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Studies on Pharmacologically Active Principles from Indonesian Crude Drugs. I. Principle Prolonging Pentobarbital-Induced Sleeping Time from *Curcuma xanthorrhiza* ROXB.[1)]

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Among methanol extracts of 16 species of Indonesian crude drugs, that of *Curcuma xanthorrhiza* showed a prolonging effect on pentobarbital-induced sleeping time, a hypothermic effect in terms of rectal temperature and a suppressive effect on acetic acid-induced writhing when given by oral administration to mice. (*R*)-(−)-Xanthorrhizol was identified as a principle prolonging pentobarbital-induced sleeping time. The effect of the compound on sleeping time was demonstrated to be due to inhibition of cytochrome P-450 activity.

Keywords——*Curcuma xanthorrhiza*; (*R*)-(−)-xanthorrhizol; pentobarbital-induced sleep; cytochrome P-450 inhibitor

Introduction

Screening of various crude drugs showed Sophorae Radix (kujin), Sophorae subprostratae Radix (sanzukon), Tetragonia Herba (bankyoo) and Glechomae Herba (rensenso) to have significant anti-ulcer activities,[2,3)] and some active principles were identified.[4–6)] Recently, the methanol extracts of 16 species of Indonesian crude drugs were subjected to screening for effects on general behavior, rectal temperature and stress ulcer in mice (Table I). Among them, *Curcuma xanthorrhiza* ROXB. (Zingiberaceae) rhizome and *Acorus calamus* L. (Araceae) rhizome showed significant suppressive activities. In this paper, we report the isolation of a principle prolonging pentobarbital-induced sleeping time from *C. xanthorrhiza*, and the results of a pharmacological study on the principle. *C. xanthorrhiza* ROXB. is called "temu lawak" in Indonesia (Japanese name: kusuri ukon), and its rhizome is utilized as a popular remedy for disorders of the gall bladder, liver and digestive organs in Indonesia.[7)]

Results and Discussion

Oral administration of the methanol extract of rhizomes of *C. xanthorrhiza* to mice had a significant inhibitory effect on stress ulcer formation (Table I), a hypothermic effect in terms of rectal temperature (Table I, Fig. 1), a prolonging effect on pentobarbital-induced sleeping time (Fig. 2) and a suppressive effect on acetic acid-induced writhing.

In order to isolate the active principles, the methanol extract was fractionated by the procedure shown in Chart 1, and each fraction was tested for effect on pentobarbital-induced sleeping time and on rectal temperature in mice. As shown in Chart 1, the most potent prolonging activity on sleeping time was observed in fr. 3. Thus, fr. 3 was re-chromatographed twice on silica gel to give a pure active compound, tentatively named CX-1. On the other hand, the most potent hypothermic activity was observed in fr. 1. The chemistry and

TABLE I. Effects of the Methanol Extracts of Indonesian Crude Drugs on Stress-Induced Ulcer, General Behavior and Rectal Temperature in Mice

Crude drug	Part used	Stress ulcer[a] (% control)	General behavior[e] (depression)	Rectal temp.[f] (ΔT°C)
Blumea balsamifera D.C.	Leaf	76	↓	−0.3
Leucaena glauca AUCT.	Leaf	73	↓	0.3
Moringa oleifera LAMK.	Leaf	82		0.2
Litsea odorifera VSL.	Leaf	36[b]	↓	−0.1
Pluchea indica LESS.	Leaf	80		−0.3
Ocimum sanctum L.	Leaf	69		1.0
Centella asiatica URB.	Leaf	123		0.4
Centella asiatica URB.	Herb	71		0.7
Acorus calamus L.	Rhizome	43[b,c]	↓↓↓	−3.6
Curcuma xanthorrhiza ROXB.	Rhizome	37[b]	↓↓↓	−2.8
Porthulaca oleraceae L.	Herb	104		1.3
Artocarpus integra MERR.	Leaf	54		0.7
Vitex trifolia L.	Leaf	117		0.5
Strychnos ligustrina BL.	Lignum	103[d]	—[g]	—[g]
Moschosma polystachyum BENTH.	Herb	33		0
Lepidium sativum L.	Seed	76	↓	0.2

Methanol extracts of crude drugs (2.0 g/kg body weight) were orally administered to mice. *a*) Mean of 8 mice. *b*) $p<0.01$ compared with control. *c*) 0.5 g/kg. *d*) 0.1 g/kg. *e*) An arrow indicates behavioral depression (3 mice were observed for 6 h). *f*) Rectal temperature of 3 mice was measured for 5 h. *g*) All mice were dead within 30 min after administration of the sample.

