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Cholagogic Action of the Essential Oil Obtained from *Curcuma xanthorrhiza* ROXB.

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The cholagogic effects of the essential oils obtained by steam distillation from the rhizome of *Curcuma longa* L. (*C. longa*) and *Curcuma xanthorrhiza* ROXB. (*C. xanthorrhiza*), which have traditionally been used as cholagoues in Indonesia, were studied using anesthetized rats. Oral administration of the essential oils caused a persistent increase of bile secretion, the essential oil of *C. xanthorrhiza* being slightly more active than that of *C. longa*. The active principle of the essential oil of *C. xanthorrhiza* isolated by silica gel column chromatography was shown to contain *d*-camphor by GC-Mass and TLC. The essential oil and *d*-camphor both caused a long lasting increase of bile secretion involving increases both in the amount of total bile acids and in the solid matter weight in the excreted bile.

These results suggest that the cholagogic effect of the essential oil is attributable to *d*-camphor contained in it and that the increase in bile secretion induced either by the essential oil or *d*-camphor is partly due to the increase in the total bile acids in the excretive bile.

Keywords——cholagogic effect; essential oil; curcumin; camphor; *Curcuma longa* L.; *Curcuma xanthorrhiza* ROXB.; bile salt dependent bile secretion

The rhizome of *Curcuma longa* L. (*C. longa*), called "Ukon" in Japan, is frequently used in Chinese medicine as aromatic stomachics and cholagogues, and many pharmacological studies on the rhizome have been reported.[1-7] Although the rhizome of *Curcuma xanthorrhiza* ROXB. (*C. xanthorrhiza*), called "Kusuri Ukon" in Japan, has traditionally been used as a cholagogues in Indonesia,[2,3] very few pharmacological studies have been done on it there. It has been reported that curcumin, isolated from the rhizome of *C. longa*, and the essential oil, obtained by steam distillation, showed a cholagogic effect and that both principles seemed to play a major role in this effect.[4,5]

It has also been reported that *p*-tolylmethylcarbinol, isolated from the essential oil from the rhizome of *C. longa*, was an active principle responsible for the cholagogic activity.[6-8] The present study was carried out to elucidate the effects of the essential oil obtained from the rhizome of *C. xanthorrhiza* and its active principles on cholagogic action.[9]

Experimental

Materials

The essential oil, obtained by steam distillation from the rhizome of *C. xanthorrhiza*, was chromatographed on a silica gel column, using *n*-hexane, benzene, ethylacetate and methanol as the elution solvent to give five fractions (Fr. I–Fr. V). The parent essential oil, the fractions I–V, curcumin, sodium dehydrocholate (Wako Pure Chemical) and *d*-camphor (Wako Pure Chemical) were each suspended in 2% C.M.C. solution just before the administration.

Methods

Pharmacological method: Male Wistar rats weighing 300–350 g were fasted for 14 h. Under anesthesia with urethane (1.5 g/kg, i.p.), the rats were laparotomized and a polyethylene cannula was inserted into the common bile duct in order to measure the rate of bile outflow, its total bile acid content and solid matter weight. Thirty min after the surgical operation when a steady state was obtained, the bile was collected for 1 h. Then each test drug was administered orally and the hourly bile secrete was collected for 5 h. Each of the collected bile samples was divided into two parts, one for the determination of the concentration of total bile acids by an enzymatic method (Sterognost-3α, Daiichi Pure Chemicals)[10-13] and the other for the measurement of the solid constituent weight by freeze drying.

The control group received an equivalent volume of 2% C.M.C. solution and, during the experimental period, the rats were kept under anesthesia. Sodium dehydrocholate was used as a standard drug.

Figures 1, 3 and 4 show the volumes of hourly biles secreted (ml/rat) (left column, before drug administration). In Fig. 5, the left diagram shows the concentration of total bile acids (mM/*l*) and the right diagram shows the excretive amount of total bile acids (mM/rat) in the hourly biles (left column, before drug administration). In Fig. 6, the left diagram shows the concentration of solid matter (mg/0.1 ml) and the right diagram shows the amount of solid dry matter (mg/rat) secreted in the hourly biles (left column, before drug administration).

