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Cholagogic Action and Characteristics of (±)-α-Terpineol-β-D-*O*-glucopyranoside, a New Monoterpenoid Glucoside

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It was shown previously that α-terpineol is the cholagogic principle in cardamom. Two monoterpenoid glucoside, (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (TAG) and (±)-α-terpineol-β-D-*O*-glucopyranoside (TG), were synthesized in order to develop new drugs whose structures are based on those of natural products. The cholagogic properties of these compounds were investigated in this study.

TG administered by the oral and intraduodendal routes in rats showed a significant cholagogic effect, while TAG showed little effect. The effect of TG was to increase both the fluid weight and the solid weight of bile. The total bile acid content was significantly increased 30 min after administration of TG as compared to that of untreated rats. An analysis of bile acid contents in the bile showed that ursodeoxycholic acid and chenodeoxycholic acid, which are known to have a dissolving effect on gallstones, were increased by TG. The analysis of biliary lipids also demonstrated that biliary cholesterol was decreased by TG. These results raise the possibility that TG may be useful as a dissolving agent for gallstones. In an acute toxicity test, the LD_{50} value for TG was about one-fourth of the value for (±)-α-terpineol, and when their molecular weights are taken into consideration, the toxicity of TG was much lower than that of (±)-α-terpineol. Since TG not only has a cholagogic effect and increases solid components of bile but also has low toxicity, it may be clinically useful as a cholagogic agent.

Keywords——(±)-α-terpineol-β-D-*O*-glucopyranoside; (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside; cholagogic property; LD_{50}; Koenigs–Knorr reaction

α-Terpineol is widely found as a component of essential oils in a variety of plants. Moreover, synthetic (±)-α-terpineol can be purchased inexpensively. α-Terpineol is known to possess biological activities, such as cholagogic action. It is, however, difficult to use it orally in large dosages because of its toxicity and its strong lilac-like odor. In our chemical and structural transformation studies aimed at beneficial usage of natural organic compounds which are abundant in nature and can be inexpensively obtained, we have synthesized new compounds through glucosidation of α-terpineol, and their biological effects are described in this report. (±)-α-Terpineol-2,6-di-*O*-acetyl-3,4-di-*O*-angeloyl-β-D-glucopyranoside, a compound related to α-terpineol glycoside, was isolated and its structure determined. During the course of determining the chemical structure, (+)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside was also found.[1] Their biological effects, however, had not been examined.

Using (±)-α-terpineol obtained from a local source, a variety of conditions for glucosidation of the third hydroxyl group were examined. As a result, (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (TAG) was obtained in high yield by heating 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide and (±)-α-terpineol together in benzene in the presence of mercuric cyanide.

The infrared (IR) absorption spectrum of TAG showed no hydroxyl group absorption but strong absorption due to the acetyl group was observed. In the proton magnetic resonance (^{1}H-NMR) spectra and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, signals due to (±)-α-terpineol and 2,3,4,6-tetra-*O*-acetyl glucopyranoside were observed, suggesting the product to be (±)-α-terpineol glucopyranoside. Based on a ^{13}C-NMR (pyridine-d_5) signal (δ_c 95.56) specific to β-glycosidic bonding of the third hydroxyl group and considering the Koenigs–Knorr reaction mechanism of the brominated glycoside with an acetyl group at position 2, it may be assumed that TAG has β-glycosidic bonding.

TG was obtained by deacetylation of TAG with 1% NaOMe–dry MeOH. Based on the ^{1}H-NMR spectrum (pyridine-d_5) of TG, signals derived from α-terpineol and the glucopyranoyl group were observed, and the signal of the anomeric proton of the β-glycosidic bond was observed at δ 4.99 (1H, d, J=7 Hz). The ^{13}C-NMR spectrum confirmed that the anomeric carbon was bound to the third hydroxyl group through β-glycosidic bonding. These results confirmed that the compound is TG.

Materials and Methods

(±)-α-Terpineol (TER) (1)——TER used was purchased from Tokyo Chemical Industry Co., Ltd. (enantiomeric purity: *ca.* 63.5% **1**, $[\alpha]_D^{31}$ −24.9°).

