Chemical Modification and Structure-activity Relationships of Pyripyropenes

2. 1,11-Cyclic Analogs

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A series of 1,11-cyclic analogs of pyripyropene A were prepared. Replacement of the 1,11-acyl groups of pyripyropenes with 1,11-cyclic acetals effectively improved *in vitro* acyl CoA : cholesterol acyltransferase (ACAT) inhibitory activity. Especially noteworthy is benzylidene acetal analog **35**, the most potent inhibitor (IC_{50} = 5.6 nM) among the derivatives prepared so far, which showed 16 times more potent inhibitory activity than pyripyropene A.

Pyripyropenes were isolated from a culture broth of *Aspergillus fumigatus* as potent and bioavailable acyl CoA : cholesterol acyltransferase (ACAT) inhibitors[1]. In the preceding paper we modified the four possible hydroxy groups in pyripyropene A (**1**) and showed structure-activity relationships[2,3]. In this paper, we describe the synthesis and structure-activity relationships of 1,11-cyclic analogs of **1**.

Chemistry

The synthetic method for preparing 1,11-cyclic analogs is illustrated in Scheme 1. Trideacetyl pyripyropene A (**2**), which was obtained from **1** by hydrolysis, was acetalized with various aldehydes, ketones, dialkyl acetals, *etc.*, in the presence of catalytic acid such as pyridinium hydrochloride or pyridinium *p*-toluenesulphoate in dimethylformamide at room temperature to afford 1,11-cyclic acetal analogs **3**~**21**. Reaction conditions and spectral data (MS, IR) of compounds **3**~**21** are shown in Table 1. Prolonged reaction time or use of a strong acid catalyst such as hydrochloric acid caused elimination of the 13-hydroxyl group to form the 5, (13)-olefin derivative **23**. Cyclic carbonate **22** was obtained by refluxing **2** with *N*,*N*-carbonyldiimidazole in tetrahydrofuran[4].

The acylation of the 7-hydroxyl group of cyclic derivatives **3**~**23** was carried out with acetic anhydride or *n*-valeric anhydride in the presence of triethylamine

Scheme 1.

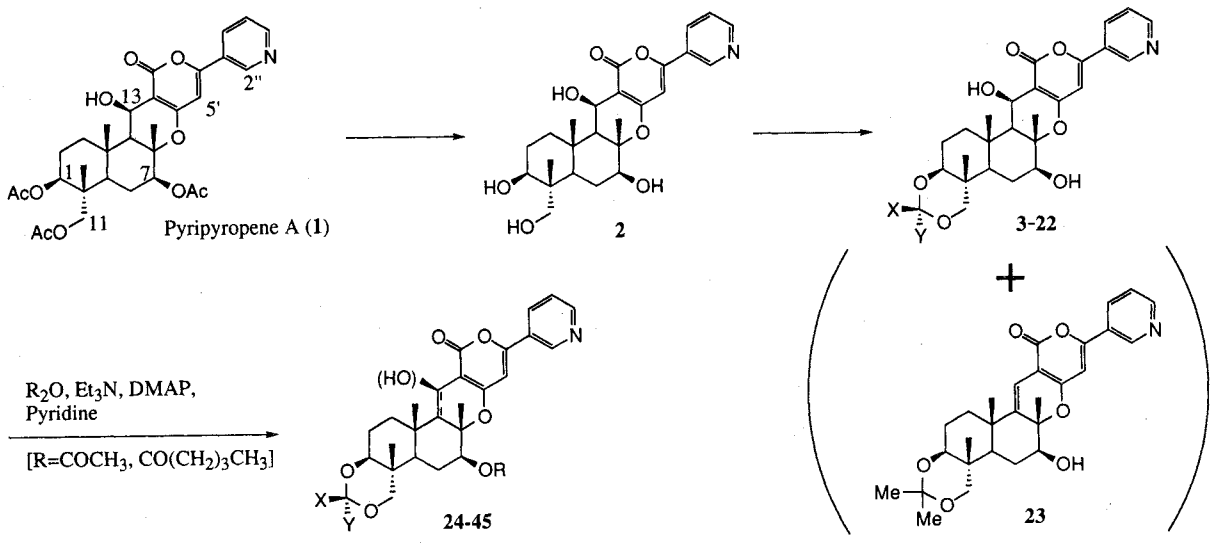


Table 1. Reagents, yield, MS and IR data for **3**~**21**.

