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CHEMICAL MODIFICATION AND STRUCTURE-ACTIVITY RELATIONSHIPS OF PYRIPYROPENES; POTENT, BIOAVAILABLE INHIBITOR OF ACYL-CoA: CHOLESTEROL *O*-ACYLTRANSFERASE(ACAT)

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Abstract: Modification and structure-activity relationships of ACAT inhibitor pyripyropene were examined. PR-109 (**7g**) showed the most potent (IC_{50} = 6 nM) inhibitory activity. PR-86 (**2e**) also had strong inhibitory activity (IC_{50} = 19 nM) and its *in vivo* activity improved 10 times better (ED_{50} = 10 mg/kg) than that of pyripyropene A.

Introduction

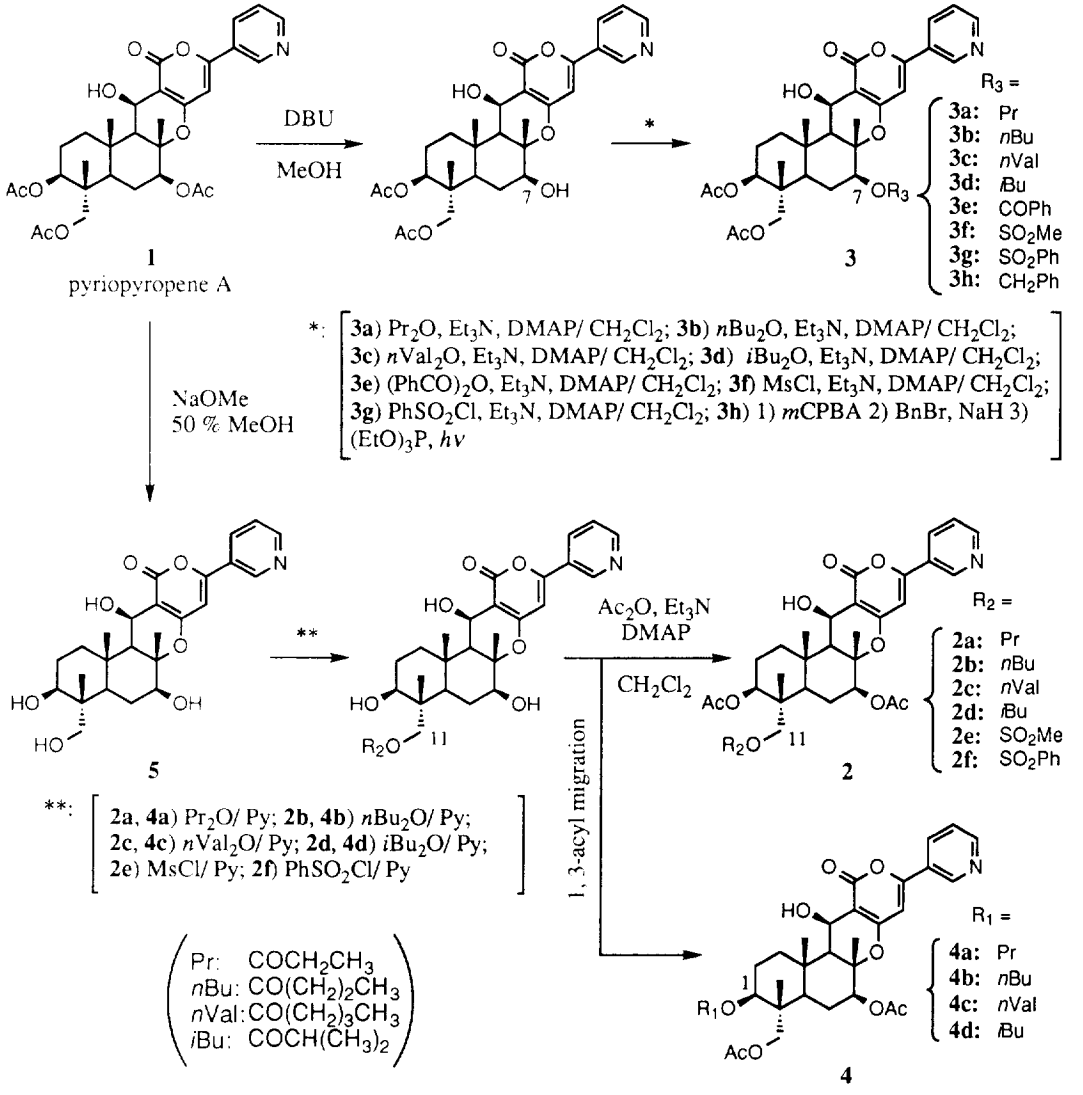
Acyl-CoA: cholesterol *O*-acyltransferase (ACAT), the enzyme responsible for intracellular esterification of cholesterol, plays a critical role in three events believed to contribute significantly to atherosclerosis: absorption of dietary cholesterol in gut, lipoprotein synthesis in liver, and accumulation of oily cholesterol esters within the macrophages and smooth muscle cells of developing arterial lesions. Inhibitors of ACAT therefore hold promise as a new type of antiatherosclerotic agents[1a-1c]. Recently, search for ACAT inhibitors from microbial origin is one of the growing areas[1d]. Pyripyropenes A (**1**), B (**2a**), C (**3a**) and D (**4a**) were isolated from the fermentation broth of *Aspergillus fumigatus* FO-1289 and shown to be potent inhibitors of ACAT (IC_{50} = 89, 270, 67 and 140 nM, respectively)[2], apparently representing the most potent naturally occurring ACAT inhibitors reported to date. Recently we determined the absolute stereochemistry of **1** by NOE difference studies, X-ray crystallographic analysis and Mosher's NMR method.[3] Importantly, **1** also proved to be orally active in hamsters with reducing cholesterol absorption, and as such they represent excellent lead compounds.

