**−**F**. This concentration-dependent increase in compounds **3**−**6** suggested that they might be dimeric and/or polymeric materials of curcumin, which were produced as A−A termination products during the antioxidation reaction.

Isolation and Structure Determination of Curcumin Dimers 3−**5.** To obtain the structural information for the peak-related compounds **3**−**6**, we planned to isolate these compounds. Fortunately, we found that the production of the same compounds **3**−**6** was observed in the absence of ethyl linoleate, which could simplify the isolation procedure by avoiding the removal step of a large amount of the lipid. Fifty vials, each of which contained 18 mg of curcumin and 360 mg of AIBN in CH_3CN, were incubated at 37 °C. After most of the AIBN was removed by decantation at low temperature, the reaction mixture was purified by MPLC and then HPLC using a cyanopropylated silica gel (CN) column with 420 nm detection to afford pure compounds **3**−**5**. The purification of compound **6** was also carried out by a similar procedure with 360 nm detection. Unfortunately, isolation of compound **6** did not succeed because of its instability; however, we found during our purification attempt that **6** was easily changed to compound **5**. This fact indicated that **6** might be a reaction intermediate during the formation of compound **5**.

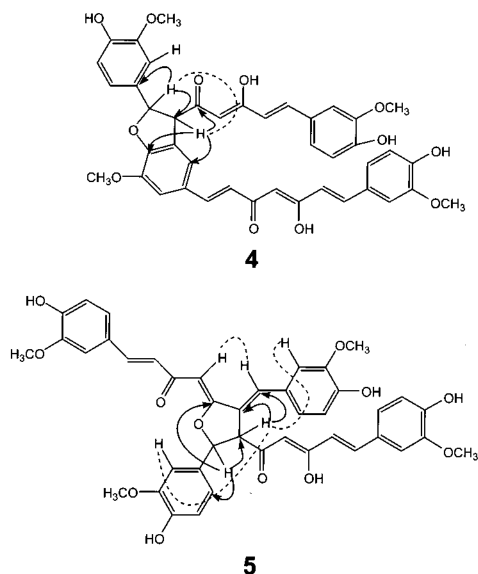

Figure 4. Selected HMBC and NOESY correlation data for compounds **4** and **5**. Arrow, H–C long-range correlation observed by HMBC; dashed line, nuclear Overhauser effect observed by NOESY.

Compound **5** was isolated as a yellow powder. Its molecular formula was established as $C_{42}H_{38}O_{22}$ from the high-resolution FABMS result [m/z 733.2278 (M − H)$^-$]. This molecular formula indicated that **5** was a dimeric product of curcumin. In the ^{1}H NMR of **5**, both signal sets due to the two curcumin moieties were observed; however, the chemical shift values of several proton signals, which were assignable to the alkyl part of curcumin, had significantly changed or disappeared, indicating that both alkyl moieties had reacted with each other. The fine structure of the coupled moiety of **5** was elucidated using the 2D NMR techniques. An analysis of the C−H long-range coupling connectivities around the coupled moiety in the HMBC spectrum revealed the presence of a furan ring, and the ring existed between the 1,2-positions of one of the curcumin parts and the 2,3-positions of the other curucmin part as shown in **Figure 4**. This structure was also supported by the correlation data in NOESY and the low-field shifted carbon signal of C-1 (δ 88.5) (**Table 2**). Thus, the structure of compound **5** was determined as depicted in **Figure 1**.

Compound **4** was also isolated as a yellow solid. Its molecular formula is the same as that of compound **5**, which was revealed by the FABMS result [m/z 733.2249 (M − H)$^-$]. Although the ^{1}H and ^{13}C NMR of **4** were similar to those of **5**, an aromatic proton signal on one of the benzene rings had disappeared, while the signal set due to the alkyl part of one of the curcumin moieties was retained when comparing with the NMR data of **5** (**Table 1**). These data indicated that **4** was an isomeric dimer of curcumin at the furan ring position. The furan moiety of **4** was revealed to be between the 1- and 2-positions of one of the curcumin moieties and the 4- and 5-positions of an aromatic ring of the other curcumin moiety based on the correlation data observed in the HMBC and NOESY of **4** as shown in **Figure 4**. Thus, the structure of compound **4** was determined as depicted in **Figure 1**.

