Antioxidative and Antiinflammatory Compounds from Tropical Gingers: Isolation, Structure Determination, and Activities of Cassumunins A, B, and C, New Complex Curcuminoids from *Zingiber cassumunar*

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Assay-guided isolation gave three new compounds, cassumunins A, B, and C, having both antioxidant and antiinflammatory activities, from the rhizomes of a tropical ginger, *Zingiber cassumunar*. Antioxidant activity and antiinflammatory activity were measured using a thiocyanate method and a 12-*O*-tetradecanoylphorbol 13-acetate-induced method on mouse ear, respectively. The antioxidant activity of cassumunins A–C is stronger than that of curcumin, and their antiinflammatory activity is also stronger than that of curcumin. Spectroscopic analysis of cassumunins A–C revealed them to be a new type of complex curcumin. Antioxidant and antiinflammatory activities of cassumunins suggested that the substituted group at the 5′-position of curcumin increased both activities.

Keywords: *Antiinflammatory antioxidants; Z. cassumunar; curcuminoid*

INTRODUCTION

Curcumin (**1**) is the main yellow pigment of turmeric (dry rhizomes of *Curcuma longa*) and has been used as a food coloring reagent in the food industry. Curcumin has received much attention with its interesting biological activities [review in Huang and Ferraro (1992)]. Curcumin is known to have antioxidant activity against the peroxidation of lipids (Sarma, 1976; Toda *et al.*, 1985). Also, most of its biological activities may be related to its antioxidant activity. Huang *et al.* (1988) reported antitumor promotion activity of curcumin, which is important for the chemoprevention of human cancer. They also clarified that its antitumor promotion activity is linked with suppression of arachidonic acid metabolism (Huang *et al.*, 1991). Arachidonic acid metabolism is well-known as a lipid peroxidation pathway in living cells. These facts indicate that curcumin works as an antioxidant even in living cells. It is widely accepted that some pathological events, such as heart disease and aging, as well as cancer, are closely related to the peroxidation reaction in living organisms [review in Halliwell and Gutteridge (1985)]. Curcumin and related compounds are expected to be used for the prevention of such diseases.

In tropical areas, many gingers are used as spices and also for traditional medicines. Curcumin is widely distributed in such ginger rhizomes (Hegnauer, 1963). We have already reported antioxidant activity of tropical ginger and the quantitative analysis of curcumin and its two analogs from the rhizomes (Jitoe *et al.*, 1992). We also isolated new curcuminoids from two tropical gingers, *Curcuma domestica* and *Curcuma xanthorrhiza* (Masuda *et al.*, 1992, 1993a). The isolated compounds have not only antioxidant activity but also antiinflammatory activity *in vivo*. Inflammation is one of the peroxidation-related events of living cells and an initial response of various diseases.

Zingiber cassumunar is a medicinal ginger and is also used as a spice like turmeric. The rhizomes also have antioxidant activity (Jitoe *et al.*, 1992) and antiinflammatory activity (Ponglux *et al.*, 1987). In the course of our research to develop effective antioxidants for living cells, we have been isolating new chemical compounds with both antioxidant and antiinflammatory activities. From the rhizomes of *Z. cassumunar*, new complex curcuminoids, cassumunins A (**2**), B (**3**), and C (**4**), were isolated by the guidance of both antioxidant and antiinflammation assays (Figure 1). We have already reported the preliminary structures of **2**–**4** (Masuda *et al.*, 1993b). In this paper, we report in detail the isolation, structure determination of the cassumunins (**2**–**4**), and their antioxidant and antiinflammatory activities.

EXPERIMENTAL PROCEDURES

Antioxidant Assays. Antioxidant assay of extracts and fractionated samples was carried out using a TLC method, and the activity of pure compounds was measured by the following thiocyanate methods.

(1) TLC Method. The method of Chang *et al.* (1983) was slightly modified. An acetone solution of each sample (10, 5, or 1 μg) was spotted on a silica gel TLC plate with a fluorescent indicator (Kieselgel 60 F254, art. 5715, E. Merck, Darmstadt). To the plate was sprayed twice 3% linoleic acid (Sigma, St. Louis, MO) in hexane solution. For 10–15 min the backgroud of the spot darkened and a fluorescent spot appeared under continuous irradiation of UV (254 nm) light using a UV lamp (UVGL-25, UVP Inc., San Gabriel, CA) placed 2.5 cm above the TLC plate. The TLC plate was observed every 5 min under continuous irradiation, and the time until each fluoresent spot disappeared was considered the induction period for lipid oxidation.

(2) Thiocyanate Method. The preparation of mixed solution and the detection of lipid peroxide were carried out according to the method described in a previous paper (Masuda *et al.*, 1992). Briefly, sample (2.7 μmol) and linoleic acid (0.10 g) were dissolved in ethanol (99.5%, 8.0 mL), a phosphate buffer (0.05 M, pH 7.0, 8.0 mL), and distilled water (3.9 mL). The solution was placed in a vial at 40 °C in the dark. Oxidation of linoleic acid was monitored by the following method. To 0.1 mL of sample solution was added 75% ethanol (9.7 mL) and 30% ammonium thiocyanate (0.1 mL). Three minutes after the addition of 0.02 M ferrous chloride in 3.5% HCl solution (0.1 mL) to the solution, the absorbance of the solution was measured at 500 nm.

