

Synthesis of (±)-Cassumunins A and B, New Curcuminoid Antioxidants Having Protective Activity of the Living Cell against Oxidative Damage

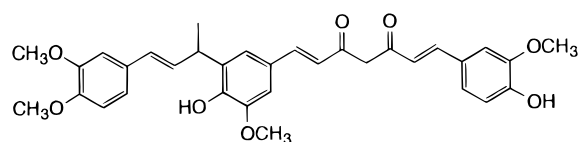
Toshiya Masuda,^{*,†} Hiroyuki Matsumura,[†] Yasuo Oyama,[†] Yoshio Takeda,[†] Akiko Jitoe,[‡] Atsuko Kida,[‡] and Kayo Hidaka[‡]

Faculty of Integrated Arts and Sciences, University of Tokushima, Tokushima 770, Japan, and Faculty of Human Life Science, Osaka City University, Osaka 558, Japan

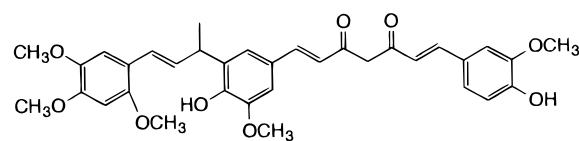
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A chemical synthesis of cassumunins A (**1**) and B (**2**), natural curcuminoid antioxidants, was developed. The synthesis was started from *o*-vanillin and after nine reaction steps resulted in 20% and 26% overall yields of **1** and **2**, respectively. The synthetic cassumunins showed stronger protective activity than curcumin against oxidative cell death induced by hydrogen peroxide in a rat thymocyte system.

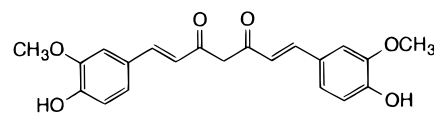
Powerful antioxidants originating from edible and medicinal plants have been extensively investigated as important inhibitory materials for the prevention of oxidative deterioration of lipids. Recently, it has been shown that peroxidation in living organisms is closely related to the initiation of some human diseases, such as cancer, coronary heart disease, and Alzheimer's disease. Ingestion of antioxidants may possibly prevent these diseases.^{1,2} In edible and medicinal plants, various types of antioxidants exist, and their chemical investigation is still in progress.³ Curcumin, a precursor to the curcuminoids, is well known as a yellow pigment from a popular spice, tumeric,⁴ and has been recognized for its useful biological activities that are related to its antioxidant activity.⁵ The activity of curcumin, however, is not strong when compared with that of vitamin E,⁶ which may limit the interest in curcumin as a useful antioxidant for human health. Recently, Osawa and co-workers found that tetrahydrocurcumin, derived from catalytic hydrogenation of curcumin, had stronger antioxidant activity than curcumin, and they also showed its efficient activity in cell membrane systems.⁷ We have screened tropical ginger species, which are used as spices or traditional medicines in some tropical areas, and have shown these gingers to be powerful antioxidant sources.⁸ New antioxidants, cassumunins and cassumunarins are found in one of these tropical gingers,^{9,10} and they have been determined to be new curcuminoid structures. We also reported that the antioxidant activity of the new curcuminoids was stronger than that of curcumin.^{11,12} In 1994, an NCI research group selected curcumin as one of the possible chemopreventive materials of cancer.¹³ Interest in such new active curcuminoids has prompted us to develop their chemical synthesis as a profitable preparation method. This paper deals with the full details of the synthesis of cassumunins A(**1**)¹⁴ and B(**2**) from commercially available *o*-vanillin. Their inhibitory activity against oxidative stress in a cell system is also reported.



