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European Journal of Pharmacology 387 (2000) 221–227

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Choleretic activity of phloracetophenone in rats: structure–function studies using acetophenone analogues

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Received 24 June 1999; received in revised form 14 October 1999; accepted 19 October 1999

Abstract

The relationship between the chemical structure and choleretic activity of phloracetophenone (2,4,6-trihydroxyacetophenone) was investigated in adult male rats. Fourteen acetophenone analogues, with different substituents on the benzene nucleus, were intraduodenally administered and bile samples were collected via a bile fistula. All of the compounds tested immediately induced choleresis. For the same number of substituents on the benzene ring, hydroxy analogues induced a greater choleresis. The number and position of hydroxy substituents on the benzene nucleus play an important role in determining choleretic activity and biliary secretion of bile acid, but had no relation to biliary excretion of cholesterol. The choleretic activity of the hydroxylated compounds was inversely related to hydrophobicity, as inferred by thin-layer chromatography (TLC). Among the hydroxylated acetophenone analogues, 2,4,6-trihydroxyacetophenone was identified as the most potent, with a choleretic activity of 231.8 ± 6.1 μl/mmol/min. It induced both a high bile flow rate and a high bile salt output and led to lower plasma cholesterol levels. This bile had a low lithogenic potential. The results suggest that a structural requirement for high choleretic activity of 2,4,6-trihydroxyacetophenone is a substituent hydroxy group at 4-position. Additional hydroxy groups at 2- and 6-positions are essential for the induction of higher an output of bile acid, and possibly, other solid materials.

Keywords: Acetophenone analogue; Choleretic; Cholesterol; (*Curcuma comosa*); Lithogenic index; Phloacetophenone

1. Introduction

The liver plays a major role in the metabolism and excretion of both endogenous and exogenous substances (Ronald et al., 1995). Several exogenous compounds including sulfobromophthalein, ethacrynic acid, and valproic acid, are not only excreted into bile, but also influence hepatic bile formation (Klaassen and Plaa, 1968; Chenderovitch et al., 1975; Dickinson et al., 1979; Watkins and Klaassen, 1981). These compounds immediately induced choleresis after acute administration. Earlier studies on the biliary excretion of bile acids and non-bile acid organic compounds have shown that chemical structure and properties are important in determining the hepatic metabolism and biliary excretion of the compounds (Millburn et al., 1967; Gurantz and Hofmann, 1984). Modification of the chemical structure would cause considerable changes in physicochemical properties, biotransformation, and pharmacological effects (Palmer et al., 1987; Berenson et al., 1988; Matoba et al., 1989). Although the mechanism underlying their choleretic effects is not clearly understood, enhanced cannalicular bile flow appears to play an important role in increasing the elimination of endogenous and exogenous substances, such as bilirubin, and may have therapeutic potential for treating cholestasis and preventing gallstone formation (Palmer et al., 1987; Berenson et al., 1988).

Recently, it has been shown that phloracetophenone (2,4,6-trihydroxyacetophenone), which is the aglucone part of a glucoside from *Curcuma comosa* (family Zingiberaceae), effectively stimulates bile secretion (Suksamrarn et al., 1997). This secretion was accompanied by an enhanced

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0014-2999/00/$ - see front matter
PII: S0014-2999(99)00743-8

biliary excretion of bile acid and decreased plasma cholesterol levels (Piyachaturawat et al., 1998). The composition of bile following 2,4,6-trihydroxyacetophenone stimulation, however, was different from that obtained after stimulation with the crude plant extract, and contained a greater concentration of cholesterol (Piyachaturawat et al., 1996). The difference has been suggested to be due to the variety of chemical compounds present in the plant extract (Piyachaturawat et al., 1996). Acetophenones are a group of compounds having a variety of analogues and it is of interest to investigate their choleretic activities. Comparison of the choleretic activity of analogues will help to determine the relative importance of the chemical structure in inducing choleretic activity, and may contribute significantly to our understanding of the molecular mechanism involved in hepatic transportation. The present study was undertaken to investigate the relation between the chemical structure of 2,4,6-trihydroxyacetophenone and its choleretic activity. Analogues with different substituents on the benzene nucleus were used.

