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ANTIOXIDATIVE CURCUMINOIDS FROM RHIZOMES OF *CURCUMA XANTHORRHIZA*

TOSHIYA MASUDA,* JUNKO ISOBE, AKIKO JITOE and NOBUJI NAKATANI

Laboratory of Food Chemistry, Faculty of Science of Living, Osaka City University, Sumiyoshi, Osaka 558, Japan

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Key Word Index—*Curcuma xanthorrhiza*; Zingiberaceae; rhizomes; curcuminoids; antioxidant activity.

Abstract—A new curcumin analogue has been isolated from the rhizomes of *Curcuma xanthorrhiza* along with four known curcuminoids, and its structure has been determined as 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,4-dione by spectral data. The new compound showed potent antioxidant activity against autoxidation of linoleic acid in a water–alcohol system.

INTRODUCTION

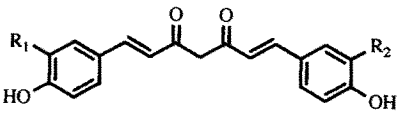
Curcuma xanthorrhiza is one of the gingers cultivated in tropical areas and used in traditional medicine and as a spice. We have investigated tropical gingers as a natural antioxidant source and already reported the possibility of existence of new antioxidants in *C. xanthorrhiza* from our antioxidant analysis [1]. We now report the isolation of a new antioxidative curcuminoid and four known curcuminoids, and report also on the antioxidant activity of the new compound.

RESULTS AND DISCUSSION

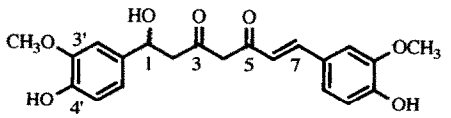
An acetone extract of fresh rhizomes of *C. xanthorrhiza* was suspended in water and extracted with hexane, methylene chloride and ethyl acetate, successively. The methylene chloride and ethyl acetate-soluble fractions showed antioxidant activity. The methylene chloride-soluble fraction was purified by repeated silica gel chromatography to give compounds **1**–**5** (see Experimental).

Compounds **1**–**3** were identified as curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), respectively, by spectroscopic methods [2]. Compound **4** was identified as 1-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-(6*E*)-6-heptene-3,5-dione, which has been reported by Uehara *et al.* from this plant [3].

Compound **5** had the molecular formula $C_{22}H_{22}O_7$ (HR EI mass spectrum; *m/z* 398.1415 $[M]^+$, calculated for $C_{22}H_{22}O_7$: 398.1364). Its UV spectrum showed a similar absorption (λ_{max} 429 nm) to that of **1**. In the aromatic proton region of its ^1H NMR, two sets of signals due to a 1,3,4-trisubstituted benzene [δ7.11 (1H, *dd*, *J* = 8.4 and 1.8 Hz), 7.05 (1H, *d*, *J* = 1.8 Hz), 6.93 (1H, *d*, *J* = 8.4 Hz)] and a 1,3,4,5-tetrasubstituted benzene [δ6.08 (2H, *s*)], three methoxyl signals, two of which have the same chemical shifts, [δ3.95 (3H, *s*), 3.94 (6H, *s*)], and two hydroxyl signals [δ5.81 (1H, *br s*), 5.78 (1H, *br s*)] were observed. The other proton signals [δ7.59 (1H, *d*, *J* = 15.8 Hz), 7.55 (1H, *d*, *J* = 15.8 Hz), 6.48 (1H, *d*, *J* = 15.8 Hz), 6.46 (1H, *d*, *J* = 15.8 Hz), 5.79 (1H, *s*)] were similar to those of **1**. The substitution positions of the three methoxyl groups and the two hydroxyl groups were determined as follows. Five oxygenated positions (methoxylated and hydroxylated positions) were shown to be at the 3- and 4-positions in the trisubstituted benzene part and the 3-, 4- and 5-positions in the tetrasubstituted benzene part by consideration of chemical shifts of aromatic protons. A NOE was observed between a methyl signal at 3.95 ppm and an aromatic signal at 7.05 ppm in



1: R_1=OCH_3, R_2=OCH_3
2: R_1=OCH_3, R_2=H
3: R_1=H, R_2=H



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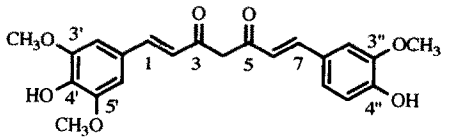