Cassumunin A (**1**)



Cassumunin B (**2**)



Curcumin

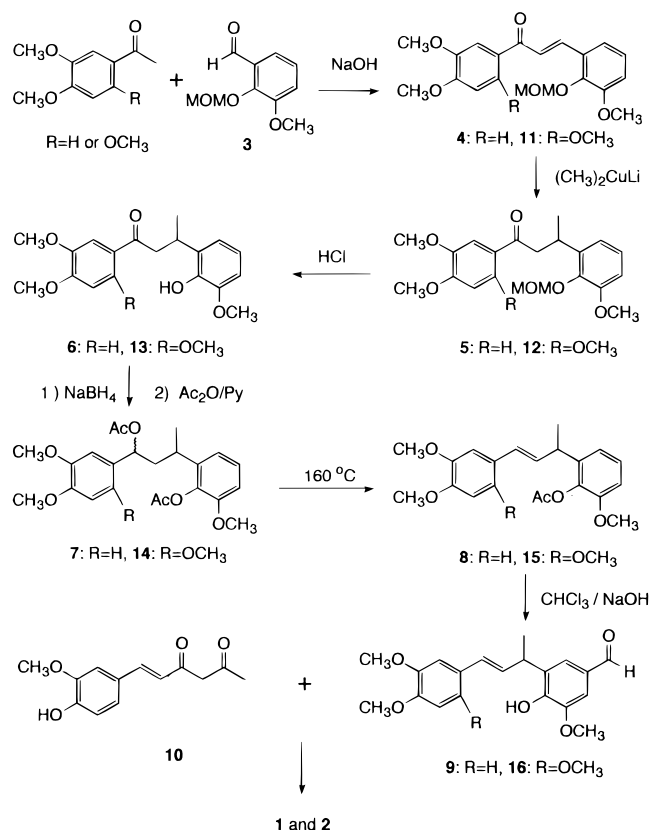
Results and Discussion

Cassumunin A (**1**) is a modified phenylbutenylated curcumin at the 5'-position of a left aromatic ring as shown. The synthetic strategy includes the preparation of the left aromatic moiety of **1**, using commercially available *o*-vanillin, by constructing the phenylbutenoid moiety from the aldehyde function of *o*-vanillin. The phenolic hydroxyl group of *o*-vanillin was protected with a methoxymethyl group, using methoxymethyl chloride in the presence of K₂CO₃, in quantitative yield. Aldol condensation of the methoxymethylated *o*-vanillin (**3**) with 3',4'-dimethoxylacetophenone and NaOH gave the chalcone **4** in 97% yield. Introduction of the C₁ unit to the β -position of the conjugated carbonyl group was achieved by dimethylcuprous iodide, giving **5** in 94% yield. The carbonyl group was reduced to a hydroxy group by treatment with NaBH₄, and the hydroxyl group was subsequently eliminated. Unfortunately, the vari-

* To whom corresponding should be addressed. Fax: +81-886-56-7244. E-mail: masuda@ias.tokushima-u.ac.jp.

[†] University of Tokushima.

[‡] Osaka City University.

Scheme 1. Synthetic route for Cassumumins A (**1**) and B (**2**); (MOM = CH₂OCH₃)

ous conditions for formation of a double bond, such as acid dehydration, dehalogenation after conversion to halide, or thermal elimination, were unsuccessful due to degradation of the methoxymethyl group to a phenol, which was spontaneously cyclized at the benzyl position under the above conditions. The methoxymethyl group of **5** was treated with 1N HCl in CH₃OH to give phenol **6** in quantitative yield. After protection of the phenolic hydroxyl group with benzyl bromide, the carbonyl group was reduced by NaBH₄. Elimination of the benzyl hydroxyl group was achieved by heating at 160 °C in dimethyl sulfoxide to form an olefin. As an alternative route, phenol **6** was directly reduced with NaBH₄ to give a diol compound. The two hydroxyl groups of the diol were protected with an acetyl group, with acetic anhydride in pyridine, giving diacetate **7** in 88% yield. This acetylation of the benzyl hydroxyl group facilitated thermo-elimination of the hydroxyl group. Diacetate **7** was heated at 160 °C for 3 h and gave olefin **8** in 84% yield, which represents the completed phenylbutenoid structure shown in cassumunin A. After the deacetylation, an aldehyde function was introduced at the appropriate position for the elongation of the alkyl chain to the aromatic ring. Various methods for aldehyde introduction to an aromatic ring have been reported; however, the methods using acidic or high-temperature conditions could not be applied because the phenolic hydroxyl group cyclized at the benzyl position. Thus, the Reimer–Teiman's protocol,¹⁵ which utilizes basic conditions, was chosen for the aldehyde introduction. Treatment of **8** with CHCl₃ in NaOH–EtOH solution at 70 °C gave the desired aldehyde **9** in 52% yield. The right-hand side of **1** and **2** was prepared from *o*-vanillin by coupling with acetylacetone. Pabon reported a one-

