Isolation and Structure Determination of Cassumunarins A, B, and C: New Anti-Inflammatory Antioxidants from a Tropical Ginger, *Zingiber cassumunar*

Toshiya Masuda[a,1,*], Akiko Jitoe[a,1] and Tom J. Mabry[b]

[a]Faculty of Human Life Science, Osaka City University, Sumiyoshi, Osaka 558, Japan
and [b]Department of Botany, University of Texas at Austin, Austin, Texas 78713

ABSTRACT: New antioxidants, cassumunarins A, B, and C, were isolated from the rhizomes of *Zingiber cassumunar*, and the structures were determined by spectroscopic methods to be complex curcuminoids. The antioxidant efficiency of cassumunarins was determined by inhibitory activity of autoxidation of linoleic acid in a buffer–ethanol system. The anti-inflammatory effect was measured by inhibition of edema formation on mouse ear induced by 12-*O*-tetradecanoylphorbol-13-acetate. Both types of activities were stronger for the cassumunarins than for the curcumin.
JAOCS 72, 1053–1057 (1995).

KEY WORDS: Anti-inflammation, antioxidant, curcuminoid, isolation, structure determination, *Zingiber cassumunar*.

Natural antioxidants are important inhibitory materials for oxidative deterioration of food lipids, and, in addition, some antioxidants are also potent in preventing the initial stage of oxidation-related diseases. In connection with our research to discover new natural antioxidants that are beneficial for human health, we have been investigating tropical ginger species, because the rhizomes of the gingers are not only used as food additives, but also as a traditional medicine in tropical areas. We previously reported efficient antioxidant activity of several tropical ginger rhizomes (1), and also clarified the chemical structures and antioxidant activity of new antioxidants isolated from two tropical gingers, *Curcuma domestica* (2) and *C. xanthorrhiza* (3). The isolated antioxidants also showed anti-inflammatory activity *in vivo* (2). Anti-inflammatory activity is an important biological property of antioxidants, which may account for the prevention of oxidative diseases (4). The rhizomes of a tropical ginger, *Zingiber cassumunar*, which are used as traditional medicine in Thailand and Indonesia, have strong antioxidant activity (1), as well as anti-inflammatory activity (5). In our previous investigation, we isolated three new complex curcuminoids as anti-inflammatory antioxidants from one of the most active fractions derived from the acetone extract of *Z. cassumunar* rhizomes (6,7). Our further investigation clarified that other active fractions contained three new additional antioxidants, and the structures were reported preliminarily in a communication (8). This paper deals with the detailed structure determination of the new compounds, cassumunarin A(**1**), B(**2**), and C(**3**), and their antioxidant and anti-inflammatory activities.

[1]Visiting scholar at University of Texas (October 1992–September 1993).

*To whom correspondence should be addressed.

EXPERIMENTAL PROCEDURES

Purification material. The silica gel used for column chromatography was silica gel C-300 (Wako Pure Chemicals, Osaka, Japan); the silica gel used for thin-layer chromatography (TLC) was Merck silica gel 60 F254 (5744) plate (Merck, Darmstadt, Germany); and the octadecanoylated silica gel (ODS) column for medium-pressure chromatography was a Lop ODS (Nomura Chemicals, Seto, Japan).

Preparation of active fraction 12. The detailed preparation method of active fraction 12 and its antioxidant and anti-inflammatory activities were reported in a previous paper (7). Briefly, the acetone extract (111 g) from fresh rhizomes (2.9 kg) of *Z. cassumunar* was treated by a solvent partition method to give an ethyl acetate-soluble fraction. Silica gel chromatography of the material in this fraction (39 g) gave 13 fractions, the twelfth of which was further analyzed.