2. Materials and methods

2.1. Chemicals

The source of the acetophenone analogues used in this study are indicated in Table 1 by the following abbreviations: Aldrich, Aldrich Chemical, (Milwaukee, WI, USA), Fluka, Fluka Chemie (Buchs, Switzerland). Fiske and Subbarow reducer, 3α-hydroxysteroid dehydrogenase and β-NAD were purchased from the Sigma (St. Louis, MO, USA).

2.2. Animal preparation

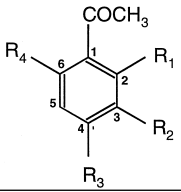
Male Wistar rats weighing 200–250 g were maintained on standard laboratory chow in a room with a 12-h dark/light cycle. After an overnight fast, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A tracheostomy was performed to facilitate breathing. The common bile duct and femoral vein were cannulated with polyethylene tubing, for collection of secreted bile and for infusion of normal saline at the rate of 1.2 ml/h, respectively. Body temperature was maintained at 37 ± 0.5°C with a heat lamp. The experimental protocol was approved by the local ethics committee and complies with the International Guiding Principles for Biomedical Research involving Animals (CIOMS/WHO).

2.3. Experimental procedure

Bile samples were collected over a 30-min period. After collection of the control sample, an individual compound dissolved in a solvent mixture (dimethylsulfoxide: ethanol:water = 25:15:60) was injected intraduodenally at various doses (100–400 μmol/kg body weight, unless otherwise indicated). Control rats were given a similar volume of solvent (0.5 ml). Hydroxy, chloro, methoxy and hydroxy–methoxy analogues of acetophenone, which are structurally related to 2,4,6-trihydroxyacetophenone, were chosen for the study. Bile samples were collected in tared test tubes for 2 h and kept for further analysis of biliary bile salt and cholesterol concentrations. Bile flow was determined gravimetrically, assuming a bile density of 1.0 g/ml. Choleretic activity of the compound was then calculated from the slope of the line relating changes in bile secretion to various doses, and expressed as secreted volume per millimole of the tested analogue (μl/mmol/min). Bile acid concentration was determined enzymatically with 3α-hydroxysteroid dehydrogenase (Turnberg and Anthony-Mote, 1969). Biliary cholesterol concentration was determined by a modification of the ferric method (Wybenga et al., 1970). Phospholipid was measured as inorganic phosphorus following the method of Fiske and Subbarow (1925). The degree of cholesterol saturation of the bile sample, or lithogenic index, was calculated according to Carey (1978).

Blood samples (0.5 ml) were collected from the femoral artery at indicated times for analysis of plasma cholesterol. In order to avoid interference with bile secretion, a separate set of rats was used for blood sampling.

Table 1
Chemical structure of acetophenone analogues



Compounds	R_1	R_2	R_3	R_4	Sources
(1) Acetophenone	H	H	H	H	Fluka
Monosubstituted compounds					
(2) 2-OH-acetophenone	OH	H	H	H	Fluka
(3) 3-OH-acetophenone	H	OH	H	H	Fluka
(4) 4-OH-acetophenone	H	H	OH	H	Fluka
(5) 2-Cl-acetophenone	Cl	H	H	H	Fluka
(6) 3-OCH_3-acetophenone	H	OCH_3	H	H	Fluka
Disubstituted compounds					
(7) 2,4-$(OH)_2$-acetophenone	OH	H	OH	H	Aldrich
(8) 2,6-$(OH)_2$-acetophenone	OH	H	H	OH	Aldrich
(9) 2-OH-6-OCH_3-acetophenone	OH	H	H	OCH_3	Aldrich
(10) 2,6-$(OCH_3)_2$-acetophenone	OCH_3	H	H	OCH_3	Aldrich
(11) 3,4-$(OCH_3)_2$-acetophenone	H	OCH_3	OCH_3	H	Aldrich
(12) 2,4-$(Cl)_2$-acetophenone	Cl	H	Cl	H	Aldrich
(13) 3,4-$(OH)_2$-acetophenone	H	OH	OH	H	Aldrich
Trisubstituted compound					
(14) 2,4,6-$(OH)_3$-acetophenone	OH	H	OH	OH	Fluka