(±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (TAG) (2)——Mercuric cyanide (3.15 g, 0.018 mol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (5 g, 0.010 mol) an TER (**1**) (1.94 g, 0.013 mol) in 50 ml of benzene. The mixture was refluxed for 30 min at 130 °C, then cooled, and 30 ml of H_2O was added to the resulting solution. The solution was extracted with AcOEt and the extract was washed with saturated sodium bicarbonate aqueous solution and then H_2O. After the extract had been dried with anhyd. $MgSO_4$, the solvent was evaporated off. The residue was chromatographed on silica gel (Kieselgel 60, Art 7734, Merck) with hexane–AcOEt (3 : 1, v/v) to give **2** as colorless crystals. Recrystallization of the crystals from Et_2O and petroleum ether (1 : 1, v/v) furnished 2.9 g of **2** as colorless needles, mp 113 °C, $[\alpha]_D^{29}$ −3.9° (c=0.45, MeOH), $[\alpha]_D^{24}$ −3.0° (c=0.46, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH 1750. ^{1}H-NMR ($CDCl_3$, δ, 400 MHz): 1.12, 1.14 (3H each, both s), 1.63 (3H, s), 1.99, 2.02, 2.05, 2.09 (3H each, all s), 5.35 (1H, m). ^{13}C-NMR (pyridine-d_5): 62.77, 69.56, 71.83, 72.26, 73.66, 80.44, 95.56, (anomeric carbon), 121.30, 133.84, 169.27, 169.76, 170.19, 170.31. *Anal.* Calcd for $C_{26}H_{36}O_{10}$: C, 59.50; H, 7.45. Found: C, 59.30; H, 7.55. MS m/z: 484 (M^+).

(±)-α-Terpineol-β-D-*O*-glucopyranoside (TG) (3)——TAG (**2**) (500 mg, 1.03 mmol) was dissolved in 5 ml of 1% NaOMe methanol solution and the solution was stirred at room temperature for 15 min. The solution was partitioned in an H_2O–*n*-butanol system. The *n*-butanol layer was concentrated under reduced pressure to yield an oily mass (299 mg). Crystallization of the oily mass from AcOEt gave colorless crystals, mp 115—121 °C, $[\alpha]_D^{24}$ 116.4° (c=1.15, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3485, no carbonyl. ^{1}H-NMR (pyridine-d_5, δ, 80 MHz): 1.33, 1.37 (3H each, both s), 1.61 (3H, s), 4.99 (1H, d, J=7 Hz, anomeric proton). ^{13}C-NMR (pyridine-d_5, δ_c): 23.01, 23.50, 24.17, 27.20, 28.89, 31.19, 44.04, 62.79, 71.61, 75.04, 77.55, 78.44, 79.27, 79.36, 98.33 (anomeric carbon), 121.44, 133.66. *Anal.* Calcd for $C_{16}H_{28}O_6$: C, 60.76; H, 8.86. Found: C, 60.58; H, 8.95. MS m/z: 316 (M^+).

Cholagogic Action——The method used to assess the cholagogic effects of TAG and TG was the same as that described in previous reports.[2,3] Male Wistar rats weighing about 300 g were divided into groups of 6. The animals were fasted for 6 h before operation, anesthetized lightly with ether, and then anesthetized with urethan (700 mg/kg, *i.p.*). Laparotomy was performed to insert a polyethylene cannula (Hibiki) into the common bile duct. Animals were allowed to recover for 1 h, and after another 30 min period, a test compound was given intraduodenally (*i.d.*) or orally (*p.o.*) as a suspension in 5% acacia. The bile secretion was measured by using a graduated cylinder to a volume of 0.01 ml at 0.5, 1, 1.5, 2, 3, 4, and 5 h after administration of TAG and TG. The percent changes in bile secretion measured at each time interval were calculated based on the bile secretion during the 30 min before administration of the test compound taken as 100%. Sodium dehydrocholate (DC-Na) served as the control drug.