Isolation of cassumunarins A, B, and C from fraction 12. The material from fraction 12 (2 g) was subjected to medium-pressure liquid chromatography (column Lop ODS, diameter, 2.4 cm, length: 23 cm; elution solvent, 55% CH_3CN in H_2O; flow rate: 10 mL/min). The eluate was collected every 120 mL, and 15 fractions were obtained. The combined material from the fourth to the sixth fractions was subjected to silica gel column chromatography (acetone/hexane, 2:3) to give a mixture of cassumunarins (0.59). Each cassumunarin [A(**1**), 17 mg; B(**2**), 14 mg; C(**3**), 15 mg] was obtained by silica gel TLC (1% methanol in CH_2Cl_2) from a 50-mg sample of the mixture.

Methods and instruments for structure determination of cassumunarins. ^{13}C Nuclear magnetic resonance (NMR) (62.5 MHz) spectra were obtained with a Bruker (Karlsruhe, Germany) AC250 spectrometer, and ^1H NMR and two-di-

mensional NMR data were obtained with a General Electric GN-500 spectrometer (Freemont, CA) (1H, 500 MHz). Ultraviolet (UV) spectra were recorded with a Varian (Palo Alto, CA) DMS100 spectrometer. All mass spectra measurements were performed in the Mass Spectrometry Facility of the University of Texas at Austin (Austin, TX).

Spectroscopic analysis of cassumunarins A–C. Cassumunarin A(**1**) $[\alpha]_D^{24}$ + 8.9° (c 1.0, $CHCl_3$). electron impact mass spectrometry (EIMS) *m/z* (rel. int.%) 558 $[M]^+$ (89), 540 (12), 381 (7), 338 (43), 219 (100), 190 (22), 177 (45). High-resolution chemical ionization mass spectrometry (CIMS) (isobutane) *m/z* 559.2298 $[M + H]^+$ (calculated for $C_{33}H_{35}O_8$: 559.2332). UV λ_{max} (CH_3OH) nm 371, 262. ^{13}C NMR ($CDCl_3$, 62.5 MHz, 19 mg/mL, reference: $CHCl_3$ 77.0 ppm) δ 33.9, 43.6, 46.6, 55.8(C×2), 55.9, 56.0, 59.7, 103.0, 109.3, 111.0, 111.1, 111.1, 114.3, 114.7, 119.7, 119.8, 120.4, 122.8, 126.9, 127.6, 130.3, 135.4, 136.2, 139.8, 144.1, 146.2, 146.7, 147.6, 147.7, 148.7, 177.1, 200.8. ^1H NMR, see Table 1 (11 mg/mL, reference: C_6H_6 7.15 ppm); two-dimensional proton–proton spectroscopy (HH-COSY) and two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) correlations, see Table 2.

Cassumunarin B(**2**) $[\alpha]_D^{24}$ −2.5° (c 1.0, $CHCl_3$). EIMS *m/z* (rel. int.%) 558 $[M]^+$ (42), 540 (5), 381 (9), 338 (32), 219 (100), 190 (59), 177 (79). High-resolution CIMS (isobutane) *m/z* 559.2308 $[M + H]^+$ (calculated for $C_{33}H_{35}O_8$: 559.2332). UV λ_{max} (CH_3OH) nm 371, 273. ^{13}C NMR ($CDCl_3$, 62.5 MHz, 16 mg/mL, reference: $CHCl_3$ 77.0 ppm) δ 35.8, 36.2, 44.8, 54.7, 55.8(C×2), 56.0(C×2), 102.0, 109.6, 110.1, 110.6, 113.2, 114.3, 114.8, 119.8, 120.4, 121.7, 122.5, 127.3, 127.7, 128.3, 132.2, 137.1, 139.5, 143.8, 146.4, 146.7, 147.6, 148.0, 148.3, 176.5, 200.5. ^1H NMR, see Table 1 (11 mg/mL, reference: acetone 2.04 ppm; HH-COSY and NOESY correlations, see Table 2.