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*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of compounds **1** and **5** (400 MHz, $CDCl_3$, ppm from TMSi as an internal standard)

H	1	5
1	7.59 *d* (15.9)	7.59 *d* (15.8)[a]
2	6.47 *d* (15.3)	6.48 *d* (15.8)[b]
4	5.80 *s**	5.79 *s**
6	6.47 *d* (15.3)	6.46 *d* (15.8)[b]
7	7.59 *d* (15.9)	7.55 *d* (15.8)[a]
2′	7.05 *d* (1.9)	6.80 *s*
5′	6.93 *d* (8.0)	—
6′	7.12 *dd* (8.0, 1.9)	6.80 *s*
2″	7.05 *d* (1.9)	7.05 *d* (1.8)
5″	6.93 *d* (8.0)	6.93 *d* (8.3)
6″	7.12 *dd* (8.0, 1.9)	7.11 *dd* (8.4, 1.8)
OMe-3′	3.95 *s*	3.94 *s*
OMe-5′	—	3.94 *s*
OMe-3″	3.95 *s*	3.95 *s*
OH-4′	5.87 *br s*	5.78 *br s*[c]
OH-4″	5.87 *br s*	5.81 *br s*[c]

J values in parentheses expressed in Hz.
* Proton signal assignable to an enol form.
[a–c] Chemical shifts with the same letter may be interchanged.

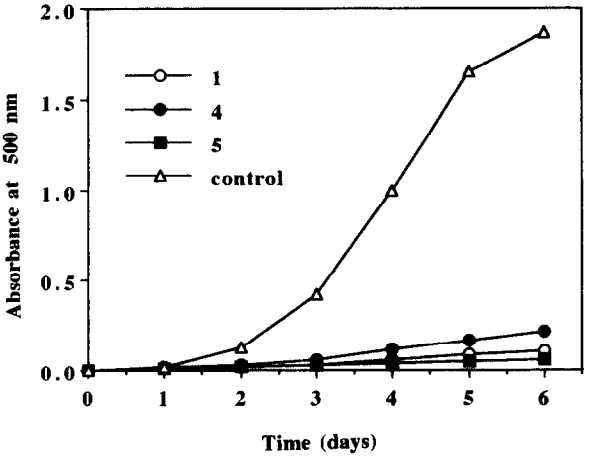

Fig. 1. Effect of compounds **1**, **4** and **5** on autoxidation of linoleic acid. Oxidation of linoleic acid was assayed by the thyocyanate method described in the Experimental.

a NOE differential spectrum, indicating that the methoxyl group was attached at the 3-position in the trisubstituted benzene ring. The substitution positions of the other methoxyl groups were on the 3- and 5-positions of the tetrasubstituted benzene part from their symmetrical nature in its ^{1}H NMR [δ6.80 (2H, *s*), 3.94 (6H, *s*)]. Thus, compound **5** was determined to be 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,5-dione.

Curcuminoids **1–3** have potent antioxidant activity [4], and their activity showed almost the same efficiency in a water–alcohol assay system [1]. We measured the activity of **4** and **5**, and compared them with that of **1**, (Fig. 1); **5** showed slightly stronger antioxidant efficiency than that of **1**, but **4** showed weaker activity than that of **1**. The observed similar efficiency of **5** to **1** was in good agreement with our previously obtained antioxidant data of **1–3** [1]. Although the high antioxidant activity of the curcumin family was explained by metal chelation on a β-diketone moiety [6, 7], recently, Cuvelier *et al.* suggested that the activity of curcumin depended on delocalization of the phenolic radical to the ketone function [8]. The stronger activity of **1** and **5** than that of **4** indicated that the delocalization of the phenolic radical to the conjugated alkyl chain is important for the antioxidant activity of curcuminoids.

EXPERIMENTAL

Plant material. Rhizomes of *C. xanthorrhiza* were cultivated and collected in Tabanan Village, Bali, Indonesia, and identified by Dr I. G. P. Tengah at Udayana University, Indonesia.

