Pure Appl. Chem., Vol. 71, No. 6, pp. 1059–1064, 1999.
Printed in Great Britain.

Meroterpenoids with various biological activities produced by fungi*

Kazuro Shiomi,† Hiroshi Tomoda, Kazuhiko Otoguro and Satoshi Ōmura

Research Center for Biological Function, The Kitasato Institute, Tokyo 108-8642, Japan

Abstract: Acyl-CoA:cholesterol acyltransferase inhibitors (pyripyropenes), acetylcholinesterase inhibitors (arisugacins), and protein farnesyltransferase inhibitors (andrastins) have been isolated from fungi. They are all biosynthesized from terpene and polyketide and called meroterpenoids (mixed polyketide-terpenoids). Although pyripyropenes and arisugacins have the same skeleton, they showed highly selective inhibition against the respective target enzymes. Pyripyropene A is biosynthesized from a sesquiterpene, a diketide, and a nicotinic acid. Andrastin A is biosynthesized from a sesquiterpene and a tetraketide. It enhanced drug accumulation and is suggested to interact with P-glycoprotein.

During the screening for bioactive compounds from microbial origin, we have found pyripyropenes A–R as acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors, arisugacins A and B as acetylcholinesterase (AChIE) inhibitors, and andrastins A–D as protein farnesyltransferase (PFT) inhibitors. They are all fungal metabolites biosynthesized from terpene and polyketide and called meroterpenoids.

Meroterpenoids are initially proposed by Cornforth as 'Compounds containing terpenoid elements along with structures of different biosynthetic origin' [1]. Recently, Simpson called meroterpenoids as more limited group, 'Compounds of mixed polyketide-terpenoid origins' [2]. We use the term 'meroterpenoids' as the definition of Simpson in this paper.

PYRIPYROPENES

ACAT plays important roles in the following three events: contribution to the atherosclerotic process; absorption of dietary cholesterol from intestines; and lipoprotein synthesis in liver and accumulation of cholesteryl esters as oil droplets within macrophages and smooth muscle cells of developing arterial lesions. Therefore, ACAT inhibitors are expected to retard the progression of atherosclerosis.

Pyripyropenes A–R were found from the culture broth of *Aspergillus fumigatus* FO-1289 as ACAT inhibitors by an enzyme assay using rat liver microsomes [3–5]. The structures were elucidated by NMR, and the absolute stereochemistry was confirmed by X-ray crystallography and Mosher's method using MTPA derivative of pyripyropene A [6]. Pyripyropenes have a common structure consisting of naphtho[2,1-*b*]pyrano[3,4-*e*]pyran and pyridine (Fig. 1, Table 1). ACAT inhibitory activity (IC_{50}) of pyripyropenes A and C was about 0.15 μM, and they were the most potent ACAT inhibitors of natural origin. ACAT inhibitory activity of pyripyropenes A–R suggested that *O*-acyl residues of R_1 and R_2 were essential for ACAT inhibition. *O*-acetyl residues of R_1 and R_3 were better than *O*-propionyl residues, and hydroxyls of R_4 were better for the inhibition. Over 300 derivatives of pyripyropenes have been synthesized. Some of them showed the inhibition at the order of nM such as PR-86, PR-45, and PR-109 (Fig. 2) [7]. From *in vivo* experiments using hamsters, PR-86 (ED_{50}, 10 mg/kg) was found to be about 10

* Invited Lecture presented at the 21st IUPAC International Symposium on The Chemistry of Natural Products (ISCNP-21), Beijing, China, 11–16 October 1998, pp. 1025–1166.
†*Corresponding author.*

Table 1 ACAT inhibitory activities of pyripyropenes (see Fig. 1 for structures)

Pyripyropene	R_1	R_2	R_3	R_4	ACAT inhibition IC_{50} (μM)
A	OAc	OAc	H	OH	0.16
B	OPr	OAc	H	OH	0.32
C	OAc	OPr	H	OH	0.15
D	OAc	OAc	Me	OH	0.74
E	H	H	H	H	399
F	H	H	Me	H	559
G	H	H	H	OH	221
H	H	H	Me	OH	270
I	OPr	OPr	Me	OH	2.45
J	OAc	OPr	Me	OH	0.85
K	OPr	OAc	Me	OH	2.65
L	OPr	OPr	H	OH	0.27
M	OAc	OPr	H	H	3.80
N	OPr	H	Me	OH	48.0
O	OAc	H	H	H	11.0
P	OPr	H	H	H	44.0
Q	OPr	H	H	OH	40.0
R	OAc	H	Me	H	78.0

Pr: propionyl.