Cassumunarin C(**3**) $[\alpha]_D^{24}$ −2.3° (c 1.0, $CHCl_3$). EIMS *m/z* (rel. int.%), 588 $[M]^+$ (100), 570 (2), 411 (6), 368 (23), 220 (96), 219 (49), 177 (47). High-resolution CIMS (isobutane) *m/z* 589.2419 (calculated for $C_{34}H_{37}O_9$: 589.2438). UV λ_{max} (CH_3OH) nm 368, 283. ^{13}C NMR ($CDCl_3$, 62.5 MHz, 17 mg/mL, reference: $CHCl_3$ 77.0 ppm) δ 35.8, 36.7, 37.3, 55.1, 55.2, 56.0(C×3), 57.3, 95.9, 101.9, 109.3, 110.4, 114.2, 114.8, 115.9, 119.4, 120.1, 120.5, 122.4, 127.5, 127.8, 128.7, 136.6, 138.5, 141.9, 143.8, 146.3, 146.8, 147.5, 148.8, 152.5, 174.8, 201.6. ^1H NMR, see Table 1 (11 mg/mL, reference: acetone 2.04 ppm); HH-COSY and NOESY correlations, see Table 2.

Evaluation of antioxidant activity of cassumunarins A–C. The antioxidant activity of the isolated cassumunarins was measured by a previously described method (7). Briefly, test sample (2.7 μmol) and linoleic acid (0.10 g) were dissolved in ethanol (99.5%, 8 mL), a phosphate buffer (0.05 M, pH 7.0, 8 mL), and distilled water (4 mL). The solution was placed in a vial at 40°C in the dark. Seventy-five percent ethanol (9.7 mL) and 30% ammonium thiocyanate (0.1 mL) were added to 0.1 mL of the sample solution. The absorbance of the solution was measured at 500 nm three minutes after the addition of 0.02 M ferrous chloride in 3.5% HCl solution (0.1 mL) to the solution.

Evaluation of anti-inflammatory activity of cassumunarins A–C. The anti-inflammatory activity of isolated cassumunarins was determined by the mouse ear method described in a previous paper (7). Briefly, an acetone solution (20 μL) of the test sample (0.6 μmol) was applied on the left ear of a mouse (male, six weeks old, Jcl:ICR mouse; CLEA Japan, Tokyo, Japan). Thirty minutes later, an acetone solution (20 μL) of 2 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was applied to both the left and right ears of the same mouse. The ear disk (0.6-cm diameter) was punched and weighed 6.5 h after TPA application. Anti-inflammatory activity was estimated by the percent inhibition, which was calculated by the following equation:

$$\text{inhibition (\%)} = \frac{\text{weight of the ear to which TPA was applied} - \text{weight of the ear to which TPA and sample were applied}}{\text{weight of the ear to which TPA was applied} - \text{weight of the ear to which only solvent was applied}} \times 100 \quad [1]$$

RESULTS AND DISCUSSION

Structural elucidation of cassumunarins A(1), B(2) and C(3) (Tables 1 and 2). In our previous investigation, fractionation of the acetone extract of *Z. cassumunar* gave three fractions (fractions 11, 12, and 13) with both antioxidant and anti-inflammatory activity. We have examined only fraction 13 and obtained new curcuminoids (7); the other fractions remain to be investigated. We found that fraction 12 contained the new anti-inflammatory antioxidants, cassumunarins A, B, and C, and succeeded in isolating them by the method described in the Experimental Procedures section.