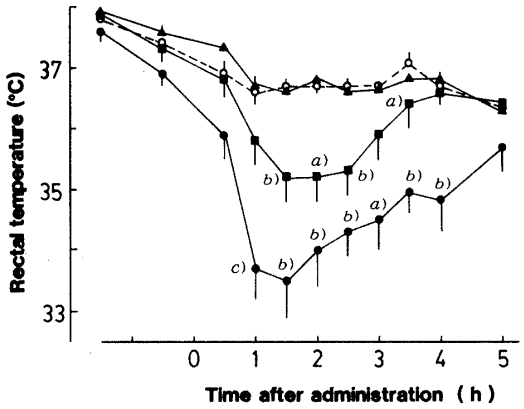

Fig. 1. Effect of the Methanol Extract of *C. xanthorrhiza* Rhizome on Rectal Temperature in Mice after Oral Administration

○—○, control; ●—●, 1.0 g/kg; ■—■, 0.5 g/kg; ▲—▲, 0.2 g/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.

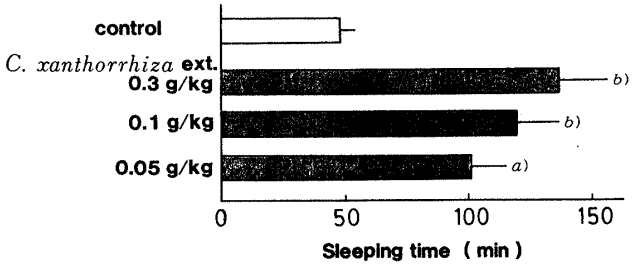

Fig. 2. Effect of the Methanol Extract of *C. xanthorrhiza* Rhizome on Pentobarbital-Induced Sleeping Time in Mice after Oral Administration

Each value represents the mean S.E. of 7 mice. *a*) $p<0.05$; *b*) $p<0.01$ compared with the control.

pharmacology of the hypothermic substance will be reported separately.

CX-1, a colorless oil, $[\alpha]_D^{25}$ −58° ($c=1.0$, $CHCl_3$), high mass spectrum (MS) *m/z*: 218.1662, Calcd 218.1668 for $C_{15}H_{22}O$, was suggested to be a sesquiterpene having a 1,3,4-substituted benzene ring system on the basis of the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. The physicochemical properties of CX-1 indicated the identity of this compound with (*R*)-(−)-xanthorrhizol (Chart 1), which has previously been isolated from *C. xanthorrhiza* ROXB.[8,9] Recently, Itokawa *et al.* isolated xanthorrhizol as an antitumor constituent from *C. xanthorrhiza*.[10] So far, there has been no report on the pharmacological