Compound **3** was also isolated as a yellow powder. Its ^{1}H NMR and MS showed the same data as those of our previous isolated dimer (*19*), which was formed by the radical reaction of curcumin at elevated temperature (70 °C). Thus, the structure was identical with structure **3** as shown in **Figure 1**.

Formation Mechanism of Curcumin Dimers. From our elucidation of the chemical structures of the isolated curcumin dimers and quantitative analytical data for all of the dimer productions, we propose a radical scavenging mechanism of curcumin during its dimerization pathway (A−A termination). As shown in **Figure 5**, curcumin traps a radical, which may be

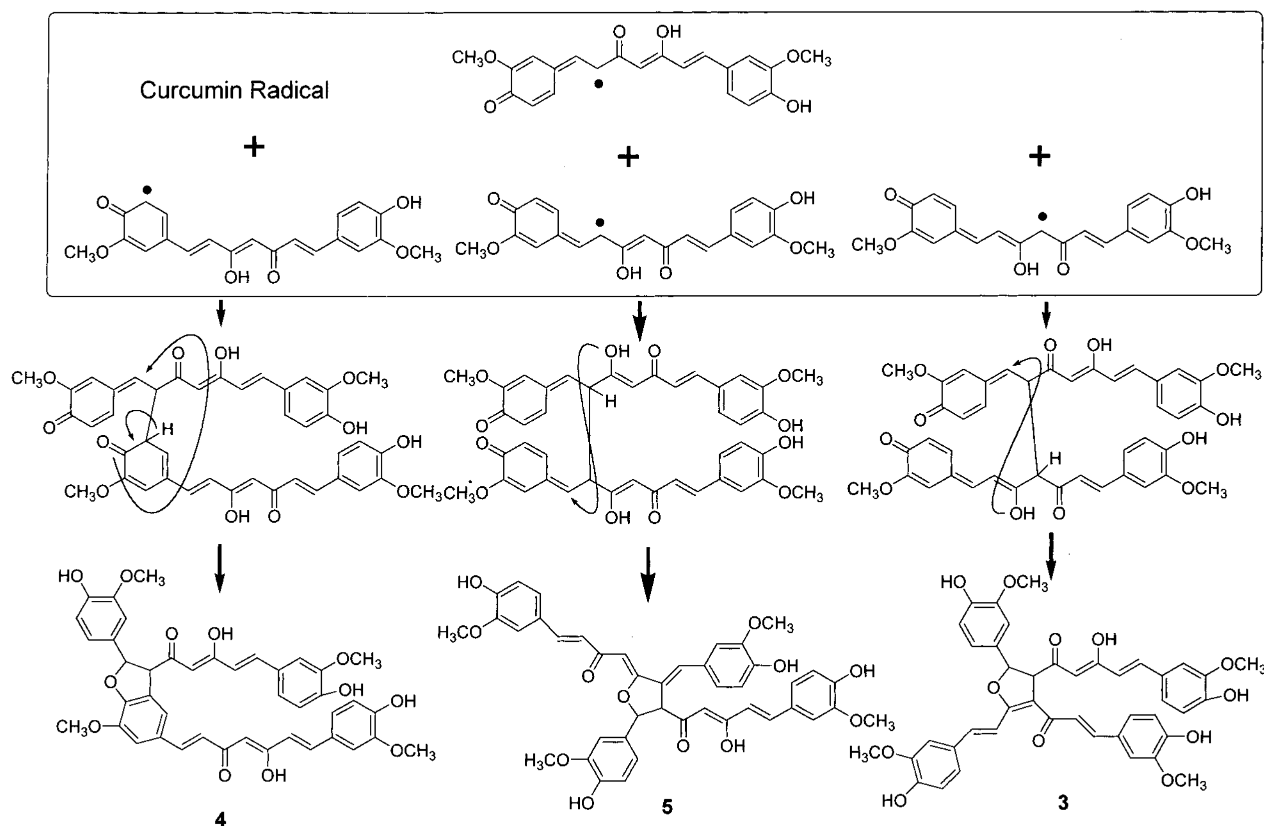

Figure 5. Proposed formation mechanism of compounds **3**–**5** via radical–radical coupling reaction.

