# Structure-activity Relationships of Pyripyropenes Fungal Acyl-CoA: Cholesterol Acyltransferase Inhibitors

Sir:

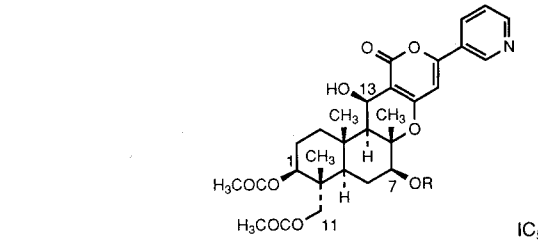
Acyl-CoA: cholesterol acyltransferase (ACAT) has been believed to be an intriguing target of inhibition as a new type of atherosclerotic agents. Pyripyropenes A to D isolated from the fermentation broth of *Aspergillus fumigatus* FO-1289 represent the most potent inhibitory activity in naturally occurring ACAT inhibitors. Pyripyropenes inhibit ACAT activity in nanomoler level of $IC_{50}$ value.[1~3] Pyripyropene A (**1**) proved to be orally active for reducing cholesterol absorption in a hamster model.[1] Recently, the stereochemistry of **1** was determined as shown in Fig. 1.[4] Among the four compounds, pyripyropene C (**2**) showed the most potent inhibition, followed by **1**. The structural difference between them lies in the 7-*O*-acyl group; **1** has an acetyl residue, and **2** does a longer propionyl one, suggesting that synthetic replacement at the 7-*O*-acyl residue is to be investigated. Here we describe the synthesis and structure-activity relationships of 7-*O*-acyl group.

Hydrolysis of pyripyropene A with 1,8-diazabicyclo-[5,4,0]undec-7-ene (DBU) in 80% methanol gave 7-OH derivative (**3**) in 52% yield. **3** was acylated by acyl anhydride and triethylamine with 4-dimethylaminopyridine (DMAP) in dichloromethane (Method A), or with acyl chloride and triethylamine in dichloromethane (Method B). Some derivatives were also prepared by appropriate carboxylic acid and DCC-DMAP in dichloromethane (Method C). The structures of the derivatives were determined by NMR analysis and MS. ACAT inhibitory activity was assessed using rat liver microsomes as an enzyme source according to our established method.[5,6]

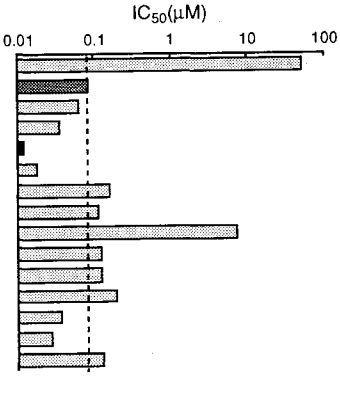
When the ACAT inhibitory activity was compared in derivatives having a linear acyl group (**1**, **2** and **4~9**), one with longer carbon length up to 5 increased the inhibitory activity, but another with longer than 7 carbons length decreased the activity. As a result, the *n*-valeryl derivative with 5 carbons length (**5**) showed the best ACAT inhibition. [Analytical data of **5**: HRFAB-MS, found 626.2945 $(M+1)^+$ calcd 626.2964, $^1H$ NMR (270 MHz, $CDCl_3$), $\delta$ 8.99 (1H, d, $J=1.7$ Hz, 2″-H), 8.68 (1H, dd, $J=1.7$, 5.0 Hz, 6″-H), 8.09 (1H, dt, $J=2$, 8 Hz, 4″-H), 7.40 (1H, dd, $J=5.0$, 8 Hz, 5″-H), 6.39 (1H, s, 5′-H), 5.01 (1H, m, 7-H), 4.99 (1H, d, $J=3.0$ Hz, 13-H), 4.78 (1H, dd, $J=5.3$, 10.9 Hz, 1-H), 3.80 (1H, d, $J=11.9$ Hz, 11-H), 3.68 (1H, d, $J=11.9$ Hz, 11′-H), 2.90 (1H, br s, 13-OH), 2.40 (2H, dt, $J=2.0$, 7.3 Hz, $COCH_2CH_2CH_2CH_3$), 2.16 (1H, m, 3-H), 2.08 (3H, s, 11-$COCH_3$), 2.04 (3H, s, 1-$COCH_3$), 1.9~1.7 (2H, m, 2 and 2′-H), 1.68 (3H, s, 14-$CH_3$), 1.7~1.5 (6H, m, 5, 8, 8′, 9 and $COCH_2CH_2CH_2CH_3$), 1.43 (3H, s, 12-$CH_3$), 1.38 (3H, m, 3′-H and $COCH_2CH_2CH_2CH_3$), 0.96 (3H, t, $J=7.4$ Hz, $COCH_2CH_2CH_2CH_3$), 0.88 (3H, s, 15-$CH_3$).] Introduction of branched acyl groups (**10~13**) resulted in decrease of the activity in comparison with the corresponding linear acyl groups (**4~6**), even though the isovaleryl derivative with 5 carbons (**13**) still showed stronger activity than **1**. Similarly, the alkene **14** with a 5 carbons chain also showed stronger activity than **1**. The activity of the aromatic derivative (**15**) was almost the same as that of the linear acyl derivatives (**7** and **8**) and the branched acyl derivatives (**10~12**).