Cassumunarin A(**1**) was isolated as yellow amorphous powder, and its molecular formula was determined by high-resolution CIMS to be $C_{33}H_{34}O_8$ (*m/z* 559.2298 $[M + H]^+$). The ^1H NMR data and the coupling systems in the HH-COSY revealed that **1** has a 3,4,5-trisubstituted cyclohexene [δ 5.95 (1H, *brd*, *J* = 10.3 Hz), 5.90 (1H, *d*, *J* = 10.3 Hz), 4.26 (1H, *brd*, *J* = 10.6 Hz), 3.41 (1H, *dt*, *J* = 10.6 and 5.4 Hz), 2.92 (1H, *t*, *J* = 10.6 Hz), 2.42 (1H, *m*), 2.34 (1H, *brdt*, *J* = 18.5 and 5.4 Hz)] and three 1,3,4-trisubstituted benzene rings [aromatic ring A: δ 6.95 (1H, *brd*, *J* = 8.3 Hz), 6.93 (1H, *d*, *J* = 2.0 Hz), 6.59 (1H, *d*, *J* = 8.3 Hz); aromatic ring B: δ 6.94 (1H, *d*, *J* = 8.2 Hz), 6.69 (1H, *dd*, *J* = 8.2 and 1.9 Hz); 6.66 (1H, *d*, *J* = 1.9 Hz); aromatic ring C: δ 6.76 (1H, *d*, *J* = 8.2 Hz), 6.53 (1H, *dd*, *J* = 8.2 and 1.8 Hz), 6.33 (1H, *d*, *J* = 1.8 Hz)]. In the NOESY spectrum of **1**, an NOE of H-3 (δ 4.26) on the cyclohexene to H-2′ (δ 6.93) or H-6′ (δ 6.95) on the aromatic ring A indicated that aromatic ring A is adjacent to the cyclohexene at the 3-position. Two NOE between H-5 (δ 3.41) and H-2‴ (δ 6.66) and between H-5 and H-6‴ (δ 6.69) also clarified that aromatic ring B is at 5-position of the cyclohexene. The aromatic ring C is determined to be attached to a *trans*-olefin [δ 7.40 (1H, *d*, *J* = 15.8 Hz), 5.79 (1H, *d*, *J* = 15.8 Hz)], which was deduced from a NOE between H-2⁗ (δ 6.33) on aromatic ring C and H-5″ (δ 7.40) on the olefin. An isolated olefinic proton (H-2″) at 5.06 ppm showed NOE to an olefinic proton

TABLE 1
^{1}H Nuclear Magnetic Resonance (NMR) Spectral Data of Cassumunarins A(1), B(2), and C(3)[a]

H	1	2	3
1	5.90 *fine separated d* (10.3)	6.00 *fine separated d* (10.3)	6.02 *fine separated d* (10.3)
2	5.95 *brd* (10.3)	5.80 *fine separated d* (10.3)	5.71 *fine separated d* (10.3)
3	4.26 *brd* (10.6)	3.87[b]	4.33 *brt* (5.7)
4	2.92 *t* (10.6)	3.41 *dd* (12.1, 5.7)	3.31 *dd* (12.1, 5.7)
5	3.41 *dt* (10.6, 5.4)	3.18 *ddd* (12.1, 10.8, 5.4)	3.15 *ddd* (12.1, 10.8, 5.7)
6e	2.34 *brdt* (18.5, 5.4)	2.51 *brdt* (18.4, 5.4)	2.51 *brdt* (18.4, 5.4)
6a	2.42 *m*	2.18 *ddd* (18.4, 10.8, 2.5)	2.21 *ddd* (18.4, 10.8, 2.5)
2′	6.93 *d* (2.0)	6.71 *d* (2.0)	6.93 *s*
5′	6.59 *d* (8.3)	6.82 *d* (8.2)	6.51 *s*
6′	6.95 *brd* (8.3)	6.74 *dd* (8.2, 2.0)	
2″	5.06 *s*	5.50 *s*	5.27 *s*
4″	5.79 *d* (15.8)	6.33 *d* (15.8)	6.29 *d* (15.8)
5″	7.40 *d* (15.8)	7.30 *d* (15.8)	7.23 *d* (15.8)
2‴	6.66 *d* (1.9)	6.81–6.84[b]	6.81 *d* (2.0)
5‴	6.94 *d*(8.2)	6.65 *d* (1)	6.62 *d* (8.1)
6‴	6.69 *dd* (8.2, 1.9)	6.65 *d* (1)	6.65 *dd* (8.1, 2.0)
2⁗	6.33 *d* (1.8)	7.20 *d* (1.9)	7.19 *br*
5⁗	6.76 *d* (8.2)	6.83 *d* (8.2)	6.83 *d* (8.2)
6⁗	6.53 *dd* (8.2, 1.8)	7.05 *dd* (8.2, 1.9)	7.03 *dd* (8.2, 2.0)
3′-OCH_3	3.50 *s*	3.67 *s*	3.79 *s*
4′-OCH_3	3.30 *s*	3.72 *s*	3.75 *s*
6′-OCH_3			3.53 *s*
3‴-OCH_3	3.21 *s*	3.77 *s*	3.75 *s*
3⁗-OCH_3	3.01 *s*	3.87 *s*	3.88 *s*
OH	5.37 *brs*	7.22 *brs*	7.19 *brs*
OH	5.72 *brs*	8.09 *brs*	8.05 *brs*

