5-Hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone: A Pancreatic Lipase Inhibitor Isolated from *Alpinia officinarum*

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A pancreatic lipase inhibitor, 5-hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone (HPH), from the rhizome of *Alpinia officinarum* (AO) was isolated and its antihyperlipidemic activity was measured. HPH inhibited a pancreatic lipase with an IC_{50} value of 1.5 mg/ml (triolein as a substrate). HPH significantly lowered the serum TG level in corn oil feeding-induced triglyceridemic mice, and reduced serum triglyceride (TG) and cholesterol in Triton WR-1339-induced hyperlipidemic mice. However, HPH did not show hypolipidemic activity in high cholesterol diet-induced hyperlipidemic mice. Based on these findings, we propose that PL inhibitors may be effective as hypolipidemic agents.

Key words *Apinia officinarum*; pancreatic lipase; antihyperlipidemic activity; 5-hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone

Lipid metabolism normally maintains an elegant balance between synthesis and degradation. When the balance is lost, hypertriglyceridemia and hypercholesterolemia may develop. This can cause a variety of serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, functional depression of some organs, *etc.*[1)]

Pancreatic lipase (PL) is a key enzyme for lipid breakdown that leads to the absorption of fatty acids.[2)] PL, one of the exocrine enzymes of pancreatic juice, catalyzes the hydrolysis of emulsified esters of glycerol and long-chain fatty acids. Short-chain fatty acids can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles.[3)] The micelles are absorbed into the mucosal cells of the intestine, and the fatty acids and monoglycerides are resynthesized into triglycerides. Dietary triglyceride is usually stored in the adipose tissue. Pharmacological agents that reduce the absorption of dietary triglyceride, thereby reducing the probability of the formation of atherosclerotic plaque, have been developed, including orlistat and clofibrate.[4)]

As part of our continuing search for biologically active antiarteriosclerosis agents from natural herbal resources, we isolated 3-methylgalangin from the rhizome of *Alpinia officinarum* (AO) as a PL inhibitor and reported that it lowered serum triglyceride level on corn oil-induced hyperlipidemic mice.[5)]

Recently we isolated a new PL-inhibitor, diarylhapanoid, from the ethylacetate fraction of AO rhizome and investigated its hypolipidemic activity.

MATERIALS AND METHODS

Materials Triton WR-1339, triolein, tributyrin, and PL were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Total cholesteol and triglyceride assay kits were from Asan Pharmaceutical Co. Ltd. (Korea). Orlistat (Xenical) was kindly donated by Dr. I. K. Kim of the Korea Food and Drug Administration.

AO (family Zingiberaceae) was purchased from Kyung Dong Market (Seoul, Republic of Korea) and identified by Dr. Nam-Je Kim, East-West Medical Research Institute, Kyung Hee Medical Center, Kyung Hee University. A voucher specimen (KHP-010507) was deposited at the Herbarium of the College of Pharmacy, Kyung Hee University.

Extraction of Herbal Medicines Each herbal medicine (5 kg) was extracted twice with water in boiling water for 2 h. These extracts were filtered and evaporated in a rotary vacuum evaporator and then lyophilized with a freeze dryer.

Isolation of 5-Hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone, a New PL-Inhibitory Compound, from the Rhizome of AO The rhizome of AO (2 kg) was extracted twice with 10 l of boiling water. After evaporation of the solvent, the extract (230 g) was suspended with 500 ml water. The suspended extract was extracted stepwise with ethyl ether, ethyl acetate, and butanol. The most potent PL-inhibitory ethyl acetate fraction (5 g) was applied to silica gel column chromatography and eluted with $CHCl_3$. We isolated two compounds (AO-6, AO-7). The AO-6 had more potent PL-inhibitory activity. To isolate AO-6 homogenously, AO-6 fraction was subjected to preparative HPLC (column: μ-Bondapak C18; solvent: 30% MeOH; elution speed 4 ml/min). The isolated compound AO-6 was identified as 5-hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone through the instrumental analysis.