Statistical analysis——Data were expressed as the mean value with standard error (%) obtained from 4–12 rats (*n*) in comparison with the values before the administration of drugs.

All results were analyzed by Student's *t*-test.

Determination of active principles——Only Fr. IV showed a stronger cholagogic effect than the essential oil itself. Therefore, Fr. IV was rechromatographed over silica gel in order to separate an active principle(s). The active principle(s) in Fr. IV were subjected to Gas Chromatography-Mass Spectrography (GC-Mass) (M-80 A, Hitachi) and thin layer chromatography using a silica gel plate (Kieselgel 60 F_{245}, Merck) (TLC) with an appropriate reference standard. The amounts of the active compound in Fr. IV and in the essential oil were measured by Gas Chromatography (GC) (063, Hitachi) using the following conditions; glass column (2% OV-101), column temperature 90°C, injection-detection temperature 180°C, carrier gas N_2 20 ml/min.

Results

1. Effects of the essential oil from *C. xanthorrhiza* and *C. longa*, and curcumin on bile secretion

As shown in Fig. 1, administration of the essential oil from *C. xanthorrhiza* and *C. longa* (300 mg/kg) caused

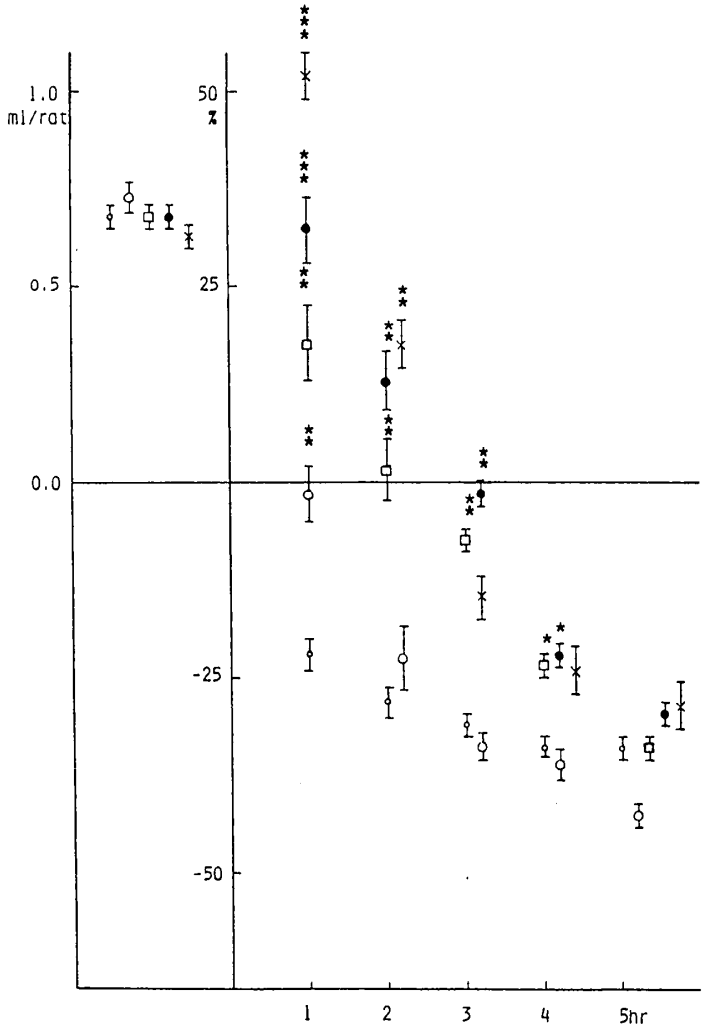

Fig. 1. Effect of the Essential Oils Obtained from *C. domestica* (*C. longa*) and *C. xanthorrhiza*, Curcumin and Sodium Dehydrocholate on Bile Secretion in Rats

