Communications to the Editor

PYRIPYROPENES, HIGHLY POTENT INHIBITIORS OF ACYL-CoA: CHOLESTEROL ACYLTRANSFERASE PRODUCED BY *Aspergillus fumigatus*

Sir:

Acyl-CoA: cholesterol acyltransferase (ACAT) is a key enzyme for cholesteryl ester accumulation in atherogenesis, lipoprotein formation in livers and cholesterol absorption from intestines. Therefore, ACAT is focused as one of the new inhibition targets for the treatment or prevention of atherosclerosis and hypercholesterolemia. During our course of screening for ACAT inhibitors of microbial origin, purpactins[1], new cyclodepsipeptides[2] and glisoprenins[3] were isolated from fungal strains. Our continuous search has led to the discovery of highly potent ACAT inhibitors termed pyripyropene produced by *Aspergillus fumigatus* FO-1289. The present communication describes the production, isolation and some biochemical and biological activities of these compounds.

Pyripyropenes were isolated from the fermentation broth (60 liters) of *A. fumigatus* FO-1289 by a combination of solvent extraction, silica gel column chromatography, ODS column chromatography and HPLC (ODS and silica gel columns). Finally, pure pyripyropenes A (45 mg), B (4.6 mg), C (4.5 mg) and D (4.1 mg) were obtained as white powders[4].

The molecular formula of pyripyropene A was determined to be $C_{31}H_{37}NO_{10}$ and those of pyripyropenes B, C and D were all $C_{32}H_{39}NO_{10}$ on

Table 1. Inhibition of ACAT activity by pyripyropenes in an enzyme assay using rat liver microsomes.

Compound	IC_{50} (nM)
Pyripyropene A	58
Pyripyropene B	117
Pyripyropene C	53
Pyripyropene D	268
CL-283,546	1,300

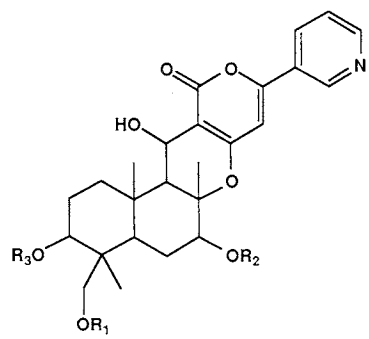
Table 2. Inhibition of cholesterol absorption in Golden Syrian Hamster by pyripyropene A[a].

Pyripyropene A		Inhibition of cholesterol absorption (%)
Exp. 1	25 mg/kg	32±7[b]
Exp. 2	10	3±9
	50	28±8
	75	46±5

[a] Group of four animals (Golden Syrian Hamster, 7~8 weeks) received 5 ml dosing solution (pyripyropene A) per kg body weight by gavage. One hour after dosing, the animals received 2 μCi of [^{3}H]-cholesterol. After 12 hours, the radioactivity (plasma plus liver) was determined by liquid scintillation spectrometer.

[b] Values are mean±standard deviation.

Fig. 1. Structures of pyripyropenes.



Compound	R_1	R_2	R_3
Pyripyropene A	CH_3CO-	CH_3CO-	CH_3CO-
B	CH_3CH_2CO-	CH_3CO-	CH_3CO-
C	CH_3CO-	CH_3CH_2CO-	CH_3CO-
D	CH_3CO-	CH_3CO-	CH_3CH_2CO-

the basis of HREI-MS. Similar UV spectra of pyripyropenes were observed with two maxima at 231 (ε 24,300) and 320 nm (13,400) in MeOH. Eventually, the structures of pyripyropenes were determined as shown in Fig. 1 by spectroscopic analyses mainly including various NMR techniques. They have a unique structure in common consisting of three parts, pyridine, α-pyrone and sesquiterpene. The details will be reported in the near future[5].

ACAT inhibitory activity was studied using rat liver microsome as an enzyme source according to our established method[1,2]. The drug concentrations causing 50% ACAT inhibition (IC_{50}) are nanomolar level (53~268 nM) as shown in Table 1. Among them, pyripyropene C is the most potent ACAT inhibitor with an IC_{50} of 53 nM. Under the same conditions, the IC_{50} value of CL 283,546, a synthetic ACAT inhibitor[6], shows micromolar level (1.3 μM), indicating over 20-fold larger value than those of pyripyropenes A and C. To our knowledge, pyripyropenes A and C are the most potent ACAT inhibitors in this assay system.

Table 2 shows the effect of pyripyropene A on cholesterol absorption after a single oral administration in an acute model using hamsters. The cholesterol absorption was inhibited in a dose-dependent fashion.

Thus, pyripyropenes are demonstrated to be highly potent ACAT inhibitors. Detailed studies on pyripyropenes will be reported in the near future[4,5].

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