The relative hydrophobicity (R_f value) of the hydroxylated analogues was determined by using thin-layer chromatography (TLC, silica gel 60 F_{254}, Merck, Darmstadt, Germany). The compounds were dissolved in methanol and spotted on the TLC plates. Plates were then developed in a mixture of chloroform and methanol (40:1, v/v) and the color was developed with anisaldehyde–sulfuric acid reagent heated at 110°C for 3 min and R_f values were determined.

2.4. Statistical analysis

All data are expressed as means ± S.E.M. All groups were first compared using one-way analysis of variance (ANOVA) and then the significance of the difference between means was calculated by the Student–Neuman–Keuls test. The level of significance was accepted at $P < 0.05$.

3. Results

The structure of acetophenone analogues used in this study are listed in Table 1.

3.1. Effect on bile flow rate

Fig. 1 shows the choleretic activity of various acetophenone analogues, calculated from the slope of the dose–response curve for bile secretion. Following a single intraduodenal administration of acetophenone analogues, the bile flow rate increased and peaked at 30 min. The peak bile flow rate increased as a function of the administered dose, which was given at 100–300 μmol/kg body weight and accounted for approximately 20–80% of the maximum response. Acetophenone, which is the parent compound without any substituents on its benzene ring, had a low choleretic activity of approximately 27.4 ± 4.6 μl/mmol/min. The introduction of one hydroxy group into the benzene ring of acetophenone markedly increased bile flow rate. Among three different monohydroxy analogues, 2-hydroxyacetophenone had lowest choleretic activity (96.5 ± 7.1 μl/mmol/min) as compared to that of 3- and 4-hydroxy acetophenones (174.0 ± 7.8 and 170.7 ± 4.6 μl/mmol/min, respectively). However, replacement of the hydroxy group with a chloro or methoxy group resulted in a similarly low bile flow rate, suggesting that the hydroxy group and its position were both important for inducing bile secretion. The increased bile flow rates induced by all monosubstituted analogues were statistically significant ($P < 0.05$) when compared to the acetophenone parent compound.

Compounds with more hydroxy groups at different positions on the benzene nucleus also induced different effects on bile flow rate. 2,4-Dihydroxyacetophenone induced a bile flow rate of approximately 207.0 ± 6.5 μl/mmol/min that was much higher than that induced by 2,6-dihydroxyacetophenone, with a bile flow rate of approximately 122.4 ± 4.2 μl/mmol/min. Both of these increases were statistically significant ($P < 0.05$). Replacing the hydroxy group with either methoxy or chloro groups on the benzene nucleus lowered choleretic activity.