∘ : Control (2% C.M.C.) (p.o.) (n=7), ○ : Curcumin 300mg/kg (n=4), □ : *C. domestica(longa)* 300mg/kg (n=7), ● : *C. xanthorrhiza* 300mg/kg (n=8), × : Dehydrocholate-N_a 100mg/kg (n=7), * : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$.

a persisting increase in bile secretion. On the other hand, curcumin (300 mg/kg) induced only a transient increase in bile secretion. The durations of the cholagogic effect induced by the two essential oils were approximately the same as that of sodium dehydrocholate (100 mg/kg). The cholagogic effect induced by the essential oil from *C. xanthorrhiza* was slightly greater than that from *C. longa*.

2. Fractionation of the essential oil from *C. xanthorrhiza*

In order to obtain cholagogically active principles of the essential oil, the oil was fractionated by silica gel column chromatography into five fractions (Fr. I–Fr. V, yields 70.2, 6.6, 7.4, 7.9 and 4.8%, respectively). Fr. I–Fr. IV gave one to three clear spots on TLC, but Fr. V gave no clear spots. The yield of each fraction and their TLC patterns are shown in Fig. 2.

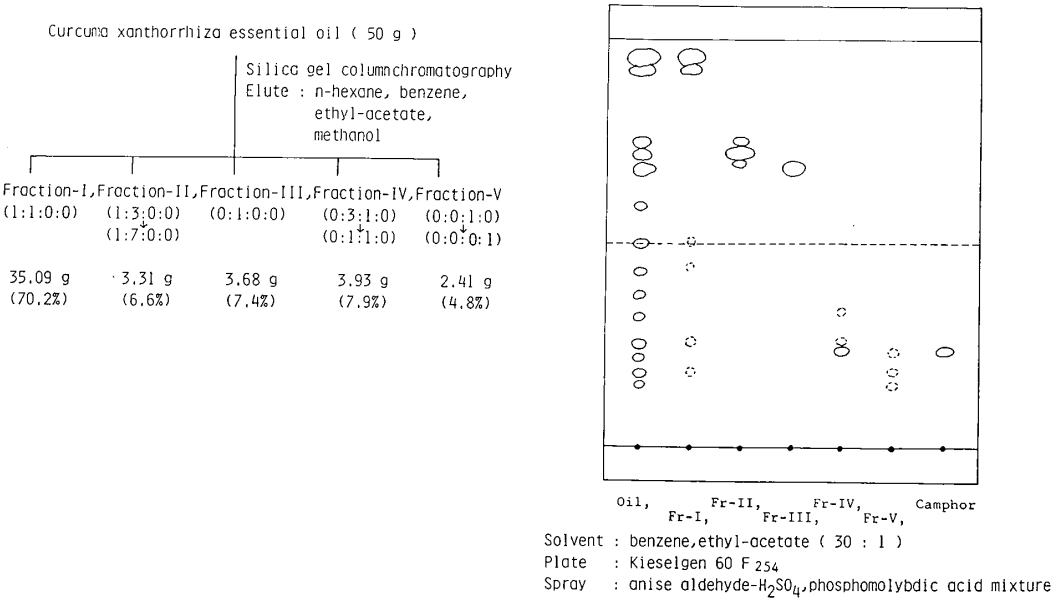

Fig. 2. Flow Diagram of Fractions of the Essential Oil from *C. xanthorrhiza* (left) and Thin Layer Chromatography Patterns of the Fractions and *d*-Camphor (right)

3. Effect of each fraction separated from the essential oil on bile secretion

Administration of Fr. I, Fr. II, Fr. IV and Fr. V (300 mg/kg) induced an increase in the bile secretion, but at the same dose Fr. III did not. Of these fractions, only Fr. IV induced a cholagogic effect which was stronger and more persistent than that of the essential oil at 300 mg/kg. These results are shown in Fig.3.

