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Studies on Pharmacologically Active Principles from Indonesian Crude Drugs. II. Hypothermic Principle from *Curcuma xanthorrhiza* ROXB.[1)]

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Germacrone was identified as a hypothermic principle of *Curcuma xanthorrhiza* ROXB. When orally administered to mice, germacrone showed the following activities in addition to the hypothermic effect: prolongation of pentobarbital-induced sleeping time, suppression of spontaneous motor activity, suppression of acetic acid-induced writhing, and inhibition of stress-induced ulcer.

Keywords——*Curcuma xanthorrhiza*; suppressive activity; germacrone; hypothermic activity

Introduction

In the preceding paper,[2)] we reported that oral administration of the methanol extract of *Curcuma xanthorrhiza* ROXB. showed significant activities: a prolonging effect on pentobarbital-induced sleeping time, a hypothermic effect in terms of rectal temperature, an inhibitory effect on stress ulcer formation and an analgesic effect on acetic acid-induced writhing. Isolation of (*R*)-(−)-xanthorrhizol as a principle prolonging pentobarbital-induced sleeping time was also reported. However, (*R*)-(−)-xanthorrhizol showed no effect on mice except for the prolongation of sleeping time. As reported previously, the most potent hypothermic activity was observed in the nonpolar fraction on column chromatography of the methanol extract of *C. xanthorrhiza*. In this paper, we report on the isolation of a hypothermic principle, and the suppressive activities of the principle.

Results and Discussion

Oral administration of methanol extract (1.0 g/kg body weight) of *C. xanthorrhiza* lowered the body temperature (−3.2 °C) in mice. The methanol extract was fractionated by silica gel column chromatography, and the hypothermic activity of each fraction was tested, as shown in Chart 1. The most potent hypothermic activity was observed in fr. 1. A pure active compound, tentatively named CX-2, was obtained by two further column chromatographies of the fraction.

CX-2, colorless needles, mp 53—55 °C, high mass spectrum (MS) *m/z*: 218.1687, Calcd 218.1669 for $C_{15}H_{22}O$, seemed to be a sesquiterpene. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra and the other physicochemical properties of CX-2 indicated the identity of this compound with germacrone[3,4)] (Chart 1).

On oral administration to mice at 100—200 mg/kg, germacrone showed a significant hypothermic effect, and its activity at 200 mg/kg corresponded to that of chlorpromazine at

