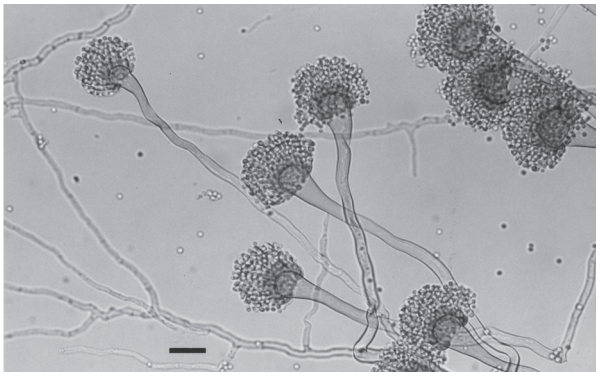
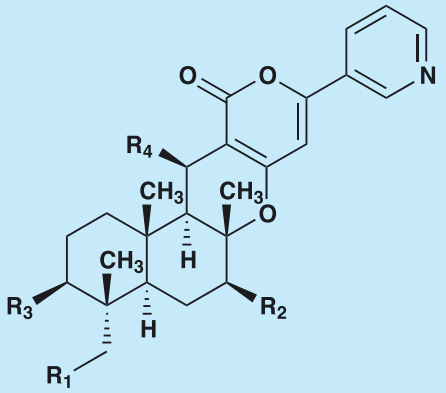
Pyripyropene ©

1. Discovery, producing organism and structures[1-6)]

Pyripyropenes were isolated from the culture broth of the fungal strain FO-1289 and found by an enzyme assay to be inhibitors of acyl-CoA:cholesterol acyltransferase (ACAT). The structures were elucidated by spectral analysis, including NMR[3,5)]. The relative and absolute stereochemistries were confirmed by NOE and X-ray crystallographic analysis for pyripyropenes A[4)] and E[5)]. Pyripyropenes have a common structure consisting of pyridine, α-pyrone and sesquiterpene moieties.



Aspergillus fumigatus FO-1289
Bar: 20 μm



Pyripyropene	R_1	R_2	R_3	R_4
A	$-OCOCH_3$	$-OCOCH_3$	$-OCOCH_3$	$-OH$
B	$-OCOCH_2CH_3$	$-OCOCH_3$	$-OCOCH_3$	$-OH$
C	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OCOCH_3$	$-OH$
D	$-OCOCH_3$	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OH$
E	$-H$	$-H$	$-OCOCH_3$	$-H$
F	$-H$	$-H$	$-OCOCH_2CH_3$	$-H$
G	$-H$	$-H$	$-OCOCH_3$	$-OH$
H	$-H$	$-H$	$-OCOCH_2CH_3$	$-OH$
I	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	$-OH$
J	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	$-OH$
K	$-OCOCH_2CH_3$	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OH$
L	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	$-OCOCH_3$	$-OH$
M	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OCOCH_3$	$-H$
N	$-OCOCH_2CH_3$	$-H$	$-OCOCH_2CH_3$	$-OH$
O	$-OCOCH_3$	$-H$	$-OCOCH_3$	$-H$
P	$-OCOCH_2CH_3$	$-H$	$-OCOCH_3$	$-H$
Q	$-OCOCH_2CH_3$	$-H$	$-OCOCH_2CH_3$	$-OH$
R	$-OCOCH_3$	$-H$	$-OCOCH_2CH_3$	$-H$

2. Physical data (Pyripyropene A)[3)]

White powder. $C_{31}H_{37}NO_{10}$; mol wt 583.24. Sol. in MeOH, EtOAc, $CHCl_3$. Insol. in H_2O, hexane.

3. Biological activity[1,2,4,7-15,20,22,23)]

1) Enzyme assay for ACAT inhibition[1,2,7)]

ACAT inhibitory activity (See also "Purpactin") was tested in an enzyme assay using rat liver microsomes. Pyripyropenes are the most potent ACAT inhibitors of microbial origin.