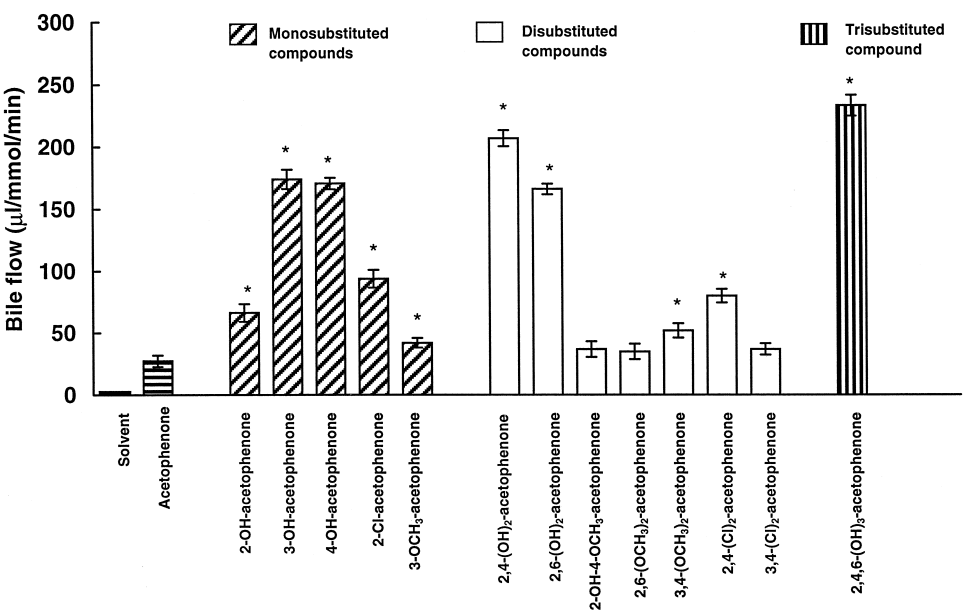


Fig. 1. Effect of acetophenone analogues on choleretic activity (μl/mmol/min). The bile samples were collected at 30 min after administration of the compound at various doses, intraduodenally. Choleretic activity was calculated from the slope of the dose–response curve for bile flow. Values are means ± S.E.M. from eight to ten animals. * $P < 0.05$ significantly different from acetophenone.

Their activities were slightly higher than that of acetophenone, the parent compound. In contrast, an increase in the number of hydroxy groups induced a higher bile flow rate and 2,4,6-trihydroxyacetophenone had the greatest choleretic activity at 231.8 ± 6.4 μl/mmol/min (Fig. 1).

3.2. Effect on bile acid concentration and output

Fig. 2A and B shows the relative effect of the acetophenone analogues on the concentration and output of bile acid after administration of the compounds at a dose of 300 μmol/kg body weight. In all instances, for monosubstituted compounds, the concentration of bile acid was inversely related to the bile flow rate although the output was not significantly different compared to that of the solvent control at corresponding time. 2-Hydroxyacetophenone slightly decreased the concentration of bile acid ($76.3 \pm 2.1\%$ of control) as compared to the control before administration of the compound. In contrast, the corresponding 3- and 4-hydroxy analogues markedly decreased the secreted concentration of bile acid to 51.9 ± 2.5 and $58.0 \pm 1.4\%$ of control, respectively. Chloro and methoxy analogues slightly lowered the concentration of secreted bile acid, similar to the 2-hydroxy analogue.

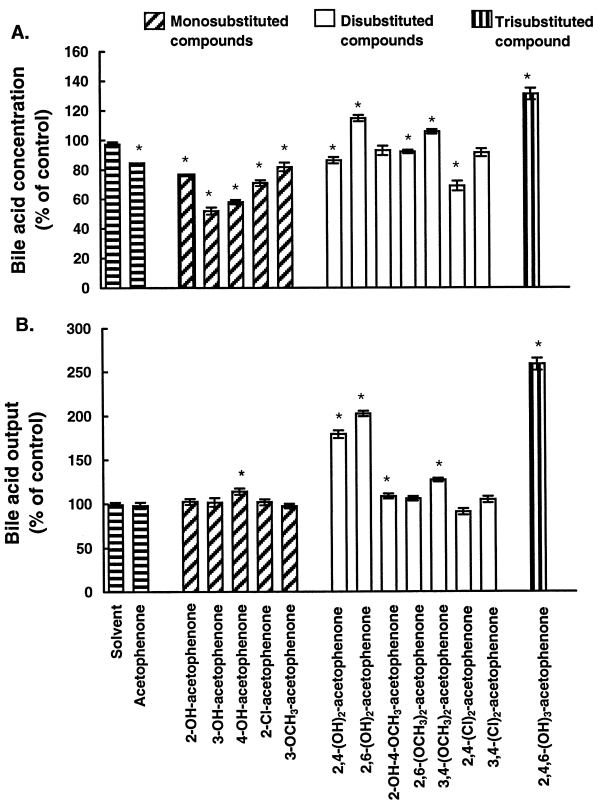


Fig. 2. Effect of acetophenone analogues on bile acid concentration (A) and output (B). The bile samples were collected at 30 min after administration of the compounds at a dose of 300 μmol/kg body weight, intraduodenally. Values are means ± S.E.M. from eight to ten animals. $^{*}P < 0.05$ significantly different from solvent control at corresponding time.

