Anti-inflammatory Effect of Zingiber cassumunar Roxb. and Its Active Principles

Yukihiro Ozaki,* Nobuo Kawahara and Masatoshi Hārada
Division of Pharmacognosy and Phytochemistry, National Institute of Hygienic Sciences, Setagaya-ku, Tokyo 138, Japan. Received March 12, 1991

The present study was carried out to elucidate the anti-inflammatory effect of the methanol extract obtained from the rhizomes of Zingiber cassumunar Roxb. and its active principles. The methanol extract was partitioned between ether and water, and then the ether-soluble fraction was extracted with n-hexane. The n-hexane-soluble fraction was chromatographed and part of the fraction was rechromatographed by silica gel column. Three compounds were isolated from the n-hexane-soluble fraction and the chemical structures of these compounds were identified as (E)-1-(3,4-dimethoxyphenyl)but-1-ene, (E)-1-(3,4,4-dimethoxyphenyl)butadiene and zerumbone. The anti-inflammatory activity of these fractions was investigated on carrageenin-induced edema in rats, as well as on acetic acid-induced vascular permeability and writhing symptoms in mice. The methanol extract (p.o.) showed both anti-inflammatory activity and analgesic activity. These activities shifted successively to ether-soluble and n-hexane-soluble fractions and to (E)-1-(3,4-dimethoxyphenyl)but-1-ene. These results suggest that the anti-inflammatory action and analgesic action of Zingiber cassumunar is the result of the (E)-1-(3,4-dimethoxyphenyl)but-1-ene that it contains.

Keywords: anti-inflammatory effect; carrageenin-induced edema; vascular permeability; Zingiber cassumunar; (E)-1-(3,4-dimethoxyphenyl)but-1-ene

The rhizomes of Zingiber cassumunar Roxb. (Z. cassumunar) are used in Indonesian folk medicine for the treatment of colic, diarrhea, vermifuge, pain, rheumatism, as an analgetic for the uterus, etc.1-5 We previously reported that the methanol extract obtained from the rhizomes of Z. cassumunar caused a lasting increase in bile secretion when orally administered to anesthetized rats, and that the cholagogic effect of the extract was attributable to the essential oil it contains.6

Although some pharmacological studies of Z. cassumunar have been reported,7 there have been very few anti-inflammatory studies on this subject.

On the basis of these uses in folk medicine, the present study was carried out to elucidate the anti-inflammatory effect of a 70% methanol extract obtained from the rhizomes of Z. cassumunar and to identify the active principle(s).

Experimental

Materials Fresh rhizomes of Z. cassumunar were cultivated in the Bandung region and reflushed with 70% methanol three times for 6h each time. The solution was filtered through filter paper and evaporated to give the extract under vacuum.

The extract was dissolved in ether and extracted with water three times. The ether phase was separated and evaporated to dryness under vacuum. The ether-soluble fraction was then dissolved in n-hexane and extracted with methanol three times. The n-hexane phase was separated and evaporated to dryness under vacuum.

As the n-hexane-soluble fraction showed three spots distinct from the methanol soluble fraction on thin layer chromatography (TLC) (Kieselgel 60 F254, Merck), the fraction was chromatographed on a silica gel column, using an elution solvent of n-hexane and ethylacetate, to monitor these spots. The solvent system used was n-hexane : ethylacetate (50 : 1), and spots on the plate were detected under ultraviolet light. Three compounds were isolated from the n-hexane-soluble fraction and these compounds showed only a large spot on the thin layer plate, respectively. These compounds gave two oily compounds and one crystal as colorless needles. By using

Z. cassumunar 70% MeOH ext.

<table>
<thead>
<tr>
<th>ether layer</th>
<th>shaken with ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>aq. layer</td>
<td>(56.9%)</td>
</tr>
<tr>
<td>n-hexane layer</td>
<td>(16.7%)</td>
</tr>
<tr>
<td>shaken with MeOH</td>
<td>(26.3%)</td>
</tr>
<tr>
<td>MeOH layer</td>
<td></td>
</tr>
<tr>
<td>chromatographed on SiO2 (n-hexane : AcOE)</td>
<td></td>
</tr>
<tr>
<td>50 : 1</td>
<td></td>
</tr>
<tr>
<td>chromatographed on SiO2 (n-hexane : CHCl3)</td>
<td></td>
</tr>
<tr>
<td>4 : 1</td>
<td></td>
</tr>
<tr>
<td>(1 : 1.3%)</td>
<td></td>
</tr>
<tr>
<td>3 : 1</td>
<td></td>
</tr>
<tr>
<td>(2 : 1.3%)</td>
<td></td>
</tr>
<tr>
<td>1 : 1</td>
<td></td>
</tr>
<tr>
<td>(3 : 0.6%)</td>
<td></td>
</tr>
</tbody>
</table>

