Pergamon

Tetrahedron Letters, Vol. 35, No. 7, pp. 981-984, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(93)E0411-C

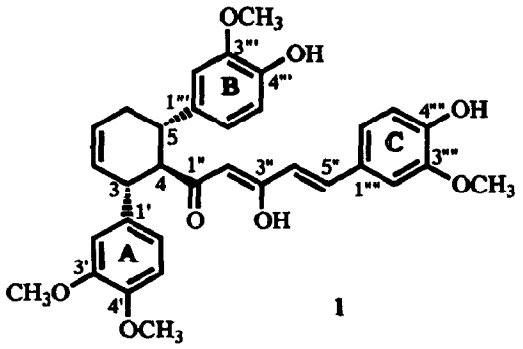
Novel Antioxidants, Cassumunarin A, B, and C, from *Zingiber cassumunar*

Akiko Jitoe, Toshiya Masuda,* and Tom J. Mabry*

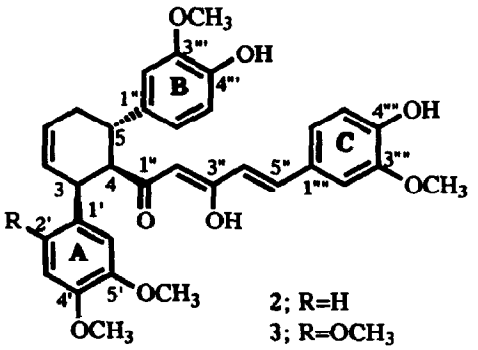
Department of Botany, University of Texas at Austin, Austin, TX 78713, USA

Abstract: The most active antioxidant fraction from the organic extract of *Zingiber cassumunar* rhizomes was found to contain three potent antioxidants, cassumunarin A, B, and C, which were shown by spectral methods to represent a new type of complex curcumin.

Curcumin, a major pigment of *Curcuma* plants (*Zingiberaceae*),[1] has remarkable tumor preventive properties as well as antioxidant,[2] and antiinflammatory activities,[3] all of which may be related.[4] The ethyl acetate-soluble fraction of the acetone extract of the rhizomes of an Indonesian medicinal ginger, *Zingiber cassumunar* Roxb. (*Zingiberaceae*), showed both potent antioxidant and antiinflammatory activities.[5,6] Novel complex curcuminoids, cassumunarin A, B, and C, were isolated from the most active fraction with the guidance of the antioxidant and antiinflammatory assays. This communication deals with the structural determination of cassumunarin A, B, and C (structures 1-3 show relative not absolute stereochemistry).