Compound		IC_{50} (μM)	Compound		IC_{50} (μM)
Pyripyropene	A	0.058	Pyripyropene	K	2.65
	B	0.117		L	0.27
	C	0.053		M	3.80
	D	0.268		N	48.0
	E	399		O	11.0
	F	559		P	40.0
	G	221		Q	40.0
	H	270		R	78.0
	I	2.45	CL-283,546*		1.30
	J	0.85			

*Synthetic ACAT inhibitor having a urea structure[7)]

2) Selectivity in inhibition of ACAT isozymes.

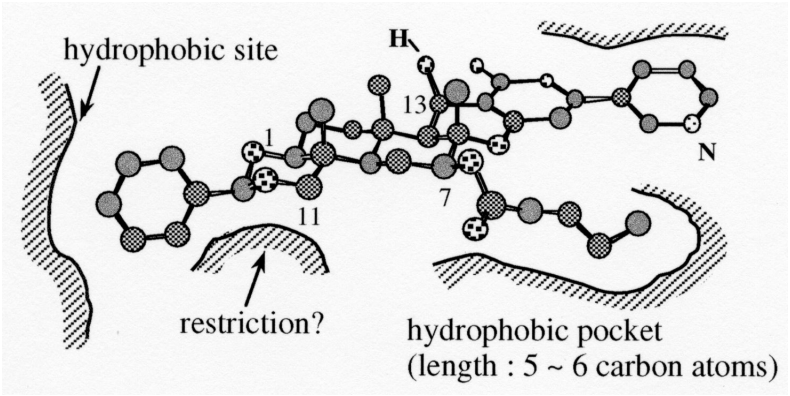
Recently two ACAT isozymes, ACAT 1 and ACAT 2, have heer identified in various mammals. ACAT 1 is ubiquitous and is expressed more strongly in sebaceous glad, steroidogenic tissues and macrophages, while ACAT 2 is expressed in the bver and intestine.

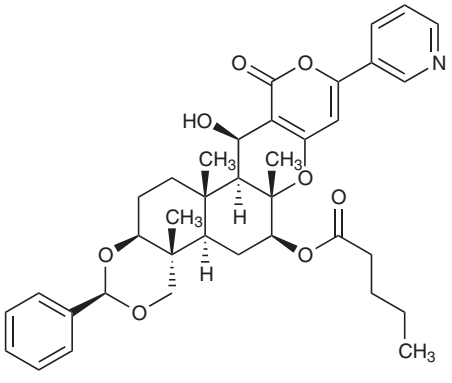
		IC_{50}(nM)	
		ACAT 1	ACAT 2
Pyripyropene	A	>80	0.07
	B	48	2.0
	C	32	0.36
	D	38	1.5

Cell-based assays were established by using ACAT 1- and ACAT 2- expressing CHO cells to evaluate the selectivity of ACAT inhibitors toward the two isozymes. Pyripyropenes were found to be the first inhibitors selective toward the ACAT 2 isozyme. In particular, pyripyropene A shows the highest selectivity among ACAT inhibitors so far reported. Research for more potent and selective pyripyropene derivatines is extensively studied for development of a new type of anti-atheroscrelatie agents.

3) Structure-activity relationship[8-12)]

Comparison of ACAT inhibitory activity among natural pyripyropenes and synthetic derivatives[8-12)] revealed the structure-activity relationship. A proposed model for binding of a derivative PR-109 to ACAT is shown below.

