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Structures of Cassumunin A, B, and C, New Potent Antioxidants from Zingiber cassumunar

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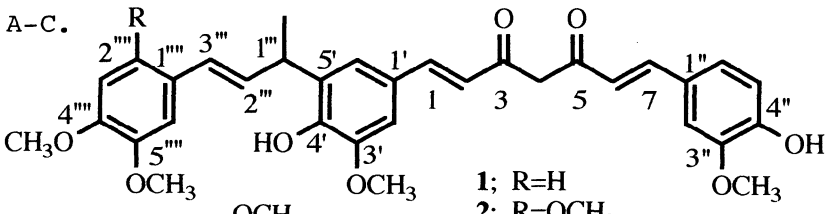
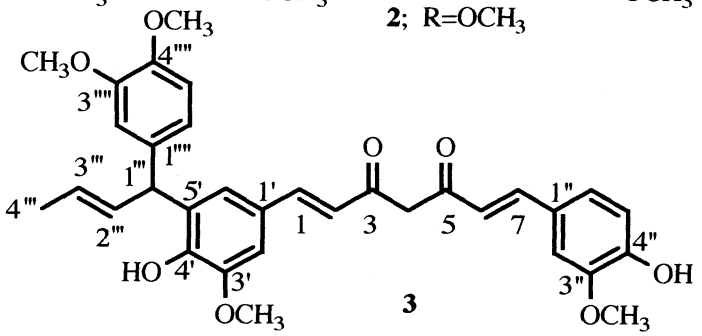
Three new potent antioxidants have been isolated from the rhizomes of Zingiber cassumunar. Their structures have been established from spectral data. These compounds also showed inhibitory activity against inflammation induced by a tumor promoter, TPA.

It is well known that the peroxidative reaction in living cells is closely related to the initiation of several diseases such as cancer and allergy. For prevention of the peroxidation-related diseases, powerful antioxidants are needed and have been screened from natural sources. We have been investigating natural antioxidants from tropical medicinal plants and also been examining their antiinflammatory activity as an initial response in oxidative damaged cells. In the course of our investigation,[1] we isolated novel antioxidants, cassumunin A-C (**1**, **2**, and **3**, respectively) from rhizomes of Zingiber cassumunar (Zingiberaceae), which have been used as a tropical traditional medicine possessing antiinflammation properties.[2] This communication deals with the structure determination of cassumunin A-C.



1; R=H
2; R=OCH_3



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Chromatographic separation of the acetone extract of the fresh rhizomes collected in Indonesia, January 1991, was done with the guidance of antioxidant and antiinflammatory assays, giving cassumunin A-C from the most active fraction.

Cassumunin A (**1**),[3] $C_{33}H_{34}O_8$. A maximal absorption at 426 nm in the UV spectrum indicated **1** had a highly conjugated structure, which was elucidated by the combination of NOESY, HH-COSY and CH-COSY data. A tetrasubstituted benzene (aromatic A) was conjugated with a trans olefin [NOE, H-6'/H-1], and the olefin was also conjugated with a carbonyl group in an enolated β-diketone moiety [δ 101.0, 183.2, 183.4], which was confirmed by the presence of down-field shifted olefinic protons [H-1, δ 7.58] and an NOE between H-2 and the enolic methine proton [H-4, δ 5.81]. Another NOE between the enolic methine proton and H-6 indicated the enolated β-diketone moiety was conjugated with the other trans olefin, which was also conjugated with a trisubstituted benzene (aromatic B) [NOE, H-6"/H-7]. ^{1}H NMR and HH-COSY data also indicated the presence of a 1,3-disubstituted butenyl group. The 3- and 1-positions of the butenyl group were attached to the 5-position of the tetrasubstituted benzene (aromatic A) [NOE, H-6'/CH_3-1"'] and the 1-position of another trisubstituted benzene (aromatic C) [NOE, H-2""/H-3"'], respectively. Finally, the substituted positions of four phenolic methoxyl and two hydroxyl groups were determined by NOEs [H-2' δ 6.96/δ 3.95, H-2" δ 7.04/δ 3.95, H-3"" δ 6.80/δ 3.87, H-6"" or -5" δ 6.94/δ 3.89] and MS fragment ions, revealing that