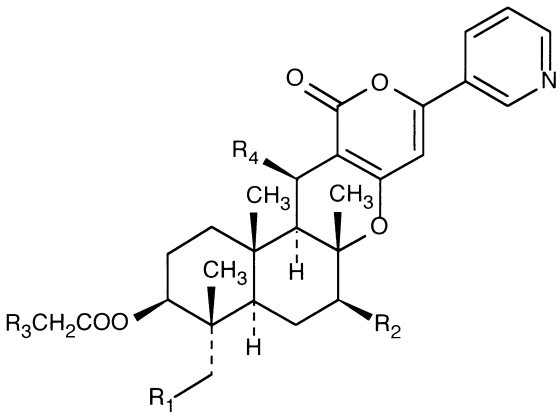

Fig. 1 Structures of pyripyropenes (see Table 1 for R_1–R_4).

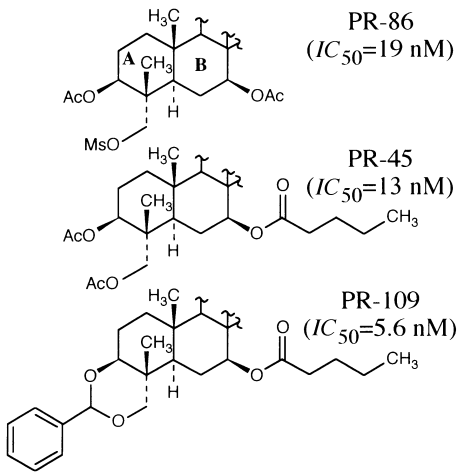

Fig. 2 Pyripyropene derivatives and their ACAT inhibitory activities.

times more effective than pyripyropene A (ED_{50}, about 100 mg/kg) in inhibition of cholesterol absorption from intestines.

The chromophore of pyripyropenes, naphtho[2,1-*b*]pyrano[3,4-*e*]pyran, is a unique one. Arisugacin-territrem group compounds, which are shown below, are the only compounds having the same chromophore. Therefore, we are interested in the biosynthesis of pyripyropenes. Incorporation of ^{13}C-labeled precursors and degradation experiments revealed that a primer nicotinic acid condensed with two acetates in a head-to-tail fashion to form the rings D and E, which was linked with a sesquiterpene (rings A and B) to form the chromophore (Fig. 3). Then, three acetyl residues were introduced into the chromophore [8].

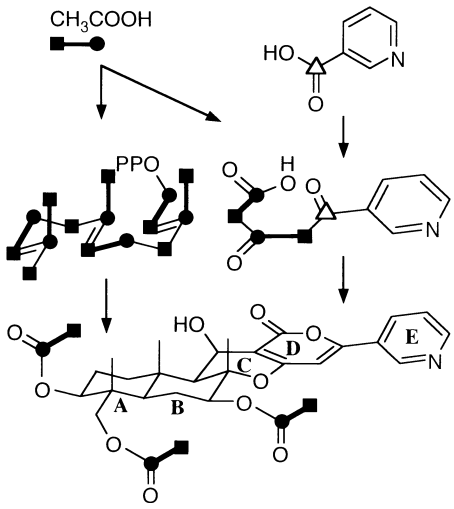

Fig. 3 Biosynthesis of pyripyropene A.

Table 2 AChE inhibitory activities of arisugacins (see Fig. 4 for structures)

				IC_{50} (μM)	
	R_1	R_2	R_3	AChE	BuChE
Arisugacin A	OCH_3	OCH_3	H	1.0	>21 000
Arisugacin B	H	OCH3	H	25.8	>516 000
Territrem A	–OCH_2O–		OCH_3	nt	nt
Territrem B	OCH_3	OCH_3	OCH_3	7.6	>20 000
Territrem C	OCH_3	OH	OCH_3	6.8	>26 000
Tacrine				200	12.0

nt: not tested.

ARISUGACINS

An AChE inhibitor, tacrine, is used for the treatment of Alzheimer's disease, though it suffers from dose-limiting side-effects. Clinical data of selective and nonselective AChE inhibitors suggested that the butyrylcholinesterase (BuChE) inhibition may be associated with peripheral side-effects. In the course of screening for selective AChE inhibitors, we isolated arisugacins A and B together with territrems B and C from *Penicillium* sp. FO-4259 [9–11]. Territrems were isolated as tremorgenic substances and lately reported to show AChE inhibition [12,13]. The structures of arisugacins were elucidated by NMR (Fig. 4, Table 2) [14]. Arisugacins and territrems have a common structure consisting of naphtho[2,1-*b*]pyrano[3,4-*e*]pyran and benzene. Though both pyripyropene group and arisugacin-territrem group have the same chromophore, they showed highly selective inhibition against the respective target enzymes as