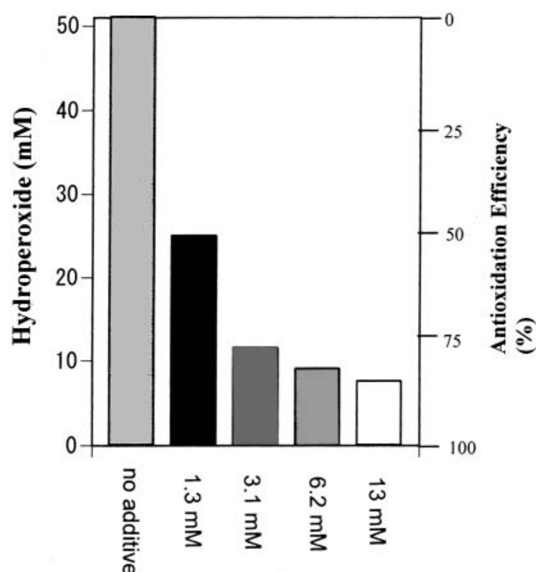

Figure 6. Antioxidant activity of the different concentrations of curcumin.

the ethyl linoleate hydroperoxyl radical. The curcumin radical produced reacts next with another curcumin radical at both 2-positions by a radical–radical coupling reaction. The coupling product is not very stable because two aromatic moieties have an unstable quinoid structure as shown in the center line of **Figure 5**. Thus, the subsequent addition of a hydroxyl group at the 3-position of one of the curcumin parts occurs smoothly at the 1-position of the other curumin moiety to produce a furan structure. The remaining quinoid structure was finally enolated to form the stable compound **5**. When radical–radical coupling occurs between the 2-position of one of the curcumins and another position of the other curucmin, a similar furan structure may be produced at different positions. As shown in the left line of **Figure 5**, the 5-position of the aromatic moiety of the curcumin radical reacts at the 2-position of the other curcumin radical and then produces compound **4** after subsequent enolation and cyclization reactions. Similarly, a coupling reaction at the 4-position of the curcumin radical with the 2-position of the other curcumin radical also produces compound **3** as shown in the right line of **Figure 5**. Quantitative analysis of the accumulation of each compound during the 4-h antioxidation revealed that the production of **5** was almost twice as high as those of compounds **3** and **4**. These facts indicate that production of **5** is the main pathway for the radical scavenging antioxidation of curcumin. Our previous results showed that the curcumin radical reacted only at the 3′-position with the lipid hydroperoxyl radical (*18*); however, radicalic dimerization of curcumin, oberved in this investigation, occurred mainly at the 2-position and any dimerization product at the 3′-position of curcumin was not found. The curcumin radical might not react at the 3′-position, whereas it may be the most stable radical position, with another curcumin radical because of the steric hindrance of both curcumin radicals.

Antioxidant Mechanism of Curcumin against Lipid Oxidation. During our studies of the antioxidation mechanism of curcumin, we found two types of radical terminations, which included the dimer formation (A–A termination) and the formation of the coupling product between curcumin and the lipid hydroperoxide (AOOS termination). From the quantitative data for the production of these termination products, the formation rate of the AOOS temination products was almost constant regardless of the curcumin concentration, while the rate of the dimer production depended on the concentration. The lowest concentration (1.3 mM) of curcumin used in this study reduced about 50% of the lipid hydroperoxide formation relative to that of the control experiment (**Figure 6**). This concentration of curcumin produced a very little amount of dimers (**3**–**5**) as compared with their amounts in the reaction with higher concentrations of curcumin and also compared with the amount of the coupling products (**1**). These facts indicated that the antioxidant mechanism of curcumin, which showed about 50% reduction of the oxidation, consisted mainly of the AOOS termination pathway. A higher concentration of curcumin