step synthesis of curcumin using the acetylacetone–boron complex to aldol condensation with 2 equiv of vanillin.¹⁶ We applied conditions with an excess amount of acetylacetone to give diketone **10** in 51% yield. After **10** was converted to its boron complex, aldehyde **9** was coupled with the boron complex in the presence of amine. *n*-Butylamine was chosen in Pabon's curcumin synthesis;¹⁶ however, piperidine was found to give a better yield (57%). Thus, cassumunin A (**1**) was synthesized in 20% overall yield from commercially available *o*-vanillin in nine steps. Synthetic **1** was identical to naturally occurring **1** in all respects (NMR, MS). Synthesis of **2** was accomplished with nearly the same procedures, using 2',4',5'-trimethoxyacetophenone instead of 3',4'-dimethoxyacetophenone as a starting acetophenone, in 26% overall yield from *o*-vanillin.

We examined the effect of synthetically obtained **1** and **2** on oxidative stress in a living cell system. Rat thymocytes were chosen as easily obtainable cells, and oxidative stress was created by adding hydrogen peroxide according to Oyama's protocol.¹⁷ Both **1** and **2** showed protective activity against H₂O₂-induced cell death. The activity efficiency was estimated and compared with curcumin using their IC₅₀ values (**1**, 0.17 μM; **2**, 0.22 μM; and curcumin, 1.02 μM). These IC₅₀ data indicated that both **1** and **2** are nearly five times more effective than curcumin.

Experimental Section

General Experimental Procedures. Melting points were taken on Yanaco micro-melting point apparatus and are uncorrected. ¹H NMR spectra were recorded with TMS as an internal standard at 400 MHz on a JEOL α-400 spectrometer or a JEOL EX-400 spectrometer. IR spectra were recorded on a Perkin–Elmer FTIR 1720 spectrometer or Shimadzu IR-400 spectrometer. MS were obtained with a Hitachi M-2000 spectrometer or a JEOL JMS–SX102A spectrometer. Si gel TLC was performed with Merck Si gel 60 F₂₅₄ plates. Column chromatography was carried out with Merck Si gel 60. Elemental analyses were obtained from the Instruments Center, Faculty of Pharmaceutical Science, University of Tokushima or from Tokyo Kasei Co., Ltd. (Tokyo, Japan). All reagents and solvents were purchased from Wako Pure Chemicals (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan) and used without further purification.

Preparation of 2-O-Methoxymethyl-*o*-vanillin (3**).** To a solution of *o*-vanillin (22 g) in Me₂CO (220 mL) were added anhydrous K₂CO₃ (44 g) and methoxymethyl chloride (18 mL), successively. The mixture was stirred for 3 h at 23 °C. To the mixture was added CH₂Cl₂ (500 mL), and then the mixture was filtered to remove K₂CO₃. The filtrate was washed with saturated NaCl solution, dried over Na₂SO₄, and concentrated. The residue was crystallized with ether–hexane, giving **3** (28.5 g, quantitative yield) as colorless needles, mp 53.5–54.0 °C; ¹H NMR δ (CDCl₃) 10.48 (1H, s), 7.45 (1H, dd, *J* = 7.8 Hz), 7.16 (2H, m), 5.24 (2H, s), 3.89 (3H, s), 3.57 (3H, s); IR (film) ν_{max} 1680; EIMS *m/z* 196 [M]⁺; anal. C 60.93%, H 6.06%, calcd for C₁₀H₁₂O₄, C, 61.22%, H, 6.16%.