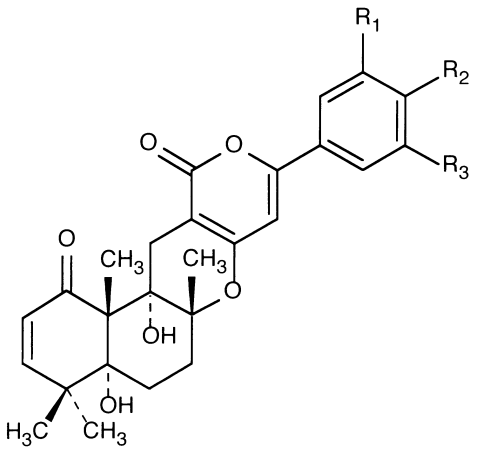

Fig. 4 Structures of arisugacins (also see Table 2).

Table 3 Enzyme inhibition of pyripyropene and arisugacin

	IC_{50} (μM)	
	ACAT	AChE
Pyripyropene A	0.16	>170
Arisugacin A	165	0.001

shown in Table 3. Arisugacin A inhibited AChE with an IC_{50} value of 1.0 nM, but did not inhibit BuChE even at 20 μM. It is expected to be effective for the treatment of Alzheimer's disease.

ANDRASTINS

Ras proteins are subjected to post-translational farnesylation at a cysteine residue, the fourth from the *C*-terminus, in which PFT is involved. Inhibition of PFT would alter membrane localization and activation of Ras proteins, and PFT is thought to be a good target for cancer chemotherapy.

Andrastins were isolated from the cultured broth of *Penicillium* sp. FO-3929 as PFT inhibitors [15,16]. Though ^{1}H-^{1}H COSY and HMBC revealed rings A, B and C of andrastin A, remaining part could not be assigned. The keto-enol tautomerism at ring D made hard to elucidate the structure, and it was revealed by ^{13}C-^{13}C spin decoupling experiments using ^{13}C-labeled andrastin A. The absolute configuration of the *p*-bromobenzoyl derivative of andrastin A was elucidated by X-ray crystallographic analysis, and its skeleton was shown to be *ent*-5α,14β-androstane (Fig. 5) [17,18]. Though inhibitory activity of andrastins against PFT was moderate (IC_{50} = 13–47 μM), another activity of andrastin A was found

	R_1	R_2
Andrastin A	OAc	CHO
Andrastin B	OAc	CH_2OH
Andrastin C	OAc	CH_3
Andrastin D	= O	CH_3

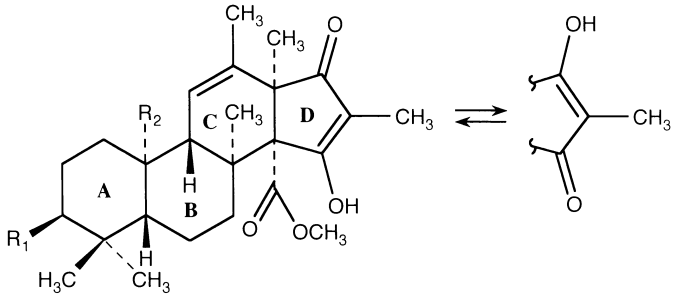

Fig. 5 Structures of andrastins.

lately. It enhanced the cytotoxicity of vincristine in vincristine-resistant cells. It is suggested that andrastin A directly interacts with P-glycoprotein and inhibits the efflux of anti-tumor agents in drug resistant cells [19].

We were interested in the biosynthesis of andrastins, because those skeletons were enantiomer of androstanes. Incorporation of ^{13}C-labeled acetates into andrastin A revealed that the acetate arrangement was the same as that of citreohybridones having the same skeletons as andrastins. From the biosynthetic study of citreohybridones [20], the following pathway was suggested as the biosynthesis of andrastin A (Fig. 6). A farnesyl pyrophosphate was cyclized to form an enantiomer of drimane. 3,5-Dimethylorsellinate (ring D) derived from tetraketide was combined with drimane, and they form ring C. Ring D changed from cyclohexane to cyclopentane by a rearrangement. Thus, an enantiomer of 5α,14β-androstane skeleton was made. Many compounds have been reported as derived from farnesylorsellinate [2]. However their absolute configurations have not been studied. Therefore, some of their configuration may be different from reported one, because andrastin A was derived from an enantiomer of drimane.

