

## Inhibition of Diacylglycerol Acyltransferase by Phenylpyropenes Produced by *Penicillium griseofulvum* F1959

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**Phenylpyropenes A, B, and C, isolated from *Penicillium griseofulvum* F1959, inhibited DGAT in rat liver microsomes with IC<sub>50</sub> values of 78.7±1.6, 21.7±0.2, and 11.04±0.2 μM, respectively. In addition, a kinetic analysis using a Lineweaver-Burk plot revealed that phenylpyropene C was a noncompetitive inhibitor of DGAT. The apparent Michaelis constant (K<sub>m</sub>) value and inhibition constant (K<sub>i</sub>) value were calculated to be 8 μM and 10.4 μM, respectively. Moreover, phenylpyropene C inhibited triglyceride formation in HepG2 cells.**

**Keywords:** *Penicillium griseofulvum* F1959, diacylglycerol acyltransferase (DGAT), phenylpyropene, triglyceride, hepG2 cell

Triglycerides are quantitatively the most important storage form of energy for eukaryotic cells. However, the excess accumulation of triglycerides in certain organs and tissues causes serious disease of obesity, type 2 diabetes, fatty liver, and hypertriglyceridemia [7]. Moreover, it is associated with tissue dysfunction referred to as lipotoxicity. Therefore, the control of triglyceride synthesis is expected on treatment and prevention for these diseases.

Triglycerides are synthesized from diacylglycerol and fatty acyl-CoA in a reaction catalyzed by acyl-CoA: diacylglycerol acyltransferase (DGAT; E.C. 2.3.1.20), a microsomal enzyme that catalyzes the transfer of the acyl residue from acyl-CoA to diacylglycerol [1, 2]. Therefore, the enzyme is important for triglyceride metabolism such as intestinal fat absorption, lipoprotein assembly, the regulation of plasma triglyceride concentrations, and fat storage in adipocytes [3, 9]. An increase in the expression and activity levels of DGAT may lead to a decrease in systemic insulin

sensitivity and energy homeostasis [11]. In fact, the study using DGAT-deficient mice has provided a better understanding of the relationship between triglyceride synthesis and the diseases of obesity and diabetes. These mice have reduced body fat and are resistant to diet-induced obesity through the mechanism that increases energy expenditure [9], and tended to have low levels of plasma glucose and insulin [3]. Therefore, DGAT inhibition may be a worthwhile strategy for treating obesity and type 2 diabetes.

In the course of searching for DGAT inhibitors from natural sources, phenylpyropenes A, B, and C were isolated from a fermentation broth of *Penicillium griseofulvum* F1959, and the phenylpyropenes showed inhibitory activities against DGAT in rat liver microsomes. The fungal strain *Penicillium griseofulvum* F1959 was isolated from a soil sample collected at Ulsan, Korea, and it was deposited in the Korean Collection for Type Culture (KCTC), Korea, as KCTC 0387BP. Taxonomic studies of the strain and fermentation procedure were described in our previous report [6], and we also reported phenylpyropenes as inhibitors of the enzyme acyl-coenzyme A: cholesterol acyltransferase (ACAT) [6, 8]. In brief, the fermentation broth was extracted with ethyl acetate (EtOAc). The extract was fractionated by open column chromatography on silica gel and semipreparative HPLC to afford three active constituents, **1**, **2**, and **3**. They were identified as phenylpyropenes A (**1**), B (**2**), and C (**3**) by spectroscopic analyses (Fig. 1), including mass spectrometry (MS) and nuclear magnetic resonance (NMR) [6, 8]. Their structures consist of three common parts, α-pyrone, sesquiterpene motif, and phenyl ring, and they contain three, two, and one OAc moieties, respectively (Fig. 1). The OAc moieties and OH group of phenylpyropene A (**1**) was replaced by the methyl group and hydrogen group, respectively, in phenylpyropene C (**3**).