Synthesis of Chalcones **4 and **11**.** To a solution of **3** (15 g) and 3,4-dimethoxyacetophenone (13.8 g) in

C₂H₅OH (30 mL) was added 1.4 N NaOH solution (C₂H₅-OH-H₂O 4:5, 70 mL) at 23 °C. The mixture was stirred for 16 h, then poured into saturated NaCl and extracted 3 times with CH₂Cl₂. The combined CH₂Cl₂ layer was dried over anhydrous Na₂SO₄ and evaporated. The residue was crystallized with ether, giving **4** (26.6 g, 97%) as a yellow powder. Yellow plates of **4** were obtained by recrystallization of the powder with CH₃-OH: mp 108.0–109.0 °C; ¹H NMR δ (CDCl₃) 8.21 (1H, d, *J* = 16 Hz), 7.69 (1H, dd, *J* = 7.2, 2 Hz), 7.62 (1H, d, *J* = 2 Hz), 7.60 (1H, d, *J* = 16 Hz), 7.31 (1H, d, *J* = 7.2 Hz), 7.11 (1H, t, *J* = 7.2 Hz), 6.97 (1H, d, *J* = 8.0 Hz), 6.92 (1H, d, *J* = 8.0 Hz), 5.16 (2H, s), 3.95 (6H, s), 3.83 (3H, s), 3.60 (3H, s); IR (film) ν_{max} 1657; EIMS *m/z* 358 [M]⁺; anal. C 66.82%, H 5.99%, calcd for C₂₀H₂₂O₆, C 67.02%, H 6.19%.

By similar procedures using 2',4',5'-trimethoxyacetophenone instead of 3',4'-dimethoxyacetophenone, **11** (12.2 g) was obtained in 84% yield from **3** (7.3 g): mp 108.5–109.5 °C; ¹H NMR (CDCl₃) 8.13 (1H, d, *J* = 16 Hz), 7.62 (1H, d, *J* = 16 Hz), 7.39 (1H, s), 7.23 (1H, d, *J* = 7.8 Hz), 7.16 (1H, t, *J* = 7.8 Hz), 6.94 (1H, d, *J* = 7.8 Hz), 6.54 (1H, s), 5.16 (2H, s), 3.97 (3H, s), 3.93 (3H, s), 3.89 (3H, s), 3.85 (3H, s), 3.61 (3H, s); IR (CHCl₃) ν_{max} 1650; EIMS *m/z* 388 [M]⁺; anal. C 65.07%, H 6.24%, calcd for C₂₁H₂₄O₇, C 64.94%, H 6.23%.

Synthesis of Ketones 5 and 12. To a solution of CuI (2.4 g) in dry ether (48 mL) was added dropwise CH₃Li (1.5 N solution in ether) at 0 °C under N₂ atmosphere. After stirring 5 min, a THF solution (15 mL) of **4** (3.0 g) was added dropwise to the solution for 10 min. After stirring for 5 min at 0 °C, the excess (CH₃)₂CuLi was decomposed by CH₃COOH and then by 1N HCl. The mixture was extracted three times with CH₂Cl₂. The CH₂Cl₂ layers were combined, dried over Na₂SO₄, and evaporated. The residue was purified by Si gel column chromatography (ethyl acetate–hexane, 1:2), giving **5** (2.95 g, 94%) as colorless powder. Colorless prisms of **5** were obtained by recrystallization with CH₃OH–H₂O: mp 95.5–97.0 °C; ¹H NMR δ (CDCl₃) 7.64 (1H, dd, *J* = 8.4, 2.0 Hz), 7.53 (1H, d, *J* = 1.6 Hz), 7.06 (1H, t, *J* = 8.0 Hz), 6.89 (1H, dd, *J* = 8.8, 1 Hz), 6.87 (1H, d, *J* = 8.8 Hz), 6.80 (1H, dd, *J* = 8.0, 1.2 Hz), 5.12 (2H, s), 3.98 (1H, m), 3.94 (3H, s), 3.93 (3H, s), 3.84 (3H, s), 3.57 (3H, s), 3.30 (1H, dd, *J* = 16, 4.4 Hz), 3.06 (1H, dd, *J* = 16, 9.2 Hz), 1.30 (3H, d, *J* = 7.2 Hz); IR (film) ν_{max} 1673; EIMS *m/z* 374 [M]⁺; anal. C 67.24%, H 7.08%, calcd for C₂₁H₂₆O₆, C 67.36%, H 7.00%.