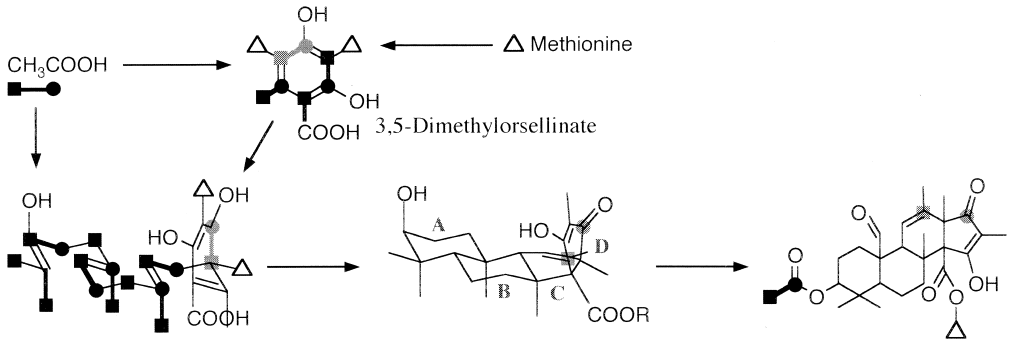

Fig. 6 Biosynthesis of andrastin A.

REFERENCES

1 J. W. Cornforth. *Chem. Br.* **4**, 102–106 (1968).

2 T. J. Simpson. *Chem. Soc. Rev.* **16**, 123–160 (1987).

3 H. Tomoda, Y. K. Kim, H. Nishida, R. Masuma, S. Ōmura. *J. Antibiot.* **47**, 148–153 (1994).

4 H. Tomoda, N. Tabata, D.-J. Yang, H. Takayanagi, H. Nishida, S. Ōmura. *J. Antibiot.* **48**, 495–503 (1995).

5 H. Tomoda, N. Tabata, D.-J. Yang, I. Namatame, H. Tanaka, S. Ōmura. *J. Antibiot.* **49**, 292–298 (1996).

6 H. Tomoda, H. Nishida, Y. K. Kim, R. Obata, T. Sunazuka, S. Ōmura, J. Bordner, M. Guadllana, G. Dormer, A. B. Smith III. *J. Am. Chem. Soc.* **116**, 12097–12098 (1994).

7 R. Obata, T. Sunazuka, Z. Li, Z. Tian, Y. Harigaya, N. Tabata, H. Tomoda, S. Ōmura. *J. Antibiot.* **49**, 1133–1148 (1996).

8 H. Tomoda, N. Tabata, Y. Nakata, H. Nishida, T. Kaneko, R. Obata, T. Sunazuka, S. Ōmura. *J. Org. Chem.* **61**, 882–886 (1996).

9 S. Ōmura, F. Kuno, K. Otoguro, T. Sunazuka, K. Shiomi, R. Masuma, Y. Iwai. *J. Antibiot.* **48**, 745–746 (1995).

10 F. Kuno, K. Otoguro, K. Shiomi, Y. lwai, S. Ōmura. *J. Antibiot.* **49**, 742–747 (1996).

11 K. Otoguro, F. Kuno, S. Ōmura. *Pharmacol. Ther.* **76**, 45–54 (1997).

12 K. H. Ling, C.-K. Yang, F.-T. Peng. *Appl. Environ. Microbiol.* **37**, 355–357 (1979).

13 P. F. Dowd, F. C. Peng, J. W. Chen, K. H. Ling. *Entomol. Exp. Appl.* **65**, 57–64 (1992).

14 F. Kuno, K. Shiomi, K. Otoguro, T. Sunazuka, S. Ōmura. *J. Antibiot.* **49**, 748–751 (1996).

15 S. Ōmura, J. Inokoshi, R. Uchida, K. Shiomi, R. Masuma, T. Kawakubo, H. Tanaka, Y. lwai, S. Kosemura, S. Yamamura. *J. Antibiot.* **49**, 414–417 (1996).

16 R. Uchida, K. Shiomi, J. Inokoshi, H. Tanaka, Y. Iwai, H. Takayanagi, S. Ōmura. *J. Antibiot.* **49**, 1278–1280 (1996).

17 K. Shiomi, R. Uchida, J. Inokoshi, H. Tanaka, Y. Iwai, S. Ōmura. *Tetrahedron Lett.* **37**, 1265–1268 (1996).

18 R. Uchida, K. Shiomi, J. Inokoshi, T. Sunazuka, H. Tanaka, Y. Iwai, H. Takayanagi, S. Ōmura. *J. Antiblot.* **49**, 418–424 (1996).

19 M.-C. Rho, M. Toyoshima, M. Hayashi, R. Uchida, K. Shiomi, K. Komiyama, S. Ōmura. *J. Antibiot.* **51**, 68–72 (1998).

20 S. Kosemura, H. Miyata, S. Yamamura, K. Albone, T. J. Simpson. *J. Chem. Soc. Perkin Trans. 1*, 135–139 (1994).