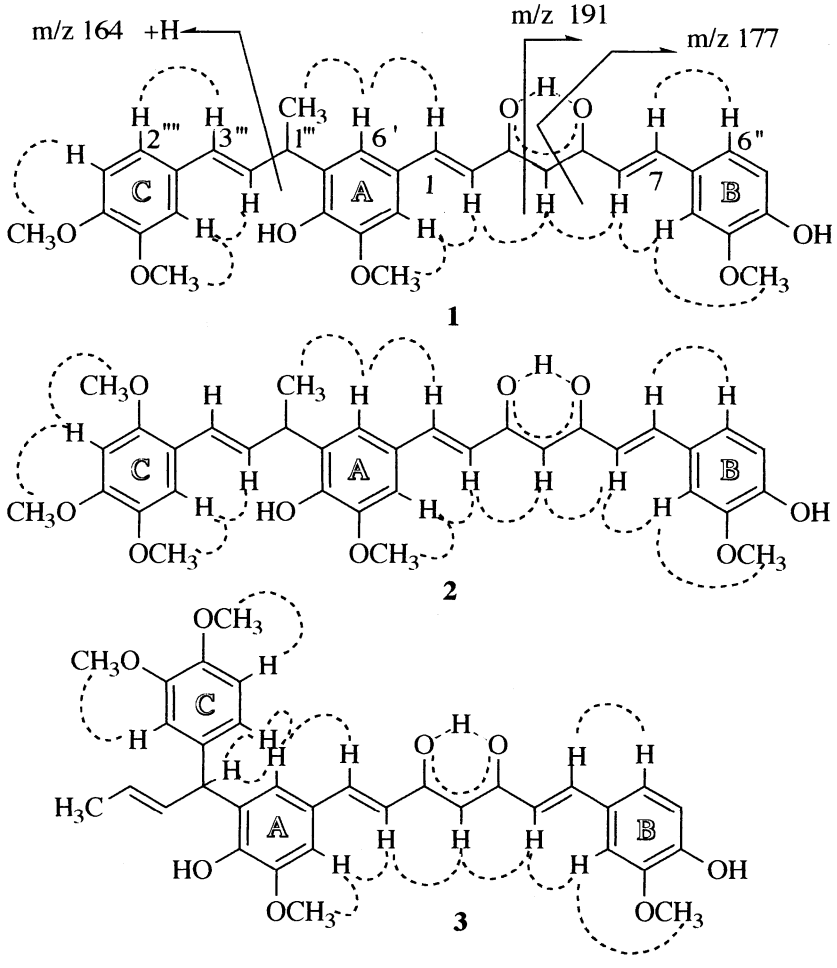

Fig. 1. Selected NOEs and MS fragments.

four methoxyl groups were at the 3-positions on aromatics A and B, at the 4- and 5-positions on aromatic C, and the other oxygenated positions were hydroxylated. Thus, the structure of **1** should be expressed as structural **1**.

Cassumunin B (**2**),[4] $C_{34}H_{36}O_9$. The ^1H NMR data were similar to those of **1** except that the signals due to a 1,2,4,5-tetrasubstituted benzene were observed instead of those due to a 1,3,4-trisubstituted benzene. One additional methoxyl signal was also observed, which was determined to be substituted to the 2-position of the tetrasubstituted benzene (aromatic C) [NOE, H-3"" δ 6.50/δ 3.82 and 3.85]. Thus, the structure of **2** should be expressed as structural **2**.

Cassumunin C (**3**),[5] $C_{33}H_{34}O_8$. The spectral data were similar to those of **1** except for the butene moiety. The homo decoupling technique revealed the presence of 1,1-disubstituted (2E)-butene. The NOESY spectrum of **3** clarified that the 1-position of the butene was attached to the 5-position of the tetrasubstituted benzene (aromatic A) [NOE, H-1"'/H-6'] and the 1-position of the trisubstituted benzene (aromatic C) [NOE, H-1"'/H-6""]. Thus, the structure of **3** should be expressed as structural **3**.

The antioxidant activity of cassumunin A-C (**1-3**) was determined by the inhibition of autoxidation of linoleic acid. The inhibitory effect of three compounds (135 μM) relative to control; cassumunin A (**1**): 98%, cassumunin B (**2**): 90%, cassumunin C (**3**): 97%,[1] showing cassumunin A-C (**1-3**) have stronger or equal antioxidant activity compared with that of curcumin (90%), the diarylheptanoid moiety of cassumunin A-C (**1-3**).[6] Cassumunin A-C (**1-3**) (0.6 μmol) also showed inhibitory activity against the inflammation induced by a tumor promoter, TPA (12-O-tetradecanoylphorbol-13-acetate) (2 μg) on mouse ears.[7,8] Detailed biological activities of cassumunin A-C will be reported in a full paper.

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References

1)A. Jitoe, T. Masuda, I. G. P. Tengah, D. N. Suprapta, I. W. Gara, and N. Nakatani, J. Agric. Food Chem., **40**, 1337 (1992).

2)"Medicinal Plants," ed by D. Ponglux, S. Wongseripipatana, T. Phadungcharoen, N. Ruangrungsri, and K. Likhitwitayawuid, Victory Power Point, Bangkok, Thailand (1987), p. 275.