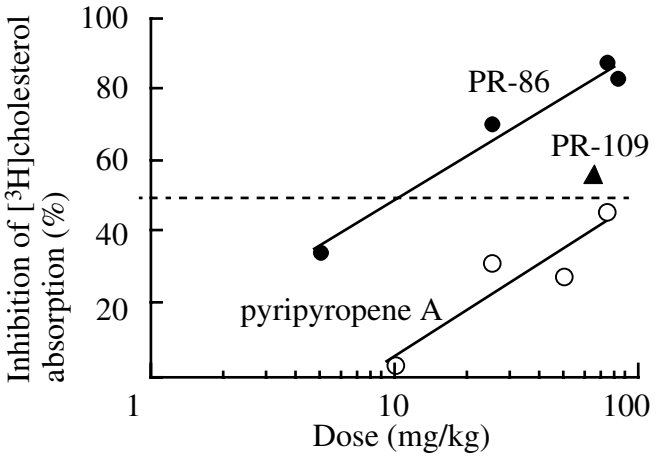


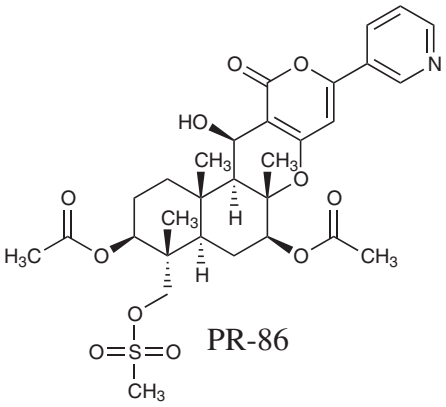


PR-109

4) *In vivo* evaluation[4)]

In vivo efficacy of pyripyropene A and synthetic derivatives were evaluated in a hamster model. PR-86 showed more potent inhibition of cholesterol absorption from the intestines (ED_{50}, 10 mg/kg) than pyripyropene A (ED_{50}, about 100 mg/kg).





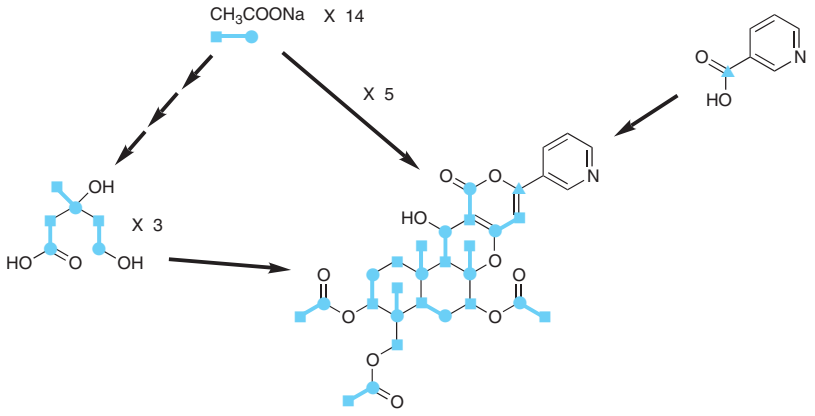
5) Inhibition of multidrug resistant P-glycoprotein by a semi-synthetic analog of pyripyropene.[14,15)]

7-*O*-Benzoylpyripyropene (7-*O*-BzP) (6.25 μg/ml) effectively reverses P-glycoprotein-related MDR by interacting directly with P-glycoproteins in drug resistant VJ-300 and P388/ADR cells.