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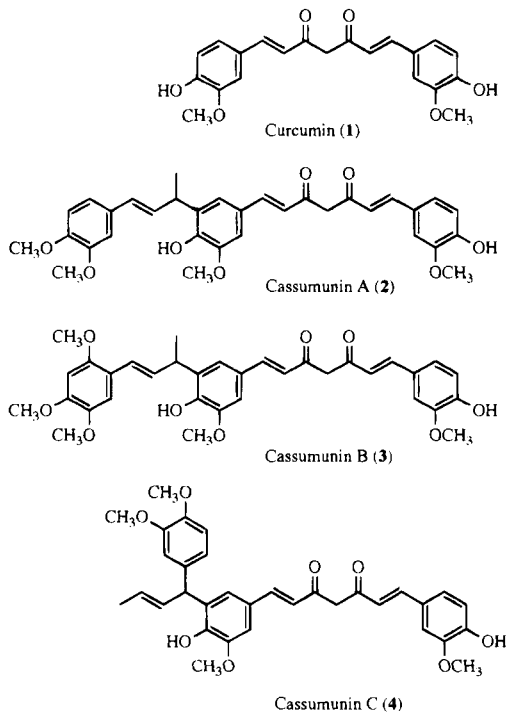


Figure 1. Structures of curcumin (**1**) and casumunins A (**2**), B (**3**), and C (**4**).

Antiinflammation Assay (TPA-Induced Method). This assay was carried out using a method described in a previous paper (Masuda *et al.*, 1993a; Hirota *et al.*, 1990). Briefly, an acetone solution of the sample (20 μL) was applied on the left ear of a mouse (male, 6 weeks old, Jcl:ICR mouse, CLEA Japan, Tokyo, Japan). Thirty minutes later, an acetone solution (20 μL) of an inflammation inducer, 12-*O*-tetradecanoylphorbol 13-acetate (TPA, 2 μg) 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. Antiinflammatory activity was estimated by the percent inhibition of the ear edema, which was calculated using the following equation:

$$\text{inhibition (\%)} = \{[(\text{wt of the ear to which TPA was applied}) - (\text{wt of the ear to which TPA and sample were applied})]/[(\text{wt of the ear to which TPA was applied}) - (\text{wt of the ear to which only vehicle was applied})]\} \times 100$$

Plant Material. Cultivated rhizomes of *Z. cassumunar* Roxb. were collected in a field near Tabanan village, Bali, Indonesia, in January 1991. Identification of the rhizomes was made by Dr. Tengah, Udayana University, Indonesia. The rhizomes are continuously cultivated in a research field of the Institute of Tropical Agriculture, University of Ryukyu, Taketomi, Okinawa, Japan.

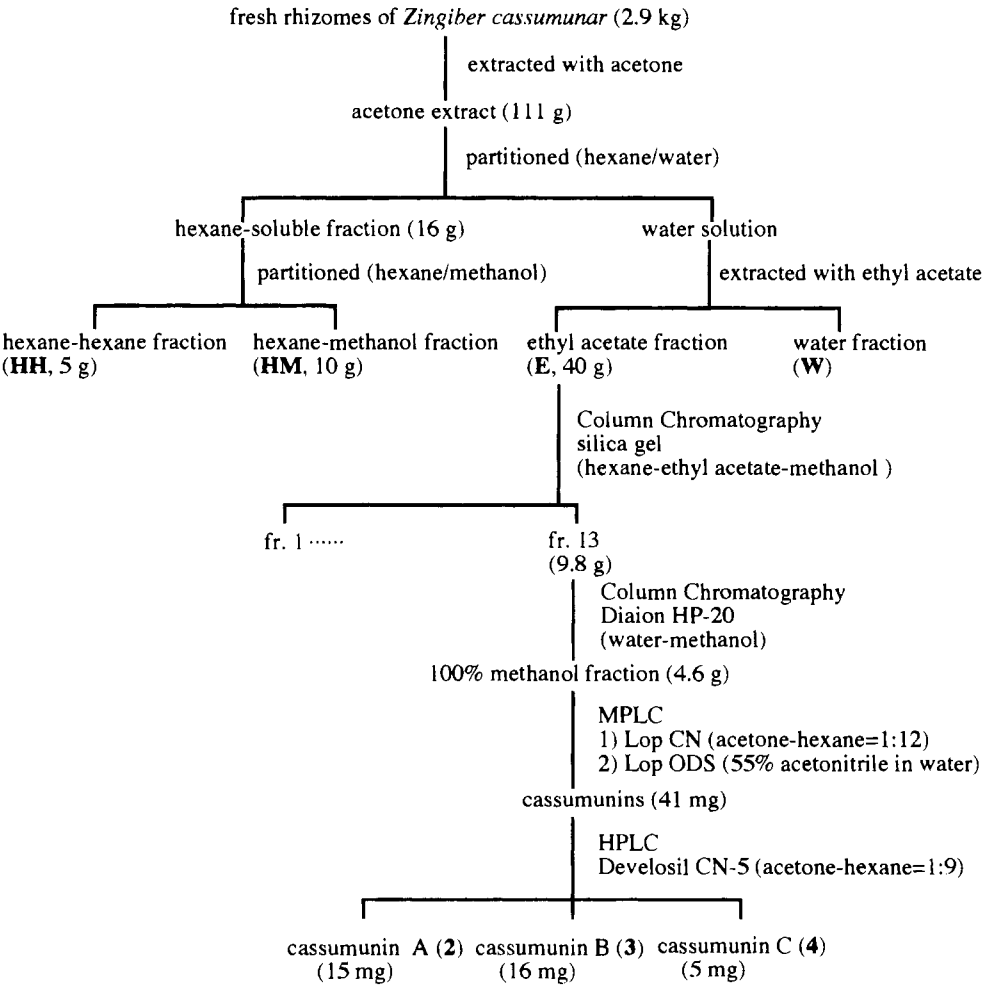
Extraction and Fractionation of Rhizomes (Figure 2). The fresh rhizomes of *Z. cassumunar* (2.9 kg) were soaked in acetone (5 L) at room temperature for 9 days. After filtration, the residue was extracted twice more with acetone. The combined acetone solutions were evaporated under reduced pressure at room temperature to give an acetone extract (111 g). The acetone extract was suspended in water (2 L) and extracted with hexane (2 L), and the hexane solution was evaporated to give a hexane-soluble fraction (16 g). The fraction was partitioned with hexane (500 mL) and methanol (500 mL). Evaporation of each obtained fraction gave a hexane–hexane fraction (**HH**, 5 g) and a hexane–methanol fraction (**HM**, 10 g), respectively. The residual water solution of the hexane extraction was then extracted six times with ethyl acetate (1 L). Evaporation of the combined ethyl acetate solution gave an ethyl acetate soluble fraction (**E**, 40 g). Twenty milliliters of the residual water solution was evaporated to give a water fraction (**W**, 0.5 g). The ethyl acetate fraction (39 g) was subjected to silica gel column chromatography (850 g of Waco gel C-300, Waco Pure Chemicals, Osaka, Japan) eluted with hexane–ethyl acetate (9:1, 17 L), hexane–ethyl acetate (2:1, 17 L), hexane–ethyl acetate (1:1, 10 L), and 15% MeOH in ethyl acetate (6 L), successively. The eluate was collected every 500 mL and combined according to TLC analytical results to produce 13 separated fractions (yields: fraction 1, 0.5 g; fraction 2, 0.5 g; fraction 3, 2.8 g; fraction 4, 0.2 g; fraction 5, 0.6 g; fraction 6, 3.8 g; fraction 7, 6.7 g; fraction 8, 2.3 g; fraction 9, 1.0 g; fraction 10, 4.0 g; fraction 11, 5.6 g; fraction 12, 2.2 g; fraction 13, 10.4 g). The antioxidant and antiinflammatory activities of the fractions are summarized in Tables 1 and 2, respectively.