Characteristics of the Cholagogic Effect——The characteristics of the cholagogic effect of each test compound administered (*i.d.*) were examined in bile samples.

a) Solid Weight of Bile: The weight of dried bile (solid weight of bile) was measured by the same method as that reported in previous papers.[2,3] The solid weight of the bile obtained during 30 min before and 0.5, 1, 3, and 5 h after the *i.d.* administration of each test compound was determined. The percent changes in solid weight of the bile obtained at each time interval were calculated as follows: % solid weight = solid weight of bile in drug groups/solid weight of bile 30 min before administration of drugs × 100.

b) Bile Acids in Bile: The bile secretion was measured according to the previous method.[2,3] Bile acids in the bile

collected during 30 min before and 30 and 60 min (the times at which greatest cholagogic action was seen) after the injection were analyzed with an high performance liquid chromatography (HPLC) bile acid analysis system (Nihon Bunko).

Following the assay of 4 kinds of bile acids in bile, the contents were totalled (total bile acids). The percent changes in bile acids determined at each time interval were calculated based on the classified bile acids and total bile acids measured in the bile during 30 min before the injection taken as 100%.

c) Cholesterol (Chol) and Phospholipids (PL) in Bile: Chol and PL in the bile collected during 30 min before and 30 and 60 min after injection were measured by the same procedure as described previously.[2,3] The percent changes in Chol and PL at each time interval were calculated based on the value obtained during 30 min before injection taken as 100%.

Acute Toxicity Study——Each compound was administered (*p.o.*) to groups of 10 male dd-K mice weighing about 25 g. From the number of deaths during a 7 d observation period after the administration of drug, LD_{50} value for each compound were calculated by the Litchfield–Wilcoxon method. Food and water were supplied *ad libitum* during the observation period.

Results

Cholagogic Action

The bile secretion was increased by about 40% at 30 and 60 min after *i.d.* injection of TG as compared to the untreated group (control group) (Fig. 1). The cholagogic effect was statistically significant 5 h after injection of TG as compared to that of a positive control drug, DC-Na; the effect of DC-Na was transient while that of TG was lasting. The bile secretion was increased slightly between 30 min and 5 h after the injection of TAG, but it was not

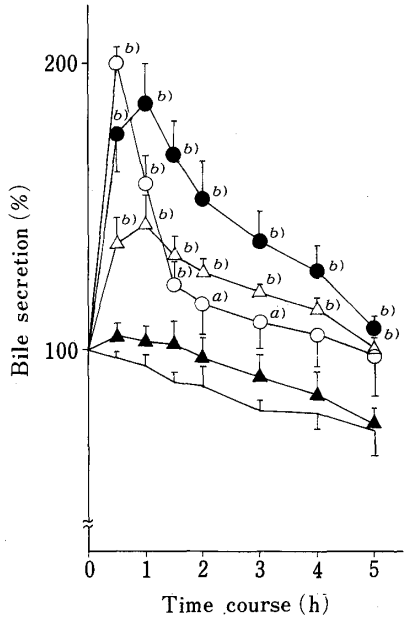

Fig. 1. Effects of (±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, (±)-α-Terpineol-β-D-*O*-glucopyranoside, (±)-α-Terpineol and Sodium Dehydrocholate on Bile Secretion in Rats

——, control; —●—, (±)-α-terpineol 100 mg/kg; —△—, (±)-α-terpineol-β-D-*O*-glucopyranoside 200 mg/kg; —▲—, (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 200 mg/kg; —○—, sodium dehydrocholate 100 mg/kg.

Each value is the mean with standard error obtained from 6 rats. Significantly different from the control at *a*) $p<0.05$, *b*) $p<0.01$. Drugs were administered *i.d.* at 0 h.

