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A novel radical terminated compound produced in the antioxidation process of curcumin against oxidation of a fatty acid ester

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Abstract

Isolation and structure elucidation of a radical coupling product between curcumin and a lipid under atmospheric conditions was successful for the first time. The compound has a novel tricyclic structure including a peroxyl linkage.

Keywords: phenolics; lipids; peroxidation; radicals and radical reaction.

Natural phenolic antioxidants from medicinal or edible plants have recently received much attention as a promising material for reducing the risk of oxidation-induced diseases.[1] Curcumin (**1**) (Fig. 1) is a famous biologically active phenolic which originated from a medicinal plant, *Curcuma longa*; it is also used as a natural yellow pigment. Curcumin is well-known for having a potent antioxidant activity,[2] and the various related activities in biological systems have been extensively investigated,[3] resulting in over 600 papers appearing worldwide during the past five years. The National Cancer Institute of the United States also selected curcumin as one of the possible chemopreventive materials of cancer, and clinical applicability studies have continued.[4] Although the antioxidant mechanism of curcumin has attracted much attention, it has not yet been well understood.[5,6] The non-enzymatic antioxidant process of the phenolic in biological systems is thought to be divided into the following two stages: [7] (1) S-OO$^{\cdot}$+AH⇆SOOH+A$^{\cdot}$; and (2) A$^{\cdot}$→non-radical materials, where S is the substance for oxidation, AH is the phenolic antioxidant, and A$^{\cdot}$ is the antioxidant radical. Although the first stage is a reversible process, the second stage is irreversible, and it must produce stable radical-terminated compounds by reaction with another radical species from surrounding substances, such as a lipid hydroperoxide. Structural information of the terminated compounds would afford an important contribution to the antioxidant mechanism of phenolic antioxidants. During the course of our studies for the antioxidant mechanism of

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curcumin, we succeeded in isolating a radical terminated compound (**2**), which was accumulated during lipid oxidation, and determining its structure with a novel tricyclic system.

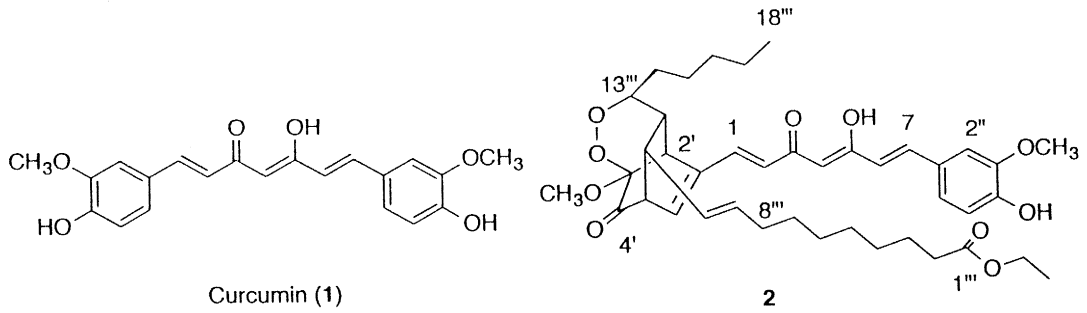

Fig. 1. Structure of curcumin (**1**) and compound **2**. Position number is assigned based on the original numbering system of curcumin and ethyl linoleate

Antioxidative reaction of curcumin was carried out in linoleic acid ethyl ester in the presence of a radical initiator, AIBN [2,2′-azobis(isobutyronitrile)]. Three milligrams of curcumin were dissolved in 3 g of ethyl linoleate in a 40 ml straight vial (∅=40 mm), and 3 ml of an AIBN (600 mg)–CH_3CN solution were added to the vial. The vial was incubated at 40°C for 4 h under atmosphere. After the excess amount of AIBN and the lipid were precipitated at −28°C, the supernatant was collected and subjected to liquid chromatography using a CN column (LOP CN 24S, 20% EtOAc in hexane containing 0.2% CH_3COOH) and an ODS column (Daisopak ODS-AP, 85% CH_3CN), successively. Final purification of **2** was carried out by silica gel TLC (EtOAc:hexane=1:2), affording pure **2** (7 mg) as the main product from a total of 510 mg of curcumin.

