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ANTI-OXIDATIVE AND ANTI-INFLAMMATORY CURCUMIN-RELATED PHENOLICS FROM RHIZOMES OF *CURCUMA DOMESTICA*

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Key Word Index—*Curcuma domestica*; Zingiberaceae; rhizome; curcuminoid; phenolic; anti-inflammatory activity; antioxidant activity.

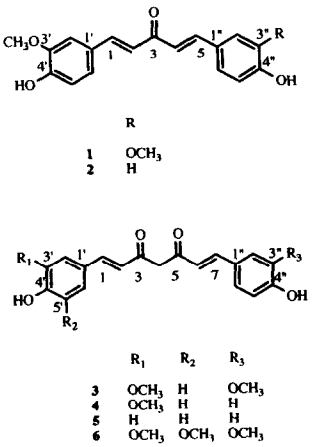
Abstract—Two new natural phenolics were isolated from the rhizomes of *Curcuma domestica* along with four known curcuminoids. The structures of the former were determined to be 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one and 1-(4-hydroxy-3-methoxyphenyl)-5-(-4-hydroxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one, respectively, by spectral data and syntheses. Anti-oxidant activity of the phenolics was determined by the inhibition of autoxidation of linoleic acid in a water–alcohol system. Anti-inflammatory activity of the isolated compounds was determined on mouse ears by using a tumour promoter, TPA (12-*O*-tetradecanoylphorbol-13-acetate) as an inducer.

INTRODUCTION

In the course of our investigation of anti-oxidative and anti-inflammatory natural products [1–3], we have studied the constituents in the rhizomes of *Curcuma domestica* Val. (Zingiberaceae), which have been used, not only as yellow colouring agent, but also for traditional medicine in Asia. It is well known that the rhizomes of *C. domestica* have potent anti-oxidative and anti-inflammatory activities, and the activities have been explained by the effect of a main constituent, curcumin. We have reported that two known analogues of curcumin exist in the rhizomes in about 60% quantity of curcumin, on the basis of our HPLC analysis [1], and our further investigation of the anti-oxidant activity of curcumin and the two analogues indicated the existence of additional potent anti-oxidative compounds in the rhizomes [1]. Recently, Huang *et al.* suggested that anti-oxidant activity and anti-inflammatory activity of curcumin related to its anti-tumour promoter activity [4]. Interest in the activities of curcumin-related compounds prompted us to investigate phenolic constituents in the rhizomes of *C. domestica*. This paper deals with the isolation of new curcumin-related phenolics (**1**, **2**) and three curcuminoids (**3**–**6**) from the rhizomes, and determination of their anti-oxidant activity and anti-inflammatory activity.

RESULTS AND DISCUSSION

Compounds **1**–**6** were isolated by repeated silica gel chromatography, with the guidance of their yellow colour, from the EtOAc-soluble part of the MeOH extract from dry rhizomes of *C. domestica* (See Experimental). Compound **1** has a molecular formula $C_{19}H_{18}O_5$ established by HR mass spectrum. In the ^{1}H NMR of **1**, half of the signals for the molecular formula were observed, indicating that **1** had a symmetrical structure. In the ^{1}H NMR of **1**, signals assignable to two sets of tri-substituted benzenes [δ6.93 (2H, *d*, J=8.0 Hz], 7.13 (2H, *d*, J=1.9 Hz), 7.18 (2H, *dd*, J=8.0 and 1.9 Hz)] and two sets of down-field shifted olefinic signals [δ6.91 (2H, *d*, J=16.0 Hz), 7.66 (2H, *d*, J=16.0 Hz)] were observed, which suggested the presence of two *trans*-cinnamoyl groups. However, the molecular formula indicated that there was only one carbonyl group in **1**. Thus, the carbonyl function (δ188.8) must be shared by the two



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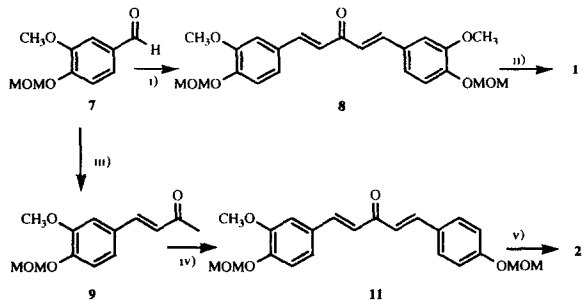

Scheme 1. Syntheses of **1** and **2**. MOM, methoxymethyl group; (i) acetone, NaOH; (ii) HOAc, H_2O; (iii) acetone (excess), NaOH; (iv) **10**, NaOH; (v) HOAc, H_2O.