4. Identification of active compound contained in Fr. IV

Fr. IV, which showed the strongest and most persisting cholagogic effect, was subjected to GC-Mass and TLC with a corresponding reference standard. It was found that Fr. IV contained 45.2% of camphor (3.9% camphor in the essential oil). Camphor isolated from Fr. IV was mixed with pure *d*-camphor, and then the mixture and pure *d*-camphor were separately reacted with 2,4-dinitrophenylhydrazine on a water bath under a reflux condenser to give crystals of the 2,4-dinitrophenylhydrazone of *d*-camphor, which melted between 175° and 179°C. It was concluded that the optical active camphor obtained from Fr. IV is *d*-form.

5. Effects of Fr. IV and *d*-camphor on the concentration of total bile acids and solid matter weight in bile

As shown in Fig. 4, Fr. IV (at doses of 100 and 300 mg/kg) and *d*-camphor (at doses of 30 and 100 mg/kg) gave a persisting cholagogic effect. The effect induced either by Fr. IV and *d*-camphor was more persistent than that induced by 100 mg/kg of sodium dehydrocholate. The cholagogic effects of Fr. IV at doses of 100 and 300 mg/kg were approximately the same as those of *d*-camphor at 30 and 100 mg/kg, respectively.

As shown in Fig. 5, the concentrations of total bile acids induced by Fr. IV (at 300 mg/kg) and *d*-camphor (at 100 mg/kg) in the 1st, 3rd and 5th hourly biles were the same as that of the control, and those in the 2nd and 4th hourly biles were lower than in the control. On the other hand, the amounts of total bile acids in the bile induced by Fr. IV and *d*-camphor increased with the increase in the bile flow. The increases of the excreted amount of total bile acids induced by Fr. IV and *d*-camphor were approximately the same

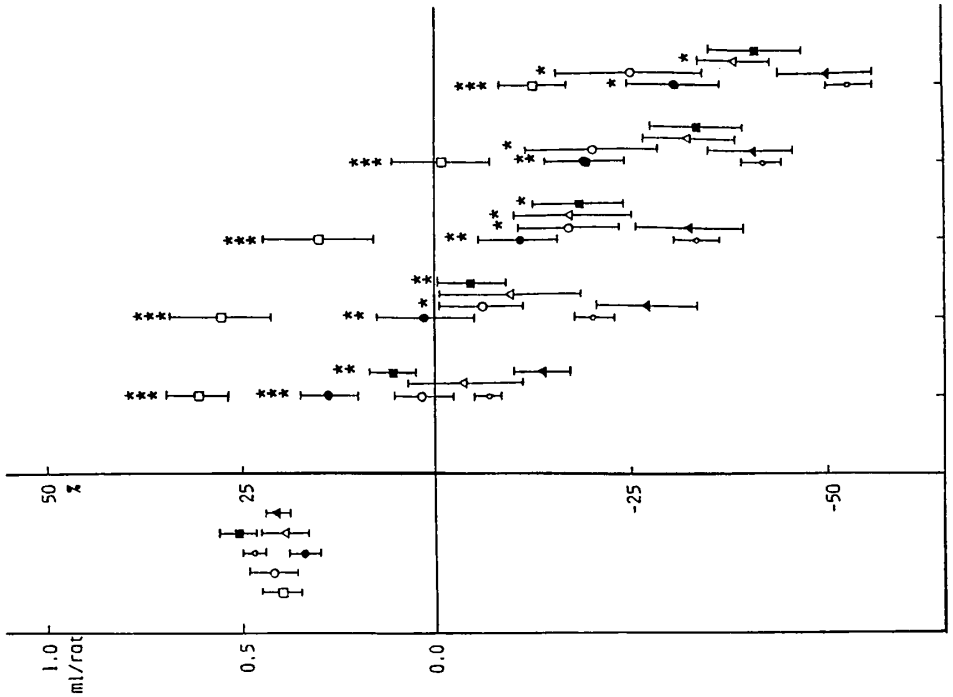

Fig. 3. Effect of the Essential Oil from *C. xanthorrhiza* and the Fractions on Bile Secretion in Rats

∘ : Control (2% C.M.C.) (p.o.) (n=12), ●: Essential oil 300mg/kg (n= 9), ○: F—I 300mg/kg (n= 5), △: F—II 300mg/kg (n= 5), ▲: F—III 300mg/kg (n= 6), □: F-IV 300mg/kg (n= 8), ■: F—V 300mg/kg (n= 5). * : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$.