[a]δ (ppm), **1** in C_6D_6, **2** and **3** in acetone-d_6 (500 Mhz); coupling constant in parentheses.
[b]Overlapped with other signals.

TABLE 2
Correlations Observed in COSY and NOESY Spectra of Cassumunarin A(1), B(2), and C(3)[a]

	Correlated proton in COSY			Correlated proton in NOESY		
H	**1**	**2**	**3**	**1**	**2**	**3**
1	H-6e	H-2, 6e	H-2, 6e			
2		H-1,3	H-1, 3		H-6′	H-2′
3	H-4, 6a	H-2, 4, 6a	H-2, 4, 6a	H-2′, 5, 6′	H-6′	
4	H-3, 5	H-3, 5	H-3, 5	H-6a, 2″	H-6a, 2‴	H-6a, 2″
5	H-4, 6a, 6e	H-4, 6a, 6e	H-4, 6a, 6e	H-3, 2‴, 6‴	H-6′, 2‴, 6‴	H-2′, 2‴
6e	H-1, 5	H-1, 5, 6a	H-1, 5, 6a			
6a	H-3, 5	H-3, 5, 6e	H-3, 5, 6e	H-4, 2‴, 6‴	H-4, 2‴, 6‴	H-4, 2‴
2′				H-3, 3′-OCH_3	3′-OCH_3	H-2, 5, 3′-OCH_3
5′	H-6′			4′-OCH_3	4′-OCH_3	4′-OCH_3, 6′-OCH_3
6′	H-5′			H-3	H-2, 3, 5	
2″				H-4, 4″	H-4, 4″	H-4, 4″
4″	H-5″	H-5″	H-5″	H-2″, 6⁗	H-2″, 2⁗, 6⁗	H-2″, 2⁗, 6⁗
5″	H-4″	H-4″	H-4″	H-2⁗		
2‴		H-6‴	H-6‴	H-5, 6a, 3‴-OCH_3	H-5, 6a, 3‴-OCH_3	H-5, 6a, 3‴-OCH_3
5‴	H-6‴					
6‴	H-5‴	H-2‴	H-2‴	H-5, 6a	H-5, 6a	
2⁗	H-6⁗	H-6⁗	H-6⁗	H-5″, 3⁗-OCH_3	H-4″, 3⁗-OCH_3	H-4″, 3⁗-OCH_3
5⁗	H-6⁗	H-6⁗	H-6⁗			
6⁗	H-2⁗, 5⁗	H-2⁗, 5⁗	H-2⁗, 5⁗	H-4″	H-4″	H-4″
3′-OCH_3				H-2′	H-2′	H-2′
4′-OCH_3				H-5′	H-5′	H-5′
6′-OCH_3						H-5′
3‴-OCH_3				H-2‴	H-2‴	H-2⁗
3⁗-OCH_3				H-2⁗	H-2⁗	H-2⁗

[a]COSY, two-dimensional correlated spectroscopy; NOESY, two-dimensional nuclear Overhauser enhancement spectroscopy.