Using similar procedures, **12** (10.2 g) was synthesized in 98% yield from **11** (10.0 g): mp 105.0–106.0 °C; ¹H NMR δ (CDCl₃) 7.36 (1H, s), 7.04 (1H, t, *J* = 7.8 Hz), 6.86 (1H, d, *J* = 7.8 Hz), 6.77 (1H, d, *J* = 7.8 Hz), 6.48 (1H, s), 5.09 (2H, s), 3.98 (1H, m), 3.92 (3H, s), 3.88 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 3.58 (3H, s), 3.32 (1H, dd, *J* = 17, 4 Hz), 3.20 (1H, dd, *J* = 17, 9 Hz), 1.26 (3H, d, *J* = 6.8 Hz); IR (CHCl₃) ν_{max} 1660; EIMS *m/z* 404 [M]⁺; anal. C 65.13%, H 6.96%, calcd for C₂₂H₂₈O₇, C 65.33%, H 6.98%.

Synthesis of Phenols 6 and 13. To a solution of **5** (8.0 g) in CH₃OH (240 mL) was added 1N HCl (136 mL). The mixture was stirred at 60 °C for 5 min, then poured into H₂O and extracted four times with CH₂Cl₂. The CH₂Cl₂ layer was dried over Na₂SO₄ and purified by Si gel column chromatography (EtOAc–hexane, 1:1),

giving **6** (7.1 g, quantitative yield) as viscous oil: ¹H NMR δ (CDCl₃) 7.67 (1H, dd, *J* = 8.0, 2.0 Hz), 7.56 (1H, d, *J* = 2 Hz), 6.88 (1H, t, *J* = 8.0 Hz), 6.83 (1H, dd, *J* = 7.8, 1.5 Hz), 6.83 (1H, d, *J* = 7.8 Hz), 6.81 (1H, d, *J* = 7.8 Hz), 6.05 (1H, br s), 3.97 (3H, s), 3.96 (3H, s), 3.90 (3H, s), 3.80 (1H, m), 3.42 (1H, dd, *J* = 16, 4 Hz), 3.02 (1H, dd, *J* = 16, 5 Hz), 1.33 (3H, d, *J* = 7 Hz); IR (film) ν_{max} 3442, 1673; HRMS *m/z* 330.1474 (calcd for C₁₉H₂₂O₅, 330.1466).

By similar procedures, **13** (6.4 g) was synthesized in 84% yield from **12** (8.5 g): mp 104.5–105.5 °C; ¹H NMR δ (CDCl₃) 7.34 (1H, s), 6.80 (2H, m), 6.70 (1H, m), 6.48 (1H, s), 6.26 (1H, br s), 3.79 (1H, m), 3.42 (1H, dd, *J* = 17, 5 Hz), 3.23 (1H, dd, *J* = 17, 7 Hz), 1.30 (3H, d, *J* = 7.3 Hz); IR (CHCl₃) ν_{max} 3500, 1660; EIMS *m/z* 360 [M]⁺; anal. C 66.42%, H 6.73%, calcd for C₂₀H₂₄O₆, C 66.65%, H 6.71%.