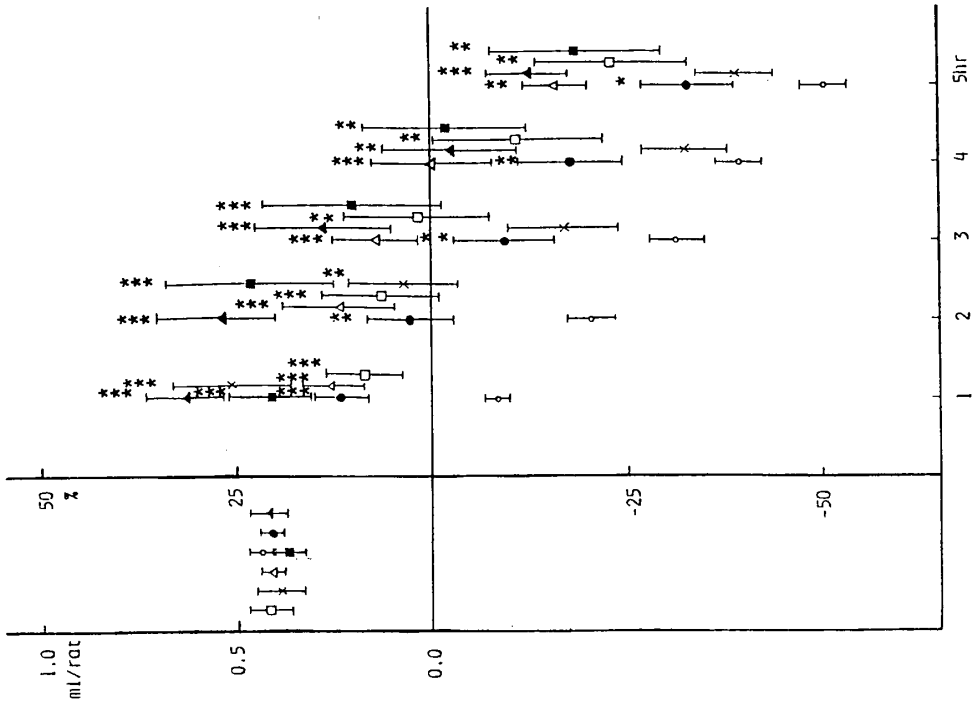

Fig. 4. Effect of the Essential Oil from *C. xanthorrhiza*, Fraction IV, *d*-Camphor and Sodium Dehydrocholate on Bile Secretion in Rats

∘ : Control (2% C.M.C.) (p.o.) (n=12), ●: Essential oil 300mg/kg (n=11), △: F—IV 100mg/kg (n= 6), ▲: F—IV 300mg/kg (n= 7), □: Camphor 300mg/kg (n= 5), ■: Camphor 100mg/kg (n= 7), ×: Dehydrocholate-N_a 100mg/kg (n= 7). * : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$.