Compound	Reagents		Yield (%)	MF	MS (FAB (+))		IR (KBr) cm^{-1}
	Reagent	cat.			Found	Calcd	
3	1,1-Dimethoxyethane	A	27	$C_{27}H_{33}O_7N$	484.2343 (M+H)	484.2335	1680, 1580
4	Isopropenylmethyl ether	A	64	$C_{28}H_{35}O_7N$	498.2486 (M+H)	498.2491	1700, 1640
5	Propionaldehyde	B	39	$C_{28}H_{35}O_7N$	498.2488 (M+H)	498.2491	1700, 1580
6	Acrolein diethyl acetal	B	32	$C_{28}H_{33}O_7N$	496.2346 (M+H)	496.2335	1700, 1580
7	Isobutylaldehyde	B	35	$C_{29}H_{37}O_7N$	512.2650 (M+H)	512.2648	1700, 1580
8	Trimethylacetaldehyde	B	17	$C_{30}H_{39}O_7N$	526.2808 (M+H)	526.2804	1700, 1580
9	1,1,1-Trimethoxymethane	A	21	$C_{27}H_{33}O_8N$	500.2310 (M+H)	500.2284	1710, 1570
10	Benzaldehyde dimethylacetal	B	83	$C_{32}H_{35}O_7N$	546.2500 (M+H)	546.2491	1700, 1580
11	(1,1-Dimethoxyethyl)benzene	B	85	$C_{33}H_{37}O_7N$	560.2636 (M+H)	560.2648	1700, 1580
12	*o*-Tolaldehyde	A	49	$C_{33}H_{37}O_7N$	560.2656 (M+H)	560.2648	1700, 1580
13	*m*-Tolaldehyde	B	39	$C_{33}H_{37}O_7N$	560.2665 (M+H)	560.2648	1700
14	*p*-Tolaldehyde	A	18	$C_{33}H_{37}O_7N$	560.2616 (M+H)	560.2648	1700
15	*o*-F-Benzaldehyde	A	19	$C_{32}H_{34}O_7NF$	564.2427 (M+H)	564.2397	1700
16	*m*-F-Benzaldehyde	B	13	$C_{32}H_{34}O_7NF$	564.2447 (M+H)	564.2397	1700, 1580
17	*p*-F-Benzaldehyde	B	12	$C_{32}H_{34}O_7NF$	560.2386 (M+H)	564.2397	1700
18	*p*-Methoxybenzaldehyde	A	88	$C_{33}H_{37}O_8N$	576.2591 (M+H)	576.2597	1700
19	*p*-Nitrobenzaldehyde	C	4	$C_{32}H_{34}O_9N_2$	591.2330 (M+H)	591.2342	1700, 1520
20	Cyclopentanone	C	20	$C_{30}H_{37}O_7N$	524.2681 (M+H)	524.2648	1700
21	1,1-Dimethoxycyclohexane	B	63	$C_{31}H_{39}O_7N$	538.2805 (M+H)	538.2804	1700

A: Pyridinium hydrochloride, B: pyridinium *p*-toluenesulfonate, C: *p*-toluenesulfonic acid.

Table 2. Yield, MS and IR data for **24**~**46**.