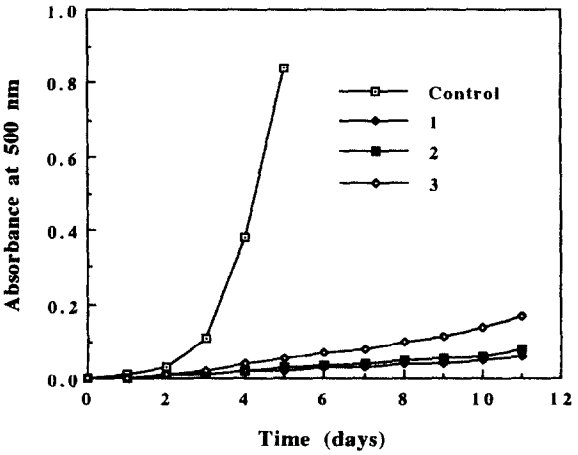

Fig. 1. Effect of **1**–**3** (each 5.4 μmol) on autoxidation of linoleic acid. Oxidation of linoleic acid was assayed by the thiocyanate method.

cinnamoyl groups. From the chemical shifts of protons on the tri-substituted benzene moiety, the methoxyl and the hydroxyl groups [δ3.97 (6H, *s*), 5.83 (2H, *s*), respectively] should be attached at the benzene. The attached positions of the methoxyl and the hydroxyl groups were determined to be at the 3- and 4-positions, respectively, by a NOE observation between the protons at 7.13 ppm and the methoxyl protons (δ3.97) in the NOE difference spectrum of **1**. Thus, the structure of **1** was 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one. Compound **1** was isolated from a natural source for the first time to our knowledge, although **1** has already been synthesized by Ramanan *et al.* [5]. We also synthesized **1** starting from vanillin as shown in Scheme 1.

Compound **2** has a molecular formula $C_{18}H_{16}O_4$ established by HR mass spectrum. Although the ^{1}H NMR of **2** was similar to that of **1**, one methoxyl signal was absent compared with that of **1**, and the signals due to a di-substituted benzene [δ7.53 (2H, *d*, J = 8.2 Hz), 6.88 (2H, *d*, J = 8.2 Hz)] were observed, instead of the signals due to a tri-substituted benzene in **1**. The substituted position of the methoxyl group in **2** was determined to be at the 3-position of the tri-substituted benzene by an NOE observation, between the proton at 7.12 ppm and the methoxyl protons (δ3.95) in the NOE difference spectrum. Thus, **2** was 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one. The structure was also confirmed by a synthesis starting from vanillin and *p*-hydroxybenzaldehyde as shown in Scheme 1.

Compounds **3**–**6** were identified as curcumin (**3**), demethoxycurcumin (**4**), bisdemethoxycurcumin (**5**) and 5′-methoxycurcumin (**6**), respectively, by spectroscopic methods and direct comparisons with the authentic samples previously isolated as potent anti-oxidants from *Curcuma xanthorrhiza* [2].

Anti-oxidant activity of phenolics (**1**, **2**) was determined by the inhibition of autoxidation of linoleic acid and was compared with that of **3**, data being shown in Fig. 1. Phenolics **1** and **2** showed stronger activity than that of **3**. Some investigators have suggested that the strong anti-oxidant activity of **3** depended on the 1,3-diketone system [6, 7]. However, we have indicated that the effect of the diketone system on the anti-oxidant activity was low [2]. The present results also strongly support our suggestion.

Anti-inflammatory activity of the isolated compounds (**1**–**6**) was determined by the inhibition of edema induced by a tumour promoter, TPA (12-*O*-tetradecanoyl-phorbol-13-acetate), on mouse ears. This assay method has been reported as a first screening method for anti-tumour promoters [8], and was also used by Huang *et al.* in their investigation of anti-tumour promoter activity of **3** [4]. As shown in Table 2, each 0.6 μmol of all isolated compounds (**1**–**6**) showed anti-inflammatory activity. Three curcuminoids (**4**–**6**) showed stronger activity than that of **3**. Compound **5** had the strongest activity among the isolated curcuminoids. The related phenolic **1** showed comparable activity with that of **3**, while the phenolic **2** showed weaker activity than that of **3**. Recently, Ramanan *et al.* examined the anti-inflammatory activity of synthetic **1** by use of the carrageenan-induced paw edema in rats [5]. They reported that **1** had much weaker activity than that of **3**, and suggested that the 1,3-diketone system that existed in **3** was essential for the increased activity. However, from the present result that **1** showed comparable activity with that of **3**, the effect of their structural difference in the ketone system on the activity in the assay system used would be small. Our results also suggest that the substituted methoxyl group in the benzene part of each compound might affect the increased or decreased activity not only of curcuminoids (**3**–**6**) but also of phenolics (**1**, **2**).