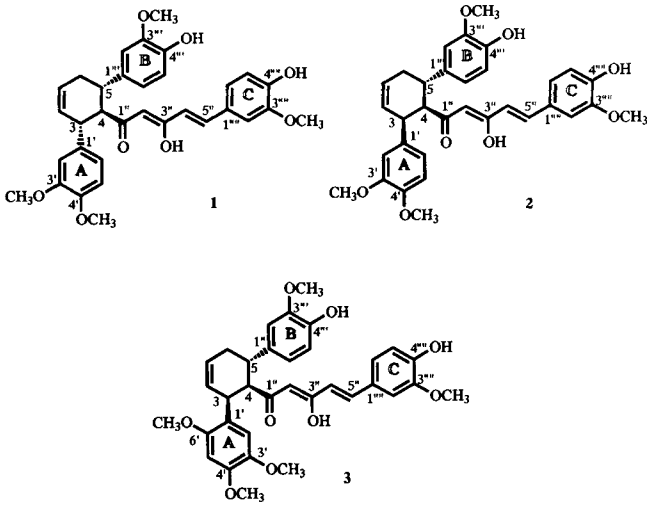
(H-4″, δ 5.79) and H-4 (δ 2.92) on the cyclohexene. The NOE results and the presence of two carbonyl groups (δ 177.1 and 200.8 in ^{13}C NMR of **1**), indicated that an enolated β-diketone moiety should exist between the olefin and the cyclohexene. This partial structure also was supported by an absorption maxima at 371 nm in the UV spectrum and a fragment ion at *m/z* 219 in the EIMS of **1**. The substituted positions of four methoxyl groups (δ 3.50, 3.30, 3.21, 3.01) were determined to be the 3- and 4-positions of aromatic ring A, 3-position of aromatic ring B, and 3-position of aromatic ring C from NOE of each methoxyl group's protons to H-2′ (δ 6.93), H-5′ (δ 6.59), H-2‴ (δ 6.66), and H-2⁗ (δ 6.33), respectively. The other functional groups on aromatic rings B and C should be hydroxyl groups (δ 5.37, 5.72). The relative stereochemistry of the three substituents on the cyclohexene were determined to be all *pseudo equatorial*, which was deduced from the diaxial coupling constant of H-4 (J = 10.6 Hz) and two NOE (H-3/H-5, H-4/H-6a)(Table 2). Thus, cassumunarin A can be expressed as structure **1** in Scheme 1.

Cassumunarin B(**2**) has a molecular formula $C_{33}H_{34}O_8$, based on high-resolution CIMS results (*m/z* 559.2308 [M + H]$^+$). Similar spectroscopic data of **2** to those for **1** indicated that **2** is an isomer of **1**. HH-COSY data of **2** revealed that **2** has the same partial structures as **1**, deduced from the following proton coupling connectivities [a 3,4,5-trisubstituted cyclohexene: δ 6.00 (1H, *d*, J = 10.3 Hz), 5.80 (1H, *d*, J = 10.3 Hz), 3.87 (1H, overlapped with other signals), 3.41 (1H, *dd*, J = 12.1 and 5.7 Hz), 3.18 (1H, *ddd*, J = 12.1, 10.8, and 5.4 Hz), 2.51 (1H, *brdt*, J = 18.4 and 5.4 Hz), 2.18 (1H, *ddd*, J = 18.4, 10.8, and 2.5 Hz), aromatic ring A: δ 6.82 (1H, *d*, J = 8.2 Hz), 6.74 (1H, *dd*, J = 8.2 and 2.0 Hz), 6.71 (1H, *d*, J = 2.0 Hz), aromatic ring B: δ 6.81–6.84 (1H, overlapped with other signals), 6.65 (2H, *d*, J = 1.0 Hz), aromatic ring C: δ 7.20 (1H, *d*, J = 1.9 Hz), 7.05 (1H, *dd*, J = 8.2 and 1.9 Hz), 6.83 (1H, *d*, J = 8.2 Hz), a *trans*-olefin: δ 7.30 (1H, *d*, J = 15.8 Hz), 6.33 (1H, *d*, J = 15.8 Hz)]. The NOE obtained in the NOESY spectrum of **2** also indicated that these partial structures should be connected similarly as in **1**. The difference from **1** is the stereochemistry on the cyclohexene ring, which was indicated by the coupling constants of the protons on the cyclohexene. The stereochemistry of the substituents on the cyclohexene was determined to be *pseudo axial*, *pseudo equatorial*, and *pseudo equatorial* at 3-, 4-, and 5-positions, respectively, by the coupling constant of H-4 (*dd*, J = 12.1 and 5.7 Hz) and NOE (H-4/H-6a, H-5/H-6′) (Table 2). Thus, cassumunarin B can be expressed as structure **2** in Scheme 1.