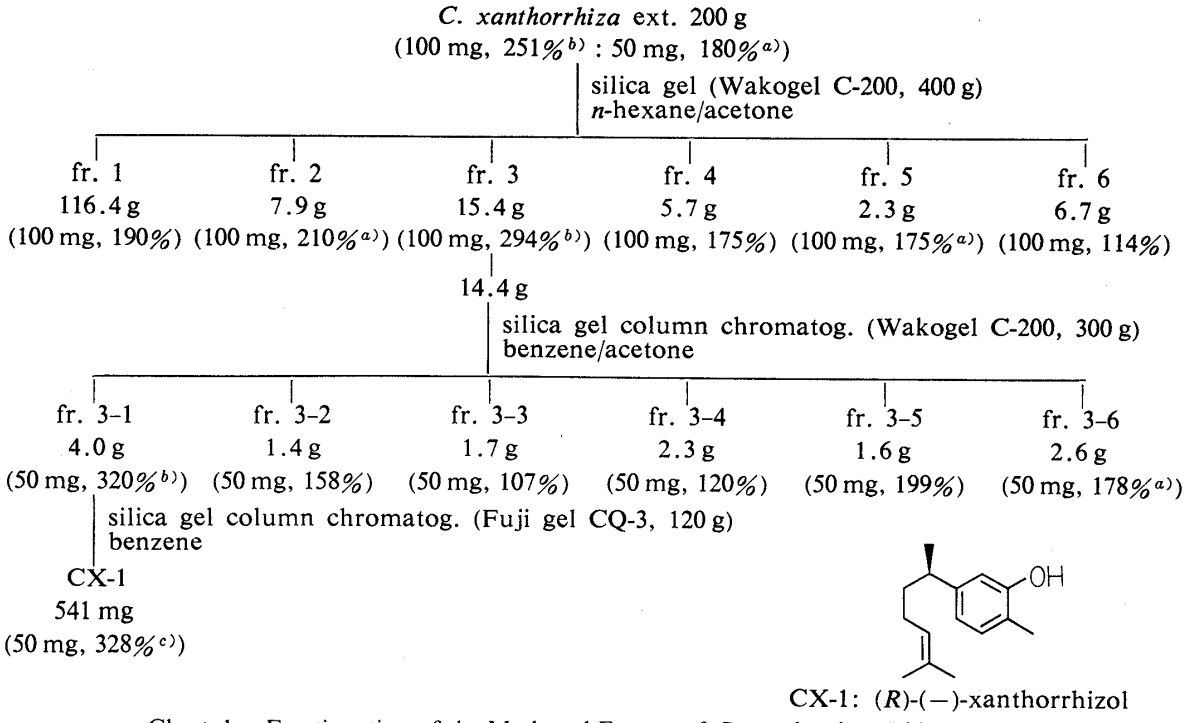

Chart 1. Fractionation of the Methanol Extract of *C. xanthorrhiza* Rhizome, and the Prolonging Activity on Pentobarbital-Induced Sleeping Time

Every 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.

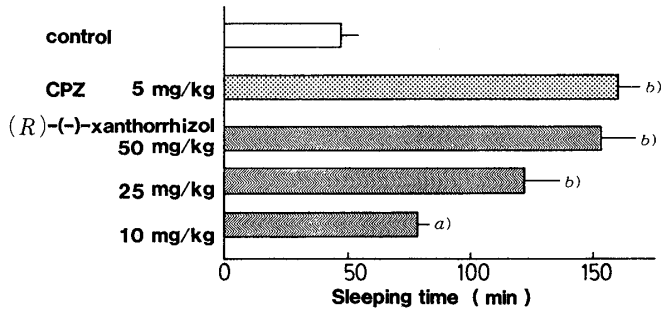

Fig. 3. Effect of (*R*)-(−)-Xanthorrhizol on Pentobarbital-Induced Sleeping Time in Mice after Oral Administration

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

activities of xanthorrhizol.

On oral administration to mice, xanthorrhizol at 50 mg/kg prolonged the pentobarbital-induced sleeping time (328%), and its activity was almost the same as that of chlorpromazine at 5 mg/kg (Fig. 3). However, xanthorrhizol showed no significant effect on general behavior, did not inhibit stress ulcer formation (250 mg/kg, *p.o.*), and showed no hypothermic action (500 mg/kg, *p.o.*), analgesic action (500 mg/kg, *p.o.*), or anti-convulsive action (500 mg/kg, *p.o.*) (the data are not shown in this paper). These results suggested that the prolonging effect of this compound on sleeping time might be due to liver damage or metabolic inhibition. Oral administration of xanthorrhizol (50 mg/kg) did not alter the plasma glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) levels within 6 h, indicating that acute liver damage was not induced by this compound at the dose which prolonged pentobarbital-induced sleeping time. On the other hand, *in vitro*, xanthorrhizol showed typical type I difference spectra in mouse liver microsomes (Fig. 4), and potently inhibited both hepatic benzphetamine *N*-demethylase and 7-ethoxycoumarin *O*-deethylase activities (Fig. 5). Its IC_{50} in the case of benzphetamine *N*-demethylase activity was 0.12 mM, and the inhibitory activity was about ten times stronger than that of SKF-525A (IC_{50} was nearly 1 mM) used as a reference drug. These results indicated that xanthorrhizol interacts with