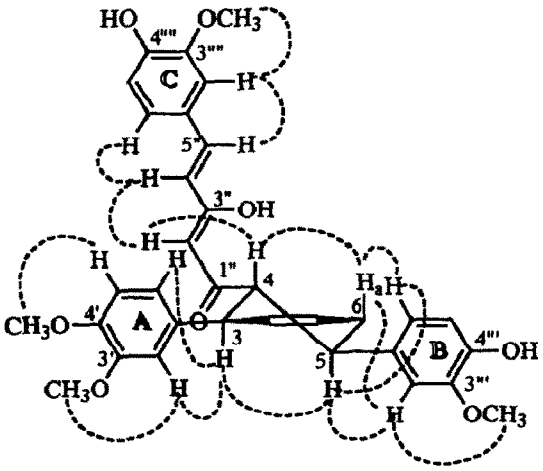
1



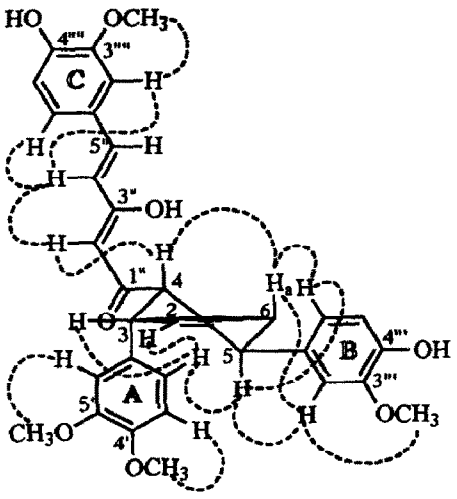
2; R=H
3; R=OCH_3

Cassumunarin A (1),[7] EIMS provided m/z 558 $[M]^+$ for a molecular formula of $C_{33}H_{34}O_8$, which was confirmed by HRCIMS, 559.2298 ($[M+H]^+$, 3.4 mmu dev.). The ^1H NMR and the HH-COSY data indicated the presence of one 3, 4, 5-trisubstituted cyclohexene ring and three 1, 3, 4-trisubstituted benzene rings. On the basis of the NOESY spectrum (NOE, H-3/H-2', H-3/H-6', H-5/H-2''', H-5/H-6''', H-6a/H-2''', H-6a/H-6'''), both the 3- and 5-positions of the cyclohexene ring were attached to the 1-positions of 1, 3, 4-trisubstituted benzene rings (designated aromatics A and B) (Fig. 1). The other 1, 3, 4-trisubstituted benzene ring (aromatic

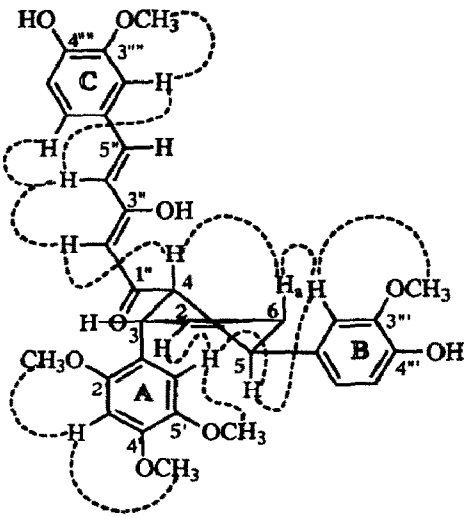
C) was attached to an isolated *trans* olefin at the 1-position (NOE, H-4"/H-6"", H-5"/H-2"") and the olefin was also attached to the enol form of the β-diketone moiety (δ 103.0, 177.1, 200.8 and NOE, H-2"/H-4"). This conclusion was confirmed for **1** by the UV absorption (λ_{max} 371 nm) and the fragment ion in the EI mass spectrum (*m/z* 219). The NOESY spectrum also clarified that the 4-position of the cyclohexene ring was substituted by the β-diketone moiety bearing the styryl group (NOE, H-4/H-2"). The positions of four phenolic methoxyl groups at the 3- and 4-positions on aromatic A and the 3-positions on aromatics B and C, were determined by NOEs in the NOESY and the NOEDIF spectra (NOE, H-2'/3'-OMe, H-5'/4'-OMe, H-2"'/3"'-OMe, H-2""/3""-OMe). Thus, the 4-positions on aromatics B and C were hydroxylated. The stereochemistry of all three substituents on the cyclohexene ring was determined to be *pseudoequatorial* on the basis of the *J* value of H-4 (*t*, 10.6 Hz) and NOEs (H-3/H-5, H-4/H-6a).



1



2



3

Fig. 1. Selected NOEs of 1, 2, and 3 obtained by NOESY and NOEDIF spectra.

Cassumunarin B (2),[8] $C_{33}H_{34}O_8$ (EIMS, 558, $[M]^+$. HRCIMS, 559.2308, $[M+H]^+$, 2.4 mmu dev.). The spectral data of **2** were similar to those of **1**. The difference from **1** was the stereochemistry on the cyclohexene ring, which was shown on the basis of the *J* values of H-4 (*dd*, 12.1 and 5.7 Hz) and NOEs (H-4/H-6a, H-5/H-2') to be *pseudoaxial, pseudoequatorial,* and *pseudoequatorial* at the 3-, 4-, and 5-positions, respectively.

Cassumunarin C (3),[9] $C_{34}H_{36}O_9$ (EIMS, 588, $[M]^+$. HRCIMS, 589.2419, $[M+H]^+$, 1.9 mmu dev.). The spectral data of **3** were similar to those of **2** except for the presence of a 1, 2, 4, 5-tetrasubstituted benzene ring instead of a 1, 3, 4-trisubstituted benzene ring, and one additional phenolic methoxyl group. Thus, **3** has three methoxyl groups at the 2-, 4-, and 5-positions on aromatic A. (NOE, H-3'/2'-OMe, H-3'/4'-OMe, H-6'/5'-OMe) The stereochemistry on the cyclohexene ring in **3** was the same as in **2**, which were determined by the *J* values for **3** of H-4 (*dd*, 12.1 and 5.7 Hz) and NOEs (H-4/H-6a, H-5/H-6').