In this paper, we describe the chemical modification and biological activity of pyripyropenes.

Chemistry

Pyripyropene A (**1**) has four hydroxy groups (acetylated at 1-, 7- and 11-, free 13-hydroxy), which were possible for chemical modification. First, after selective removal of the 7-*O*-acetyl residure from **1** in the treatment with 1, 8-diazabicyclo[5, 4, 0]undec-7-ene (DBU) in methanol, different acyl, alkyl, and alkylsulfonyl groups were introduced into the position (acyl anhydride or alkylsulfonyl chloride, triethylamine, 4-dimethylaminopyridine (DMAP) in dichloromethane) to obtain **3a-3g**. To introduce benzyl group to the position (**3h**), pyridine was protected as *N*-oxide followed by alkylation with benzyl bromide, and *N*-oxide was removed

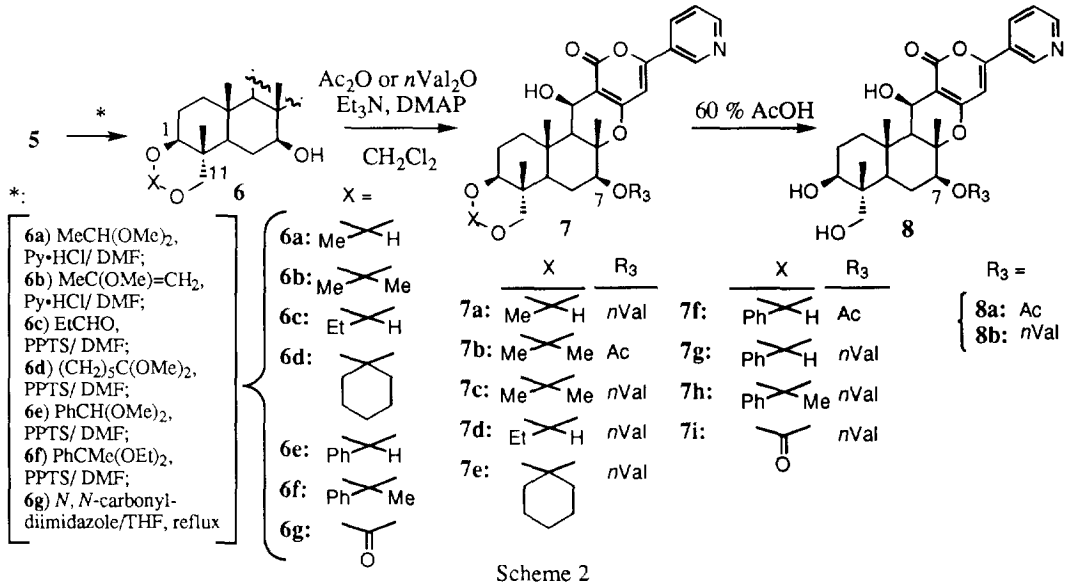
by irradiation in the presence of triethylphosphite[4]). Next, three acetyl groups of **1** were removed to afford tetraol (**5**) in the treatment with sodium methoxide in 50 % methanol. Selective acylation (a stoichiometric acyl anhydride in pyridine) at 11-position followed by diacetylation (acetic anhydride, triethylamine, DMAP in dichloromethane) gave the mixture of **2a-2d**, and **4a-4d** which were obtained by 1, 3-acyl migration. Furthermore, selective alkylsulfonylation (alkylsulfonyl chloride in pyridine) followed by diacetylation gave 1-*O*-alkylsulfonyl-1, 7-di-*O*-acetyl derivatives (**2e** and **2f**) (Scheme 1).