Compound	Yield (%)	MF	MS			IR (KBr) cm^{-1}
				Found	Calcd	
24	100	$C_{33}H_{41}O_7N$	EI (+)	563.2892 (M+)	563.2883	1740
25	61	$C_{30}H_{37}O_8N$	FAB (+)	540.2616 (M+H)	540.2597	1710, 1250
26	57	$C_{34}H_{33}O_8N$	FAB (+)	588.2615 (M+H)	588.2597	1700, 1580
27	83	$C_{33}H_{43}O_8N$	FAB (+)	582.3062 (M+H)	582.3066	1730, 1700
28	88	$C_{37}H_{43}O_8N$	FAB (+)	630.3089 (M+H)	630.3066	1730, 1710
29	65	$C_{32}H_{41}O_8N$	FAB (+)	568.2905 (M+H)	568.2910	1730
30	96	$C_{38}H_{45}O_8N$	FAB (+)	644.3218 (M+H)	644.3223	1700, 1740
31	77	$C_{36}H_{47}O_8N$	FAB (+)	622.3370 (M+H)	622.3379	1700
32	54	$C_{35}H_{45}O_8N$	FAB (+)	630.3059 (M+Na)	630.3042	1710
33	9	$C_{31}H_{37}O_9N$	FAB (+)	568.2560 (M+H)	568.2546	1740
34	66	$C_{33}H_{43}O_8N$	FAB (+)	582.3071 (M+H)	582.3066	1740, 1710
35	63	$C_{33}H_{41}O_8N$	FAB (+)	580.2913 (M+H)	580.2921	1720
36	57	$C_{34}H_{45}O_8N$	FAB (+)	596.3218 (M+H)	596.3223	1740, 1720
37	60	$C_{35}H_{47}O_8N$	FAB (+)	610.3370 (M+H)	610.3380	1740, 1700
38	58	$C_{32}H_{41}O_9N$	FAB (+)	584.2847 (M+H)	584.2860	1740, 1700
39	71	$C_{38}H_{45}O_8N$	FAB (+)	644.3160 (M+H)	644.3223	1730, 1720
40	52	$C_{38}H_{45}O_8N$	FAB (+)	644.3194 (M+H)	644.3223	1720
41	64	$C_{38}H_{45}O_8N$	FAB (+)	644.3170 (M+H)	644.3223	1700
42	40	$C_{37}H_{42}O_8NF$	FAB (+)	648.2976 (M+H)	648.2972	1720
43	40	$C_{37}H_{42}O_8NF$	FAB (+)	648.3026 (M+H)	648.2972	1730, 1710
44	50	$C_{37}H_{42}O_8NF$	FAB (+)	648.2932 (M+H)	648.2972	1740
45	95	$C_{38}H_{45}O_9N$	FAB (+)	660.3180 (M+H)	660.3172	1720
46	51	$C_{37}H_{42}O_{10}N_2$	FAB (+)	675.2945 (M+H)	675.2917	1710

(Et_3N) and 4-dimethylaminopyridine (DMAP) as base in dry dichloromethane (CH_2Cl_2) to give compounds **24**~**46**. Yield and spectral data (MS, IR) for compounds **24**~**46** are shown in Table 2.

Cleavage of isopropylidene derivatives **25** and **27** by treatment with 60% aq. acetic acid[5] yields compounds **47** and **48**, respectively.

^1H NMR data for compounds **3**~**47** are shown in Table 3.

There are two possible conformations for 6-membered

Table 3-1. ^{1}H NMR data for **3**~**17**.