Cassumunarin C(**3**) has a molecular formula $C_{34}H_{36}O_9$, which was obtained by high-resolution CIMS (*m/z* 589.2419 [M + H]$^+$). ^1H NMR data of **3** was similar to those for **2**. However, signals for a 1,2,4,5-tetrasubstituted benzene ring were observed instead of the signals for a 1,3,4-trisubstituted benzene ring in **2**, and an additional methoxyl signal was also detected. The additional methoxyl group (δ 3.53) must be at the 6-position of aromatic ring A (this numbering is based on the numbering system used in structure **2**) by a NOE between the methoxyl signal and H-5′ (δ 6.51). The stereochemistry of the cyclohexene ring of **3** was determined to be the same as that of **2** from the coupling constant of H-4 (*dd*, J = 12.1 and 5.7 Hz) and NOE (H-4/H-6a, H-5/H-2′) (Table 2). Thus, cassumunarin C can be expressed as structure **3** in Scheme 1.

Antioxidant and anti-inflammatory activities of cassumunarins A, B, and C. Antioxidant activity of newly isolated compounds was measured by inhibition of autoxidation of linoleic acid in a buffer–ethanol system, and the oxidation of linoleic acid was detected by a thiocyanate method (Fig. 1). As shown in Figure 1, all isolated cassumunarins (2.7 μmol) inhibited the accumulation of lipid hydroperoxide. The antioxidant efficiency of cassumunarins was much stronger than that of curcumin, a potent antioxidant widely distributed in ginger species. Cassumunarin A showed slightly stronger antioxidant activity than the other cassumunarins.

Anti-inflammatory activity of cassumunarins was determined on mouse ear by using a tumor promoter, TPA, as the inducer. The efficiency of each compound was estimated and compared by the inhibition of edema formation (Table 3).



SCHEME 1

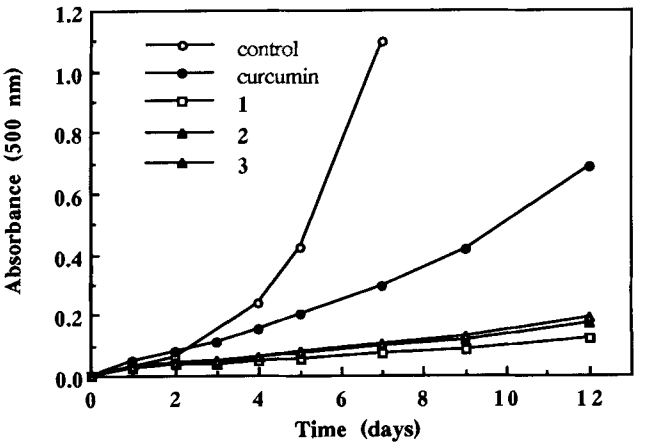


FIG. 1. Effect of cassumunarins A(**1**), B(**2**), C(**3**) and curcumin on autoxidation of linoleic acid.