Scheme 1

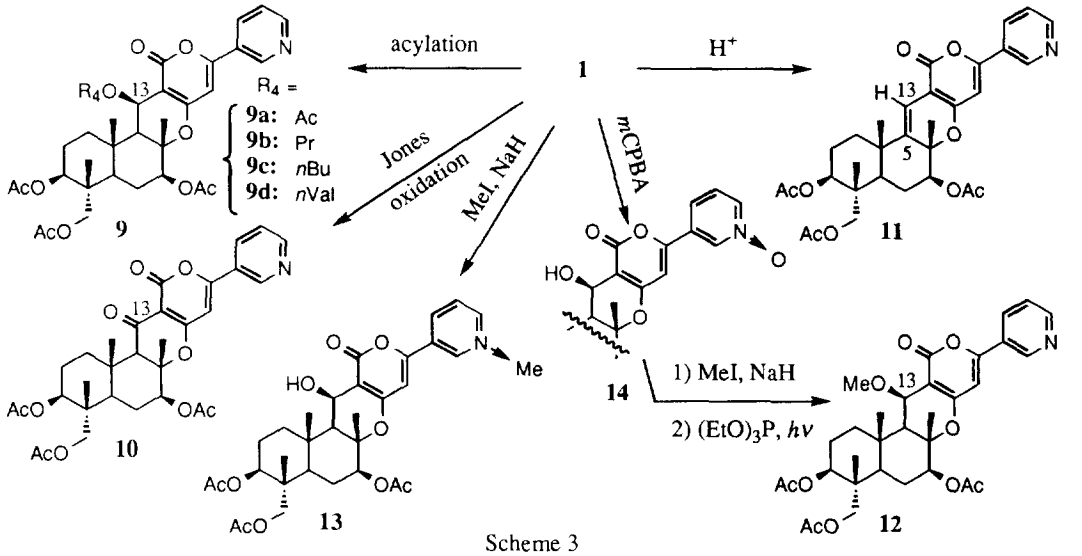
1, 11-Cyclic acetal-7-*O*-acyl derivatives (**7a-7h**) were prepared from **5** in the treatment with corresponding aldehyde or dimethylacetal in the presence of catalytic amount of pyridine•HCl or pyridinium-*p*-

toluenesulfonate (PPTS) in DMF, followed by acetylation. 1, 11-Cyclic carbonate (**7i**) was prepared from **5** in the treatment with *N*, *N*-carbonyldiimidazole in THF at reflux, followed by acylation. Cleavage of cyclic acetal of **7** gave 7-*mono*-*O*-acylated derivatives (**8a** and **8b**) (Scheme 2).



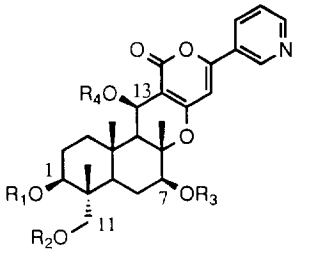
Scheme 2

On the other hand, **1** was treated with excess acyl anhydride in the presence of DMAP to obtain 13-*O*-acyl derivatives (**9a-9d**). Jones oxidation of **1** gave 13-keto derivative (**10**). Treatment of **1** with HCl under anhydrous condition gave olefin derivative (**11**). Pyridine of **1** was protected as *N*-oxide (**14**) in the treatment with 3-chloroperoxybenzoic acid (*m*CPBA) followed by methylation (methyl iodide, sodium hydride), and deprotection (*hν*, triethylphosphite) to obtain 13-*O*-methyl derivative (**12**). Without protection of pyridine, methylation of **1** gave *N*-methyl derivative **13** (Scheme 3).