1: (E)-1-(3, 4-dimethoxyphenyl)but-1-ene
2: (E)-1-(3, 4-dimethoxyphenyl)butadiene
3: zerumbone

Fig. 1. Flow Diagram of Fractionation of the Methanol Extract Obtained from Z. cassumunar

Here, (%) indicates percentage yield calculated on the basis of the methanol extract obtained from Z. cassumunar.

© 1991 Pharmaceutical Society of Japan
infrared (IR), ultraviolet (UV) and nuclear magnetic resonance (NMR) analyses, the chemical structures of these compounds were identical with \((E)-1-(3,4\text{-dimethoxyphenyl})but-1\text{-ene}\) and \((E)-1-(3,4\text{-dimethoxyphenyl})\)butadiene (two oily compounds), and zerumbone (one crystal), respectively. As shown in Fig. 1, the yields (%) were calculated on the basis of the methanol extract.

The methanol extract, each fraction, these compounds and indomethacin (Sigma, methyl cellulose (CMC) solution, and were administered orally 60–90 min before to assay for anti-inflammatory and analgesic effects. The dose for each of the fractions and the compounds were chosen based on the yields obtained from the 70% methanol extraction.

**Carrageenin-Induced Hind-Paw Edema Test** Male Wistar rats weighing 180–230 g were fasted for 16 h prior to the experiments, but were supplied with water *ad libitum*. Carrageenin (Piccinin A, Zushikagaku Lab., Inc.) was suspended in saline to make a 1% (w/v) suspension. The suspension of carrageenin (0.05 ml/animal) was injected subcutaneously into the right hind-paw 30 min after the test solutions had been administered.

The volume of the hind-paw was measured prior to administration of the test solutions by a water displacement transducer (LPU-01,1, Nihon Kohden). The hind-paw volumes were measured 30 min and 1 h after the suspension of carrageenin had been administered and then at intervals of 1 h for up to 6 h.

Control rats were treated similarly except that they received an oral dose of 2% CMC solution alone. The results were expressed as the percentage increase in hind-paw volume due to swelling, as compared with the initial hind-paw volume.

**Acetic Acid-Induced Vascular Permeability Test** Male ddY mice weighing 20–25 g were fasted for 2 h prior to experiments, but were supplied with water *ad libitum*. Four percent pontamine sky blue solution in saline (w/v) was injected intraperitoneally into the tail vein 40 min after the administration of test solutions. After 30 min, a 1% acetic acid solution in saline (v/v) was injected intraperitoneally, and after 20 min, the mice were killed by dislocation of the neck and the abdominal wall was cut to expose the entrails. After washing of the entrails with saline, the washing were filtered through glass wool and collected in test tubes. To clear any turbidity due to protein, 0.1 ml of 1 N NaOH solution was added to each tube, and the absorbance was read at 590 nm in a spectrophotometer (model 200-10, Hitachi). Control mice were treated similarly, except that they received an oral dose of 2% CMC solution alone.

The vascular permeability effects were expressed in terms of the amount of total dye (µg/animal) which leaked into the intraperitoneal cavity.

**Acetic Acid-Induced Writhing Test** Male ddY mice weighing 20–25 g were fasted for 2 h, but were supplied with water *ad libitum*. A 0.7% solution of acetic acid in saline (v/v) was injected intraperitoneally 85 min after the test solutions had been administered. After 5 min, the number of writhes induced by the acetic acid solution was counted for 10 min.

Control mice were treated similarly, except that they received an oral dose of 2% CMC solution alone.

**Statistical Analysis** Data were expressed as the mean value ± standard error. All results were analyzed for variance by Bartlett’s method, and significant differences were subsequently examined by Duncan’s method.