We tested the DGAT inhibitory effect of phenylpyropenes using microsomes prepared from rat livers (male Sprague-Dawley rat), according to the methods previously reported

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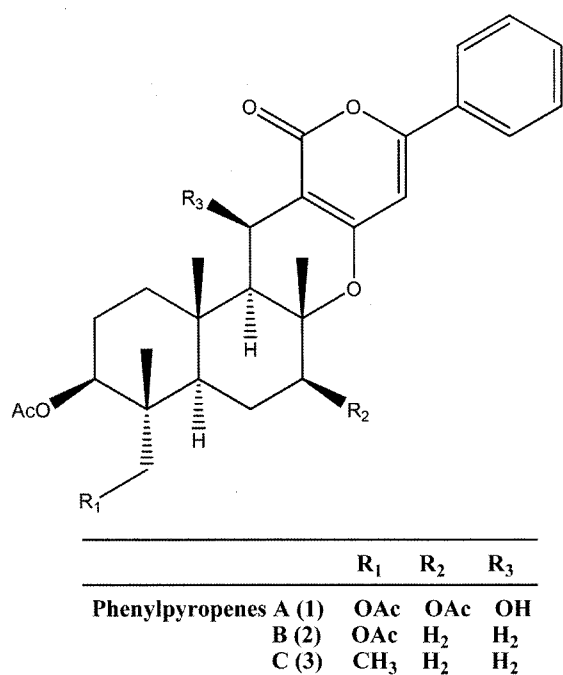


Fig. 1. Structures of phenylpyropenes A, B, and C isolated from *Penicillium griseofulvum* F1959.

by Coleman [5]. In brief, phenylpyropenes were incubated with microsomes, [ $^{14}\text{C}$ ] oleoyl-CoA and *sn*-1, 2-dioleoylglycerol as substrates. After a 10 min enzymatic reaction, the [ $^{14}\text{C}$ ] triglyceride products were extracted with heptane from the reactants, and the radioactivity of the heptane layer was measured by a scintillation counter. Phenylpyropenes A, B, and C inhibited DGAT activity in a concentration-dependent fashion with  $\text{IC}_{50}$  values of  $78.7 \pm$