Isolation. Fresh rhizomes (702 g) were crushed and soaked in Me_2CO (1.5 l × 2) for 9 days. After filtration, the extract was concd. The residue (24 g) was resuspended in H_2O, extracted with hexane, CH_2Cl_2 and EtOAc, successively, and concd. The CH_2Cl_2-sol. fr. (2.6 g) was filtered with C_6H_6–Me_2CO–CH_2Cl_2 (5:1:1) to give a mixt. of compounds **1–3** (131 mg), which was purified by silica gel TLC (5% MeOH in CH_2Cl_2) to give pure **1–3**. The mother liquor was subjected to silica gel CC (C_6H_6–Me_2CO 5:1 ~ 0:1) to separate into 10 frs. Compound **4** (3.5 mg) was isolated from fr. 10 after silica gel TLC (Me_2CO–hexane, 1:20). Compound **5** (4 mg) was isolated from fr. 7, after silica gel TLC (Me_2CO–hexane 1:10).

Curcumin (**1**). UV λ_{max}^{MeOH} nm: 423. EI-MS *m/z* 368 $[M]^+$. ^{1}H NMR see Table 1.

Demethoxycurcumin (**2**). EI-MS *m/z* 338 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.61 (1H, *d*, *J* = 15.9 Hz, H-1 or H-7), 7.59 (1H, *d*, *J* = 15.9 Hz, H-1 or H-7), 7.45 (2H, *d*, *J* = 8.1 Hz, H-2″ and H-6″), 7.12 (1H, *dd*, *J* = 7.8 and 1.8 Hz, H-6′), 7.05 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.93 (1H, *d*, *J* = 7.8 Hz, H-5′), 6.88 (2H, *d*, *J* = 8.1 Hz, H-3″ and H-5″), 6.48 (1H, *d*, *J* = 15.9 Hz, H-2 or H-6), 6.47 (1H, *d*, *J* = 15.9 Hz, H-2 or H-6), 5.86 (2H, *br s*, OH), 5.80 (1H, *s*, H-4), 3.95 (3H, *s*).

Bisdemethoxycurcumin (**3**). EI-MS *m/z* 308 $[M]^+$. ^{1}H NMR (Me_2CO-d_6) δ7.61 (2H, *d*, *J* = 15.9 Hz, H-1 and H-7), 7.55 (4H, *d*, *J* = 8.6 Hz, H-2′, H-6′, H-2″ and H-6″), 6.89 (4H, *d*, *J* = 8.6 Hz, H-3′, H-5′, H-3″ and H-5″), 5.97 (1H, *s*, H-4).

1-*Hydroxy*-1,7-*bis*(4-*hydroxy*-3-*methoxyphenyl*)-(6E-6-*heptene*-3,4-*dione* (**4**). Pale yellow powder, mp 84–88°. UV λ_{max}^{MeOH} nm: 372. EI-MS *m/z*: 368 $[M-H_2O]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.30 (1H, *d*, *J* = 15.6 Hz, H-7), 7.03 (1H, *dd*, *J* = 7.9 and 1.8 Hz, H-6′ or H-6″), 7.0 (4H, *m*, arom.), 6.90 (1H, *d*, *J* = 8.4 Hz, H-5′ or H-5″), 6.44 (1H, *d*, *J* = 15.8 Hz, H-6), 5.60 (1H, *s*, H-4), 5.38 (1H, *dd*, *J* = 14.0 and 3.1 Hz, H-1), 3.95 (3H, *s*, Me), 3.93 (3H, *s*, Me), 2.93 (1H, *dd*, *J* = 16.6 and 14.0 Hz, H-2), 2.63 (1H, *dd*, *J* = 16.6 and 3.1 Hz, H-2).

1-(4-*Hydroxy*-3,5-*dimethoxyphenyl*)-7-(4-*hydroxy*-3-*methoxyphenyl*)-(1E,6E)-1,6-*heptadiene*-3,4-*dione* (**5**). Yellow powder, mp 145–146°. UV λ_{max}^{MeOH} nm: 429. HR EI-MS *m/z*: 398.1425 $[M]^+$ (calc. for $C_{22}H_{22}O_7$: 398.1364). ^{1}H NMR see Table 1.

Antioxidant assay. The method of ref. [5] was slightly modified. A mixt. of sample (2 mg) in 4 ml 99.5% EtOH, 4.1 ml of 2.53% linoleic acid in 99.5% EtOH, 8 ml of 0.05 M Pi buffer (pH 7) and 3.9 ml of dist. H_2O was placed in a vial (50 ml) with a screw cap and placed in an oven at 40° in the dark. Oxidation of linoleic acid was monitored by the following method. To 0.1 ml of this sample soln was added 9.7 ml of 75% EtOH and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition

of 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% HCl to the reaction mixt. the A of the red colour was measured at 500 nm.

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