5-Hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone (HPH) Light-yellow liquid, $[\alpha]_D^{20}$ −13.9° (c=1.15, $CHCl_3$). IR, ν_{max}^{Kbr} 3400 (H-bond), 1700 (carbonyl), 1600, 1500 (benzene ring) cm^{-1}. Electrospray ionization mass spectroscopy (ESI-MS) (m/z): 328 $[M]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.29 (2H, dd, J=5.6, 2.0 Hz, H-2″,6″), 7.18 (1H, ddd, J=5.6, 5.6, 2.0 Hz, H-4″), 7.17 (2H, ddd, J=5.6, 5.6, 2.0 Hz, H-3″,5″), 6.82 (1H, d, J=8.0 Hz, H-5′), 6.70 (1H, d, J=2.0 Hz, H-2′), 6.67 (1H, dd, J=8.0, 2.0 Hz, H-6′), 4.40 (1H, m, H-5), 2.90 (2H, t, J=8.8 Hz, H-1), 2.75 (2H, t, J=8.8 Hz, H-2), 2.70 (1H, m, H-7a), 2.60 (1H, m, H-7b), 2.54 (2H, d, J=7.6 Hz, H-4), 1.76 (1H, m, H-6a), 1.63 (1H,m, H-6b). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 210.98 (C-3),

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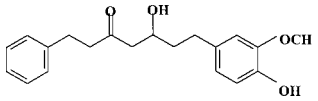

146.24 (C-3′), 143.57 (C-4′), 140.50 (C-1″), 133.57 (C-1′), 128.45 (C-3″,5″), 128.15 (C-2″,6″), 126.14 (C-4″), 120.81 (C-6′), 114.15 (C-5′), 110.96 (C-2′), 77.21 (C-5), 66.83 (C-5), 55.87 (C-3′-OMe), 49.26 (C-4), 45.04 (C-2), 38.35 (C-6), 31.46 (C-7), 29.52 (C-1).

Activity Assay of PL The enzyme activity assay was performed according to the previously reported method.[6] The reaction mixture (3.06 ml) containing 135 mM triolein (or tributyrin) emulsified in gum acasia, 2 mM sodium thioglycolate, and PL (0.6 unit using triacetin as a substrate) and the sample was adjusted to pH 8.8 with 0.1 M NaOH, incubated at 25 °C and titrated with 10 mM NaOH to adjust at the pH to 8.8. The inhibitory activity of the sample (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 mg/ml) was calculated from the titrant volume. This was done in triplicate for each experiment. The IC_{50} value was estimated by Probit's method.

Animals Male ICR mice (20—25 g) were purchased from Daehan Experimental Animal (Republic of Korea) and fed a commercial diet (Samyang, Republic of Korea). These animals were kept for at least 7 d prior to the experiments. To evaluate the hypolipidemic effect, three kinds of hyperlipidemic animal models were established, and the orlistat was used as a positive control.

First, a hyperlipidemic mouse model based on corn oil was established according to the method of Duhault *et al.*[7] Six mice were used per group. Corn oil (1 g/kg) was orally administered 2 h after each sample or orlistat was administered orally. Two hours after the administration of corn oil, blood samples of mice were drawn by cardiac puncture under ether anesthesia.

Second, a hyperlipidemic mouse model based on Trition WR-1339 was established according to the method of Kusama *et al.*[8] Triton WR-1339 was injected at the end of the regular 16-h fasting period as a 10% solution in saline at a dose of 200 mg/kg body weight into the tail veins of mice under light ether anesthesia. Six mice were used per group. These mice were anesthetized with ether 18 h after Triton WR-1339 injection, and 1—1.5 ml of blood was withdrawn by cardiac puncture. Sera were obtained by centrifugation (1500×***g***, 10 min). Tested samples and orlistat were administered orally once a day for 3 d. The final administration of the samples was performed 1 h before Triton WR-1339 injection.