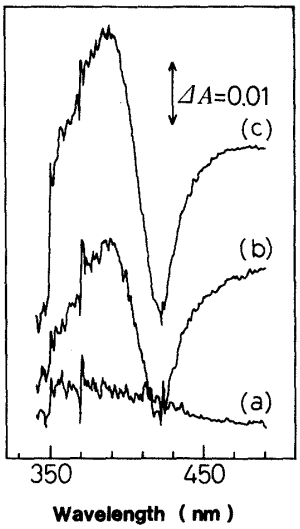

Fig. 4. Spectral Change on Binding of (*R*)-(−)-Xanthorrhizol to Mouse Liver Microsomes

Microsomes were suspended in 100 mM Na–K phosphate buffer (pH 7.4) at a concentration of 3 mg protein/ml. The concentration of (*R*)-(−)-xanthorrhizol was (a) 0 mM, (b) 1 mM or (c) 2 mM.

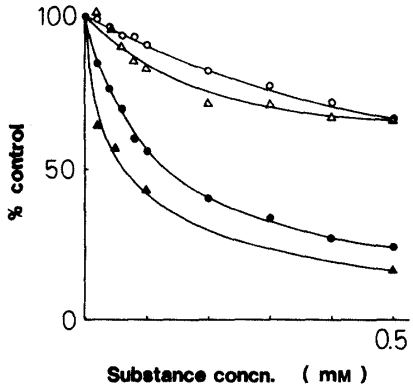

Fig. 5. Effect of (*R*)-(−)-Xanthorrhizol on the *in Vitro* Metabolism of Benzphetamine and 7-Ethoxycoumarin by Mouse Liver Microsomes

Benzphetamine *N*-demethylase activity: ○—○, SKF-525A; ●—●, (*R*)-(−)-xanthorrhizol. 7-Ethoxycoumarin *O*-deethylase activity: △—△, SKF-525A; ▲—▲, (*R*)-(−)-xanthorrhizol. The 100% control corresponded to 7.0 nmol HCHO/mg/min or 5.0 nmol 7-OH coumarin/mg/min. Each value represents the mean of two or four determinations.

cytochrome P-450 to inhibit the metabolism of pentobarbital.

Thus, it has become evident that xanthorrhizol makes no direct contribution to the suppressive actions of *C. xanthorrhiza*. The content of xanthorrhizol in the methanol extract was so high (11.7% as determined by gas-liquid chromatography (GLC)) that its inhibitory effect on hepatic drug metabolism may contribute to the action of *C. xanthorrhiza* as a crude drug.

Experimental

Materials——Sixteen species of Indonesian crude drugs and the methanol extract of *C. xanthorrhiza* ROXB. were supplied by P.T. Eisai Indonesia. The crude drugs (70—500 g) were extracted with methanol (1—9 l) three times at room temperature, and the extract was evaporated under reduced pressure at 40 °C.

Pharmacological and Biochemical Assays——The methanol extracts of Indonesian crude drugs were suspended in saline with 5% olive oil–2% Tween 80–5% gum arabic, and xanthorrhizol was suspended with 4% Tween 80–5% gum arabic. Chlorpromazine was used as a control drug. Groups of 5 to 10 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. Ultraviolet (UV) spectra were measured on a Hitachi 557 double-wavelength double-beam Spectrophoto meter.

Effect on Stress-Induced Ulcer: Mice were fastened for about 6 h, and test 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.[11] 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.