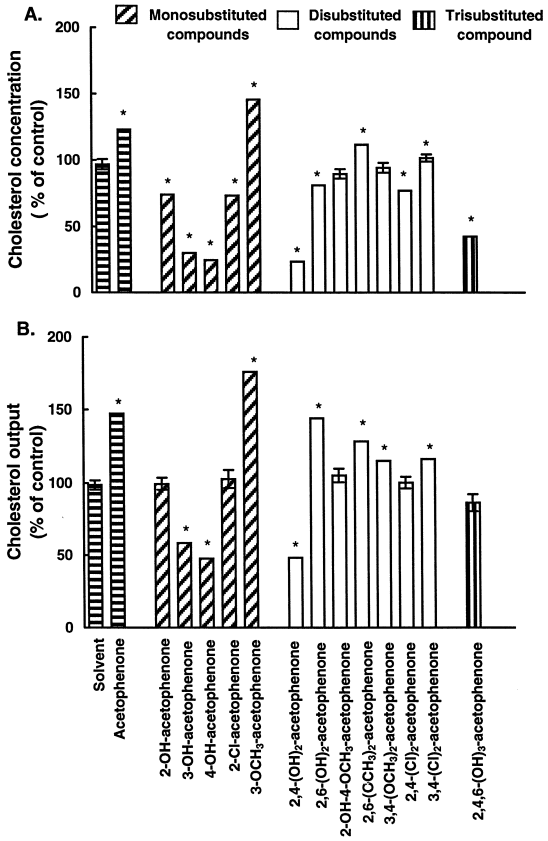


Fig. 3. Effect of acetophenone analogues and derivatives on cholesterol concentration (A) and output (B). The bile samples were collected at 30 min after administration of the compounds at a dose of 300 μmol/kg body weight, intraduodenally. Values are means ± S.E.M. from eight to ten animals. $^{*}P < 0.05$ significantly different from solvent control at corresponding time.

Modulation of the number and position of hydroxy groups affected both the concentration and output of bile acid. The concentration of bile acid induced by 2,4-dihydroxy analogues ($86.3 \pm 2.1\%$ of control) was higher than that induced by the 4-hydroxy analogue. However, it was much lower than that induced by the 2,6-dihydroxy analogue ($114.8 \pm 2.2\%$ of control), which had the same number and type of substituents but at different positions. Moreover, the output of bile acid induced by the 2,4-dihydroxy analogue ($179.1 \pm 4.4\%$ of control) was also notably lower than that induced by the 2,6-dihydroxy analogue ($202.6 \pm 3.1\%$ of control), indicating the importance of the substituted position. The highest secreted concentration and output of bile acid were obtained following 2,4,6-trihydroxyacetophenone administration, namely, $131.2 \pm 4.0\%$ and $258.8 \pm 6.9\%$ of control, respectively.

3.3. Effect on biliary secretion of cholesterol

Fig. 3A and B shows the effect of acetophenone analogues on biliary cholesterol concentration and output after administration of the compounds at a dose of 300 μmol/kg

Table 2
Effect of hydroxyacetophenone analogues on lithogenic index and plasma cholesterol. Values are means ± S.E.M.