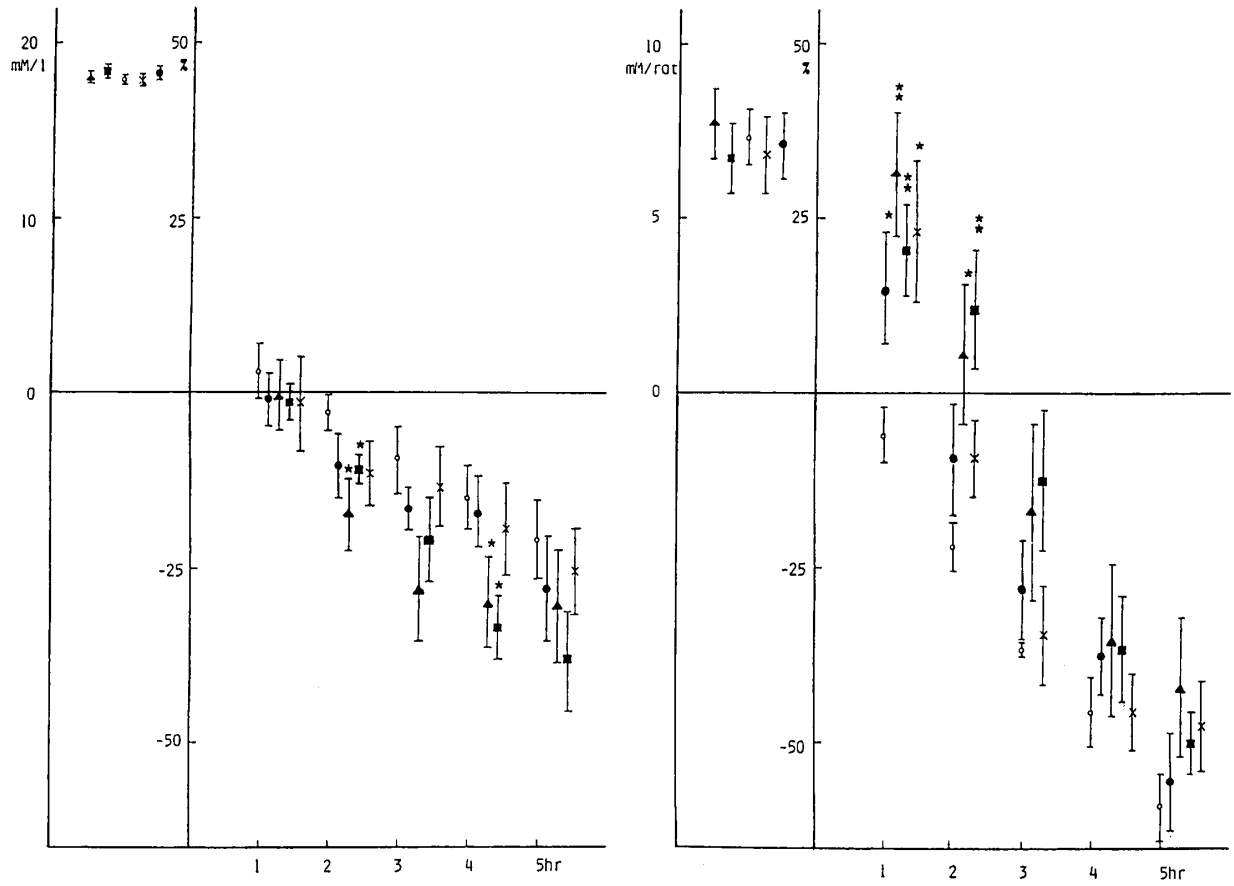

Fig. 5. Effect of the Essential Oil from *C. xanthorrhiza*, Fraction IV, *d*-Camphor and Sodium Dehydrocholate on the Concentration of Total Bile Acids (left) and their Excretive Amount (right) in the Bile of Rats

○ : Control (2% C.M.C.) (p.o.) (n=8), ● : Essential oil 300mg/kg (n=8), ▲ : F—IV 300mg/kg (n=7), ■ : Camphor 100mg/kg (n=7), × : Dehydrocholate-N_a 100mg/kg (n=7), * : $p<0.05$, ** : $p<0.01$.

as that induced by sodium dehydrocholate at dose of 100 mg/kg.

