Anti-inflammatory sesquiterpenes from *Curcuma zedoaria*

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From the methanolic extract of the rhizome of *Curcuma zedoaria*, we isolated anti-inflammatory sesquiterpene furanodiene (**1**) and furanodienone (**2**) along with new sesquiterpene compound **3** and known eight sesquiterpenes, zederone (**4**), curzerenone (**5**), curzeone (**6**), germacrone (**7**), 13-hydroxygermacrone (**8**), dehydrocurdione (**9**), curcumenone (**10**), and zedoaronediol (**11**). Their structures were elucidated on the basis of spectroscopic data. The anti-inflammatory effect of isolated components on 12-*O*-tetradecanoylphorbol-13- acetate (TPA)-induced inflammation of mouse ears were examined. Compounds **1** and **2** suppressed TPA-induced inflammation of mouse ears by 75% and 53%, respectively, at a dose of 1.0 μmol. Their activities are comparable to that of indomethacin, the normally used anti-inflammatory agent.

Keywords: anti-inflammatory; *Curcuma zadoaria*; 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema

Zedoary (*Curcuma zedoaria* Roscoe, Zingiberaceae) is cultivated as a vegetable, spice, and perfumery material in Southeast Asian countries. The rhizome of this plant has been used as a stimulant, stomachic, carminative, diuretic, anti-diarrheal, anti-emetic, anti-pyretic, and depurator, and also as an ointment for ulcers, wounds, and other skin disorders.[1)] Recently, Yoshikawa *et al* have revealed that chemical constituent of *C. zedoaria* showed vasorelaxant, heptatoprotective, and inhibitory activity of NO production.[2)-4)] The zedoary is used to the treatment of the skin disease, however the study of the anti-inflammatory effect of chemical constituents from *C. zedoaria* has not been studied so far except that of dehydrocurdione (**9**) by Yoshioka *et al*.[5)] Therefore, we tried to isolate sesquiterpens from zedoary and subject on anti-inflammatory test. For isolating sesquiterpens like dehydrocurudione, we used the reddish spot on thin layer chromatography with spraying of anisaldehyde/sulfuric acid as index.

In the present paper, we describe the structure of isolated eleven sesquiterpenes from *C. zedoaria* and their anti-inflammatory effect on the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema of mouse ears.

The zedoary rhizome (cultivated in Okinawa, Japan) was soaked in methanol, and the resulting methanol extract was concentrated *in vacuo*. The concentrate was successively partitioned between benzene and water, and then ethyl acetate and water. The benzene-soluble part showed anti-inflammatory activity (IE of 34% at a dose of 2 mg). The benzene-soluble part was chromatographed on silica gel to give 20% ethyl

acetate in hexane eluate as an active fraction. Further purification with preparative thin layer chromatography gave compounds **1** and **2** as anti-inflammatory compounds. Spectral analysis of **1** and **2** were indicative to be furanodiene[6] and furanodienone, [7] respectively. Comparison of the spectral data of **1** and **2** with the reference data confirmed the structure of **1** and **2**.

Compounds **1**, **2**, and **5**-**7** were also isolated from 20% ethyl acetate in hexane eluate and identified as germacrane type sesquiterpene, curzerenone (**5**),[8] curzeone (**6**),[9]germacrone (**7**).[10] From 30% EtOAc in hexane eluate gave dehydrocurdione (**9**),[11] 40% EtOAc in hexane eluate gave zederone (**4**), [12] and curcumenone (**10**),[13] and 50% EtOAc in hexane eluate afforded 13-hydroxygermacrone (**8**).[9] The EtOAc soluble part afforded zedoaronediol (**11**).[14]

These constituents (**4**-**11**) were identified by comparison of their physical and spectral data with reported values (Figure 1). [8]-[14]