Treatments	R_f[a]	Number of animals	Body weight (g)	Lithogenic index (% of control)	Plasma cholesterol (mg%)
Control		10	229.4 ± 4.0	102.0 ± 5.9	62.5 ± 1.9
Solvent		12	227.8 ± 5.1	92.9 ± 6.2	57.6 ± 2.1
4-OH-acetophenone	0.35	9	231.8 ± 4.5	78.3 ± 10.9	49.7 ± 2.3
2,4-$(OH)_2$-acetophenone	0.40	8	230.5 ± 5.2	59.3 ± 7.7	52.0 ± 2.8
2,6-$(OH)_2$-acetophenone	0.49	8	228.8 ± 5.1	126.9 ± 14.7	48.2 ± 2.8[b]
2,4,6-$(OH)_3$-acetophenone	0.07	9	230.6 ± 3.8	65.3 ± 5.3	49.4 ± 2.6[b]

[a]Relative hydrophobicity is determined by TLC using chloroform:methanol 40:1 as solvent.
[b]$P < 0.01$ significantly different from the solvent control at corresponding time.

body weight. Although it was thought that the compounds, because of their ability to generate a high bile flow rate, would reciprocally modulate the concentration of cholesterol secreted, there was no obvious relation between the number of hydroxy groups on the benzene ring of acetophenone and the concentration of cholesterol in biliary secretion. Thirty minutes after administration of acetophenone, the parent compound, the concentration and output of cholesterol were significantly increased to 122.9 ± 3.6% and 147.2 ± 8.1% of control, respectively. Among the monosubstituted compounds, 2-hydroxyacetophenone slightly decreased the cholesterol concentration to 74.0 ± 3.9 whereas 3- and 4-hydroxyacetophenones markedly decreased the cholesterol concentration to 29.7 ± 2.7% and 24.2 ± 2.4% of control, respectively. In contrast, 3-methoxy-acetophenone markedly increased the concentration of cholesterol to 145.3 ± 2.2% of control and the cholesterol output was also markedly elevated. Replacement of hydroxy with a chloro group slightly changed the concentration and output of cholesterol, effects comparable to those of 2-hydroxyacetophenone. For disubstituted compounds, administration of 2,4-dihydroxyacetophenone decreased the concentration and output of cholesterol to 23.3 ± 2.7% and 48.1 ± 5.7% of control, respectively. With 2,6-dihydroxyacetophenone, the cholesterol concentration decreased to 80.9 ± 4.4% of control but output increased to 144.3 ± 6.7% of control. However, both the concentration and output of cholesterol were decreased following administration of 2,4,6-trihydroxyacetophenone.

3.4. Effect on plasma cholesterol and lithogenic index

It was apparent that some hydroxylated analogues induced pronounced choleretic effects and modified the biliary composition of bile acid and cholesterol differently, which primarily affected the lithogenicity of bile. As shown in Table 2, the lithogenic index was markedly increased by 2,6-dihydroxyacetophenone. In contrast, it was significantly decreased by 2,4-dihydroxy and 2,4,6-trihydroxyacetophenone. As the acetophenone analogues variably altered the secretion of biliary lipids, it was worthwhile to investigate their effects on plasma lipid levels. Following administration of the hydroxylated acetophenone analogues, only 2,6-dihydroxy and 2,4,6-trihydroxyacetophenones caused significant decreases in plasma cholesterol levels ($P < 0.05$).

The relative hydrophobicity (R_f value) of some of the hydroxylated acetophenone analogues was also determined. It was found that 2,4,6-trihydroxyacetophenone was the most hydrophilic and 2,6-dihydroxyacetophenone the most hydrophobic.

4. Discussion

The present study explored the relationships between a number of the structural features associated with the acetophenone core of 2,4,6-trihydroxyacetophenone and choleretic activity. Among 14 acetophenone analogues, it was evident that 2,4,6-trihydroxyacetophenone, the aglucone of a naturally occurring compound from *C. comosa*, was the most effective in inducing choleresis. The structural requirement of 2,4,6-trihydroxyacetophenone for stimulation of bile flow was the strong polar hydroxy group at the 4-position on the benzene nucleus. More hydroxy groups at 2- and 6-positions were essential for inducing a higher secretion of bile salt and cholesterol, and possibly, other solid materials.