As shown in Fig. 6, the concentration of solid matter in the bile induced by Fr. IV (at 300 mg/kg) and *d*-camphor (at 100 mg/kg) was the same as that of the control. On the other hand, the excreted amount of solid matter in the bile induced by Fr. IV or by *d*-camphor increased with the increase in the bile flow. The increase in the excreted amount of solid matter in the bile induced by Fr. IV or *d*-camphor was more persisting than that by sodium dehydrocholate at a dose of 100 mg/kg.

Discussion

In the present study, the essential oils from the rhizome of *C. longa* and *C. xanthorrhiza*, which have traditionally been used as cholagogues in Indonesia, were found to show a persistent cholagogic effect. On the other hand, curcumin, which was contained in the rhizomes of both plants, showed a weaker cholagogic effect than the two essential oils.

It had been reported that the essential oil and curcumin, contained in the rhizome of *C. longa*, showed a cholagogic effect and that the effect induced by the essential oil was due to *p*-tolylmethylcarbinol contained in it.[4-7]

From these results, the essential oils seem to be at least partly responsible for the cholagogic effect induced by the rhizomes of *C. longa* and *C. xanthorrhiza*.

The essential oil from *C. xanthorrhiza* showed a slightly stronger cholagogic effect than that from *C. longa*. Therefore, to elucidate the active principle(s) in the material, the essential oil was chromatographed on a silica gel column using the elution solvent described to give five fractions (Fr. I–Fr. V). Of these Frs., only Fr. IV was found to be more active than the essential oil itself. Fr. IV was then rechromatographed to