TABLE 3
Inhibitory Effect of Cassumunarins A(1), B(2), and C(3) on 12-*O*-Tetradecanoylphorbol-13-Acetate (TPA)-Induced Edema of Mouse Ears

Treatment				
Left ear	Right ear	n[a]	D ± SE (mg)[b]	Inhibition (%)
	TPA (2 μg)	6	16.6 ± 0.6	
Curcumin (0.6 μmol) + TPA (2 μg)	TPA (2 μg)	5	9.2 ± 1.3	56
1 (0.6 μmol) + TPA (2 μg)	TPA (2 μg)	5	12.3 ± 2.1	75
2 (0.6 μmol) + TPA (2 μg)	TPA (2 μg)	5	10.3 ± 1.4	62
3 (0.6 μmol) + TPA (2 μg)	TPA (2 μg)	5	10.3 ± 0.8	62

[a]n = Number of mice.
[b]D = means of weight differences between right and left ears.

Table 3 shows that all cassumunarins (0.6 μmol) have anti-inflammatory activity. The activity efficiency of cassumunarins (A, 75%; B, 62%; C, 62%) was stronger than that of curcumin (56%), and the activity of cassumunarin A was the strongest among all samples tested.

Curcumin, a potent antioxidant in tropical gingers (9,10), also has potent anti-inflammatory activity (11,12). Cassumunarins A–C have the same partial structure at the 4-position of the cyclohexene that occurs in curcumin. This partial structure contributes efficient antioxidant and anti-inflammatory activities to the cassumunarins and curcumin. Recently, curcumin received much attention because of its biological effect for cancer prevention (13), and most of these activities are probably related to its antioxidant activity *in vivo* (14). Cassumunarins showed stronger antioxidant and anti-inflammatory activities than those of curcumin, suggesting that the cassumunarins may have importance for such diseases as cancer.

ACKNOWLEDGMENTS

This work at the University of Texas was supported by the National Institutes of Health (GM-35710) and The Robert A. Welch Foundation (F-130), and the work at Osaka City University was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan and the Takano Foods Research Foundation.

REFERENCES

1. Jitoe, A., T. Masuda, I.G.P. Tengah, D.N. Suprapta, I.W. Gara and N. Nakatani, *J. Agric. Food Chem. 40*:1337 (1992).
2. Masuda, T., A. Jitoe, J. Isobe, N. Nakatani and S. Yonemori, *Phytochemistry 32*:1557 (1993).
3. Masuda, T., J. Isobe, A. Jitoe and N. Nakatani, *Ibid. 31*:3645 (1992).
4. Nakadate, T., S. Yamamoto, E. Aizu and R. Kato, *Gann 75*:214 (1984).
5. Ponglux, D., S. Wongseripipatana, T. Phadungcharoen, N. Ruangrungsri and K. Likhiwitayawuid, in *Medicinal Plants*, Victory Point, Bangkok, 1987, p. 275.
6. Masuda, T., A. Jitoe and N. Nakatani, *Chemistry Lett. 189* (1993).
7. Masuda, T., and A. Jitoe, *J. Agric. Food Chem. 42*:1850 (1994).
8. Jitoe, A., T. Masuda and T.J. Mabry, *Tetrahedron Lett. 35*:981 (1994).
9. Sharma, O.P., *Biochem. Pharmacol. 25*:1811 (1976).
10. Toda, S., T. Miyase, H. Arichi, H. Tanizawa and Y. Takino, *Chem. Pharm. Bull. 33*:1725 (1985).
11. Srimal, R.C., and B.N. Dhawan, *J. Pharm. Pharmac. 25*:447 (1973).
12. Mukhopadhyay, A., N. Basu, N. Ghatak and P.K. Gutral, *Agents and Actions 12*:4 (1982).
13. Ammon, H.P.T., and M.A. Wahl, *Planta Med. 57*:1 (1991).
14. Huang, T.T., R.C. Smart, C.Q. Wong and A.H. Conney, *Cancer Res. 48*:5941 (1988).

[Received October 14, 1994; accepted June 6, 1995]