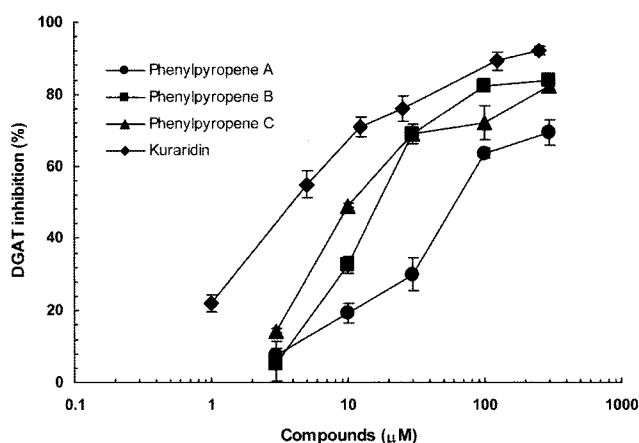


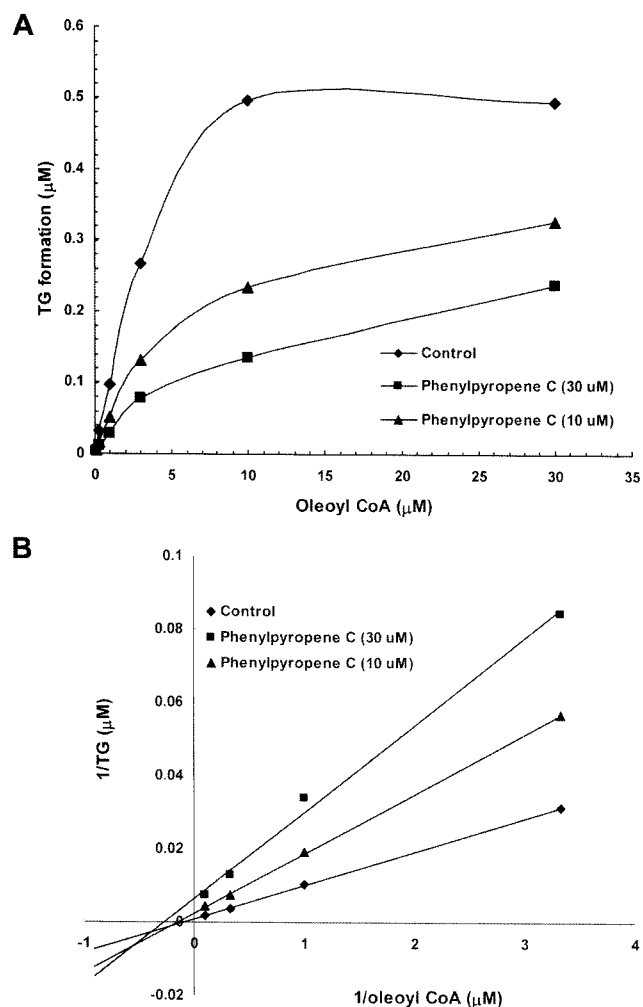
Fig. 2. Inhibitory effect of phenylpyropenes A, B, and C on rat liver microsomal DGAT.

The enzyme reaction was carried out in the presence of phenylpyropenes A (●), B (■), and C (▲) at  $37^\circ\text{C}$  for 10 min. The kuraridin (◆) was used as a positive control. The data are presented as the means of three independent experiments performed in duplicate.

1.6,  $21.7 \pm 0.2$ , and  $11.04 \pm 0.2 \mu\text{M}$ , respectively (Fig. 2). The inhibitory effects of these compounds (1–3) were confirmed using kuraridin as a positive control, which inhibited DGAT activity with an  $\text{IC}_{50}$  value of  $9.8 \mu\text{M}$  in the assay system [4]. Phenylpyropene C (3) showed more potent DGAT inhibitory activity than phenylpyropene A (1) or B (2). This result suggests that the presence of the methyl group on the R1 position of the compounds is important for their DGAT inhibition (Fig. 1).

Because DGAT uses oleoyl-CoA as a substrate, we performed experiments using this substrate to determine DGAT enzyme kinetic parameters, including apparent  $K_m$ , apparent  $V_{\text{max}}$ , dissociation constant of enzyme-inhibitor complex ( $K_i$ ), and the type of enzyme inhibition with phenylpyropene C (3). For the kinetic study, DGAT assay was carried out with varying [ $^{14}\text{C}$ ] oleoyl-CoA concentration (0.1–100  $\mu\text{M}$ ) and constant concentration of *sn*-1,2-dioleoylglycerol (100  $\mu\text{M}$ ) to determine the reaction velocity and apparent  $V_{\text{max}}$  in the absence or presence of 10 and 30  $\mu\text{M}$  of phenylpyropene C (3). As shown in Fig. 3A, phenylpyropene C (3) decreased the apparent  $V_{\text{max}}$  by 48% (30  $\mu\text{M}$ ) and 66% (10  $\mu\text{M}$ ) compared with the control. The nature of DGAT inhibition by phenylpyropene C (3) was assessed by plotting the reciprocal of the reaction velocity versus the reciprocal of the substrate oleoyl-CoA concentration [Lineweaver-Burk plot (LB plot)] (Fig. 3B). Apparent  $K_m$  was calculated based on the intercept on the negative X-axis of the LB plot, and the value was found to be 8 mM for [ $^{14}\text{C}$ ] oleoyl-CoA as a substrate (Fig. 3B). The apparent  $V_{\text{max}}$  (for control) and the apparent  $V_{\text{max}}^i$  (for 10 and 30  $\mu\text{M}$  phenylpyropene C) were calculated from the intercept on the Y-axis of the LB plot. The  $K_i$  was calculated based on the formula  $V_{\text{max}}^i = V_{\text{max}} / (1 + [I] / K_i)$ , where  $[I]$  is the concentration of phenylpyropene C (3). Based on this equation, the  $K_i$  was found to be 10.4  $\mu\text{M}$ . The LB plot of DGAT inhibition by phenylpyropene C (3) showed a noncompetitive pattern of enzyme inhibition. The kinetic parameters were calculated using the Enzyme Kinetics! Pro (version 2.36, ChemSW) program.