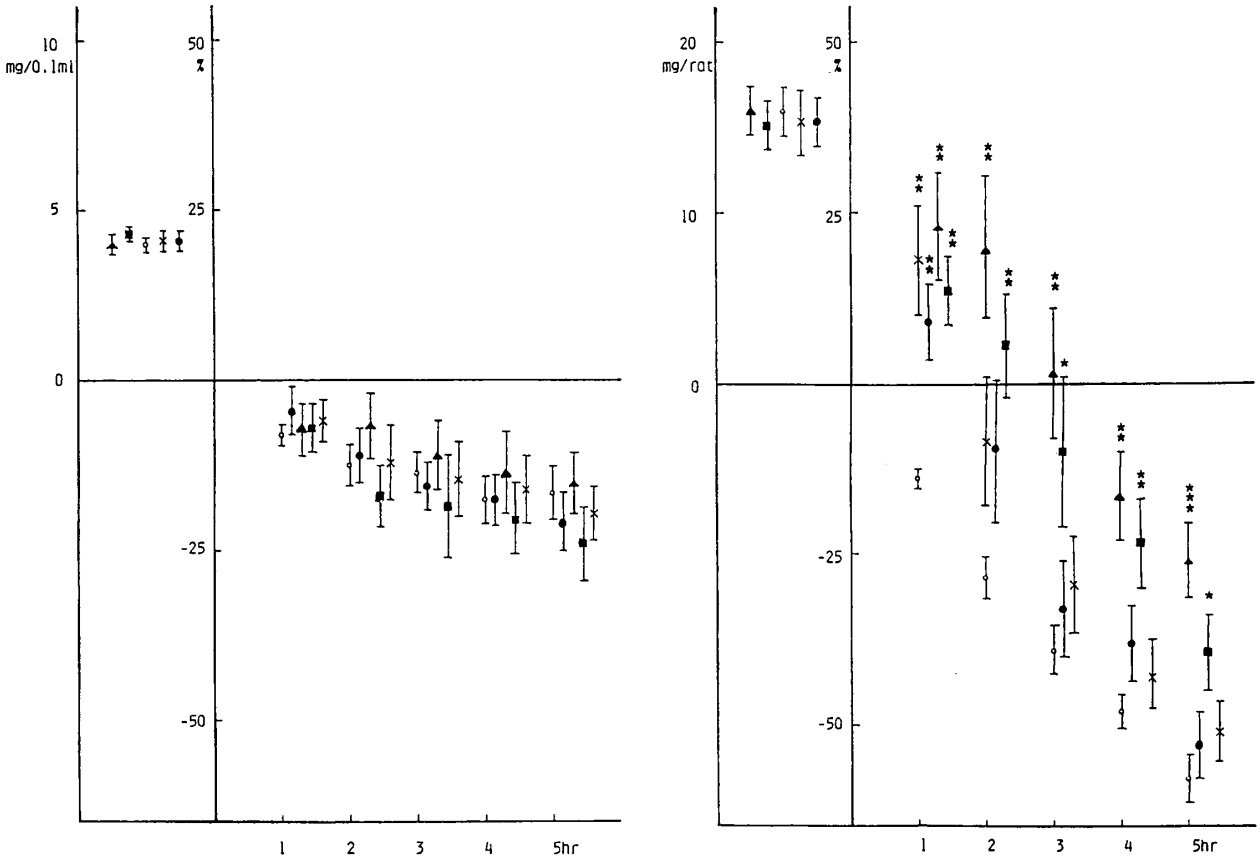

Fig. 6. Effect of the Eessential Oil from *C. xanthorrhiza*, Fraction IV, *d*-Camphor and Sodium dehydrocholate on the Concentration of Solid Dry Matter (left) and Their Excretive Amount (right) in the Bile of Rats

∘ : Control (2% C.M.C.) (p.o.) (n=8), ● : Essential oil 300mg/kg (n=8), ▲ : F—IV 300mg/kg (n=7), ■ : Camphor 100mg/kg (n=7), × : Dehydrocholate-N_a 100mg/kg (n=7). * : $p<0.05$, ** : $p<0.01$.

give an active principle which was identified as *d*-camphor by means of GC-Mass and TLC. The amounts of the active compound in Fr. IV and that in the essential oil were measured by GC.

It was found that Fr. IV and the essential oil contained *d*-camphor in the amount of 45.2 and 3.9%, respectively. Both Fr. IV and *d*-camphor (at a dose corresponding to the amount contained in Fr. IV) induced persisting cholagogic effects of approximately the same strength and duration.

It has been reported that, of various components contained in crude drugs, iridoids, coumarins, monoterpenes and phenolic compounds generally induce cholagogic effects.[14-20] Mörsdorf reported that monoterpenes, menthol, anethole, camphor, borneol, fenchone, camphene, pinene and cineol exhibited effective cholagogic activity of moderate duration when given orally to male rats, that borneol and menthol first increased bile flow and then decreased it and that camphor and menthone were cholagogic compounds which induced a very persistent effect: increased bile flow being observed even after 5 h following oral administration.[17]

From these results, it is concluded that the cholagogic effect, induced by the essential oil, is due to the *d*-camphor it contains and the essential oil is at least responsible in exerting the cholagogic effect given by the rhizome of *C. xanthorrhiza*.

As regards the mode of cholagogic effect of Fr. IV and *d*-camphor, it is well known that there are two mechanisms involved in bile secretion: one is bile salt dependent canalicular bile secretion and the other is bile salt independent bile secretion.[21-25] In the case of the former, the increase in bile flow is related to an increase of bile salt synthesis by the liver: it induces a higher secretion of bile salt in the bile, which then induces an increase in bile flow. In the latter case, the increase in bile flow is related to an increase in active sodium transport mediated by Na^+-K^+-ATPase system: it decreases the concentration of bile salt in the

bile, and then increases the bile flow.

In the present study, the concentration of total bile acids and that of solid matter induced by Fr. IV and *d*-camphor were found to be approximately the same as those of the control, except in the 2nd and 4th hourly biles. While the excreted amounts of total bile acids and that of solid matter in the bile induced by Fr. IV and *d*-camphor increased with the increase in the excretive bile flow, although these increases were small compared with the percent increase in the excretive bile flow.

Therefore, it is considered that the cholagogic effect induced by Fr. IV and *d*-camphor is at least partly due to the bile salt dependent bile secretion.

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