Synthesis of Diacetates 7 and 14. To a solution of **6** (7.1 g) in CH₃OH (300 mL) was added NaBH₄ (3.5 g) at 23 °C. After stirring for 10 min at 23 °C, the mixture was poured into H₂O, extracted four times with CH₂Cl₂, dried over Na₂SO₄, and concentrated. To the residue were added Ac₂O (56 mL) and pyridine (56 mL) at 23 °C. After standing for 3 h, the solvent was removed *in vacuo*, and the residue was purified by Si gel column chromatography (EtOAc–hexane, 1:2) to give diacetate **7** as a 3:1 mixture of stereoisomers (7.9 g, 88%): colorless oil, ¹H NMR δ (CDCl₃) major isomer 7.19 (1H, t, *J* = 7.8 Hz), 6.93–6.78 (5H, m), 5.62 (1H, br t, *J* = 7.2 Hz), 3.88 (3H, s), 3.87 (3H, s), 3.82 (3H, s), 2.82 (1H, m), 2.31 (1H, m), 2.23 (3H, s), 2.00 (3H, s), 1.92 (1H, m), 1.39 (3H, d, *J* = 7.2 Hz); IR (film) ν_{max} 1765, 1735; EIMS *m/z* 416 [M]⁺; anal. C 66.57%, H 6.69%, calcd for C₂₃H₂₈O₇, C 66.33%, H 6.78%.

By similar procedures, **14** (7.2 g) was synthesized in 97% yield from **13** (6.0 g): a 1:1 mixture of stereoisomers; ¹H NMR δ (CDCl₃) 7.15 (1H, t, *J* = 8 Hz), 7.14 (1H, t, *J* = 8 Hz), 6.88 (1H, d, *J* = 8 Hz), 6.86 (1H, d, *J* = 8 Hz), 6.80 (1H, d, *J* = 8 Hz), 6.79 (1H, s), 6.78 (1H, d, *J* = 8 Hz), 6.77 (1H, s), 6.50 (1H, s), 6.49 (1H, s), 6.16 (1H, dd, *J* = 9, 4 Hz), 6.04 (1H, t, *J* = 6 Hz), 3.89 (3H, s), 3.88 (3H, s), 3.84 (3H, s), 3.82 (3H, s), 3.81 (6H, s), 3.79 (3H, s), 3.78 (3H, s), 2.94 (1H, m), 2.79 (1H, m), 2.29 (3H, s), 2.25–2.15 (3H, m), 2.11 (3H, s), 2.10 (3H, s), 2.00 (3H, s), 1.88 (1H, m), 1.12 (3H, d, *J* = 7 Hz), 1.11 (3H, d, *J* = 7 Hz); IR (CHCl₃) ν_{max} 1760, 1725. HRMS *m/z* 446.1948 (calcd for C₂₄H₃₀O₈, 446.1941).

Synthesis of Olefins 8 and 15. The DMSO solution (40 mL) of **7** (7.8 g) was heated for 1 h at 160 °C. After cooling, DMSO was removed *in vacuo*, and the residue was purified by Si gel column chromatography (EtOAc–hexane 1:2) to give **8** (5.6 g, 84%). Colorless cubes of **7** were obtained by recrystallization with EtOAc–hexane: mp 78.0–79.0 °C; ¹H NMR δ (CDCl₃) 7.26 (1H, d, *J* = 1.2 Hz), 7.18 (1H, t, *J* = 8 Hz), 6.92–6.85 (3H, m), 6.79 (1H, d, *J* = 8.0 Hz), 6.35 (1H, d, *J* = 16 Hz), 6.19 (3H, dd, *J* = 16, 5.6 Hz), 3.88 (3H, s), 3.87 (3H, s), 3.83 (3H, s), 3.77 (1H, m), 2.30 (3H, s), 1.42 (3H, d, *J* = 6.8 Hz); IR (film) ν_{max} 1765; EIMS *m/z* 356 [M]⁺; anal. C 70.82%, H 6.79%, calcd for C₂₁H₂₄O₅, C 70.76%, H 6.79%.