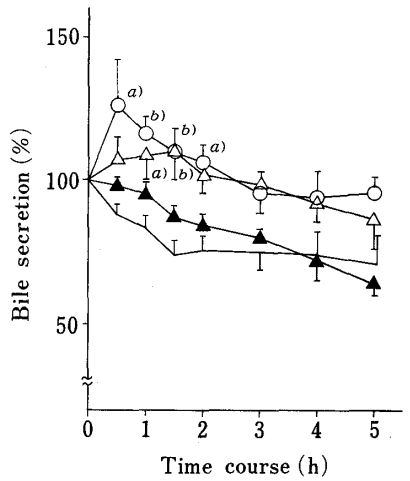

Fig. 2. Effects of (±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, (±)-α-Terpineol-β-D-*O*-glucopyranoside and Sodium Dehydrocholate on Bile Secretion in Rats

——, control; —△—, (±)-α-terpineol-β-D-*O*-glucopyranoside 200 mg/kg; —▲—, (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 200 mg/kg; —○—, sodium dehydrocholate.

Each value is the mean with standard error obtained from 5 rats. Significantly different from the control at *a*) $p<0.05$, *b*) $p<0.01$. Drugs were administered *p.o.* at 0 h.

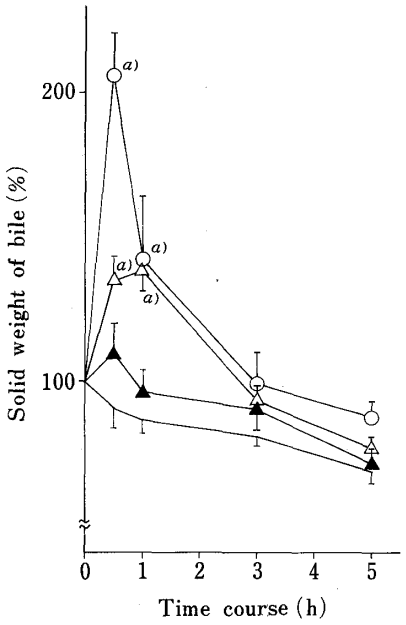

Fig. 3. Effects of (±)-α-Terpineol-β-D-O-glucopyranoside, (±)-α-Terpineol-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside and Sodium Dehydrocholate on Solid Weight of Bile

——, control, —△—, (±)-α-terpineol-β-D-O-glucopyranoside; —▲—, (±)-α-terpineol-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside; —○—, sodium dehydrocholate.

Each value is the mean with standard error obtained from 6 rats. Significantly different from the control at *a*) $p<0.01$.

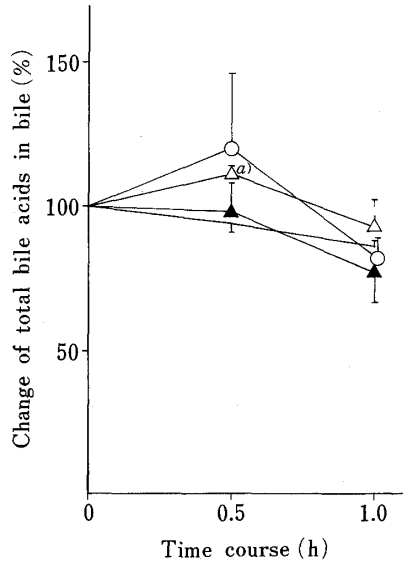

Fig. 4. Effects of (±)-α-Terpineol-β-D-O-glucopyranoside, (±)-α-Terpineol-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside and Sodium Dehydrocholate on the Amount of Total Bile Acids in Bile

——, control; —△—, (±)-α-terpineol-β-D-O-glucopyranoside; —▲—, (±)-α-terpineol-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside; —○—, sodium dehydrocholate.

Each value is the mean with standard error obtained from 6 rats. Significantly different from the control at *a*) $p<0.05$.