4. Biosynthesis of pyripyropene A[16,24,25)]

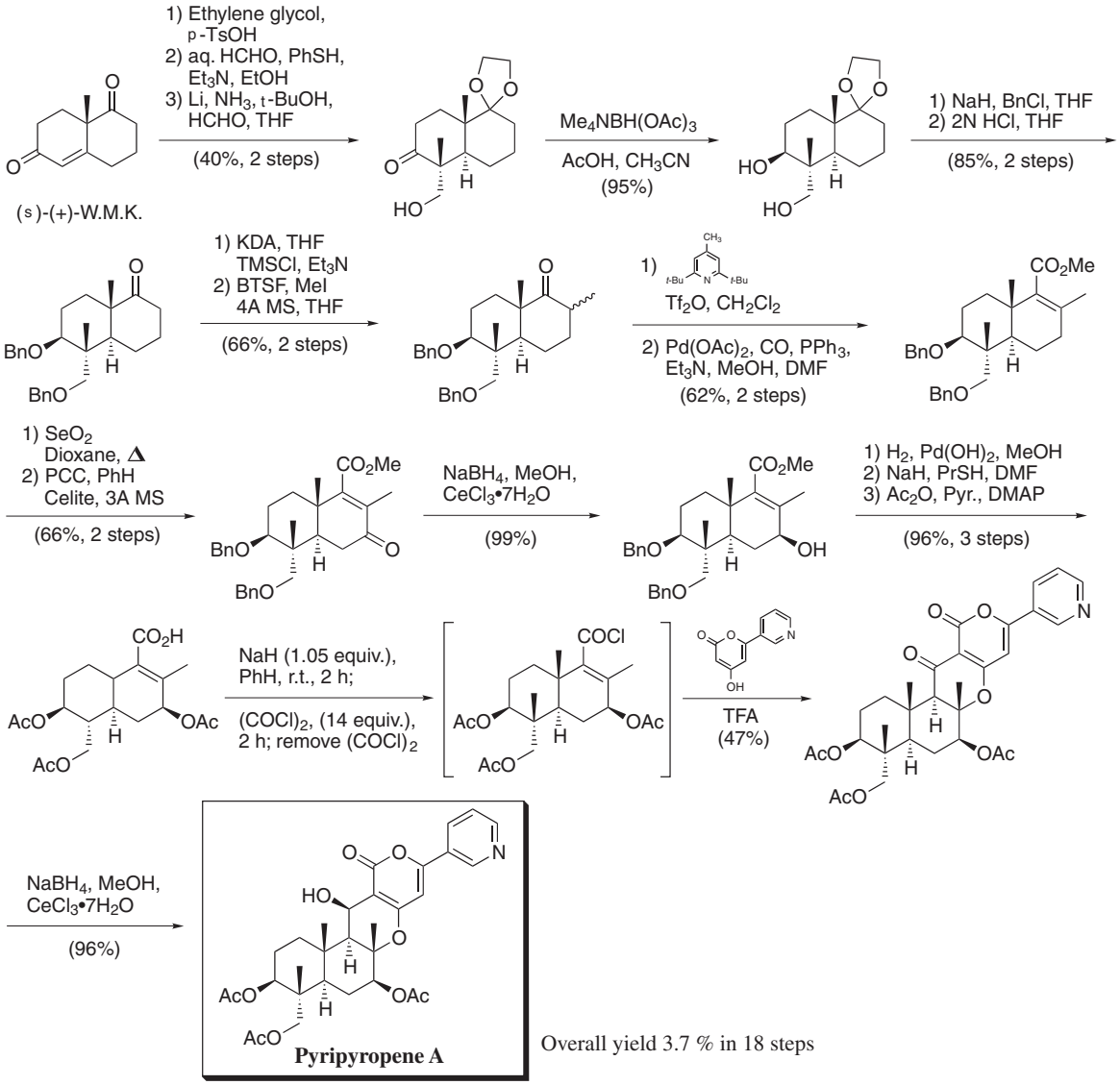
Incorporation of ^{13}C-labeled precursors and degradation experiments revealed that a nicotinic acid primer condenses with two acetates in a head-to-tail fashion forming the pyridino-α-pyrone moiety, which is linked with a sesquiterpene forming the core skeleton. Then, three acetyl residues are introduced into the core. This is the first demonstration that an intact nicotinic acid works as an acyl primer unit for oligoketide formation in fungal secondary metabolites.

The biosynthetic gene cluster for pyripyropene was identified and the biosynthetic enzyme activities were demonstrated.



5. Total synthesis[17,18,21,26)]

The total syntheses of pyripyropenes have been reported by several groups. The following scheme is Ōmura's approach.[17)] (See Appendix-I).



Overall yield 3.7 % in 18 steps

6. References

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