To determine the effect of phenylpyropene C (3) on cellular triglyceride formation, incorporation of [ $^{14}\text{C}$ ] acetic acid into cellular lipids was measured in an intact cell assay using HepG2 cells. The experimental procedure followed the methods reported previously [10]. HepG2 cells were cultured in a 24-well plate at the density of  $1-10 \times 10^5$  cells/ml/well, and cells were treated with phenylpyropene C (3) at varying concentrations and incubated for 1 h at  $37^\circ\text{C}$ . The incorporation of [ $^{14}\text{C}$ ] acetic acid into cellular triglyceride was determined by the addition of 0.25  $\mu\text{Ci}$  [ $^{14}\text{C}$ ] acetic acid complexed with BSA (bovine serum albumin, fatty acid free; Sigma). Incubation was carried out for 6 h at  $37^\circ\text{C}$ , and the total lipids were extracted by  $\text{CHCl}_3$ -MeOH (2:1, v/v) and separated on TLC using hexane-ether-acetic acid (70:30:1, v/v) as a

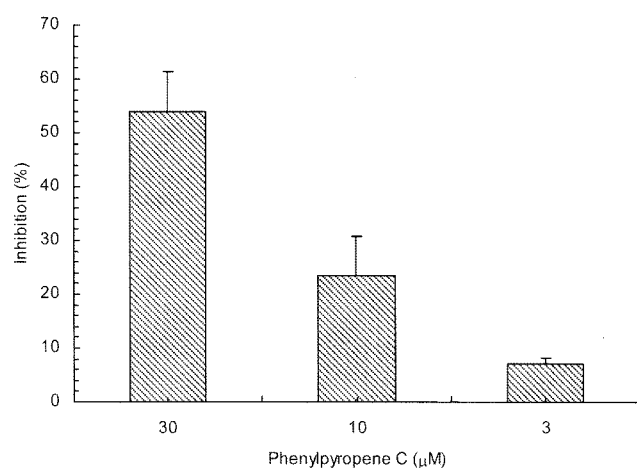


**Fig. 3.** The characteristics of enzyme inhibition by phenylpyropene C.

**A.** The initial velocity plot showing the dependence of DGAT activity on  $[1-^{14}\text{C}]$  oleoyl-CoA. DGAT activity was measured at varying concentrations of  $[1-^{14}\text{C}]$  oleoyl-CoA (0.1–30  $\mu\text{M}$ ) in the absence (control) and presence of phenylpyropene C (10  $\mu\text{M}$  and 30  $\mu\text{M}$ ). **B.** Lineweaver-Burk plot of inhibition kinetics of DGAT for phenylpyropene C. The reciprocal of the rate of formation of triglyceride was plotted against the reciprocal of  $[1-^{14}\text{C}]$  oleoyl-CoA concentration. The data are presented as the means of three independent experiments performed in duplicate.

developing solvent. The amounts of triglyceride were analyzed with a bio-imaging analyzer (BAS-1500; Fuji Poto Film Co. Ltd.). Phenylpyropene C (**3**) inhibited  $[1-^{14}\text{C}]$  acetic acid incorporation into triglyceride in a dose-dependent manner with an  $\text{IC}_{50}$  value of  $27.8 \pm 7.4 \mu\text{M}$  (Fig. 4). The compound did not show cytotoxicity at the concentrations employed in this study (data not shown).