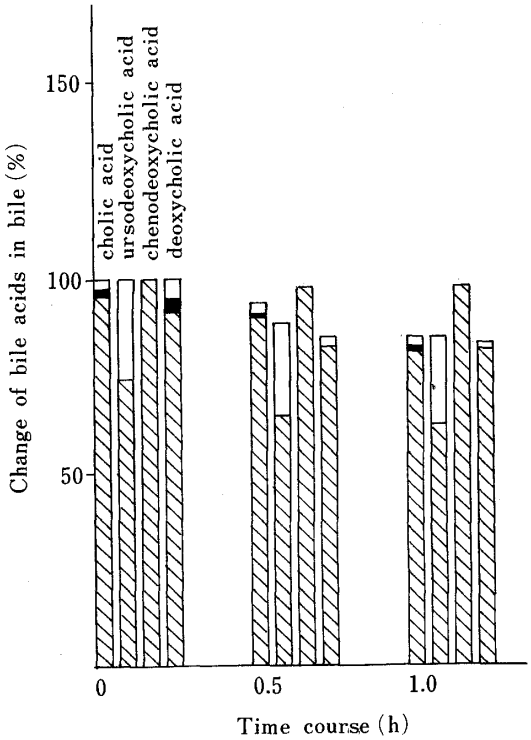

Fig. 5-1. Change of Bile Acids in Bile in Untreated Rats

□, free type; ■, glycine conjugation type; ▨, taurine conjugation type.

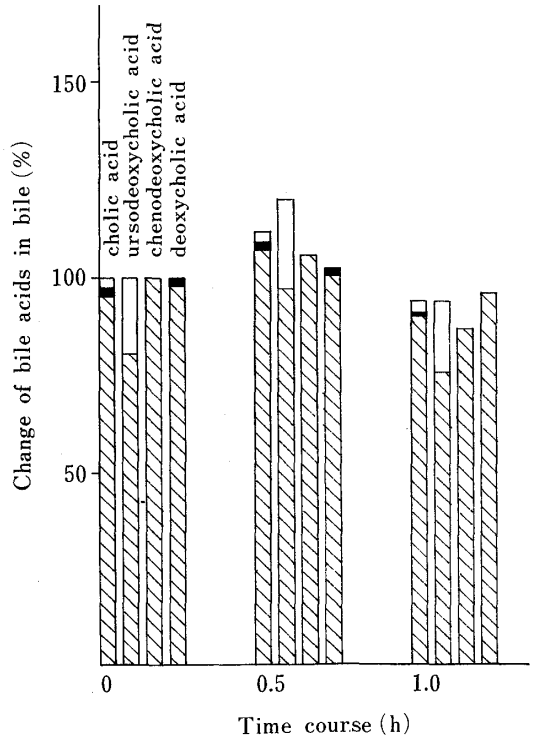

Fig. 5-2. Change of Bile Acids in Bile after Intraduodenal Administration of (±)-α-Terpineol-β-D-O-glucopyranoside

□, free type; ■, glycine conjugation type; ▨, taurine conjugation type

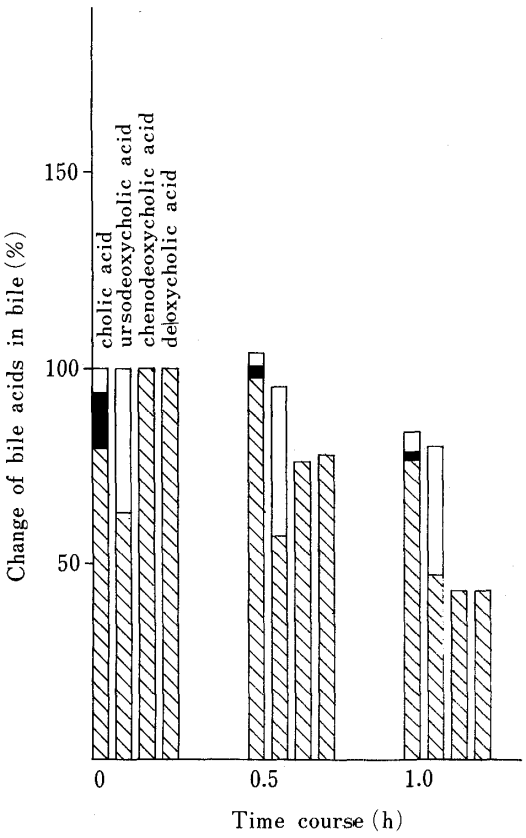

Fig. 5-3. Change of Bile Acids in Bile after Intraduodenal Administration of (±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside

□, free type; ■, glycine conjugation type; ▨, taurine conjugation type.