Purification of Cassumunins A (2), B (3), and C (4) (Figure 2). Fraction 13 (9.8 g) of the silica gel chromatography was subjected to a polystyrene resin, Diaion HP-20 (Mitsubishi Kasei, Tokyo, Japan), column chromatography eluted with water that increased in methanol volume. The eluent with 100% methanol was evaporated (4.6 g). The residue was purified by medium-pressure liquid chromatography (MPLC) using a cyanopropylated silica gel (Lop CN, Nomura Chemicals, Seto, Japan) eluted with acetone–hexane (1:12) and an octadecanoylated silica gel (Lop ODS, Nomura Chemical) eluted with 55% acetonitrile in water, successively, to give a mixture of cassumunins. Finally, the mixture (2 mg per injection, total 41 mg) was separated by preparative HPLC [column: Develosil CN-5, 10 × 250 mm (Nomura Chemical); flow rate, 10 mL/min] eluted with acetone–hexane (1:9) to give cassumunins A (15 mg, retention time = 40 min), B (16 mg, retention time = 51 min), and C (5 mg, retention time = 31 min).

Instruments for Structure Determination. UV work was performed on a Hitachi 220A and NMR on a JEOL GX-400 (^{1}H for 400 MHz, ^{13}C for 100 MHz). 2D NMR was carried out with JEOL-supplied pulse sequences for HH-COSY-(VCOSYN), CH-COSY(VCHSHF), and NOESY(VNOEN). Mass spectrometry was done on a Hitachi M-2000 and optical rotation on a Union PM-101. CD studies were made with a Jasco J-500E.

RESULTS

Fractionation of the Extract of *Z. cassumunar* and Isolation of Cassumunins A (2), B (3), and C (4). Our previous research suggested that the antioxidant of *Z. cassumunar* was a moderately polar substance soluble in dichloromethane or ethyl acetate (Jitoe *et al.*, 1992). To prepare the antioxidant active fraction, a solvent fractionation of the acetone extract of the rhizomes was carried out as described under Experimental Procedures. Antioxidant activity of the fractionated samples was measured using a TLC method, and the efficiency of the activity was compared with the induction period of the sample for lipid oxidation (Table 1). The ethyl acetate fraction (**E**, 10 μg) and the hexane–methanol fraction (**HM**, 10 μg) showed antioxidant activity, and the efficiencies were 55 and 25 min, respectively, for the induction period. However, the hexane–hexane fraction (**HH**) and the water fraction (**W**) showed no induction period under the conditions used. The activity of the ethyl acetate fraction (**E**) was much stronger than that of the hexane–methanol fraction (**HM**). The antiinflammatory activity of each fraction was measured and estimated by the percent inhibition of each sample (Table 2). The strongest antiinflammatory activity was also observed in the ethyl acetate fraction (**E**, 62% inhibition). The ethyl acetate fraction (**E**) is expected to contain antiinflammatory and antioxidative compounds, and further fractionation of the ethyl acetate fraction was carried out. The fractionation was performed using silica gel column chro-



All materials were stored in the dark at -20°C throughout this purification.