In each corresponding class of mono-, di- and tri-substituents, hydroxyacetophenone analogues were more effective in inducing bile secretion (Fig. 1). Their choleretic activities were apparently dependent on both the position and the number of hydroxy substituents on the benzene nucleus. In monohydroxylated acetophenone analogues, it appeared that a substitutent hydroxy at the 2-position on the benzene nucleus of acetophenone induced a mild secretion of bile whereas the corresponding 3- and 4-hydroxy analogues markedly increased bile secretion to a similar degree. The variability in the choleretic efficacy of these different chemical structures might be partially related to their physicochemical properties. In earlier studies, the choleresis of exogenous compounds has been shown to be mainly due to the osmotic activity of the compound and/or its metabolites in bile (Combes, 1965; Klaassen and Plaa,

1968; Chenderovitch et al., 1975; Watkins and Klaassen, 1981). Biotransformation of several compounds by conjugation with glucuronic acid, glutathione, as well as sulfate has been demonstrated to essentially augment the biliary excretion of the compounds (Combes, 1965; Millburn et al., 1967; Klaassen and Plaa, 1968; Chenderovitch et al., 1975; Watkins and Klaassen, 1981). Moreover, a compound containing a strongly polar group or potentially ionizable group on a molecule, which is an important site for direct conjugation, would enhance its biliary excretion (Combes, 1965; Millburn et al., 1967; Klaassen and Plaa, 1968; Watkins and Klaassen, 1981; Shimamura et al., 1994). However, the biliary excretion of xenobiotics and metabolites does not always induce choleresis (Klaassen and Watkins, 1997). In the present study, it was not clear how and to what extent the acetophenone analogues were biotransformed and excreted by the liver. However, the earlier observation regarding the significance of polar hydroxy groups might account for the observed results in the present study, particularly the lower choleretic activity of 2-hydroxyacetophenone. The relatively lower effectiveness of the 2-hydroxy substituent of acetophenone might be due to intramolecular bonding between the 2-hydroxy group and keto group and the steric effects of the acetyl group, which caused it to be less effective for conjugation. The polarity of the compound was indeed low, as determined by TLC (Table 2). In contrast, 4-hydroxy acetophenone, which had a stronger hydroxy group and higher hydrophilicity, possessed a much higher choleretic activity. Consistent with the results derived from application of this physicochemical property, an increased hydrophobicity caused by replacement of a hydroxy group with a methoxy or chloro group, reduced the choleretic efficacy of the compound. A decreased hydrophobicity of the compounds, due to the addition of more hydroxy groups induced greater choleretic activity. Therefore, the differences in the physicochemical properties of the acetophenone compounds could, at least in part, account for the variability of the observed effects in the present study, although it is likely that other factors such as hepatic uptake, biotransformation and cannalicular transport also contributed. It was also evident that the choleretic efficacy of 2,4-dihydroxyacetophenone was much higher than that of 2,6-dihydroxyacetophenone. The lower choleretic activity of 2,6-dihydroxyacetophenone might also be associated with the intramolecular bonding and a steric effect of the acetyl group, hindering the adjacent hydroxy group and rendering the 6-hydroxy group less polar. The 2,4,6-trihydroxyacetophenone analogue, which was the most polar compound used, had the highest choleretic activity. Thus the presence of the hydroxy substituent at the 4-position, rather than at the 2- or 2- and 6-positions, was considered to be important for choleretic activity.