By similar procedures, **15** (7.0 g) was synthesized in quantitative yield from **14** (7.0 g): mp 118.0–119.0 °C; ¹H NMR δ (CDCl₃) 7.17 (1H, t, *J* = 8 Hz), 6.93 (1H, s),

6.92 (1H, d, $J = 8$ Hz), 6.84 (1H, d, $J = 8$ Hz), 6.74 (1H, d, $J = 16$ Hz), 6.15 (1H, dd, $J = 16, 7$ Hz), 3.88 (3H, s), 3.82 (3H, s), 3.81 (6H, s), 3.78 (1H, m), 2.30 (3H, s), 1.43 (3H, d, $J = 7$ Hz); IR (CHCl₃) $\nu_{\max}$ 1760. EIMS m/z 386 [M]⁺; anal. C 68.24%, H 6.80%, calcd for C₂₂H₂₆O₆, C 68.38, H 6.78%.

Synthesis of Aldehydes 9 and 16. To a solution of **8** (695 mg) in CH₃OH (4 mL) was added 1N NaOH (4 mL) at 23 °C. After standing for 30 min, the mixture was poured into 1N HCl, extracted four times with CH₂Cl₂, and concentrated. To a solution of the residue (600 mg) in C₂H₄OH (50 mL) and 6N NaOH (50 mL) was added dropwise CHCl₃ (6 mL) at 60 °C for 1 h. The mixture was poured into 1N HCl, extracted four times with CH₂Cl₂, dried over Na₂SO₄, and concentrated. The residue was purified by Si gel TLC (EtOAc–hexane, 1:2) to give **9** (345 mg, 52%). Compound **9** was obtained as a yellowish powder by crystallization from ether: mp 50.5–52.0 °C; ¹H NMR δ (CDCl₃) 9.75 (1H, s), 7.40 (1H, s), 7.31 (1H, s), 6.91 (1H, br s), 6.90 (1H, brd, $J = 8$ Hz), 6.80 (1H, d, $J = 8$ Hz), 6.49 (1H, d, $J = 16$ Hz), 6.32 (1H, s), 6.23 (1H, dd, $J = 16, 7$ Hz), 4.17 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 1.49 (3H, d, $J = 7.5$ Hz); IR (film) $\nu_{\max}$ 3387, 1681. HRMS m/z 342.1454 (calcd for C₂₀H₂₂O₅, 342.1465).

By similar procedures, **16** (565 mg) was synthesized in 59% yield from **15** (1.0 g): ¹H NMR δ (CDCl₃) 9.82 (1H, s), 7.42 (1H, d, $J = 1.5$ Hz), 7.30 (1H, d, $J = 1.5$ Hz), 6.97 (1H, s), 6.78 (1H, d, $J = 16$ Hz), 6.39 (1H, br s), 6.28 (1H, dd, $J = 16, 7$ Hz), 4.10 (1H, m), 3.96 (3H, s), 3.88 (3H, s), 3.83 (3H, s), 3.81 (3H, s), 1.49 (3H, d, $J = 6.8$ Hz); IR (CHCl₃) $\nu_{\max}$ 3525, 1680; HRMS m/z 372.1546 (calcd for C₂₁H₂₄O₆, 372.1573).

Synthesis of Diketone 10. To a solution of vanillin (2.8 g) and (*n*-BuO)₃B (1.3 mL) was added a acetylacetone–borate complex, which was prepared from acetylacetone (3.8 mL) and B₂O₃ (1.8 g) at 70 °C. After cooling until 85 °C, *n*-butylamine (0.7 mL) was added dropwise at 85 °C. After standing at 100 °C for 1 h, 15 mL of 1N HCl was added at 50 °C to the solution. The mixture was allowed to stand for 0.5 h at 50 °C and extracted three times with EtOAc. The EtOAc layer was washed with H₂O and saturated NaCl, successively, and evaporated. The residue was purified by Si gel column chromatography (EtOAc–hexane, 1:2), giving **10** as pale yellow needles (2.2 g, 51%): mp 142.0–142.5 °C; ¹H NMR δ (CDCl₃) 7.53 (1H, d, $J = 16$ Hz), 7.09 (1H, dd, $J = 8, 2$ Hz), 7.06 (1H, d, $J = 2$ Hz), 6.92 (1H, d, $J = 8$ Hz), 6.32 (1H, d, $J = 16$ Hz), 5.83 (1H, br s), 5.62 (1H, s), 3.94 (3H, s), 2.15 (3H, s); IR (CHCl₃) $\nu_{\max}$ 3520; anal. C 66.39%, H 6.09, calcd for C₁₃H₁₄O₄, C 66.66%, H 6.02%.