Figure 2. Isolation procedure of cassumunins A (**2**), B (**3**), and C (**4**).

Table 1. Antioxidant Activity of Fractions from Rhizomes of *Z. cassumunar*

		induction period (min) for lipid peroxidation		
expt	sample	1 μg/spot	5 μg/spot	10 μg/spot
1	α-tocopherol	–[a]	65	–
	curcumin	–	100	–
	HH	–	<10	<10
	HM	–	<10	25
	E	–	40	55
	W	–	<10	<10
2	α-tocopherol	40	–	–
	curcumin	60	–	–
	fraction 1	<15	<15	–
	fraction 2	<15	<15	–
	fraction 3	<15	<15	–
	fraction 4	<15	<15	–
	fraction 5	<15	<15	–
	fraction 6	<15	<15	–
	fraction 7	<15	<15	–
	fraction 8	<15	15	–
	fraction 9	<15	25	–
	fraction 10	<15	15	–
	fraction 11	60	85	–
	fraction 12	50	80	–
	fraction 13	40	65	–

[a] –, not examined.

Table 2. Inhibitory Effect of Fractions (1 mg) from Rhizomes of *Z. cassumunar* against TPA (2 μg)-Induced Inflammation on Mouse Ears

	treatment				
expt	left ear	right ear	n[a]	D[b] ± SE (mg)	inhibition (%)
1		TPA	5	16.7 ± 1.4	
	HH + TPA	TPA	5	3.5 ± 1.2	21
	HM + TPA	TPA	5	8.9 ± 1.2	53
	E + TPA	TPA	5	10.4 ± 1.0	62
	W + TPA	TPA	5	7.9 ± 2.7	47
2		TPA	6	16.6 ± 1.1	
	fraction 1 + TPA	TPA	5	1.8 ± 1.1	11
	fraction 2 + TPA	TPA	5	2.6 ± 1.1	16
	fraction 3 + TPA	TPA	5	0.3 ± 0.9	2
	fraction 4 + TPA	TPA	5	0.3 ± 0.5	2
	fraction 5 + TPA	TPA	5	0.5 ± 1.1	3
	fraction 6 + TPA	TPA	5	4.3 ± 1.9	26
	fraction 7 + TPA	TPA	5	7.8 ± 1.4	48
	fraction 8 + TPA	TPA	5	7.9 ± 1.7	48
	fraction 9 + TPA	TPA	5	5.3 ± 1.4	32
	fraction 10 + TPA	TPA	5	11.9 ± 0.9	72
	fraction 11 + TPA	TPA	5	14.2 ± 0.4	86
	fraction 12 + TPA	TPA	5	14.2 ± 0.5	86
	fraction 13 + TPA	TPA	5	15.2 ± 0.7	92

[a] Number of mice. [b] Means of weight differences between right and left ears.

matography, giving 13 fractions (fractions 1–13). The antioxidant and antiinflammatory activities of each fraction are also summarized in Tables 1 and 2, respectively. Each 5 μg of fractions 11, 12, and 13 showed strong antioxidant activity (induction periods of 85, 80,

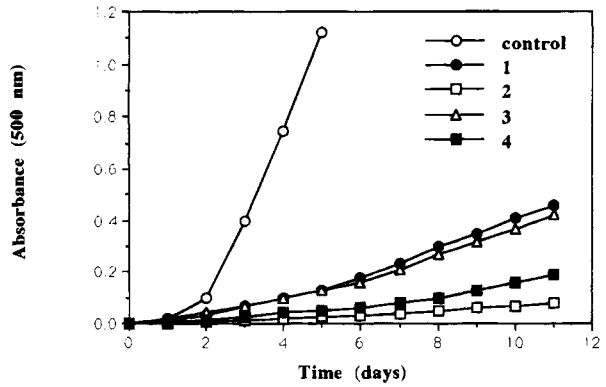


Figure 3. Effect of **1**–**4** (2.7 μmol) on autoxidation of linoleic acid (thiocyanate method).

and 65 min, respectively), while fractions 8, 9, and 10 showed weak activity (induction periods of 15, 25, and 15 min, respectively). The other fractions showed no activity under the conditions used. Fractions 10, 11, 12, and 13 also showed very strong antiinflammatory activity (inhibition of 72%, 86%, 86%, and 92%, respectively), with the activity of fraction 13 was the strongest. Fractions 7 and 8 also showed antiinflammatory activity, but the efficiency was moderate (inhibition of both, 48%). These results let us purify fraction 13 as one of the most active fractions for both antioxidant and antiinflammatory activities. In the fraction, several yellow substances were observed using silica gel TLC analysis. These compounds seem to be irreversibly absorbed in normal silica gel, similar to curcumin (Tønnesen and Karsen, 1986). To isolate these compounds, we chose a styrene polymer and alkylated silica gels as chromatographic supports rather than normal silica gel. Cynanopropylated silica gel (CN) was found to be especially effective for isolating the compounds. By a combination of these chromatographies, three yellow compounds, which were named cassumunins A, B, and C, were isolated.