### Results

#### Effect of Methanol Extract Obtained from *Z. cassumunar*

The methanol extract (at 3 g/kg) showed a lasting inhibition of the edema induced by carrageenin during the 6 h period. The inhibitory potency was about the same as that of indomethacin (at 10 mg/kg) (Fig. 2). In the preliminary experiment, the lower dose of methanol extract (at 1 g/kg) did not show a significant inhibitory effect on the edema (data not shown in the figure).

The same doses of methanol extract also reduced dose-dependently the number of writhes induced by acetic acid. The inhibitory potency induced by the extract (at 3 g/kg) was about the same as that of indomethacin (at 10 mg/kg) (Table I).

The methanol extract (at doses of 0.3 and 1 g/kg) inhibited dose-dependently the increase of dye leakage induced by acetic acid. Indomethacin (at 10 mg/kg) inhibited the dye leakage with a potency about the same as that of the methanol extract (at 1 g/kg) (Table II).

#### Effects of Each Fraction Obtained from the Methanol Extract

The ether-soluble fraction obtained from the
TABLE IV. Effect of the n-Hexane-Soluble Fraction, the Methanol-Soluble Fraction and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (g/kg p.o.)</th>
<th>No. of animals</th>
<th>Amount of leaked dye (μg/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% CMC)</td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Z. cassumunar n-hexane layer</td>
<td>0.2</td>
<td>8</td>
<td>439.2 ± 19.2</td>
</tr>
<tr>
<td>MeOH layer</td>
<td>0.3</td>
<td>8</td>
<td>305.0 ± 23.8</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.01</td>
<td>14</td>
<td>274.9 ± 25.8</td>
</tr>
</tbody>
</table>

a) Significantly different from the control at p<0.001. b) Not significantly different from indomethacin.

TABLE V. Effect of (E)-1-(3,4-Dimethoxyphenyl)but-1-ene, (E)-1-(3,4-Dimethoxyphenyl)butadiene and Zerumbone from the n-Hexane-Soluble Fraction and Indomethacin on the Increased Vascular Permeability and Writhing Induced by Acetic Acid in Mice

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (g/kg p.o.)</th>
<th>No. of animals</th>
<th>Amount of leaked dye (μg/animal)</th>
<th>No. of writhes (in 10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% CMC)</td>
<td></td>
<td></td>
<td>6</td>
<td>245.1 ± 23.0</td>
</tr>
<tr>
<td>(E)-1-(3,4-Dimethoxyphenyl)but-1-ene</td>
<td>0.016</td>
<td>6</td>
<td>130.0 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>(E)-1-(3,4-Dimethoxyphenyl)butadiene</td>
<td>0.016</td>
<td>6</td>
<td>224.7 ± 28.4</td>
<td></td>
</tr>
<tr>
<td>Zerumbone</td>
<td>0.008</td>
<td>6</td>
<td>269.0 ± 19.1</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.01</td>
<td>6</td>
<td>136.5 ± 27.8</td>
<td></td>
</tr>
</tbody>
</table>

a, c) Significantly different from the control at p<0.05 and p<0.01, respectively. b) Not significantly different from indomethacin.

From these results, it was suggested that the pharmacologically active principle(s) passed into the n-hexane-soluble fraction.

As shown in Table V, among the compounds isolated from the n-hexane-soluble fraction, only (E)-1-(3,4-dimethoxyphenyl)but-1-ene (at 0.016 g/kg) inhibited the increase in dye leakage and it also reduced the number of writhes induced by acetic acid. On the other hand, (E)-1-(3,4-dimethoxyphenyl)butadiene (at 0.016 g/kg) and zerumbone (at 0.008 g/kg) did not significantly inhibit the increase of dye leakage and neither compound reduced the number of writhes induced by acetic acid.

The inhibitory potency induced by (E)-1-(3,4-dimethoxyphenyl)but-1-ene was about the same as that of indomethacin (at 10 mg/kg).

**Discussion**

In the present study, it was found that the 70% methanol extract obtained from Z. cassumunar significantly inhibited edema induced by carrageenin (at 3 g/kg). The increase in dye leakage induced by acetic acid (at 1 g/kg) and the number of writhes induced by acetic acid (at 3 g/kg). It was also found that the inhibitory potency induced by the extract was about the same as that of indomethacin at 10 mg/kg.