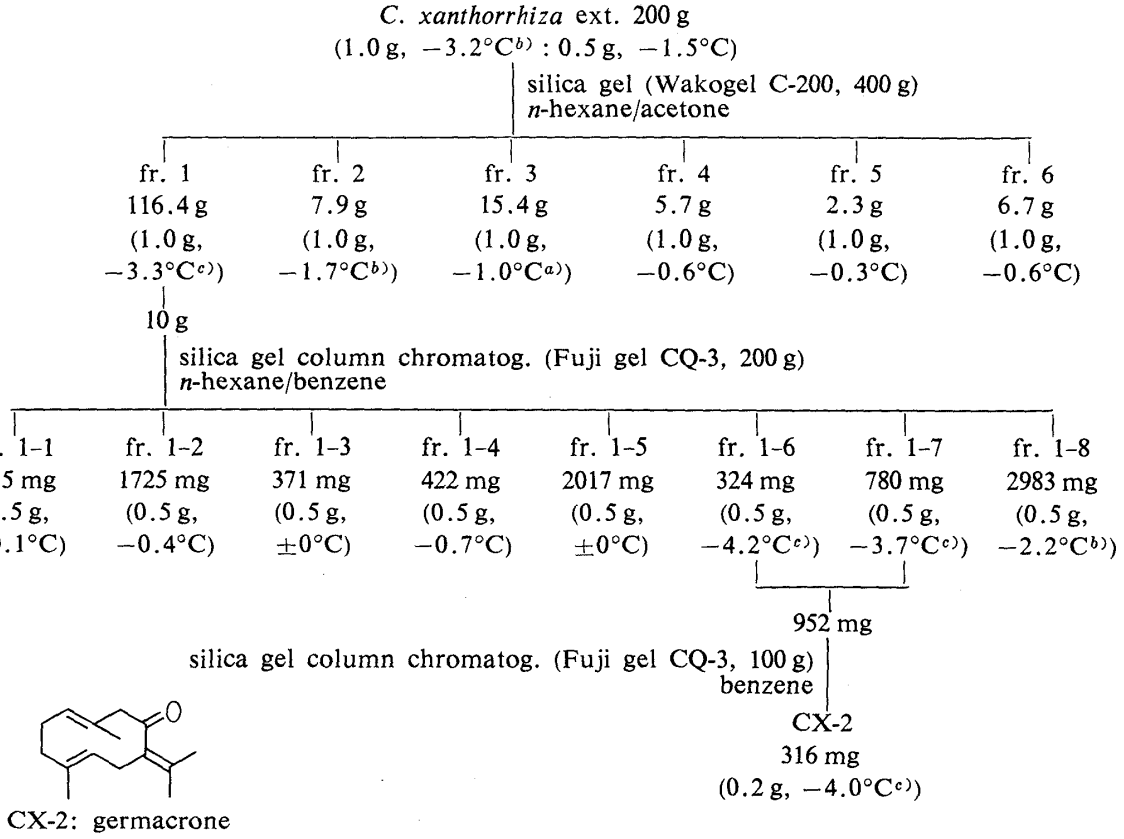

Chart 1. Fractionation of the Methanol Extract of *C. xanthorrhiza* Rhizome, and the Hypothermic Activity

Each fraction was orally administered to a group of 6—8 mice. ( ) indicates dose per kg body weight and activity. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ compared with the control.

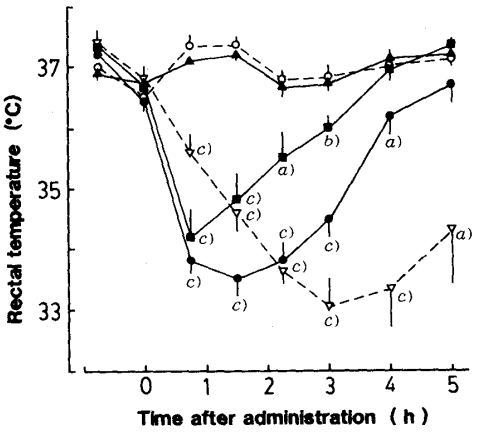

Fig. 1. Effect of Germacrone on Rectal Temperature in Mice after Oral Administration

○---○, control; ▽---▽, chlorpromazine (10 mg/kg); ●—●, germacrone (200 mg/kg); ■—■, germacrone (100 mg/kg); ▲—▲, germacrone (50 mg/kg). Each value represents the mean S.E. of 8 mice. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ compared with the control.

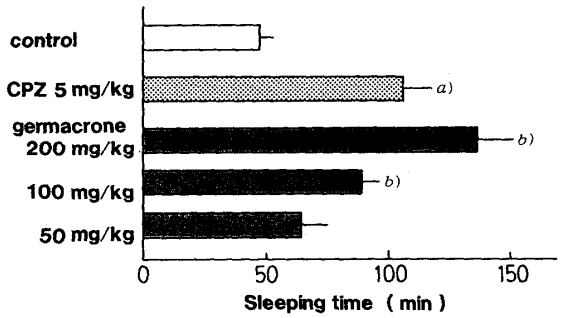

Fig. 2. Effect of Germacrone on Pentobarbital-Induced Sleeping Time in Mice by after Administration

Each value represents the mean S.E. of 8 mice. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ compared with the control.

10 mg/kg (Fig. 1). Germacrone also showed several significant effects on mice: prolongation of pentobarbital-induced sleeping time (Fig. 2), a suppressive effect on spontaneous motor activity as determined with a wheel cage (Fig. 3), a suppressive effect on acetic acid-induced

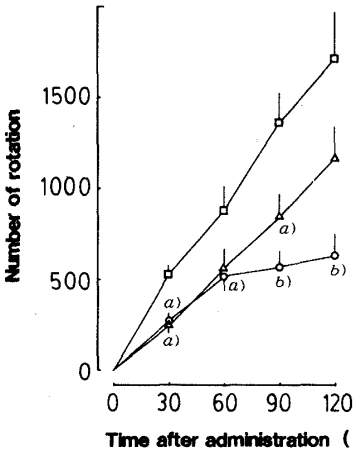

Fig. 3. Effect of Germacrone on Motor Activity (Wheel Cage) of Mice after Oral Administration

□—□, control; ○—○, 200 mg/kg; △—△, 100 mg/kg. Each value represents the mean S.E. of 7 mice. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ compared with the control.