In conclusion, phenylpropenes inhibited the DGAT activity of rat liver microsomes. Among them, phenylpyropene C (**3**) inhibited triglyceride formation in HepG2 cells and showed a non-competitive pattern of enzyme inhibition in



**Fig. 4.** Effect of phenylpyropene C on triglyceride formation in HepG2 cells.

The cells were incubated with  $[1-^{14}\text{C}]$  acetic acid (0.25  $\mu\text{Ci}$ , 9  $\mu\text{M}$ ) in the presence or absence of phenylpyropene C at varying concentrations for 6 h at  $37^\circ\text{C}$ . The total lipids were extracted and separated on TLC. The amounts of radioactivity of triglyceride were analyzed with a bio-imaging analyzer. Results are expressed as mean  $\pm$  SE ( $n=3$ ).

kinetic study. This study reports DGAT inhibitory effect of phenylpropenes for the first time. Therefore, these compounds may be useful for the design of new DGAT inhibitors leading to anti-obesity and anti-type 2 diabetic agents.

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## REFERENCES

- Bell, P. M. and R. A. Coleman. 1980. Enzymes of glycerolipid synthesis in eukaryotes. *Annu. Rev. Biochem.* **19**: 459–487.
- Cases, S., S. J. Smith, Y. W. Zheng, H. M. Myers, S. R. Lear, E. Sande, *et al.* 1998. Identification of a gene encoding an acyl CoA: Diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Natl. Acad. Sci. USA* **95**: 13018–13023.
- Chen, H. C., S. J. Smith, Z. Ladha, D. R. Jenson, L. D. Ferreira, L. K. Pulawa, *et al.* 2002. Increased insulin and leptin sensitivity in mice lacking acyl CoA: Diacylglycerol acyltransferase 1. *J. Clin. Invest.* **109**: 1049–1055.
- Chung, M. Y., M. Rho, J. S. Ko, S. Y. Ryu, K. H. Jeune, K. Kim, H. S. Lee, and Y. K. Kim. 2004. *In vitro* inhibition of diacylglycerol acyltransferase by prenylflavonoids from *Sophora flavescens*. *Planta Med.* **70**: 258–260.

5. Coleman, R. A. 1992. Diacylglycerol acyltransferase and monoacylglycerol acyltransferase from liver and intestine. *Methods Enzymol.* **209**: 98–103.
6. Kwon, O. E., M. C. Rho, H. Y. Song, S. W. Lee, M. Y. Chung, J. H. Lee, Y. H. Kim, H. S. Lee, and Y. K. Kim. 2002. Phenylpyropene A and B, new inhibitor of acyl-CoA: Cholesterol acyltransferase produced by *Penicillium griseofulvum* F1959. *J. Antibiot.* **55**: 1004–1008.
7. Lehner, R. and A. Kuksis. 1996. Biosynthesis of triacylglycerols. *Prog. Lipid Res.* **35**: 169–201.
8. Rho, M. C., H. S. Lee, K. T. Chang, H. Y. Song, O. E. Kwon, S. W. Lee, J. S. Ko, S. G. Hong, and Y. K. Kim. 2002. Phenylpyropene C, a new inhibitor of acyl-CoA: Cholesterol acyltransferase produced by *Penicillium griseofulvum* F1959. *J. Antibiot.* **55**: 211–214.
9. Smith, S. J., S. Cases, D. R. Jenson, H. C. Chen, E. Sande, B. Tow, et al. 2000. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking DGAT. *Nat. Genet.* **25**: 87–90.
10. Tomoda, H., K. Igarashi, J. C. Cyong, and S. Omura. 1991. Evidence for an essential role of long chain acyl-CoA synthetase in animal cell proliferation. *J. Biol. Chem.* **266**: 4214–4219.
11. Yu, Y. and H. Ginsberg. 2004. The role of acyl-CoA: Diacylglycerol acyltransferase (DGAT) in energy metabolism. *Annu. Rev. Med.* **36**: 252–261.