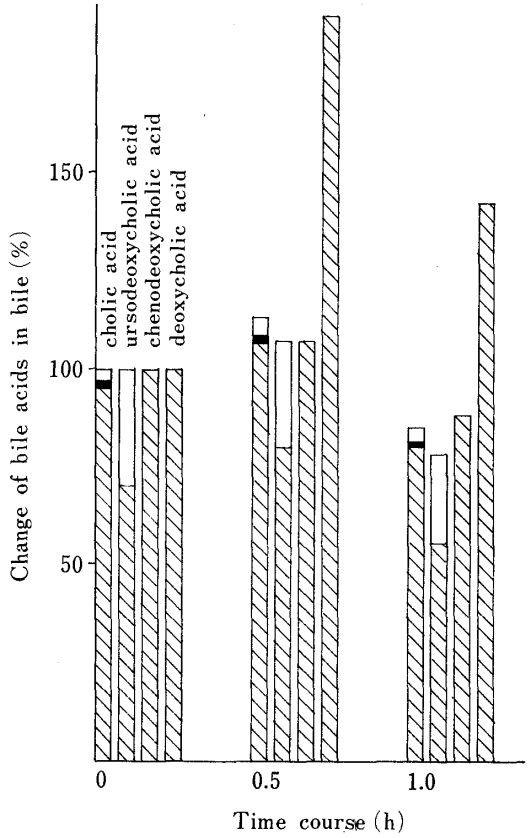

Fig. 5-4. Change of Bile Acids in Bile after Intraduodenal Administration of Sodium Dehydrocholate

□, free type; ■, glycine conjugation type; ▨, taurine conjugation type.

significantly different from the control.

The bile secretion was increased significantly between 1 and 2 h after the *p.o.* administration of TG as compared to the control. The effect of TG was almost the same as that of DC-Na. There was almost no difference in bile secretion between TAG and the control group (Fig. 2).

Characteristics of the Cholagogic Effect

The increase in solid weight of bile was accompanied by a similar increase in the volume of bile in the DC-Na, TG and TAG groups (Fig. 3). The total bile acids were increased significantly at 30 min after the injection of TG as compared to the control (Fig. 4). However, the rate of increase was smaller than that of bile secretion.

In comparison to the decrease in all bile acids in the control group (Fig. 5-1), cholic acid and chenodeoxycholic acid were increased by about 10% at 30 min after an injection of TG, and deoxycholic acid by about 20% (Fig. 5-2). Cholic acid was increased by about 5% at 30 min after the injection of TAG, but other bile acids were decreased (Fig. 5-3). Deoxycholic acid was increased significantly at 30 and 60 min after the injection of DC-Na (Fig. 5-4). Chol and PL in the bile were increased by about 20% and about 5%, respectively, at 30 min after the *i.d.* administration of DC-Na. Chol, however, decreased with the lapse of time in the control, TG and TAG groups. PL was increased slightly at 30 min after an injection of TAG, but it decreased in the control and TG groups as time passed (Figs. 6 and 7).

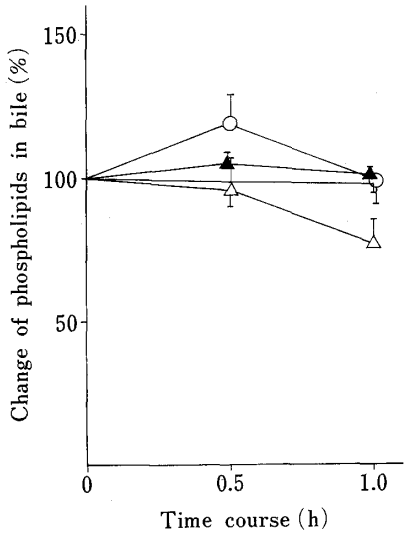

Fig. 6. Effects of (±)-α-Terpineol-β-D-*O*-glucopyranoside, (±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside and Sodium Dehydrocholate on the Amount of Phospholipids in Bile

——, control; —△—, (±)-α-terpineol-β-D-*O*-glucopyranoside; —▲—, (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside; —○—, sodium dehydrocholate.