It is well known that irritating compounds sometimes cause pseudo inhibition of the edema induced by carrageenin. But the methanol extract also inhibited the increase in dye leakage induced by acetic acid and the number of writhes induced by acetic acid.

From these results, it seems likely that the methanol extract does not have an irritating effect, but both anti-inflammatory and analgesic effects. Therefore, it was considered...
worthwhile to elucidate the anti-inflammatory activity of the extract and to isolate its active principles.

The ether-soluble fraction obtained from the methanol extract inhibited both edema induced by carrageenin and the increase in dye leakage induced by acetic acid, but the water-soluble fraction did not. As the anti-inflammatory activity had been concentrated in the ether-soluble fraction, this was further fractionated, based on the results of an anti-inflammatory activity assay using the experimental model of dye leakage induced by acetic acid.

The activity shifted successively to the n-hexane-soluble fraction. Since the potency of the inhibitory effects induced by these fractions was approximately the same as that of a fixed dose of indomethacin in all experiments, it is considered that the anti-inflammatory activity had been almost entirely in the n-hexane-soluble fraction. Then, the active principle was isolated from this fraction and its chemical structure was identified as (E)-1-(3,4-dimethoxyphenyl)but-1-ene.

(E)-1-(3,4-dimethoxyphenyl)but-1-ene inhibited the increase in dye leakage induced by acetic acid and also reduced the number of writhes induced by acetic acid.

These results suggest that the anti-inflammatory effect of the methanol extract is due to its (E)-1-(3,4-dimethoxyphenyl)but-1-ene content and also that it may exert an analgesic effect.

It is very interesting that (E)-1-(3,4-dimethoxyphenyl)but-1-ene showed an anti-inflammatory effect, but (E)-1-(3,4-dimethoxyphenyl)butadiene did not, suggesting the butene moiety in their chemical structure is important in producing the anti-inflammatory effect.

Kuroyanagi et al. and Tuntiwachwuttikul et al. reported that (E)-1-(3,4-dimethoxyphenyl)but-1-ene and (E)-1-(3,4-dimethoxyphenyl)butadiene were isolated from a chloroform-soluble or hexane-soluble extraction of the rhizome of Z. cassumunar, respectively. And, there were some reports in which zerumbone was isolated from the rhizome of Zingiber zerumbet (L.) Sm., but there have been few reports in which it was isolated from the rhizome of Z. cassumunar.

Kanjnapothi et al. reported that (E)-4-(3,4-dimethoxyphenyl)but-3-ene-1-ol was isolated from a hexane-soluble extract of Z. cassumunar and that it exhibited a dose-dependent relaxant effect on the uterus of nonpregnant rats, and also that it may act by mechanisms similar to that of papaverine. But, there have been few reports about the pharmacological effects of (E)-4-(3,4-dimethoxyphenyl)but-1-ene, especially anti-inflammatory and analgesic effects.

It is well known that the development of edema induced by carrageenin and the increase in vascular permeability induced by acetic acid correspond to the early exudative stage of inflammation, one of the important processes of inflammatory pathology. (E)-1-(3,4-dimethoxyphenyl)but-1-ene inhibited the edema induced by carrageenin and the increase of the vascular permeability induced by acetic acid in the present study, which shows that it exerts an anti-inflammatory effect at an early exudative stage of inflammation.

Brown et al. then reported that many centrally acting drugs inhibited the edema induced by carrageenin in the hind-paw of rats.

Although neither the fractions nor the (E)-1-(3,4-dimethoxyphenyl)but-1-ene, at the doses used in this experiment (0.016 g/kg), was found to have any apparent effect on the central nervous system or on toxicity in mice and rats, these reports suggest that the anti-inflammatory effects of the compound may be partly exerted through the central nervous system.

Acknowledgments The author is indebted to Dr. S. Soedigdo, Professor at the Institute Technology of Bandung, for collection of the rhizomes of Z. cassumunar. This work was supported in part by a grant from the Special Coordinating Funds for Promoting Science and Technology (1984–1987) of the Science and Technology Agency of Japan.

References