Antioxidant Activity of Cassumunins A (2), B (3), and C (4). The antioxidant assay of cassumunins A (**2**), B (**3**), and C (**4**) (each purity is over 95% in ^{1}H NMR) was carried out in an ethanol–water system using linoleic acid as the substrate for autoxidation; the data are shown in Figure 3. Figure 3 shows all cassumunins (**2**–**4**, 2.7 μmol each) inhibited the accumulation of linoleic acid hydroperoxide, which is detectable by the thiocyanate method used. The antioxidant efficiency increased in the order cassumunin A (**2**), cassumunin C (**4**), cassumunin B (**3**), and the reference sample of curcumin (**1**) under these conditions. Cassumunin A (**2**) showed the strongest activity among all samples tested, and cassumunin C (**4**) showed efficiency comparable to that of **2**. Although cassumunin B (**3**) showed weaker activity than **2** and **4**, it had a slightly stronger activity than curcumin.

Antiinflammatory Activity of Cassumunins A (2), B (3), and C (4). The antiinflammatory activity of cassumunins A–C (**2**–**4**) was measured on mouse ear using TPA as the inducer. The efficiency was judged by the inhibition of edema formation. The data are summarized in Table 3. Table 3 shows cassumunins A–C (0.6 μmol) have antiinflammatory activity, and their efficiency (inhibition of 83%, 76%, and 75%, respectively) is stronger than that of curcumin (51% inhibition). Cassumunin A (**2**) showed the strongest activity among all samples tested.

Structure Determination of Cassumunins A (2), B (3), and C (4) (Tables 4–6; Figure 4). Cassumunin A (**2**) was isolated as a yellow amorphous powder. The molecular formula of **2** was determined from high-resolution mass spectral evidence (*m/z* 558.2224 [M$^+$]) to be $C_{33}H_{34}O_8$. The structure was elucidated by 2D NMR techniques, such as HH-COSY, CH-COSY, and NOESY. The HH-COSY data of **2** indicated the presence of six proton–proton coupling systems due to a 1,3,4,5-substituted benzene [aromatic A, δ 6.96 (1H, d, J = 1.8 Hz, H-2′) and 7.07 (1H, d, J = 1.8 Hz, H-6′)], two 1,3,4-substituted benzenes [aromatic B, δ 6.94 (1H, d, J = 7.9 Hz, H-5″), 7.04 (1H, d, J = 1.8 Hz, H-2″), and 7.12 (1H, dd, J = 7.9 and 1.8 Hz, H-6″); aromatic C, δ 6.80 (1H, d, J = 7.9 Hz, H-5‴″), 6.91 (1H, dd, J = 7.9 and 1.8 Hz, H-6‴″), and 6.94 (1H, d, J = 1.8 Hz, H-2‴″)], a 1,3-substituted 1-butenyl group [δ 6.40 (1H, d, J = 15.9 Hz, H-1‴), 6.29 (1H, dd, J = 15.9 and 6.7 Hz, H-2‴), 4.05 (1H, quint, J = 6.7 Hz, H-3‴), and 1.46 (3H, d, J = 6.7 Hz, H-4‴)], and two isolated *trans* olefins [olefin A, δ 7.58 (1H, d, J = 15.9 Hz, H-1) and 6.47 (1H, d, J = 15.9 Hz, H-2); olefin B, δ 7.59 (1H, d, J = 15.9 Hz, H-7) and 6.47 (1H, d, J = 15.9 Hz, H-6)]. The two NOEs between H-2′ (δ 6.96) and H-2 (δ 6.47) and between H-6′ (δ 7.07) and H-1 (δ 7.58) indicated that aromatic A is conjugated to olefin A. Also, aromatic B was indicated to be conjugated with olefin B by the NOEs between H-2″ (δ 7.04) and H-6 (δ 6.47) and between H-6″ (δ 7.12) and H-7 (δ 7.59). Compound **2** has two carbonyl groups revealed by carbon signals at 183.4 and 183.2 ppm in the ^{13}C NMR of **2**. The carbonyl groups constitute an enol form of a β-diketone system conjugated to both olefins A and B like that found in curcumin (**1**), the fact of which was derived from UV absorption maxima at 426 nm in the UV spectrum of **2** similar to that of curcumin and which is also supported by the downfield-shifted proton signals of H-1 (δ 7.58) and H-7 (δ 7.59) and the NOE observation of the enol methine proton (H-4, δ 5.81) with H-2 and H-6. The NOEs between H-2‴″,H-6‴″ in aromatic C and H-2‴,H-1‴ in the butenyl group, respectively, indicated that the aromatic C is attached to the butenyl group. The butenyl group was also attached to the 5-position of aromatic A, which was determined by the NOEs between the methyl protons (δ 1.46) of the butenyl group and H-6′ (δ 7.07) in aromatic A. The substituted positions of the three phenolic methoxyl groups (δ 3.95, 3.95, and 3.87) were determined by the NOEs with H-2′, H-2″, and H-5‴″, respectively, to be the 3-position of aromatic A, the 3-position of aromatic B, and the 4-position of aromatic C. One methoxyl group (δ 3.89) and two hydroxyl groups (δ 5.85 and δ 6.03) were determined to be attached to the 3-position of aromatic C, the 4-position of aromatic A, and the 4-position of aromatic B, respectively, by the fragment ion peaks (*m/z* 164, 177, and 191) which resulted from the cleavages

Table 3. Inhibitory Effect of 1–4 (0.6 μmol) against TPA (2 μg)-Induced Inflammation on Mouse Ears

treatment				
left ear	right ear	n^a	$D^b \pm$ SE (mg)	inhibition (%)
	TPA	6	17.5 ± 0.9	
1 + TPA	TPA	5	8.9 ± 0.9	51
2 + TPA	TPA	5	14.5 ± 1.0	83
3 + TPA	TPA	5	13.3 ± 1.2	76
4 + TPA	TPA	5	13.1 ± 1.3	75

[a] Number of mice. [b] Means of weight differences between right and left ears.