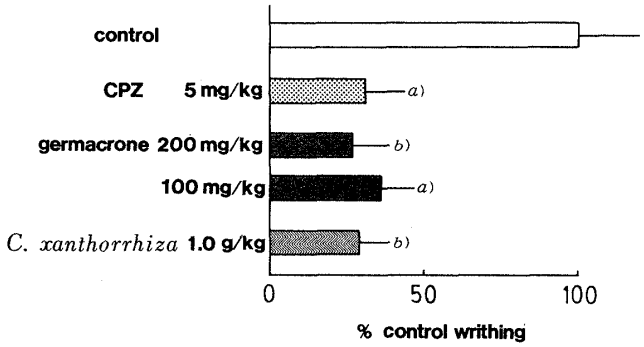

Fig. 4. Effect of Germacrone on Acetic Acid-Induced Writhing in Mice after Oral Administration

Each value represents the mean S.E. of 8 mice. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ compared with the control.

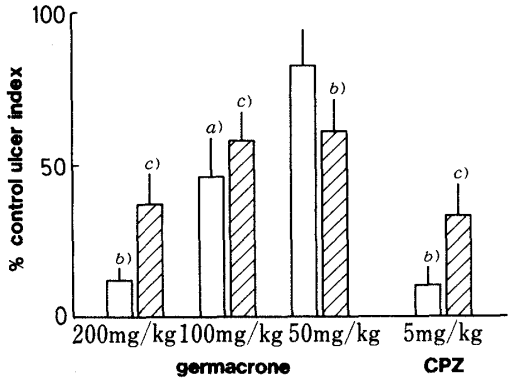

Fig. 5. Effect of Germacrone on Stress-Induced Ulcer in Mice after Oral Administration

Each value represents the mean S.E. of 8 mice. CPZ, chlorpromazine. □, *p.o.*; ▨, s.c. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ compared with the control.

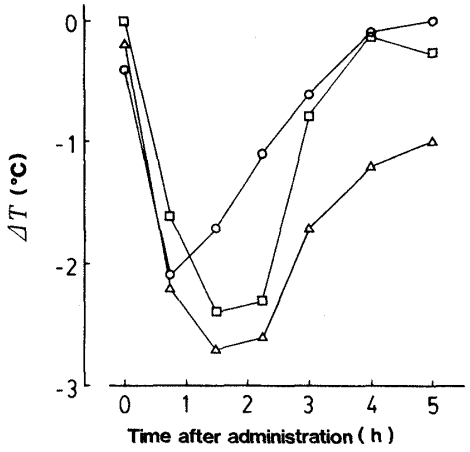

Fig. 6. Comparative Effect of *p.o.*, i.p. and i.v. Administrations of Germacrone on Rectal Temperature in Mice

○—○, *p.o.*; △—△, i.p.; □—□, i.v. Each value represents the mean of 8 mice. Ordinate: temperature difference from the control.

writhing (Fig. 4), and an inhibitory effect on stress-induced ulcer (Fig. 5). Moreover, on oral administration to mice at 200 mg/kg, germacrone depressed the hyperactivity induced by methamphetamine (3 mg/kg i.p.). Germacrone showed no lethal toxicity on oral administration to mice at 750 mg/kg. These results indicate that germacrone has a depressive effect on the central nervous system (CNS).

The content of germacrone in the methanol extract of *C. xanthorrhiza* was as high as 5.3% (determined by gas-liquid chromatography (GC)), so that the suppressive activity in methanol extract of *C. xanthorrhiza* might be mainly due to that of germacrone. Germacrone gave almost the same hypothermic effect when given by oral, intravenous and intraperitoneal routes at 100 mg/kg (Fig. 6).

On the other hand, germacrone 4,5-epoxide has been found widely in *Curcuma* species and is believed to be a key intermediate in the biogenetic pathway of many sesquiterpenes,[4,5] although this compound was not detected in gas-liquid chromatographic analysis of the

methanol extract of *C. xanthorrhiza* in our experiment. Watanabe *et al.*[6] reported an inhibitory effect of germacrone 4,5-epoxide on stress-induced ulceration. In a comparison of the suppressive activities of germacrone and germacrone 4,5-epoxide, the former showed slightly lower activities than the latter in hypothermia and prolongation of pentobarbital-induced sleeping time, but greater inhibitory activity against stress-induced ulceration.

Experimental

Materials——The methanol extract of *C. xanthorrhiza* was supplied by P. T. Eisai Indonesia, and the extraction was done as described in the preceding paper.[2]

Pharmacological Assays——Fractionated samples and germacrone were suspended in saline with 5% olive oil–2% Tween 80–5% gum arabic. Chlorpromazine was used as a reference drug. Groups of 5 to 8 male ddY-strain mice (5 to 6 weeks old, 24—30 g) were used. Student's *t*-test was employed for the statistical evaluation of experimental data.