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR: 400 or 60 MHz, ^{13}C NMR: 100 MHz, TMS as int. standard. EI and HREI-MS: 70 eV, direct inlet.

Plant material. The rhizomes of *Curcuma domestica* were collected in Iriomote Island, Japan, in October 1989, and were identified by one of the authors, Dr S.

Table 1. ^{1}H and ^{13}C NMR data of **1** and **2** (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR)

	1		**2**	
Position	H ($CDCl_3$)	C ($CDCl_3$)	H ($CDCl_3$)	C (acetone-d_6)
1	7.66 *d* (16.0)	143.2	7.67[b] *d* (15.9)	143.1[d]
2	6.91 *d* (16.0)	123.4[a]	6.92[c] *d* (15.9)	123.8[e]
3		188.8		188.8
4	6.91 *d* (16.0)	123.4[a]	6.96[c] *d* (15.9)	124.1[e]
5	7.66 *d* (16.0)	143.2	7.69[b] *d* (15.9)	143.5[d]
1′		127.5		127.7[f]
2′	7.13 *d* (1.9)	109.9	7.12 *d* (2.0)	111.8
3′		146.9		148.8
4′		148.3		150.2
5′	6.93 *d* (8.0)	114.9	6.94 *d* (7.8)	116.2
6′	7.18 *dd* (8.0, 1.9)	123.3[a]	7.18 *dd* (7.8, 2.0)	124.2[e]
1″		127.5		128.1[f]
2″	7.13 *d* (1.9)	109.9	7.53 *d* (8.2)	131.1
3″		146.9	6.88 *d* (8.2)	116.9
4″		148.3		160.7
5″	6.93 *d* (8.0)	114.9	6.88 *d* (8.2)	116.9
6″	7.18 *dd* (8.0, 1.9)	123.3[a]	7.53 *d* (8.2)	131.1
MeO at 3′	3.97 *s*	56.0	3.95 *s*	56.4
MeO at 3″	3.97 *s*	56.0		
HO at 4′	5.83 *s*		5.88 *s*	
HO at 4″	5.83 *s*		5.32 *brs*	

[a–f]Interchangeable values.
Coupling constants (*J* in Hz) are given in parentheses.

Table 2. The inhibitory effect of compounds **1–6** (0.6 μmol) on TPA (2 μg)-induced edema of mouse ears

Compound	*n*[a]	*D*[b]/mg ± s.e.	% inhibition	Significance
control	10	16.6 ± 0.9[c]		
1	5	8.7 ± 1.4	52	$P<0.01$
2	5	5.3 ± 0.4	32	$P<0.01$
3	10	9.7 ± 1.1	58	$P<0.01$
4	5	11.0 ± 1.8	66	$P<0.01$
5	6	13.3 ± 0.9	80	$P<0.01$
6	6	11.5 ± 1.3	69	$P<0.01$

[a]*n*, number of mice; [b]*D*, (wt of TPA applied ear) − (wt of TPA + sample applied ear); [c]*D*, (wt of TPA applied ear) − (wt of vehicle applied ear).

Yonemori. A voucher specimen is deposited at the Institute of Tropical Agriculture, University of the Ryukyus.

Extraction and isolation. The air-dried rhizomes of *C. domestica* (300 g) were powdered and extracted successively with *n*-hexane (1 l) and MeOH (1 l) at room temp. The MeOH extract (25.8 g) was filtered with EtOAc to give an EtOAc-soluble fraction (13.8 g). The fraction was separated into eight fractions by silica gel column chromatography eluted with hexane–acetone (4:3–0:1). Fraction 4 was purified by silica gel TLC (3% MeOH in CH_2Cl_2) to give **1** (8.0 mg) and **2** (4.0 mg). Fraction 6 was purified by silica gel TLC (hexane–acetone, 4:3) to give **6** (6.0 mg). Fraction 3 (20 mg; 2.1 g) was purified by silica gel TLC (2% MeOH in CH_2Cl_2) to give **3** (8 mg), **4** (2 mg) and **5** (2 mg).