Compound **2** showed a pseudomolecular ion at *m/z* 705 in negative FABMS. The high-resolution mode of the MS clarified its molecular formula as $C_{41}H_{54}O_{10}$ [*m/z* 705.3616 $(M–H)^+$; calcd for $C_{41}H_{53}O_{10}$: 705.3639]. The obtained formula suggested that **2** was an oxidative coupling compound consisting of curcumin, ethyl linoleate, and a molecular oxygen. Although both signal sets, which originated from curcumin and ethyl linoleate, were observed in the ^1H NMR, signal sets due to one of the trisubstituted benzene rings from curcumin and an olefin from the linoleate disappeared, indicating that the double bond of the linoleate reacted with the benzene part of curcumin. The structure of the coupling part in **2** was elucidated by 2D NMR. From a proton–proton coupling connectivity due to H2′–H12′′′–H11′′′–H5′′–H6′′ found in the HH-COSY spectrum and six carbon–proton long-range coupling connectivities in the HMBC spectrum, which are illustrated in Fig. 2, a bicyclo[2,2,2] structure, including a carbonyl group at the 4′-position (δ 201.7), was revealed (Table 1). The presence of a little

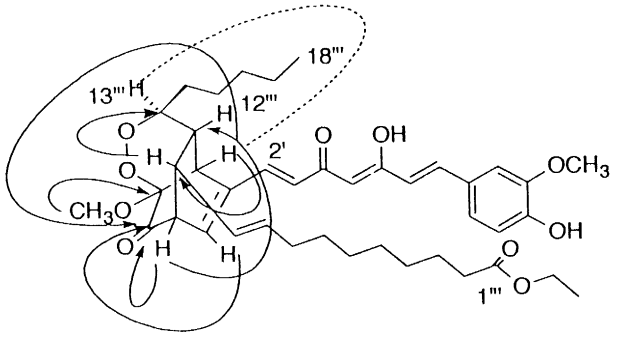

Fig. 2. Selected correlations observed in HMBC (arrow) and NOESY (dashed line) of **2**

Table 1
^{1}H and ^{13}C NMR spectral data of **2** (δ in $CDCl_3$, 500 MHz for ^{1}H and 125 MHz for ^{13}C)

position	^{1}H *	^{13}C	position	^{1}H *	^{13}C
1	7.37(d, 16.0)	136.6	3'''	1.56-1.64(m)	24.9
2	6.26(d,16.0)	124.0	4'''	1.22-1.33(m)	28.9**
3		181.1	5'''	1.22-1.33(m)	29.0**
4	5.79(s)	101.9	6'''	1.22-1.33(m)	29.1**
5		185.0	7'''	1.22-1.33(m)	29.1**
6	6.47(d, 16.0)	121.8	8'''	1.94(brq, 6.5)	32.2
7	7.60(d, 16.0)	141.4	9'''	5.46(dt, 15.5,6.5)	132.4
1'		140.8	10'''	5.20(brdd,15.5,8.5)	130.6
2'	3.21(dd, 3.0,2.0)	44.0	11'''	2.85(dt, 8.5,2.5)	41.9
3'		95.3	12'''	1.95(m)	41.3
4'		201.7	13'''	4.36(brd, 6.5)	85.1
5'	3.31(dd, 6.5,2.5)	56.0	14'''	1.50-1.56 (m)	29.9
				1.35-1.42 (m)	
6'	6.45(brdd,6.5,2.0)	131.6	15'''	1.22-1.33(m)	25.4
1''		127.5	16'''	1.22-1.33(m)	31.6
2''	7.05(d, 2.0)	109.6	17'''	1.22-1.33(m)	22.4
3''		146.8	18'''	0.88(brt, 7.0)	14.0
4''		148.0	3'-OCH_3	3.48(s)	53.6
5''	6.94(d, 8.0)	114.8	3''-OCH_3	3.95(s)	56.0
6''	7.13(dd, 8.0,2.0)	123.1	4''-OH	5.90(brs)	
1'''		173.8	1'''-$\underline{CH_2}CH_3$	4.12(q, 7.3)	60.2
2'''	2.28(t, 7.5)	34.3	1'''-$CH_2\underline{CH_3}$	1.25(t,7.3)	14.2

* Coupling pattern and coupling constant (J in Hz) in parentheses.