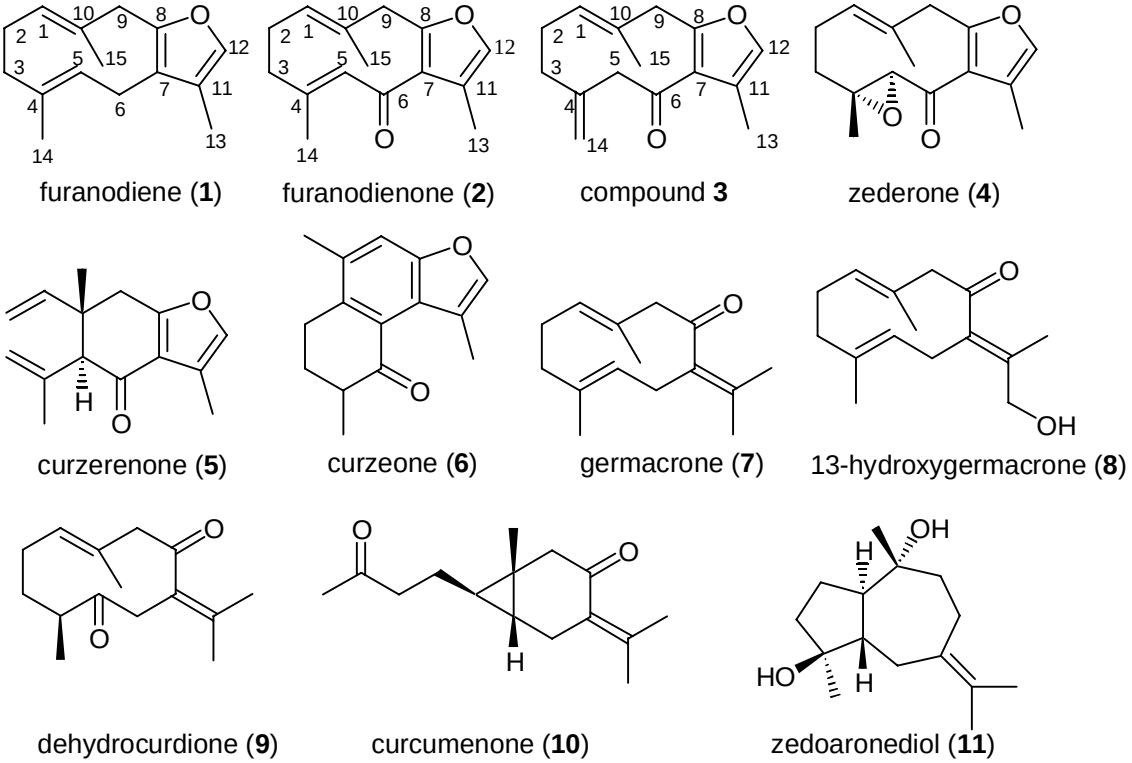

Fig. 1. Sesquiterpenes from *Curcuma zedoaria*

Compound **3** was newly isolated compound. The HR-EIMS analysis of **3** gave the molecular formula of $C_{15}H_{18}O_2$ (*m/z* 230.1285) which showed same molecular formula that of **2**. The IR spectrum of **3** showed the presence of an α, β-unsaturated carbonyl group (1682 cm^{-1}). The ^{13}C-NMR spectrum of **3** showed a signal assignable to carbonyl group (δ 200.0) and eight signals due to four C=C bonds (δ 152.9-117.1), suggesting that **3** was a bicycle compound. Signals assignable to two methyl groups (δ

1.61 and 1.94), four methylene groups (δ 3.38, 3.38, 2.33, and 2.25), an *exo*-methylene group (δ 5.06 and 4.91, each 1H), and two methine groups (δ 7.01 and 5.40) were observed in the ^{1}H-NMR spectrum of **3** (Table 1).

Table 1. ^{1}H-and ^{13}C-NMR Data for **3**

position	^{13}C		^{1}H
1	130.8	CH	5.40, (1H, dd, *J* = 7.8, 7.2 Hz)
2	28.5	CH_2	2.25, (2H, m)
3	35.1	CH_2	2.33, (2H, br.s)
4	142.3	C	
5	52.9	CH_2	3.38, (2H, br.s)
6	200.0	C	
7	119.5	C	
8	152.9	C	
9	38.3	CH_2	3.38, (2H, br.s)
10	131.5	C	
11	125.7	C	
12	137.5	CH	7.01, s
13	8.6	CH_3	1.94, s
14	117.1	CH_2	4.91, s 5.06, s
15	17.2	CH_3	1.61, s

Making comparison of the ^{1}H- and ^{13}C-NMR data between **2** and **3**, compound **2** has an *exo*-methylene moiety and lacks of one methyl group. This compound is an isomer of **2**, in the structure of which one of the C=C bond is located between C-4 and C-5 by an analysis of the H-H COSY, and HMBC spectra of **3** (Figure 2).

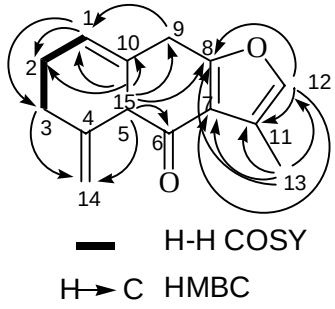

Fig. 2. H-H COSY and HMBC correlation of **3**

Compounds **1**-**11** were subjected to the mouse ear anti-inflammatory test. As shown in Table 2, **1** and **2** suppressed TPA-induced inflammation by up to 75% and 53%, respectively, at a dose of 1 μmol application. Among the furan containing germacrane type sesquiterpenes, furanodiene (**1**) and furanodienone (**2**) showed potent

anti-inflammatory activity. Oxidized compound **4** and compound **3** which possesses *exo*-methylene moiety lost the activity.