3)^1H NMR (400 MHz, $CDCl_3$)δ 1.46 (3H, d, J=6.7 Hz, CH_3-1"'), 3.87 (3H, s, 4""-OMe), 3.89 (3H, s, 5""-OMe), 3.95 (3H, s, 3"-OMe), 3.95 (3H, s, 3'-OMe), 4.05 (1H, quintet, J=6.7 Hz, H-1"'), 5.81 (1H, s, H-4), 5.85

(1H, brs, OH), 6.03 (1H, brs, OH), 6.29 (1H, dd, J=15.9, 6.7 Hz, H-2"'), 6.40 (1H, d, J=15.9 Hz, H-3"'), 6.47 (2H, d, J=15.9 Hz, H-2 and H-6), 6.80 (1H, d, J=7.9 Hz, H-3""), 6.91 (1H, dd, J=7.9, 1.8 Hz, H-2""), 6.94 (1H, d, J=1.8 Hz, H-6""), 6.94 (1H, d, J=7.9 Hz, H-5"), 6.96 (1H, d, J=1.8 Hz, H-2'), 7.04 (1H, d, J=1.8 Hz, H-2"), 7.07 (1H, d, J=1.8 Hz, H-6'), 7.12 (1H, dd, J=7.9, 1.8 Hz, H-6"), 7.58 (1H, d, J=15.9 Hz, H-1), 7.59 (1H, d, J=15.9 Hz, H-7). ^{13}C NMR (100 MHz, $CDCl_3$)δ 19.8, 35.6, 55.9, 56.0 (Cx2), 56.2, 101.0, 107.4, 108.9, 109.7, 111.3, 114.9, 119.2, 121.7 (Cx2), 121.9, 122.8, 127.0, 127.7, 128.6, 130.8, 132.0 (Cx2), 140.4, 141.0, 145.3, 146.8, 146.9, 147.9, 148.5, 149.1, 183.2, 183.4. HR-MS m/z 558.2224 (M^+, $C_{33}H_{34}O_8$: 558.2252), 191.0715 ($C_{11}H_{11}O_3$: 191.0708), 177.0575 ($C_{10}H_9O_3$: 177.0551) 164.0828 ($C_{10}H_{12}O_2$: 164.0837). UV λmax (MeOH) nm 426, 297 (sh), 260.

4) ^{1}H NMR (400 MHz, $CDCl_3$)δ 1.47 (3H, d, J=6.7 Hz, CH_3-1"'), 3.82 (3H, s, 2"" or 4""-OMe), 3.85 (3H, s, 5""-OMe), 3.89 (3H, s, 4"" or 2""-OMe), 3.95 (6H, s, 3'-OMe and 3"-OMe), 4.07 (1H, quintet, J=6.7 Hz, H-1"'), 5.80 (1H, s, H-4), 5.84 (1H, brs, OH), 6.03 (1H, brs, OH), 6.79 (1H, dd, J=15.9, 6.7 Hz, H-2"'), 6.47 (2H, d, J=15.9 Hz, H-2 and H-6), 6.50 (1H, s, H-3""), 6.78 (1H, d, J=15.9 Hz, H-3"'), 6.93 (1H, d, J=7.9 Hz, H-5"), 6.95 (1H, d, J=1.8 Hz, H-2'), 6.98 (1H, s, H-6""), 7.05 (1H, d, J=1.8 Hz, H-2"), 7.09 (1H, d, J=1.8 Hz, H-6'), 7.12 (1H, dd, J=7.9, 1.8 Hz, H-6"), 7.58 (1H, d, J=15.9 Hz, H-1), 7.59 (1H, d, J=15.9 Hz, H-7). HR-MS m/z 588.2298 (M^+, $C_{34}H_{36}O_9$: 588.2357). UV λmax (MeOH) nm 426, 314, 260.

5) ^{1}H NMR (400 MHz, $CDCl_3$)δ 1.75 (3H, d, J=6.7 Hz, H-4"'), 3.83 (3H, s, 3""-OMe), 3.85 (3H, s, 4""-OMe), 3.93 (3H, s, 3'-OMe), 3.95 (3H, s, 3"-OMe), 5.00 (1H, brd, J=6.7 Hz, H-1"'), 5.44 (1H, ddd, J=15.6, 6.7, 1.8 Hz, H-3"'), 5.80 (1H, s, H-4), 5.86 (1H, brs, OH), 5.90 (1H, ddd, J=15.6, 6.7, 1.8 Hz, H-2"'), 5.98 (1H, brs, OH), 6.43 (1H, d, J=15.9 Hz, H-2), 6.47 (1H, d, J=15.9 Hz, H-6), 6.75 (2H, m, H-2"" and H-6""), 6.80 (1H, d, J=7.9 Hz, H-5""), 6.93 (1H, d, J=7.9 Hz, H-5"), 6.97 (2H, s, H-2' and H-6'), 7.05 (1H, d, J=1.8 Hz, H-2"), 7.12 (1H, dd, J=7.9, 1.8 Hz, H-6"), 7.56 (1H, d, J=15.9 Hz, H-1), 7.59 (1H, d, J=15.9 Hz, H-7). HR-MS 558.2289 (M^+, $C_{33}H_{34}O_8$: 558.2252). UV λmax (MeOH) nm 426, 262, 231.

6) S. Toda, T. Miyase, H. Arichi, H. Tanizawa, and Y. Takino, Chem. Pharm. Bull., **33**, 1725 (1985).

7) The inhibitory effect of cassunumin A-C and curcumin relative to control; 83%, 76%, 75%, and 51%, respectively.

8) M. Gschwendt, K. Kittstein, G. Fürstenberger, and F. Marks, Cancer Lett., **25**, 177 (1984).

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