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 Hepatic Drug-Metabolizing Enzymes: All mice were fasted for about 18 h prior to sacrifice and hepatic microsomes were prepared by the method of Kamataki and Kitagawa.[12] The *N*-demethylase activity towards benzphetamine was estimated by determining formaldehyde production using the method of Nash.[13] The *O*-

deethylase activity towards 7-ethoxycoumarin was estimated by determining 7-hydroxycoumarin production using the method of Aitio.[14]

Chemical Analysis——Spectral data were obtained on the following instruments; high-resolution MS on a Hitachi RMU-7M and optical rotation on a JASCO DIP-181 digital polarimeter. ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) spectra were measured on a JEOL GX-270 spectrometer the chemical shifts are given on the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). GLC 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-1 ((*R*)-(−)-Xanthorrhizol): The methanol extract (200 g) of *C. xanthorrhiza* was dissolved in acetone and mixed with silica gel (Wakogel C-200, 400 g). After evaporation of the acetone, the silica gel with the methanol extract was layered over Wakogel C-200 (200 g) packed in a column. Evaporation of fr. 3 eluted with *n*-hexane–acetone (2 : 1) left a brown oil (15.4 g). This fraction (14 g) afforded CX-1 (541 mg) after repeated column chromatography on Wakogel C-200 (300 g) and Fuji gel CQ-3 (120 g) (Chart 1).

CX-1 ((*R*)-(−)-Xanthorrhizol): Colorless oil, $[\alpha]_D^{25}$ −58° (c=1.0, $CHCl_3$). High MS m/z: M^+ Calcd for $C_{15}H_{22}O$ 218.1668. Found: 218.1662. ^{1}H-NMR (in $CDCl_3$) δ: 1.18 (3H, d, J=6.9 Hz), 1.52 (3H, s), 1.55 (2H, m), 1.67 (3H, s), 1.88 (2H, m), 2.20 (3H, s), 2.60 (1H, m), 4.88 (1H, s), 5.08 (1H, t, J=6.9, 7.3 Hz), 6.59 (1H, br s), 6.67 (1H, br d, J=7.9 Hz), 7.01 (1H, d, J=7.6 Hz). ^{13}C-NMR (in $CDCl_3$) δ: 15.33 (q), 17.67 (q), 22.36 (q), 25.70 (q), 26.19 (t), 38.41 (t), 39.07 (d), 113.60 (d), 119.49 (d), 120.93 (d), 124.57 (d), 130.80 (d), 131.40 (s), 147.24 (s), 153.62 (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 and H. Shirota, *Shoyakugaku Zasshi*, **35**, 96 (1981).
3) M. Yamazaki, H. Shirota, and S. Suzuki, *Proc. Symp. WAKAN-YAKU*, **14**, 7 (1981).
4) E. Okuyama and M. Yamazaki, *Yakugaku Zasshi*, **103**, 43 (1983).
5) E. Okuyama and M. Yamazaki, *Chem. Pharm. Bull.*, **31**, 2209 (1983).
6) E. Okuyama, M. Yamazaki, and Y. Ishii, *Shoyakugaku Zasshi*, **37**, 52 (1983).
7) Y. S. Kasahara (ed.), "Medical Herb Index in Indonesia," P.T. Eisai Indonesia, Djakarta, 1986, p. 343.
8) H. Rimpler, R. Hänsel, and L. Kochendoerfer, *Z. Naturforsch.*, **25b**, 995 (1970).
9) T. K. John and G. S. Krishna Rao, *Indian J. Chem.*, **24B**, 35 (1985).
10) H. Itokawa, F. Hirayama, K. Funakoshi, and K. Takeya, *Chem. Pharm. Bull.*, **33**, 3488 (1985).
11) S. Yano and M. Harada, *Jpn. J. Pharmacol.*, **23**, 57 (1973).
12) T. Kamataki and H. Kitagawa, *Jpn. J. Pharmacol.*, **24**, 195 (1974).
13) T. Nash, *Biochem. J.*, **55**, 416 (1953).
14) A. Aitio, *Anal. Biochem.*, **85**, 448 (1987).