Synthesis of Cassumunins A and B (1 and 2, respectively). To a solution of **10** (130 mg) in EtOAc (3 mL) was added B₂O₃ (40 mg) in 80 °C bath. To the mixture were added the EtOAc solution (3 mL) of **9** (100 mg) and (*n*-BuO)₃B (0.15 mL). After stirring for 30 min, piperidine (0.02 mL) was added to the solution. After stirring for 30 min in the same bath, 0.4N HCl (1 mL) was added at 50 °C. The mixture was vigorously stirred for 30 min and extracted four times with CH₂Cl₂. The CH₂Cl₂ layer was dried over Na₂SO₄ and concentrated. The residue was purified by Si gel TLC (EtOAc–hexane, 1:2), giving **1** (93 mg, 57%): ¹H NMR δ (CDCl₃) 7.58

(2H, br d, $J = 15.6$ Hz), 7.12 (1H, br d, $J = 8$ Hz), 7.07 (1H, br s), 7.05 (1H, br s), 6.96 (1H, br s), 6.93 (1H, d, $J = 8$ Hz), 6.91 (1H, br s), 6.89 (1H, br d, $J = 8$ Hz), 6.80 (1H, d, $J = 7.6$ Hz), 6.46 (2H, d, $J = 15.6$ Hz), 6.40 (1H, d, $J = 16$ Hz), 6.29 (1H, dd, $J = 16, 7$ Hz), 6.03 (1H, s), 5.84 (1H, s), 5.81 (1H, s), 4.05 (1H, m), 3.95 (3H, s), 3.94 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 1.46 (3H, d, $J = 7$ Hz); HREIMS m/z 558.2414 (calcd for C₃₃H₃₄O₈, 558.2254).

Using similar procedures, **2** (653 mg) was synthesized in 66% from **10** (780 mg) and **16** (622 mg): ¹H NMR δ (CDCl₃) 7.59 (1H, d, $J = 16$ Hz), 7.58 (1H, d, $J = 16$ Hz), 7.12 (1H, dd, $J = 8, 2$ Hz), 7.09 (1H, d, $J = 1.5$ Hz), 7.03 (1H, d, $J = 2$ Hz), 6.98 (1H, s), 6.95 (1H, d, $J = 1.5$ Hz), 6.93 (1H, d, $J = 8$ Hz), 6.78 (1H, d, $J = 17$ Hz), 6.50 (1H, s), 6.46 (2H, d, $J = 16$ Hz), 6.29 (1H, dd, $J = 17, 8$ Hz), 6.04 (1H, br s), 5.87 (1H, br s), 5.80 (1H, s), 4.07 (1H, m), 3.92 (6H, s), 3.88 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 1.45 (3H, s, $J = 7$ Hz); IR (CHCl₃) $\nu_{\max}$ 3550, 1630; HREIMS m/z 588.2333 (calcd for C₃₄H₃₆O₉, 588.2337).

Bioassay of 1 and 2. Assay was carried out by Oyama's protocol using rat thymocytes.¹⁷ Briefly, thymocytes were dissociated from the thymus glands of 4–5-week-old rats in Tyrode's solution. To the cell suspension was added a test sample (final concentrations: 0.03 μ M, 0.1 μ M, 0.3 μ M, 1 μ M, and 3 μ M) in a small amount of DMSO and incubated for 30 min at 36 °C. To the suspension was added 30% H₂O₂ (final concentration 3 mM), and the suspension was incubated an additional 30 min. The dead cells in the suspension were stained by adding 5 mM ethidium bromide and counted with a flowcytometer. Cell viability was calculated from the number of dead cells and total cells, and the IC₅₀ value was obtained by the usual graphical method from the cell viability data.

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