Third, a hypercholesterolemic mouse model based on high-cholesterol (HC) diets was established according to the previously reported method.[9,10]

Determination of Total Serum Cholesterol, Triglyceride, and LDL Cholesterol Total cholesterol was measured by the enzyme method of Allain *et al.*[10] Serum triglycerides were measured by the method designed by Sardesai and Mannig[11] and LDL cholesterol was measured by the enzyme method of Mainard and Madec.[12]

Statistical Analysis All the data from the *in vivo* experiments were expressed as mean±S.D., and statistical significance was determined using Student's *t*-test.

RESULTS AND DISCUSSION

As part of our continuing search for biologically active antiarteriosclerosis agents from natural herbal resources, we reported that the ethyl acetate fraction of AO water extract exhibited the most potent inhibition. From this fraction, we isolated a PL inhibitor, 3-methylgalangin, from its rhizome, and reported that it lowered the serum triglyceride level on corn oil-induced hyperlipidemic mice. However, the nonpolar fraction, more than 3-methylgalangin, also showed a PL-inhibitory activity. Then, we isolated the inhibitor of PL. Isolated AO-6 exhibited the most potent PL-inhibitory activity with an IC_{50} value of 1.5 mg/ml (triolein as a substrate) (Table 1). Its PL-inhibitory potency was similar to that of 3-methylgalangin. AO-6 was confirmed to be 5-hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone (HPH) by instrumental analysis. The hypolipidemic effect of PL inhibitor HPH was measured in Triton WR-1339-induced hyperlipidemic mice. HPH showed potent hypolipidemic activity. Triglyceride and cholesterol levels were dose-dependently decreased compared with the Triton WR-1339-alone group, respectively. This compound at 50 and 100 mg/kg

Table 1. The Inhibitory Effect of Extracts and Compounds of *Alpinia officinarum* on Pancreatic Lipase Activity

Agent	IC_{50} (mg/ml)	
	Tributyrin	Triolein
Water extract	3.7	9.6
Ether Fr.	6.0	9.2
Ethylacetate Fr.	5.6	3.0
Butanol Fr.	>10	>10
Residual Fr.	>10	>10
HPH (AO-6)	3.4	1.5
AO-7	>10	>10
Orlistat	2.1	0.8

Table 2. The Effects of HPH on Serum TC, TG and HDL-Cholesterol Levels in Triton WR-1339-Induced Hyperlipidemic Mice

Group	Dose (g/kg/d)	TG level (mg/dl)	TC level (mg/dl)	HDL level (mg/dl)
Normal	—	81.1±8.5	196.1±27.0	81.1±14.7
Control	—	436.7±19.6##	299.2±21.9##	41.3±15.8#
HPH	0.05	273.8±6.8**	209.5±15.9**	52.6±7.2
	0.1	256.4±12.6**	201.3±22.8**	56.9±13.3
Orlistat	0.05	213.1±17.4**	126.2±7.1**	53.7±6.5

Significantly different from normal group ($p<0.05$). ## Significantly different from control group ($p<0.01$). ** Significantly different from control group ($p<0.01$).

Table 3. Effects of HPH on Serum TC, TG and HDL-Cholesterol Levels in Corn Oil-Induced Hyperlipidemic Mice

Group	Dose (g/kg/d)	TG level (mg/dl)	TC level (mg/dl)	HDL level (mg/dl)
Normal	—	97.3±12.4	123.3±9.7	56.3±6.8
Control	—	188.0±10.1##	137.5±12.6	56.0±1.1
HPH	0.05	159.5±10.5*	114.3±4.0	55.1±5.6
	0.1	116.8±12.7**	112.6±8.6	57.6±4.7
Orlistat	0.05	63.9±13.5**	126.4±24.3	49.0±3.5

Significantly different from control group ($p<0.01$). * Significantly different from control group ($p<0.05$). ** Significantly different from control group ($p<0.01$).

doses also exhibited potent antihyperlipidemic activity in corn oil feeding-induced hyperlipidemic mice (Table 3). However, HPH did not show hypolipidemic activity in high-cholesterol diet-induced hyperlipidemic mice (data not shown).

We believe that the hypolipidemic activity of HPH may be due to the inhibition of PL, although the effect of its long-term feeding was not investigated. Judging from these results, we propose that PL inhibitors may be effective as hypolipidemic agents.

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