Table 4. Physicochemical and Spectral Data of Cassumunins A (2), B (3), and C (4)

	cassumunin A (2)	cassumunin B (3)	cassumunin C (4)
molecular formula	$C_{33}H_{34}O_8$	$C_{34}H_{36}O_9$	$C_{33}H_{34}O_8$
high-resolution EIMS (*m/z*)	558.2224 $[M]^+$ (calcd for $C_{33}H_{34}O_8$: 558.2252)	588.2354 $[M]^+$ (calcd for $C_{34}H_{36}O_9$: 588.2357)	558.2289 $[M]^+$ (calcd for $C_{33}H_{34}O_8$: 558.2252)
$[\alpha]^{24}_D$	−11° (*c* 1.0, $CHCl_3$)	−4° (*c* 1.0, $CHCl_3$)	−13° (*c* 0.4, $CHCl_3$)
CD (MeOH)	no Cotton effect	no Cotton effect	no Cotton effect
UV λ_{max} (MeOH, nm)	426, 297 (sh), 260	426, 314, 260	426, 262
EIMS (70 eV, *m/z*)	558 (rel intensity 14%), 382 (4%), 340 (4%), 314 (9%), 191 (9%), 177 (23%), 164 (100%)	588 (rel intensity 9%), 412 (28%), 370 (15%), 344 (25%), 194 (100%), 177 (8%)	588 (rel intensity 69%), 382 (79%), 340 (81%), 314 (100%)

Table 5. ^{1}H-NMR Spectral Data[a] of Cassumunins A (2), B(3), and C (4) [δ in $CDCl_3$ (400 MHz)]

H	cassumunin A (2)	cassumunin B (3)	cassumunin C (4)
1	7.58, 1H, d (15.9)	7.58, 1H, d (15.9)	7.56, 1H, d (15.9)
2	6.47, 1H, d (15.9)	6.47, 1H, d (15.9)	6.43, 1H, d (15.9)
4	5.81, 1H, s	5.80, 1H, s	5.80, 1H, s
6	6.47, 1H, d (15.9)	6.47, 1H, d (15.9)	6.47, 1H, d (15.9)
7	7.59, 1H, d (15.9)	7.59, 1H, d (15.9)	7.59, 1H, d (15.9)
2′	6.96, 1H, d (1.8)	6.95, 1H, d (1.8)	6.97, 1H
6′	7.07, 1H, d (1.8)	7.09, 1H, d (1.8)	6.97, 1H
2″	7.04, 1H, d (1.8)	7.05, 1H, d (1.8)	7.05, 1H, d (1.8)
5″	6.94, 1H, d (7.9)	6.93, 1H, d (7.9)	6.93, 1H, d (7.9)
6″	7.12, 1H, dd (7.9, 1.8)	7.12, 1H, dd (7.9, 1.8)	7.12, 1H, dd (7.9, 1.8)
1‴	6.40, 1H, d (15.9)	6.78, 1H, d (15.9)	5.00, 1H, br d (6.7)
2‴	6.29, 1H, dd (15.9, 6.7)	6.29, 1H, d (15.9, 6.7)	5.90, 1H, ddd (15.6, 6.7, 1.8)
3‴	4.05, 1H, quint (6.7)	4.07, 1H, quint (6.7)	5.44, 1H, ddd (15.6, 6.7, 1.8)
4‴	1.46, 3H, d (6.7)	1.47, 3H, d (6.7)	1.75, 3H, d (6.7)
2⁗	6.94, 1H, d (1.8)	6.98, 1H, s	6.75, 1H
5⁗	6.80, 1H, d (7.9)	6.50, 1H, s	6.80, 1H, d (7.9)
6⁗	6.91, 1H, dd (7.9, 1.8)		6.75, 1H
3′-OMe	3.95, 3H, s	3.95, 3H, s	3.93, 3H, s
3″-OMe	3.95, 3H, s	3.95, 3H, s	3.95, 3H, s
3⁗-OMe	3.89, 3H, s	3.85, 3H, s	3.83, 3H, s
4⁗-OMe	3.87, 3H, s	3.82, 3H, s[b]	3.85, 3H, s
6⁗-OMe		3.89, 3H, s[b]	
OH	5.85, 1H, br s	5.84, 1H, br s	5.86, 1H, br s
OH	6.03, 1H, br s	6.03, 1H, br s	5.98, 1H, br s

[a] Coupling constants (*J* in hertz) in parentheses. [b] Assignments may be interchangeable.

between C-2‴ and C-3‴, between C-4 and C-5, and between C-3 and C-4, respectively, in the EI mass spectrum.