Each value is the mean with standard error obtained from 6 rats.

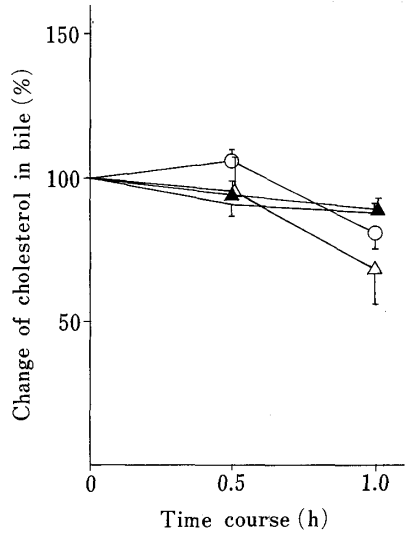

Fig. 7. Effects of (±)-α-Terpineol-β-D-*O*-glucopyranoside, (±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside and Sodium Dehydrocholate on the Amount of Cholesterol in Bile

——, control; —△—, (±)-α-terpineol-β-D-*O*-glucopyranoside; —▲—, (±)-α-terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside; —○—, sodium dehydrocholate.

Each value is the mean with standard error obtained from 6 rats.

TABLE I. Acute Toxicity of (±)-α-Terpineol, (±)-α-Terpineol-β-D-*O*-glucopyranoside, (±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside and Sodium Dehydrocholate in Mice

Compound	LD_{50} (95%, C.L., mg/kg, *p.o.*)
(±)-α-Terpineol	2830 (2290—3497)
(±)-α-Terpineol-β-D-*O*-glucopyranoside	>8000
(±)-α-Terpineol-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside	>8000
Sodium dehydrocholate	2259 (1807—2834)

C.L. = clearance.

Acute Toxicity

The oral acute toxicity of TG was lower than that of DC-Na and TER (Table I).

Discussion and Conclusion

It was shown that the cholagogic principle in cardamon is terpineol.[2] Since the characteristic odor and liquid state of terpineol are rather undesirable attributes for a drug, derivatives of terpineol were synthesized which were odorless and solid. In this report, TG and TAG, an intermediary compound between TER and TG, were examined for cholagogic action. The cholagogic effect of TG (*i.d.*) was weaker than that of TER, but was consistently found as compared to the control. TAG was less effective than TG. It was also shown that *p.o.* administration of TAG, TG or DC-Na was less effective in terms of cholagogic action as compared to *i.d.* administration of the drugs.

Since the cholagogic effects of TAG and TG administered *i.d.* were more potent than those of the compounds administered *p.o.*, the characteristics of the cholagogic effects of the compounds were investigated only after *i.d.* administration. TER had a fluid cholagogic property, whereas the cholagogic effect of TG was to increase the solid weight of bile, accompanied by a similar degree of increase in the volume of bile. The HPLC study of bile acids revealed that ursodeoxycholic acid and chenodeoxycholic acid, which have been reported[4] to have a dissolving action on gallstones, were increased. The analysis of biliary lipids indicated a decrease in Chol. The concominant administration of chenodeoxycholic acid with a preparation containing a monoterpene, which resembles TER, has been reported to improve cholelithiasis in man.[5] These results suggest that TG may be applicable as an agent for dissolving gallstones. A further investigation is in progress.

In the acute toxicity study, the LD_{50} value of TG was found to be about one-fourth of that of TER. Thus, it seems unlikely that TG is hydrolyzed by acid or pepsin in the stomach.

In conclusion, TER is an essential oil having a characteristic odor, whereas TG is an odorless solid compound. The increasing effect of TG on bile secretion is somewhat inferior to that of TER, but as compared to the fluid cholagogic action of TER, TG not only exerts a cholagogic effect but also induces an increase in the solid weight of bile. In addition, the toxicity of TG is lower than that of TER. Therefore, TG may be clinically useful as a cholagogic agent.

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