Scheme 3

Table 1 Structures and ACAT inhibitory activity of pyripyropene derivatives



Category	Compound		R_1	R_2	R_3	R_4	IC_{50} (μM)
	Pyripyropene A	**1**	Ac	Ac	Ac	H	0.089
		5	H	H	H	H	>220
	Pyripyropene D	**4a**	Pr	Ac	Ac	H	0.14
		4b	*n*Bu	Ac	Ac	H	0.20
		4c	*n*Val	Ac	Ac	H	0.62
		4d	*i*Bu	Ac	Ac	H	0.13
	Pyripyropene B	**2a**	Ac	Pr	Ac	H	0.27
		2b	Ac	*n*Bu	Ac	H	4.2
I		**2c**	Ac	*n*Val	Ac	H	>8.0
		2d	Ac	*i*Bu	Ac	H	5.9
	Pyripyropene C	**3a**	Ac	Ac	Pr	H	0.067
		3b	Ac	Ac	*n*Bu	H	0.038
	PR-45	**3c**	Ac	Ac	*n*Val	H	0.013
		3d	Ac	Ac	*i*Bu	H	0.13
		9a	Ac	Ac	Ac	Ac	5.1
		9b	Ac	Ac	Ac	Pr	23
		9c	Ac	Ac	Ac	*n*Bu	2.4
		9d	Ac	Ac	Ac	*n*Val	16
		3e	Ac	Ac	COPh	H	0.085
II		**3f**	Ac	Ac	SO_2Me	H	1.8
		3g	Ac	Ac	SO_2Ph	H	3.1
		3h	Ac	Ac	CH_2Ph	H	7.4
		6b	$>CMe_2$		H	H	>200
		6e	>CHPh		H	H	>180
		7a	>CHMe		*n*Val	H	0.025
		7b	$>CMe_2$		Ac	H	1.2
		7c	$>CMe_2$		*n*Val	H	0.086
III		**7d**	>CHEt		*n*Val	H	0.033
		7e	$>C(CH_2)_5$		*n*Val	H	0.039
		7f	>CHPh		Ac	H	0.12
	PR-109	**7g**	>CHPh		*n*Val	H	0.006
		7h	>CMePh		*n*Val	H	0.91
		7i	>C=O		*n*Val	H	2.5
		8a	H	H	Ac	H	100
		8b	H	H	*n*Val	H	79
IV	PR-86	**2e**	Ac	SO_2Me	Ac	H	0.019
		2f	Ac	SO_2Ph	Ac	H	110

Biological Results and Discussion

Structure and ACAT inhibitory activity[5] of pyripyropene derivatives are shown in Tables 1 and 2. Relationships between acyl groups of each position (R_1-R_4) in their structures and ACAT inhibitory activity were studied in comparison with **1** (Table 1, Category I). Hydrolysis of all acetyl groups (**5**) lost the activity. Introduction of a longer acyl chain into C-1 position lowered its activity but did not show clear relationships between acyl sizes and their activity (**4a-4d**). On the other hand, C-11 and C-13 positions marked structural relationships with activity. Introduction of a longer acyl chain resulted in 1/100 week activity (**2b-2d** and **9a-9d**), which suggests large acyl groups are undesirable to these positions. On the contrary, the 7-*O*-acyl derivatives which have a longer acyl chain showed stronger activity (**3a-3d**). Among them, 7-*O*-valeryl derivative PR-45 (**3c**) was found as the most potent inhibitor of this series[6].

Furthermore, 7-*O*-benzyl derivative (**3h**) was 100 times less potent than the corresponding acyl derivative (**3e**), and 7-*O*-alkylsulfonyl derivatives (**3f, 3g**) also resulted in decreasing activity. This result suggests the carbonyl group plays an important role to show potent activity (Table 1, Category II).