Cassumunin B (**3**) was also isolated as a yellow powder and showed ^{1}H-NMR data similar to those of **2**. The molecular formula ($C_{34}H_{36}O_9$) of **3**, which was derived from a high-resolution mass spectrometric result (*m/z* 588.2298 $[M^+]$), indicated that **3** has an additional methoxyl group compared with **2**. In the HH-COSY of **3**, proton−proton coupling systems due to a 1,3,4,5-tetrasubstituted benzene [aromatic A, δ 6.95 (1H, d, *J* = 1.8 Hz, H-2′) and 7.09 (1H, d, *J* = 1.8 Hz, H-6′)] and a 1,3,4-trisubstituted benzene [aromatic B, δ 6.93 (1H, d, *J* = 7.9 Hz, H-5″), 7.05 (1H, d, *J* = 1.8 Hz, H-2″), and 7.12 (1H, dd, *J* = 7.9 and 1.8 Hz, H-6″)] were very similar to those of **2**; however, signals due to a 1,2,4,5-tetrasubstituted benzene [aromatic C, δ 6.50 (1H, s, H-5⁗) and 6.98 (1H, s, H-2⁗)] were observed instead of those due to the 1,3,4-trisubstituted benzene in **2**. The same diarylheptanoid moiety to **2** was also clarified by combination of the UV spectral data (λ_{max} 426 nm) and NOEs (H-6′/H-1, H-2′/H-2, H-2/H-4, H-4/H-6, H-6/H-2″, and H-7/H-6″) in the NOESY of **3**. Also, the structure of the phenylbutenyl moiety including aromatic C is revealed by a proton coupling connectivity in the HH-COSY of **3** [δ 1.47 (3H, d, *J* = 6.7 Hz, H-4‴), 4.07 (1H, quint, *J* = 6.7 Hz, H-3‴), 6.29 (1H, dd, *J* = 15.9 and 6.7 Hz, H-2‴), 6.78 (1H, d, *J* = 15.9 Hz, H-1‴)] and the NOE between H-2⁗ and H-2‴ in the NOESY of **3**. The attached position of the phenylbutenyl moiety was determined to be the 5-position of aromatic A by the NOE between H-4‴ and H-6′. The NOEs from five phenolic methoxyl groups (δ 3.95, 3.95, 3.85, 3.82, and 3.89) to H-2′, H-2″, H-2⁗, H-5⁗, and H-5⁗, respectively, indicated that the methoxyl groups are attached at the 3-position of aromatic A, the 3-position of aromatic B, and the 3-, 4-, and 6-positions of aromatic C.

Cassumunin C (**4**) was isolated as a yellow powder and its molecular formula, $C_{33}H_{34}O_8$, was obtained by a high-resolution mass spectral result (*m/z* 558.2289 $[M^+]$) of **4**. The ^{1}H-NMR data of **4** were also similar to those of **2**, and similar proton−proton coupling systems to those of **2** [aromatic A, δ 6.97 (2H, H-2′ and H-6′); aromatic B, δ 6.93 (1H, d, *J* = 7.9 Hz, H-5″), 7.05 (1H, d, *J* = 1.8 Hz, H-2″), 7.12 (1H, dd, *J* = 7.9 and 1.8 Hz, H-6″); aromatic C, δ 6.75 (2H, H-2⁗ and H-6⁗), 6.80 (1H, d, *J* = 7.9 Hz, H-5⁗)] were clarified in HH-COSY data of **4** except for a butenyl moiety. The diarylheptanoid moiety including aromatics A and B was determined by UV (λ_{max} 426 nm), carbon signals for a β-diketone (δ 101.0, 183.1, and 183.5), and NOEs (H-6′/H-1, H-2′/H-2, H-2/H-4, H-4/H-6, H-6/H-2″, and H-7/H-6″). The proton coupling connectivity due to a 1,1-disubstituted 2-butenyl group was revealed by a homodecoupling technique in the ^{1}H NMR [δ 1.75 (3H, d, *J* = 6.7 Hz, H-4‴), 5.00 (1H, br d, *J* = 6.7 Hz, H-1‴), 5.44 (1H, ddd, *J* = 15.6, 6.7, and 1.8 Hz, H-3‴), 5.90 (1H, ddd, *J* = 15.6, 6.7, and 1.8 Hz, H-2‴)]. The attached positions of the butenyl group were determined to be the 5-position of aromatic A and the 1-position of aromatic C by the NOEs between H-6′ and H-1‴ and between H-6⁗ and H-1‴ in the NOESY of **4**.

Cassumunins A−C have an asymmetric center in their phenylbutenyl moiety and showed small optical rotation values [$[\alpha]_D$ **2**, −11° (*c* 1.0); **3**, −4° (*c* 1.0); **4**,

Table 6. ^{13}C-NMR Spectral Data of Cassumunins A (2), B (3), and C (4) [δ in $CDCl_3$ (100 MHz)]

C	cassumunin A (2)[a]	cassumunin B (3)	cassumunin C (4)
1	140.4[a]	140.4[a]	140.4[a]
2	121.7[b]	121.7[b]	121.6[b]
3	183.2[c]	183.1[c]	183.1[c]
4	101.0	101.0	101.0
5	183.4[c]	183.5[c]	183.5[c]
6	121.9[b]	121.8[b]	121.9[b]
7	141.0[a]	141.4[a]	141.0[a]
1′	127.0[d]	127.0[d]	126.8[d]
2′	107.4	107.4	107.3
3′	145.3[e]	145.3[e]	145.4[e]
4′	146.8[e]	146.8[e]	146.8[e]
5′	132.0	132.3[f]	135.8
6′	121.7	121.9[b]	120.3[b]
1″	127.7[d]	127.8[d]	127.8[d]
2″	109.7	109.7	109.7
3″	146.9[e]	146.9[e]	146.9[e]
4″	147.9[e]	147.9[e]	147.9[e]
5″	114.9	114.4	114.9
6″	122.8	123.0	122.9
1‴	128.6	122.8	46.4
2‴	132.0	132.1[f]	127.2
3‴	35.6	36.1	112.2
4‴	19.8	20.0	18.0
1⁗	130.8	118.7	130.5
2⁗	108.9	151.2	132.3
3⁗	149.1[f]	98.3	147.6[f]
4⁗	148.5[f]	149.2	148.9[f]
5⁗	111.3	143.5	111.2
6⁗	119.2	110.1	123.3[b]
OMe	55.9	56.0	55.9
	56.0	56.2	55.9
	56.0	56.7	56.0
	56.2	56.8	56.1

[a] Assignments were based on the CH-COSY spectrum. [a–f] Assignments may be interchangeable within each column.