Antioxidant activity of cassumunarin A~C (**1~3**) were measured on the basis of the inhibitory effect against the autoxidation of linoleic acid relative to the inhibitory effect of curcumin. The inhibitory effects of cassumunarin A~C (each 135 μM) were stronger (95, 94, and 93 %, respectively) than that of curcumin (135 μM, 78 %),[10] showing the novel complex curcuminoids, cassumunarin A~C, are more potent antioxidants.

ACKNOWLEDGMENT

This work at the University of Texas was supported by the National Institutes of Health (GM-35710) and the Robert A. Welch Foundation (Grant F-130). This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan for T. Masuda and A. Jitoe. A. Jitoe also thanks the Japan Society for the Promotion of Science for her Japanese Junior Scientists Fellowship.

REFERENCES AND NOTES

1. Hegnauer, R. Zingiberaceae. In *Chemotaxonomie der Pflanzen*; Birkhäuser Varlag: Basel, 1963; Vol. 2, pp 451-471.
2. Toda, S.; Miyase, T.; Arichi, H.; Tanizawa, H.; Takino, Y. *Chem. Pharm. Bull.* **1985**, *33*, 1725-1728.
3. Mukhopadhyay, A.; Basu, N.; Ghatak, N.; Gujral, P. K. *Agents and Actions* **1982**, *12*, 508-515.
4. Huang, M.-T.; Smart, R. C.; Wong, C.-Q.; Conney, A. H. *Cancer Research* **1988**, *48*, 5941-5946.
5. Jitoe, A.; Masuda, T.; Tengah, I. G. P.; Suprapta, D. N.; Gara, I. W., Nakatani, N. *J. Agric. Food Chem.* **1992**, *40*, 1337-1340.
6. Masuda, T.; Jitoe, A.; Nakatani, N. *Chem. Lett.* **1993**, 189-192.
7. Cassumunarin A (**1**): $[\alpha]^{24}_D$ +8.9° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, benzene-d_6) δ 2.34 (1H, *brdt*, *J*=18.5 and 5.4 Hz, H-6e), 2.42 (1H, *m*, H-6a), 2.92 (1H, *t*, *J*=10.6 Hz, H-4), 3.01 (3H, *s*, 3""-OMe), 3.21 (3H, *s*, 3"'-OMe), 3.30 (3H, *s*, 4'-OMe), 3.41 (1H, *dt*, *J*=10.6 and 5.4 Hz, H-5), 3.50 (3H, *s*, 3'-OMe), 4.26 (1H, *brd*, *J*=10.6 Hz, H-3), 5.06 (1H, *s*, H-2"), 5.37 (1H, *brs*, OH), 5.72 (1H, *brs*, OH), 5.79 (1H, *d*, *J*=15.8 Hz, H-4"), 5.90 (1H, fine separated *d*, *J*=10.3 Hz, H-1), 5.95 (1H, *brd*, *J* =10.3 Hz, H-2), 6.33 (1H, *d*, *J* =1.8 Hz, H-2""), 6.53 (1H, *dd*, *J*=8.3 and 1.8 Hz, H-6""), 6.59 (1H, *d*, *J*=8.3 Hz, H-5'), 6.66 (1H, *d*, *J*=1.9 Hz, H-2"'), 6.69 (1H, *dd*, *J*=8.2 and 1.9 Hz, H-6"'), 6.76 (1H, *d*, *J*=8.2 Hz, H-5"") 6.93~6.95 (3H, H-2', 6', and 5"'), 7.40 (1H, *d*, *J*=15.8 Hz, H-5").; ^{13}C NMR

(62.5 MHz, $CDCl_3$) 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.; HRCIMS (isobutane) *m/z* 559.2298 ($[M+H]^+$, $C_{33}H_{35}O_8$: 559.2332).; EI-MS *m/z* (rel. int.) 558 (89), 540 (12), 421 (3), 381 (7), 366 (36), 348 (95), 338 (43), 219 (100), 190 (22), 177 (45), 137 (20).; UV λmax (MeOH) nm 371, 262, 223.