Concerning structure-activity relationships of various 1,11-cyclic acetal derivatives (Table 1, Category III), 7-*O*-valeryl derivatives (**7a**, **7c-7e**, **7g** and **7h**) showed good or better activity than **1**, although 1, 11-cyclic carbonate (**7i**) showed much less activity. Especially, PR-109 (**7g**) was 14 times more potent than **1** as an ACAT inhibitor. 1,11-Cyclic acetal with 7-hydroxyl derivatives (**6b** and **6e**) had no activity, but 7-*O*-*n*-valeryl derivatives (**7c**, **7g**) showed potent activity, suggested that 7-*O*-*n*-valeryl group plays an important role in exhibiting potent inhibitory activity. However, 1, 11-dihydroxyl derivatives (**8a**, **8b**) had almost no activity. This suggested the substituent groups at both 1 and 11-positions are also necessary.

Methanesulfonyl derivative (**2e**) showed potent inhibitory activity, but phenyl sulfonyl derivative (**2f**) had almost no activity (Table 1, Category IV).

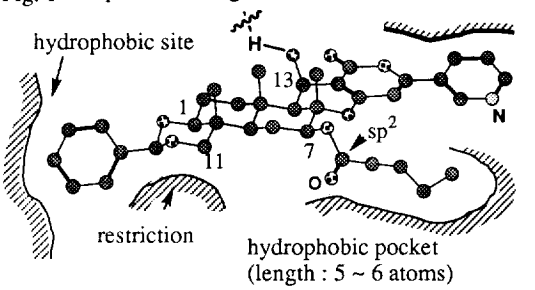
Modification of 13-hydroxy as ketone (**10**), olefin (**11**) or methoxy (**12**), resulted as week activity, suggested the importance of 13-hydroxyl to show potent activity (Table 2). Also, pyridine *N*-methyl derivative (**13**), and *N*-oxide derivative (**14**) were resulted as week activity, suggested the importance of pyridine to show potent activity.

Table 2

Compound	IC_{50} (μM)
10	2.1
11	5.8
12	38
13	2.1
14	5.8

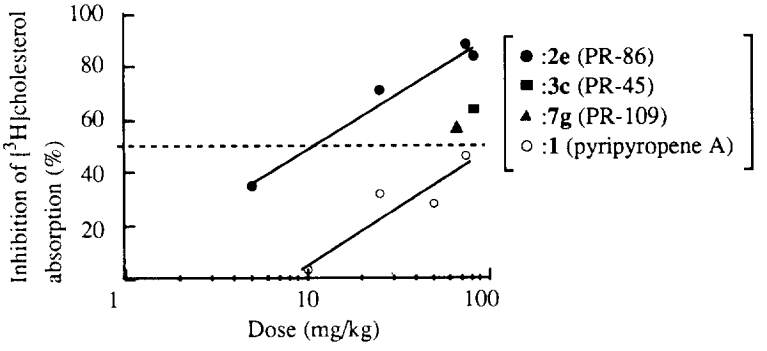
Based on the structure-activity relationships described above, we propose here the binding model of the most potent derivative PR-109 (**7g**) to ACAT (Fig. 1). The phenyl group of 1, 11-benzylidene acetal moiety is located in an equatorial position, properly fitting a hydrophobic site of ACAT. Another hydrophobic pocket might exist for 7-*O*-valeryl group. 7-*O*-Valeryl including the carboxyl group, 13-hydroxy, and pyridine moieties appear to be very important for binding to ACAT.

Fig. 1 Proposed binding model of PR-109 to ACAT



PR-45 (**3c**), PR-109 (**7g**) and PR-86 (**2e**) were selected for further *in vivo* study using hamsters[2a] (Fig. 2). The activity of PR-45 and PR-109 was almost the same as that of **1**. Remarkably, the *in vivo* efficacy of PR-86 showed improved 10 times activity with an ED50 value of 10 mg/kg via single oral administration.

Fig. 2 *In vivo* efficacy in a hamster model



In conclusion, our study on modification of four hydroxyl groups in pyripyropene revealed the importance of 7-substituent group and effectiveness of 1,11-cyclic derivatives for exhibiting potent ACAT inhibitory activity. Potent ACAT inhibitors PR-109 and PR-86 were obtained (IC_{50} = 6.8 and 19 nM respectively), and especially, PR-86 improved *in vivo* activity about 10 times than pyripyropene A (**1**).

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