−13° (*c* 0.4)]. However, they showed no Cotton effect in their CD spectra. Their absolute chemistry is still unclear.

DISCUSSION

We succeeded in isolating new compounds with both antioxidant and antiinflammatory activities. Their activity efficiencies were also shown to be stronger than those of curcumin. Cassumunins are new complex curcuminoids, and their structures were determined to be 5′-phenylbutenylated curcumins. Curcumin is a yellow pigment of the rhizomes of turmeric, and two analogs (*p*-hydroxycinnamoylferuloylmethane and *p*,*p*′-dihydroxydicinnamoylmethane) are also known (Kuroyanagi and Natori, 1970). The antioxidant activity of each compound was measured and compared by Toda *et al.* (1985). They reported that curcumin has the strongest activity among the three curcuminoids by their modified active oxygen method and also indicated that the methoxyl group near the hydroxyl group increases the antioxidant activity. 5′-Methoxylated curcumin, which we have isolated from a tropical ginger, *Curcuma xanthorrhiza*, has a slightly stronger activity than curcumin (Masuda *et al.*, 1992). The present results indicate that not only the methoxyl group but also the alkyl group at the 5′-position of curcumin would increase the antioxidant activity. Also, the activity efficiency would vary with the structure of the alkyl group at the 5′-position like that observed in the activity difference between cassumunins A and B. The antioxidant mechanism of tocopherol has been examined in detail [review in Hughes *et al.* (1992)], and the alkyl group effect on the benzene part of tocopherol to

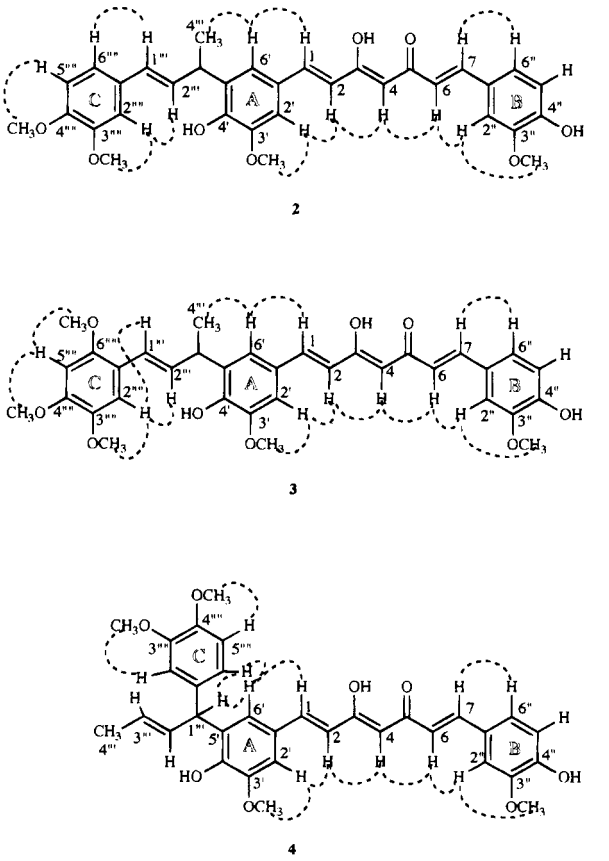


Figure 4. Selected NOEs (dashed line) observed in the NOESY spectrum of **2**–**4**.

antioxidant activity was recently reported by Barclay *et al.* (1993). However, little is known about the substituent effect on curcuminoid. We are investigating the antioxidant active structure of curcuminoids including the cassumunins.

TPA, the inflammation inducer used, is known as one of the strongest tumor promoters and shows pleiotropic effects on living organisms [review in Fujiki and Sugimura (1989)]. One of the effects of TPA is inflammation, and the inflammation is thought to be related to the generation of active oxygen species including arachidonic acid peroxide. Although the production of arachidonic acid peroxide, which is an intermediate of various prostaglandins and leukotrienes, is an enzymatic reaction, this mechanism is very similar to the autoxidation of food lipids. Some food antioxidants also inhibit arachidonic acid metabolism during the stage of the enzymatic peroxidation reaction (Nakadate *et al.*, 1984). Curcumin also inhibited arachidonic acid metabolism and showed inhibitory activity against the TPA-induced inflammation (Huang *et al.*, 1991). The activity may be related to its antioxidant activity. The antiinflammatory activity of cassumunins, as well as their antioxidant activity, is stronger than that of curcumin. They may more efficiently inhibit arachidonic acid metabolism. We have already reported that the methoxyl group on the benzene part of curcumin influenced its antiinflammatory activity. Studies on the alkyl group effect found in cassumunins are now in progress.

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