1,5-*Bis*(4-*hydroxy*-3-*methoxyphenyl*)-(1E,4E)-1,4-*pentadien*-3-*one* (**1**). Yellow powder from $CHCl_3$–hexane; mp 82–83°; UV λ_{max}^{MeOH}: 380 nm; HRMS *m/z*: 326.1099 $[M]^+$ (calcd for $C_{19}H_{18}O_5$ *m/z* 326.1153); ^{1}H and ^{13}C NMR: see Table 1.

1-(4-*hydroxy*-3-*methoxyphenyl*)-5-(4-*hydroxyphenyl*)-(1E,4E)-1,4-*Pentadien*-3-*one* (**2**). Yellow powder from $CHCl_3$–hexane; mp 161.5–162.5°; UV λ_{max}^{MeOH}: 382 nm; HRMS *m/z*: 296.1048 $[M]^+$ (calcd for $C_{18}H_{16}O_4$ *m/z* 296.1048); ^{1}H and ^{13}C NMR: see Table 1.

Curcumin (**3**), *demethoxycurcumin* (**4**), *bisdemethoxycurcumin* (**5**) *and* 5′-*methoxycurcumin* (**6**). These compounds were identified by ^{1}H NMR, MS and direct comparisons on silica gel TLC with authentic samples previously isolated. See ref. [2] for their spectral data.

Protection of the hydroxyl group of vanillin and p-hydroxybenzaldehyde. To a soln of vanillin (2 g) and dry K_2CO_3 (9 g) in DMF (30 ml) was added, dropwise, methoxymethylchloride (1.3 ml) at room temp. After stirring for 30 min, benzene (30 ml) was added to the mixt. The mixt. was filtered, and the filtrate was washed 3 times with H_2O, and once with brine. The organic layer was dried over Na_2SO_4 and concd to give 4-*O*-methoxymethylvanillin (**7**; 2.7 g, 100% yield). ^{1}H NMR (60 MHz, $CDCl_3$) δ3.52 (3H, *s*), 3.93 (3H, *s*), 5.32 (2H, *s*), 7.42 (3H, *m*), 9.90 (1H, *s*), MS *m/z* 196 $[M]^+$. By the same procedure, 4-methoxymethyloxybenzaldehyde (**10**; 2.6 g, 96%

yield) was synthesized from *p*-hydroxybenzaldehyde (2 g). ^{1}H NMR (60 MHz, $CDCl_3$) δ3.50 (3H, *s*), 5.25 (2H, *s*), 7.13 (2H, *d*, $J=8$ Hz), 7.85 (2H, *d*, $J=8$ Hz), 9.90 (1H, *s*), MS *m/z* 166 $[M]^+$.

Synthesis of **1**. To a soln of NaOH (0.4 g) in H_2O (4 ml) and EtOH (4 ml) was added a mixt. of **7** (1 g) and acetone (0.19 ml) at room temp. After stirring for 2 hr at room temp., the mixt. was extracted 3 times with CH_2Cl_2, dried over Na_2SO_4, and concd to give 4′, 4″-*O*-dimethoxymethylated **1** (**8**) as a yellow oil (1 g, 95% yield). ^{1}H NMR (60 MHz, $CDCl_3$) δ3.52 (6H, *s*), 3.94 (6H, *s*), 5.29 (4H, *s*), 6.95 (2H, *d*, $J=16$ Hz), 7.19 (6H, *m*), 7.71 (2H, *d*, $J=16$ Hz), MS *m/z* 414 $[M]^+$. Compound **8** (51 mg) was dissolved in a mixt. of HOAc (1.2 ml), H_2O (1.2 ml) and DMF (0.1 ml). After stirring for 1 hr at 80°, the mix. was extracted 3 times with CH_2Cl_2, dried over Na_2SO_4, and concd. The residue was purified by silica gel TLC (5% MeOH in CH_2Cl_2) to give **1** (17 mg, 42% yield). Identification of synthetic **1** was based on spectroscopic methods.

