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

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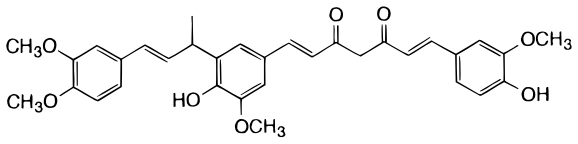
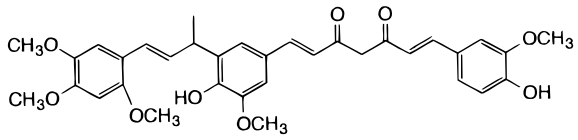
A chemical synthesis of cassumunins A (**1**) and B (**2**), natural curcuminoid antioxidants, was developed. The synthesis was started from *o*-vanillin and after nine reaction steps resulted in 20% and 26% overall yields of **1** and **2**, respectively. The synthetic cassumunins showed stronger protective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

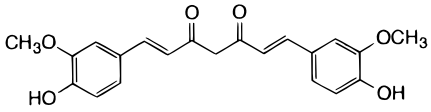
Powerful antioxidants originating from edible and medicinal plants have been extensively investigated as important inhibitory materials for the prevention of oxidative deterioration of lipids. Recently, it has been shown that peroxidation in living organisms is closely related to the initiation of some 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 coworkers 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

Cassumnin 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 K_2CO_3, 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 C_1 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 $NaBH_4$, and the hydroxyl group was subsequently eliminated. Unfortunately, the vari-

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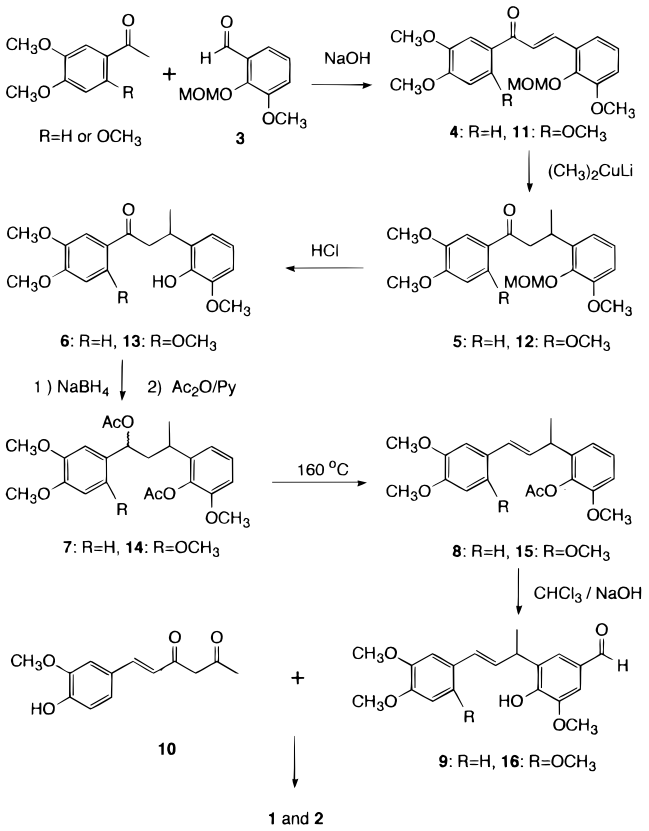
Scheme 1. Synthetic route for Cassumumins A (**1**) and B (**2**); (MOM = CH_2OCH_3)



ous conditions for formation of a double bond, such as acid dehydration, dehalogenation after conversion to halide, or thermal elimination, were unsucessful 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 CH_3OH to give phenol **6** in quantitative yield. After protection of the phenolic hydroxyl group with benzyl bromide, the carbonyl group was reduced by $NaBH_4$. 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 $NaBH_4$ 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 $CHCl_3$ 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 H_2O_2-induced cell death. The activity efficiency was estimated and compared with curcumin using their IC_{50} values (**1**, 0.17 μM; **2**, 0.22 μM; and curcumin, 1.02 μM). These IC_{50} 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. ^{1}H 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 F_{254} 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 Me_2CO (220 mL) were added anhydrous K_2CO_3 (44 g) and methoxymethyl chloride (18 mL), successively. The mixture was stirred for 3 h at 23 °C. To the mixture was added CH_2Cl_2 (500 mL), and then the mixture was filtered to remove K_2CO_3. The filtrate was washed with saturated NaCl solution, dried over Na_2SO_4, 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; ^{1}H NMR δ ($CDCl_3$) 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 $C_{10}H_{12}O_4$, 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