Table 2. Anti-inflammatory Activities of **1-11** in the Mouse Ear Inflammatory Test

Test compound	Inhibitory effect (IE %)
1	75 (± 0.9)**
2	53 (± 1.3)**
3	9 (± 1.1)
4	2 (± 0.7)
5	-2 (± 0.8)
6	18 (± 1.2)
7	7 (± 1.0)
8	9 (± 0.4)**
7	-2 (± 1.2)
9	16 (± 2.4)
11	17 (± 1.4)
indomethacin*	78 (± 0.8)**

In parentheses: A sample (1.0 μmol) was applied on one mouse ear and, after 30 min, TPA (0.5 mg) was applied to both ears of the mouse. The edema was evaluated after 7 hr, the inhibitory effect being expressed as the percentage ratio of the edema. Five mice were used for each experiment. *1.4 μmol was applied. **Significantly different, $P < 0.05$ in the Student's t-test.

Dehydrocurdione (**9**) was reported to inhibit acute inflammation of rat paw induced by carrageenan injection. However, **9** did not show anti-inflammatory activity on TPA-induced mouse ear edema at a dose of 1 μmol per ear. Compound **5-8**, **10**, and **11** did not show any inhibitory activity on mouse ear edema.

1 and **2** exhibited anti-inflammatory effect on TPA-induced mouse ear edema. This is the first finding of germacrane type sesquiterpenes showed anti-inflammatory activity. The activity is comparable to that of indomathacin, the normally used anti-inflammatory agent. The isolated amount of **2** is quite large. This may be important evidence substantiating traditional effect of this herbal medicine for the treatment of wounds and skin disorders.

EXPERIMENTAL

The mass spectra were obtained using a Jeol JMS-700 mass spectrometer. The IR spectra were recorded on a Jasco FT/IR-480 Plus IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 FT-NMR spectrometer operating at 500.1 MHz for the protons and at 125.8 MHz for carbons, with TMS as the internal standard.

Extraction and isolation

Fresh rhizomes of *C. zedoaria* (3.6 kg) were extracted with MeOH, and the extract was concentrated *in vacuo*. The aqueous solution was successively partitioned with benzene/water and EtOAc/water. Benzene and EtOAc soluble portions yielded 7.8 g and 0.6 g, respectively.

Isolation of 1-10

Benzene-soluble portion (7.8 g) was chromatographed in a silica gel (400 g) column with EtOAc/hexane by 10% stepwise elution. The 20% EtOAc/hexane eluate (1.6 g) was further purified with preparative TLC to afford furanodiene (**1**, 8 mg), furanodienone (**2**, 384 mg), compound **3** (8 mg), curzerenone (**5**, 4 mg), curzeone (**6**, 7 mg), germacrone (**7**, 10 mg). As the same method described above, the 30% EtOAc/hexane eluate (3.4 g) afforded dehydrocurdione (**9**, 33 mg), the 40% EtOAc/hexane eluate (0.88 g) afforded zedrone (**4**, 70 mg) and curcumenone (**10**, 110 mg), and the 50% EtOAc/hexane eluate (0.28 g) furnished 13-hydroxygermacrone (**8**, 22 mg).

Isolation of 11

EtOAc soluble portion (0.6 g) was chromatographed in a silica gel (30 g) column with hexane/EtOAc by 10% stepwise elution. The 70% EtOAc/hexane eluate (25 mg) was further purified with preparative TLC to afford zedoaronediol (**11**, 20 mg).

Furanodiene (1). ^{1}H-NMR ($CDCl_3$, Me_4Si) δ: 1.27 (3H, s), 1.60 (3H, s), 1.92 (3H, s), 1.76-2.30 (4H, m), 3.07 (2H, m), 3.43-3.56 (2H, m), 4.74 (1H, t, *J* = 7.2 Hz), 4.94 (1H, m), 7.07 (1H, s). ^{13}C-NMR ($CDCl_3$, Me_4Si) δ: 8.90, 16.21, 16.47, 24.36, 26.83, 39.49, 40.93, 118.89, 121.89, 127.60, 128.80, 129.04, 134.36, 135.98, 149.73.

Furanodienone (2). ^{1}H-NMR ($CDCl_3$, Me_4Si) δ: 1.30 (3H, s), 1.99 (3H, s), 2.13 (3H, s), 1.85-2.49 (4H, m), 3.70 (2H, m), 5.17 (1H, m), 5.81 (1H, s), 7.07 (1H, s). ^{13}C-NMR ($CDCl_3$, Me_4Si) δ: 9.56, 15.80, 18.99, 26.47, 40.70, 41.74, 122.24, 123.78, 130.57, 132.49, 135.44, 138.11, 145.74, 156.53, 189.81.

Compound 3. Colorless resin. HREIMS *m/z* (M^+): calcd: for $C_{15}H_{18}O_2$, 230.1307; found, 230.1285. EIMS *m/z* (rel. int.): 230 [M^+] (77), 215 (47), 175 (93), 163 (100), 122 (90). IR ν_{max}(film) cm^{-1}: 3074, 2926, 2856, 1683, 1640, 1375, 897, 752.^{1}H-NMR and ^{13}C-NMR data are shown in Table 1.

Anti-inflammatory test.
Mouse inflammatory tests were conducted by Gshwendt's method.[15] TPA was purchased from Sigma Chemical Co. This experiment complied with the regulations concerning animal experimentation and the care of experimental animals of the Faculty of Agriculture at Shinshu University.

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