Synthesis of 1-(3-*methoxy*-4-*methoxymethyloxyphenyl*)-(1E)-1-*buten*-3-*one* (**9**). To a soln of NaOH (0.4 g) in EtOH (4 ml) and H_2O (4 ml) were added **7** (1 g) and acetone (4 ml) at room temp. After stirring for 1 hr, the mix. was extracted 3 times with CH_2Cl_2, dried over Na_2SO_4, and concd. The residue was purified by silica gel column chromatography, eluted with EtOAc–hexane (1:2) to give **9** as a yellowish solid (0.74 g, 61% yield). ^{1}H NMR (60 MHz, $CDCl_3$) δ2.33 (3H, *s*), 3.52 (3H, *s*), 3.93 (3H, *s*), 5.27 (2H, *s*), 6.63 (1H, *d*, $J=15$ Hz), 7.15 (3H, *m*), 7.50 (1H, *d*, $J=15$ Hz), MS *m/z* 236 $[M]^+$.

Synthesis of **2**. To a soln of NaOH (0.04 g) in EtOH (0.8 ml) and H_2O (0.4 ml) was added a mix. of **9** (100 mg) and **10** (70 mg). After stirring for 1 hr at room temp., the mix. was extracted 3 times with CH_2Cl_2, dried over Na_2SO_4, and concd. The residue was purified by silica gel column chromatography eluted with EtOAc–hexane (1:2) to give 4′, 4″-*O*-dimethoxymethylated **2** (**11**; 110 mg, 68% yield). ^{1}H NMR (60 MHz, $CDCl_3$) δ3.47 (3H, *s*), 3.50 (3H, *s*), 3.92 (3H, *s*), 5.18 (2H, *s*), 5.26 (2H, *s*), 6.89 (2H, *d*, $J=8$ Hz), 6.97 (2H, *d*, $J=16$ Hz), 7.10 (3H, *m*), 7.57 (2H, *d*, $J=8$ Hz), 7.70 (2H, *d*, $J=16$ Hz), MS *m/z* 384 $[M]^+$. Compound **11** (38 mg) was dissolved with HOAc (1 ml) and H_2O (1 ml). After stirring for 3.5 hr at 80°, the mix. was extracted 3 times with CH_2Cl_2, dried over Na_2SO_4, and concd. The residue was purified by silica gel TLC (EtOAc–hexane (1:2) to give **2** (23 mg, 82% yield). Identification of synthetic **2** was based on spectroscopic methods.

Anti-inflammatory assay. The assay was carried out by means of the procedure of ref. [8], and slightly modified. Male Jcl:ICR mice, 6 weeks of age, were obtained from Clea Japan inc., Tokyo, Japan, and used for this assay. A sample (0.6 μmol) in acetone (20 μl) and vehicle were applied to the inner part of the left and right ear, respectively, of the same mouse. Thirty minutes after the sample application, TPA (2 μg) in acetone (20 μl) was applied to the same part of both ears. After 6.5 hr, the mouse was killed. Immediately after the killing, plugs of each ear were obtained with a punch (0.6 cm diameter) was weighed. The inhibitory effect (% inhibition) was determined using the following equation: {[wt of TPA applied ear]−[wt of sample+TPA applied ear]} × 100/{[wt of TPA applied ear]−[wt of vehicle applied ear]}. Student's *t*-test was used to determine the statistical significance.

Anti-oxidant assay. The assay was carried out according to ref. [2].

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REFERENCES

1. Jitoe, A., Masuda, T., Tengah, I. G. P., Suprapta, D. N., Gara, I. W. and Nakatani, N. (1992) *J. Agric. Food Chem.* **40**, 1337.
2. Masuda, T., Isobe, J., Jitoe, A. and Nakatani, N. (1992) *Phytochemistry* **31**, 3645.
3. Masuda, T., Masuda, K. and Nakatani, N. (1992) *Tetrahedron Letters* **33**, 945.
4. Huang, M. T., Lysz, T., Ferraro, T., Abidi, T. F., Laskin, J. D. and Conney, A. H. (1991) *Cancer Res.* **51**, 813.
5. Ramanan, P. N. and Rao, M. N. A. (1989) *Indian J. Pharm. Sci.* **51**, 207.
6. Osawa, T. and Namiki, M. (1985) *J. Agric. Food Chem.* **33**, 777.
7. Larson, R. (1988) *Phytochemistry* **27**, 969.
8. Gschwendt, M., Kittstein, K., Fürstenberger, G. and Marks, F. (1984) *Cancer Letters* **25**, 177.