C_2H_5OH (30 mL) was added 1.4 N NaOH solution (C_2H_5-OH−H_2O 4:5, 70 mL) at 23 °C. The mixture was stirred for 16 h, then poured into saturated NaCl and extracted 3 times with CH_2Cl_2. The combined CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 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 recrystalization of the powder with CH_3-OH: mp 108.0−109.0 °C; ^{1}H NMR δ ($CDCl_3$) 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 $C_{20}H_{22}O_6$, 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; ^{1}H NMR ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 1650; EIMS *m/z* 388 $[M]^+$; *anal.* C 65.07%, H 6.24%, calcd for $C_{21}H_{24}O_7$, 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 CH_3Li (1.5 N solution in ether) at 0 °C under N_2 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 $(CH_3)_2CuLi$ was decomposed by CH_3COOH and then by 1N HCl. The mixture was extracted three times with CH_2Cl_2. The CH_2Cl_2 layers were combined, dried over Na_2SO_4, 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 CH_3OH−H_2O: mp 95.5−97.0 °C; ^{1}H NMR δ ($CDCl_3$) 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 $C_{21}H_{26}O_6$, 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; ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 1660; EIMS *m/z* 404 $[M]^+$; *anal.* C 65.13%, H 6.96%, calcd for $C_{22}H_{28}O_7$, C 65.33%, H 6.98%.

Synthesis of Phenols 6 and 13. To a solution of **5** (8.0 g) in CH_3OH (240 mL) was added 1N HCl (136 mL). The mixture was stirred at 60 °C for 5 min, then poured into H_2O and extracted four times with CH_2Cl_2. The CH_2Cl_2 layer was dried over Na_2SO_4 and purified by Si gel column chromatography (EtOAc− hexane, 1:1), giving **6** (7.1 g, quantitative yield) as viscous oil: ^{1}H NMR δ ($CDCl_3$) 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 $C_{19}H_{22}O_5$, 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; ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 3500, 1660; EIMS *m/z* 360 $[M]^+$; *anal.* C 66.42%, H 6.73%, calcd for $C_{20}H_{24}O_6$, C 66.65%, H 6.71%.

Synthesis of Diacetates 7 and 14. To a solution of **6** (7.1 g) in CH_3OH (300 mL) was added $NaBH_4$ (3.5 g) at 23 °C. After stirring for 10 min at 23 °C, the mixture was poured into H_2O, extracted four times with CH_2Cl_2, dried over Na_2SO_4, and concentrated. To the residue were added Ac_2O (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, ^{1}H NMR δ ($CDCl_3$) 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 $C_{23}H_{28}O_7$, 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 streoisomers; ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 1760, 1725. HRMS *m/z* 446.1948 (calcd for $C_{24}H_{30}O_8$, 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; ^{1}H NMR δ ($CDCl_3$) 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 $C_{21}H_{24}O_5$, 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; ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 1760. EIMS m/z 386 $[M]^+$; anal. C 68.24%, H 6.80%, calcd for $C_{22}H_{26}O_6$, C 68.38, H 6.78%.

Synthesis of Aldehydes 9 and 16. To a solution of **8** (695 mg) in CH_3OH (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 CH_2Cl_2, and concentrated. To a solution of the residue (600 mg) in C_2H_4OH (50 mL) and 6N NaOH (50 mL) was added dropwise $CHCl_3$ (6 mL) at 60 °C for 1 h. The mixture was poured into 1N HCl, extracted four times with CH_2Cl_2, dried over Na_2SO_4, 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 crystalization from ether: mp 50.5−52.0 °C; ^{1}H NMR δ ($CDCl_3$) 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) ν_{max} 3387, 1681. HRMS m/z 342.1454 (calcd for $C_{20}H_{22}O_5$, 342.1465).

By similar procedures, **16** (565 mg) was synthesized in 59% yield from **15** (1.0 g): ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 3525, 1680; HRMS m/z 372.1546 (calcd for $C_{21}H_{24}O_6$, 372.1573).

Synthesis of Diketone 10. To a solution of vanillin (2.8 g) and (n-BuO)$_3$B (1.3 mL) was added a acetylacetone−borate complex, which was prepared from acetylacetone (3.8 mL) and B_2O_3 (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 H_2O 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; ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 3520; *anal.* C 66.39%, H 6.09, calcd for $C_{13}H_{14}O_4$, 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 B_2O_3(40 mg) in 80 °C bath. To the mixture were added the EtOAc solution (3 mL) of **9** (100 mg) and (n-BuO)$_3$B (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 CH_2Cl_2. The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated. The residue was purified by Si gel TLC (EtOAc−hexane, 1:2), giving **1** (93 mg, 57%): ^{1}H NMR δ ($CDCl_3$) 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 $C_{33}H_{34}O_8$, 558.2254).

Using similar procedures, **2** (653 mg) was synthesized in 66% from **10** (780 mg) and **16** (622 mg): ^{1}H NMR δ ($CDCl_3$) 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 ($CHCl_3$) ν_{max} 3550, 1630; HREIMS m/z 588.2333 (calcd for $C_{34}H_{36}O_9$, 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 μM, 0.1 μM, 0.3 μM, 1 μM, and 3 μM) in a small amount of DMSO and incubated for 30 min at 36 °C. To the suspension was added 30% H_2O_2 (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 IC_{50} value was obtained by the usual graphical method from the cell viability data.

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