Effect on Body Temperature: Rectal temperature of mice was measured with a thermister from 1 h before to 5 h after oral administration of the samples.

Effect on Pentobarbital-Induced Sleeping Time: Samples were administered orally to mice 30 min before intraperitoneal injection of sodium pentobarbital (50 mg/kg). The time required to regain the righting reflex was measured.

Effect on Motor Activity: Spontaneous motor activity of mice was measured by using a wheel cage. Mice rotating the wheel cage at a constant rate per 10 min were selected. After oral administration of samples, the number of rotations was counted at 30 min intervals for 120 min.

Effect on Acetic Acid-Induced Writhing: Samples were administered orally to mice 60 min before intraperitoneal injection of 0.7% acetic acid (0.1 ml/10 g body weight). The number of writhing movements was counted for 10 min, starting at 10 min after the injection of acetic acid.

Effect on Stress-Induced Ulcer: Mice were fasted for about 6 h, and samples were administered orally or subcutaneously 30 min before the stress. These mice were immobilized in a restraint cage and immersed to the level of the xiphoid process in a water bath at 25 °C for 18 h according to the method described by Yano and Harada.[7] The stomachs removed from sacrificed mice were fixed with 3% formalin solution, and the ulcer index was evaluated as the sum of the lesion length in the glandular portion.

Chemical Analysis——All melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded with a Hitachi EPI-G3 grating infrared spectrophotometer, and high-resolution MS with a Hitachi RMU-7M. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL GX-270 and the chemical shifts are given on the ppm scale with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). GC was carried out on a Shimadzu GC-9A apparatus equipped with a glass column (3 mm × 1.5 m, 2% OV-7) and a flame ionization detector.

Isolation of CX-2 (Germacrone): The isolation was carried out according to the preceding paper.[2] Evaporation of fr. 1, eluted with *n*-hexane, left a yellowish oil (116.4 g). This fraction (10 g) afforded CX-2 (316 mg) upon repeated column chromatography on Fujigel CQ-3 (200 and 100 g) (Chart 1).

CX-2 (Germacrone): Colorless needles, mp 53—55 °C. High MS *m/z*: M^+ Calcd for $C_{15}H_{22}O$ 218.1669. Found: 218.1687. IR ν_{max}^{KBr} cm^{-1}: 1665, 1440, 1288, 1130, 855, 535. ^{1}H-NMR (in $CDCl_3$) δ: 1.44 (3H, s), 1.63 (3H, s), 1.73 (3H, s), 1.78 (3H, s), 2.11 (3H, m), 2.36 (1H, m), 2.93 (3H, m), 3.41 (1H, d, J = 10.6 Hz), 4.72 (1H, d, J = 10.6 Hz), 4.99 (1H, d, J = 11.7 Hz). ^{13}C-NMR (in $CDCl_3$) δ: 15.60 (q), 16.74 (q), 19.90 (q), 22.33 (q), 24.13 (t), 29.26 (t), 38.15 (t), 55.92 (t), 125.46 (d), 126.74 (s), 129.40 (s), 132.69 (d), 134.98 (s), 137.35 (s), 207.78 (s).

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References and Notes

1) A part of this work was presented at the 34th Annual Meeting of The Japanese Society of Pharmacognosy, Osaka, October 1987.
2) M. Yamazaki, Y. Maebayashi, N. Iwase, and T. Kaneko, *Chem. Pharm. Bull.*, **36**, 2070 (1988).
3) M. Yoshihara, H. Shibuya, E. Kitano, K. Yanagi, and I. Kitagawa, *Chem. Pharm. Bull.*, **32**, 2059 (1984).
4) M. Kuroyanagi, A. Ueno, K. Ujiie, and S. Sato, *Chem. Pharm. Bull.*, **35**, 53 (1987).
5) Y. Shiobara, Y. Asakawa, M. Kodama, K. Yasuda, and T. Takemoto, *Phytochemistry*, **24**, 2629 (1985).
6) K. Watanabe, M. Shibata, S. Yano, Y. Cai, H. Shibuya, and I. Kitagawa, *Yakugaku Zasshi*, **106**, 1137 (1986).
7) S. Yano and M. Harada, *Jpn. J. Pharmacol.*, **23**, 57 (1973).