8. Cassumunarin B (2): $[\alpha]^{24}_D$ -2.5° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, acetone-d_6) δ 2.18 (1H, *ddd*, *J*=18.4, 10.8, and 2.5 Hz, H-6a), 2.51 (1H, *brdt*, *J*=18.4, and 5.4 Hz, H-6e), 3.18 (1H, *ddd*, *J*=12.1, 10.8, and 5.4 Hz, H-5), 3.41 (1H, *dd*, *J*=12.1, and 5.4 Hz, H-4), 3.67 (3H, *s*, 5'-OMe), 3.72 (3H, *s*, 4'-OMe), 3.77 (3H, *s*, 3"'-OMe), 3.87 (4H, 3""-OMe and H-3), 5.50 (1H, *s*, H-2"), 5.80 (1H, fine separated *d*, *J*=10.3 Hz, H-2), 6.00 (1H, fine separated *d*, *J*=10.3 Hz, H-1), 6.33 (1H, *d*, *J*=15.8 Hz, H-4"), 6.65 (2H, H-5"' and 6"'), 6.71 (1H, *d*, *J*=2.0 Hz, H-6'), 6.74 (1H, *dd*, *J*=8.2 and 2.0 Hz, H-2'), 6.81~6.84 (3H, H-3', 2"', and 5""), 7.05 (1H, *dd*, *J*=8.2 and 1.9 Hz, H-6""), 7.20 (1H, *d*, *J*=1.9 Hz, H-2""), 7.22 (1H, *brs*, OH), 7.30 (1H, *d*, *J*=15.8 Hz), 8.09 (1H, *brs*, OH).; ^{13}C NMR (62.5 MHz, $CDCl_3$) 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. ;HRCIMS (isobutane) *m/z* 559.2308 ($[M+H]^+$, $C_{33}H_{35}O_8$: 559.2332).; EIMS *m/z* (rel. int.) 558 (42), 540 (5), 421 (4), 381 (9), 366 (39), 348 (35), 338 (32), 219 (100), 190 (59), 177 (79), 137 (25).; UV λmax (MeOH) nm 371, 273, 223.

9. Cassumunarin C (3): $[\alpha]^{24}_D$ -2.3° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, acetone-d_6) δ 2.21 (1H, *ddd*, *J*=18.4, 10.8, and 2.5 Hz, H-6a), 2.51 (1H, *brdt*, *J*=18.4, and 5.4 Hz, H-6e), 3.15 (1H, *ddd*, *J*=12.1, 10.8, and 5.4 Hz, H-5), 3.31 (1H, *dd*, *J*=12.1 and 5.7 Hz, H-4), 3.53 (3H, *s*, 2'-OMe), 3.75 (6H, *s*, 4'-OMe, and 3"'-OMe), 3.79 (3H, *s*, 5'-OMe), 3.88 (3H, *s*, 3""-OMe), 4.33 (1H, *brt*, *J*=5.7 Hz, H-3), 5.27 (1H, *s*, H-2"), 5.71 (1H, fine separated *d*, *J*=10.3 Hz, H-2), 6.02 (1H, fine separated *d*, *J*=10.3 Hz, H-1), 6.29 (1H, *d*, *J*=15.8 Hz, H-4"), 6.51 (1H, *s*, H-3'), 6.63 (2H, H-5"' and 6"'), 6.81 (1H, H-2"'), 6.83 (1H, *d*, *J*=8.2 Hz, H-5""), 6.93 (1H, *s*, H-6'), 7.03 (1H, *dd*, *J*=8.2, and 2.0 Hz, H-6""), 7.19 (2H, H-2"" and OH), 7.23 (1H, *d*, *J*=15.8 Hz, H-5"), 8.05 (1H, *brs*, OH).; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 35.8, 36.7, 37.3, 55.1, 55.2, 56.0 (C×2), 56.0, 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, 136.6, 138.5, 141.9, 143.8, 146.3, 146.8, 147.5, 148.8, 152.5, 174.8, 201.6.; HRCIMS (isobutane) *m/z* 589.2419 ($[M+H]^+$, $C_{34}H_{37}O_9$: 589.2438).; EIMS *m/z* (rel. int.) 588 (100), 570 (2), 451 (1), 411 (6), 396 (17), 380 (20), 368 (23), 220 (96), 219 (49), 177 (47), 137 (12).; UV λmax (MeOH) nm 368, 283, 227.

10. The method is described in Masuda, T.; Isobe, J.; Jitoe, A.; Nakatani, N. *Phytochemistry* **1992**, *31*, 3645-3647. The data are shown in the inhibitory % after 8 days incubation at 40 °C.

(*Received in USA* 26 *October* 1993; *revised* 30 *November* 1993; *accepted* 10 *December* 1993)