In summary, the 7-*O*-acyl moiety was modified with several acyl groups in order to find more potent ACAT inhibitors than **1**. Among 15 derivatives, **5** having an *n*-valeryl group showed the most potent ACAT inhibi-

Fig. 1. Structures, synthetic preparation methods and *in vitro* ACAT inhibitory activity of pyripyropene derivatives.




| Compound | Method | R |
|---|---|---|
| **3** | - | H |
| **1** | - | $COCH_3$ |
| **2** | A | $COCH_2CH_3$ |
| **4** | A | $CO(CH_2)_2CH_3$ |
| **5** | A | $CO(CH_2)_3CH_3$ |
| **6** | A | $CO(CH_2)_4CH_3$ |
| **7** | A | $CO(CH_2)_5CH_3$ |
| **8** | A | $CO(CH_2)_6CH_3$ |
| **9** | A | $CO(CH_2)_{16}CH_3$ |
| **10** | A | $COCH(CH_3)_2$ |
| **11** | B | $CO(CH_3)_3$ |
| **12** | A | $COCH_2CH(CH_3)_2$ |
| **13** | C | $CO(CH_2)_2CH(CH_3)_2$ |
| **14** | C | $CO(E)CH=CHCH_2CH_3$ |
| **15** | B | $COC_6H_5$ |



tion with an $IC_{50}$ value of 13 nM, indicating 7-fold stronger activity than **1**. This result suggests that 7-*O*-acyl moiety of pyripyropene plays an important role to show the ACAT inhibitory activity.

RIKA OBATA
TOSHIAKI SUNAZUKA
LI ZHUORONG
HIROSHI TOMODA
SATOSHI ŌMURA*

Research Center for Biological Function,
The Kitasato Institute, and Kitasato University,
Minato-ku, Tokyo 108, Japan

(Received January 5, 1995)

## References

1) ŌMURA, S.; H. TOMODA, Y. K. KIM & H. NISHIDA: Pyripyropenes, highly potent inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. J. Antibiotics 46: 1168～1169, 1993
2) TOMODA, H.; Y. K. KIM, H. NISHIDA, R. MASUMA & S. ŌMURA: Pyripyropenes, novel inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. I. Production, isolation and biological properties. J. Antibiotics 47: 723～728, 1994
3) KIM, Y. K.; H. TOMODA, H. NISHIDA, T. SUNAZUKA, R. OBATA & S. ŌMURA: Pyripyropenes, novel inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. II. Structure elucidation of pyripyropenes A, B, C and D. J. Antibiotics 47: 729～737, 1994
4) TOMODA, H.; H. NISHIDA, Y. K. KIM, R. OBATA, T. SUNAZUKA, S. ŌMURA, J. BORDNER, M. GUADLLANA, P. G. DORMER & A. B. SMITH III: Relative and absolute stereochemistry of pyripyropene A, a potent, bioavailable inhibitor of acyl-CoA: cholesterol acyltransferase (ACAT). J. Am. Chem. Soc. 116: 12097～12098, 1994
5) TOMODA, H.; H. NISHIDA, R. MASUMA, J. CAO, S. OKUDA & S. ŌMURA: Purpactins, new inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Penicillium purpurogenum*. I. Production, isolation and physicochemical and biological properties. J. Antibiotics 44: 136～143, 1991
6) TOMODA, H.; X.-H. HUANG, J. CAO, H. NISHIDA, R. NAGAO, S. OKUDA, H. TANAKA, S. ŌMURA, H. ARAI & K. INOUE: Inhibition of acyl-CoA: cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. J. Antibiotics 45: 1202～1206, 1992