** Assignments may be interchangeable.

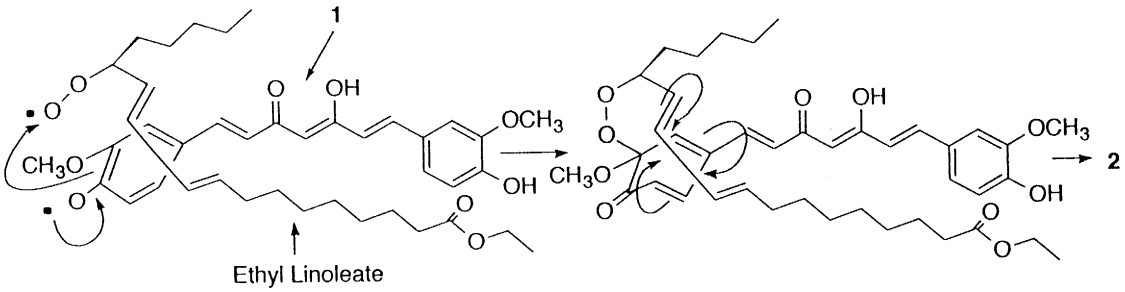

Fig. 3. Proposed formation mechanism of compound **2**

downfield-shifted monooxygenated carbon (C13‴, δ 85.1)[8] and an acetalic carbon (C3′, δ 95.3),[8] with the consideration of an oxygen count of the molecular formula, strongly indicated that a peroxyl group should exist between C13‴ and C3′. Thus, as depicted, **2** was determined to have a novel tricyclic ring structure. The stereochemistry around the tricyclic ring was deduced by proton coupling constants and an NOE observation. The phase-sensitive NOESY spectrum of **2** showed a strong correlation between

H2′ and H13′′′, suggesting that H13′′′ had the axial orientation in the conformationally restricted 1,2-dioxycyclohexane ring (Fig. 2). The stereorelationship between H12′′′ and H11′′′ was also deduced to be *trans* from the small coupling constant (J=2.5 Hz) between the two protons. Finally, attached groups to the ring system at the 11′′′ and 13′′′-positions were elucidated. A *trans* olefin [δ 5.20 (brdd, J=15.5, 8.5), 5.46 (dt, J=15.5,6.5)] was determined to be adjacent to the 11′′′-position by the chemical shift of H11′′′ (δ 2.85) and a COSY correlation with H9′′′–H10′′′–H11′′′. At the other end of the olefin (9′′′-position) and at the 13′′′-position, the remaining alkyl groups, including ethyl ester, would be attached. Although these groups should be pentyl and carboethoxyheptyl groups by considering the starting ethyl linoleate structure, the proton and carbon signals due to the corresponding alkyl chain did not separate sufficiently for determination of the attached positions. By the way, in the TOCSY spectrum (mixing time 0.12 s) of **2**, clear connectivities from the 18′′′-methyl proton (δ 0.88) to the H13′′′ (δ 4.36) and from the α-protons of ethyl ester (δ 2.28) to the olefinic protons (δ 5.46) at the 9′′′-position were observed, showing that the pentyl group is attached to the 13′′′-position and that the esteric alkyl chain is attached to the 9′′′-position. Thus, **2** should have the structure as expressed in Fig. 1. From the consideration of the obtained structure, a radical termination mechanism of curcumin in the unsaturated lipid environment was proposed, as illustrated in Fig. 3, which includes a radical coupling reaction with a lipid peroxyl radical at the 3′-position of curcumin, and subsequent stabilization by the Diels–Alder reaction with an olefin of the lipid and a diene moiety from curcumin. A coupling reaction of a phenolic with a lipid hydroperoxide has been reported as a radical termination process of vitamin E;[9] however, the formation of a tricyclic radical terminated compound has not yet been reported on various phenolic antioxidants.

References

1. Auroma, O. I. *J. Am. Oil Chem. Soc.* **1998**, *75*, 199–212.
2. Toda, S.; Miyase, T.; Arichi, H.; Tanizawa, H.; Takino, Y. *Chem. Pharm. Bull*. **1985**, *33*, 1725–1728.
3. Recent Review: Srimal, R. C. *Fitoterapia* **1997**, *68*, 483–493.
4. Kelloff, G. J.; Crowell, J. A.; Hawk, E. T.; Steele, V. E.; Lubet, S.; Greenwald, P.; Homg, W. K.; Parkinson, D. R.; Bagheri, D.; Baxter, G. T.; Blunden, M.; Doelts, M. K.; Eisenhauer, K. M.; Johnson, K.; Knapp, G. G.; Longfellow, D. G.; Malone, W. F.; Nayfield, S. G.; Sefried, H. E.; Swall, L. M.; Sigman, C. C. *J. Cell Biochem*. **1996**, *26*, 54–71.
5. Noguchi, N.; Komuro, E.; Niki, E.; Willson, R. L. *J. Jpn. Oil Chem. Soc*. **1994**, *43*, 1045–1051.
6. Without lipid, dimers of curcumin were observed as radical terminated products: Masuda, T.; Hidaka, K.; Shinohara, A.; Maekawa, T.; Takeda, Y.; Yanaguchi, Y. *J. Agric. Food Chem*. **1999**, *47*, 71–77.
7. Frankel, E. N. *Lipid Oxidation*; The Oily Press: Dundee, 1998; pp. 129–166.
8. Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett*. **1988**, *29*, 4731–4734.
9. Yamauchi, R.; Matsui, T.; Kato, K.; Ueno, Y. *Lipid* **1990**, *25*, 152–158, and references cited therein.