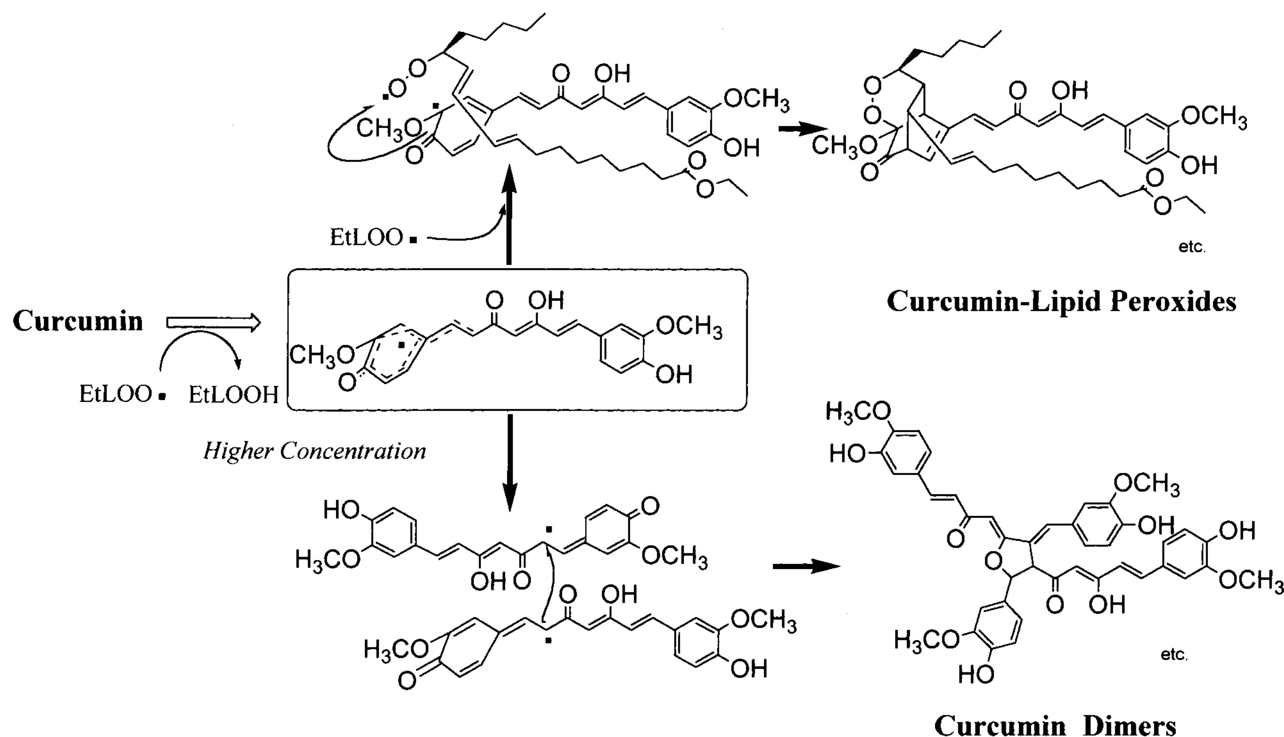

Figure 7. Chain-breaking antioxidant mechanism of curcumin against the oxidation of ethyl linoleate.

showed a stronger activity and gave a larger amount of dimers in addition to the constantly produced curcumin−lipid hydroperoxide coupling products. The results clarified that the AOOS pathway was fundamental; however, the mechanism at the high concentration of curcumin should include the additional dimeric termination pathway (A−A pathway). In **Figure 6**, the antioxidant activity of the highest concentration (13 mM) of curcumin represented by the 85% reduction of oxidation and the activity enhancement seems to reach near plateau by consideration of the activity enhancement tendency according to the curcumin concentration. The contribution of the dimerizing termination to the antioxidant mechanism of curcumin was estimated to be at least about 40% [= (85−50%)/85%] for the entire antioxidation process (**Figure 7**).

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