It is noted that differences in the number of hydroxy groups in hydroxylated acetophenone analogues also variably modulated the concentration and output of bile acid. Similar to the effect on bile flow, the higher the number of hydroxy groups, the greater the concentration and output of bile acid. As bile acid is osmotically active and its secretion induces the flow of bile, the higher bile flow rate of the polyhydroxylated analogues in the present study might be partly mediated via the secretion of bile acid. Bile acid, in addition to inducing bile flow, also stimulates the secretion of the water-insoluble amphipathics, cholesterol and phospholipid (Roda et al., 1983; Bilhartz and Dietschy, 1988). In each class of compounds, although the secreted bile salt concentration was inversely related to bile flow, there was no obvious relation between bile flow and the secreted concentration of cholesterol. Differences in the secreted concentration of cholesterol induced by acetophenones were also possibly related to an induction of secretion of different bile acid species. Although the mechanisms of cholesterol and phospholipid secretion are poorly understood, a number of studies reported the secretion of cholesterol and phospholipid to be linearly related to the hydrophobicity of bile acids (Roda et al., 1983; Bilhartz and Dietschy, 1988). Bile acid that is relatively hydrophilic, with a higher critical micellar concentration, induces less cholesterol excretion than others (Roda et al., 1983; Bilhartz and Dietschy, 1998). In this regard, the higher hydrophobicity of the bile acid secreted in response to acetophenone analogues would induce a higher cholesterol secretion, as observed following 2,6-dihydroxyacetophenone. However, further studies on the hepatic excretion of metabolites of individual compounds and bile acid species are required to clarify this point.

As the liver is the major site responsible for the disposal of cholesterol from the body, increases in the biliary excretion of either cholesterol or bile acid or both would lead to a decrease in the plasma cholesterol concentration. In the present study, 2,6-dihydroxyacetophenone, which greatly increased the excretion of both bile acid and cholesterol, was able to lower plasma cholesterol levels. Unlike 2,6-dihydroxyacetophenone, the lowering of plasma cholesterol levels by 2,4,6-trihydroxyacetophenone occurred concurrently with a great increase in the excretion of bile acid with less cholesterol. Furthermore, when the lithogenic index, or cholesterol saturation index, which is an indicator for evaluating the risk of gallstone formation, was determined, only 2,4,6-trihydroxyacetophenone induced bile with low lithogenic index whereas 2,6-dihydroxyacetophene generated bile with high lithogenic index. The property of 2,4,6 trihydroxyacetophenone found in the present study was in agreement with our earlier study (Piyachaturawat et al., 1998) in which 2,4,6 trihydroxyacetophenone was considered to have potential for use as a cholitholytic agent, in addition to use as a lipid-lowering agent.

Regarding the choleretic mechanism of acetophenone analogues, although there was a correlation between the choleretic activity and hydrophicility of the compounds in the present study, several factors involved in hepatic up-

take, biotransformation and excretion have to taken for consideration. Recently, there has been remarkable progress in research on the transportation of organic compounds in the liver (Meier, 1995; Yamazaki et al., 1996; Muller and Jansen, 1997; Sekine et al., 1998). A number of organic transporters at both sinusoidal and cannalicular sites have been reported and cloned. Some transport systems particularly at the cannalicular membrane show multiplicity in the recognition of a broad range of substrates (Meier, 1995; Yamazaki et al., 1996; Muller and Jansen, 1997; Sekine et al., 1998). In the present study, the acetophenone analogues with a slight modification of the chemical structure demonstrated different choleretic activities and affected the composition of bile differently. Further study of the interaction of these compounds with hepatic transporters in inducing changes would help to clarify these points and also provide an understanding of the molecular mechanism of our observed effects.

In conclusion, the results of the present study demonstrate that there are marked differences in the choleretic activity of acetophenone analogues and in the composition of bile secreted in response to these analogues. The extent of bile secretion and the components excreted were dependent on the number, position and type of substituents. Selective modification of the chemical structure of the compound would give a variety of pharmacological effects. Some of the structural features determine the volume of bile secreted while others are important for the secretion of solid materials. The information gained from the present study provides a rational basis for the design and selection of candidate molecules with choleretic, litholytic or/and lipid lowering activities.

Acknowledgements

This study was supported by a grant from The Thailand Research Fund (TRF-BRG 4180006). The authors thank Dr. K.K. Shida Pang for criticism and suggestions.

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