	3	**4**	**5**	**6**	**7**
2″	8.99 (d, 1.7)	9.00 (d, 2.0)	8.99 (d, 1.7)	8.99 (d, 1.7)	8.98 (s)
6″	8.68 (dd, 1.7, 4.6)	8.70 (dd, 1.4, 4.3)	8.68 (dd, 1.5, 4.8)	8.68 (dd, 1.7, 5.0)	8.66 (d, 4.0)
4″	8.09 (dt, 2.0, 7.9)	8.11 (dd, 2.0, 6.9)	8.10 (dt, 2.0, 8.6)	8.10 (dt, 2.0, 8.6)	8.10 (dd, 1.7, 7.1)
5″	7.41 (dd, 4.8, 8.1)	7.42 (dd, 5.1, 7.8)	7.41 (dd, 4.6, 7.9)	7.41 (dd, 5.3, 7.6)	7.43 (dd, 5.0, 7.9)
5′	6.49 (s)	6.51 (s)	6.50 (s)	6.50 (s)	6.52 (s)
13	4.97 (d, 4.0)	4.99 (d, 2.6)	4.98 (d, 3.0)	4.99 (s)	4.96 (d, 4.0)
7	3.77 (m)	3.81 (m)	3.79 (m)	3.82 (m)	3.79 (m)
1	3.25 (m)	3.25 (m)	3.23 (m)	3.33 (m)	3.19 (m)
11	3.76 (d, 10.2)	3.54 (d, 7.6)	3.78 (d, 10.2)	3.83 (d, 10.2)	3.76 (d, 10.2)
11′	3.23 (d, 9.9)	3.44 (d, 10.9)	3.22 (d, 9.6)	3.29 (d, 9.6)	3.18 (d, 9.9)
14-Me	1.64 (s)	1.64 (s)	1.64 (s)	1.64 (s)	1.63 (s)
12-Me	1.41 (s)	1.59 (s)	1.41 (s)	1.41 (s)	1.39 (s)
15-Me	1.13 (s)	1.11 (s)	1.11 (s)	1.15 (s)	1.08 (s)
R-C*H*<	4.72 (q, 4.9)	—	4.51 (t, 4.9)	4.99 (m)	4.29 (d, 4.6)
	Me: 1.35 (3H, d, 4.9)	Me: 1.44 (3H, s), Me: 1.41 (3H, s)	CH_3CH_2: 1.64 (2H, m), CH_3CH_2: 0.95 (3H, t, 7.4)	CH_2=C*H*–: 5.90 (1H, ddd, 5.0, 10.6, 17.2), CH_2=CH–: 5.47 (1H, d, 17.5), 5.32 (1H, d, 10.6)	Me_2CH: 2.92 (1H, m), Me_2CH: 0.93 (6H, d, 6.9)

	8	**9**	**10**	**11**	**12**
2″	8.99 (s)	9.01 (d, 1.7)	9.01 (d, 2.3)	9.00 (s)	9.03 (br s)
6″	8.68 (d, 4.0)	8.70 (dd, 1.5, 4.8)	8.69 (dd, 1.5, 4.9)	8.67 (s)	8.71 (br s)
4″	8.10 (dt, 2.0, 8.6)	8.11 (dt, 2.0, 7.6)	8.11 (dt, 2.0, 7.9)	8.10 (dd, 1.5, 7.5)	8.13 (d, 7.9)
5″	7.41 (dd, 4.5, 8.1)	7.43 (dd, 4.8, 8.3)	7.42 (m)	7.41 (m)	7.44 (s)
5′	6.50 (s)	6.51 (s)	6.51 (s)	6.51 (s)	6.52 (s)
13	4.98 (d, 3.0)	4.98 (d, 3.3)	4.99 (d, 2.3)	5.00 (d, 4.3)	4.99 (d, 4.0)
7	3.80 (m)	3.80 (m)	3.82 (m)	3.80 (m)	3.83 (m)
1	3.18 (m)	3.34 (m)	3.47 (m)	3.78 (m)	3.46 (m)
11	3.78 (d, 10.2)	3.82 (d, 10.6)	3.95 (d, 10.2)	3.72 (d, 10.6)	3.94 (d, 10.2)
11′	3.17 (d, 10.6)	3.36 (d, 10.6)	3.45 (d, 10.6)	3.65 (d, 10.9)	3.46 (m)
14-Me	1.64 (s)	1.65 (s)	1.61 (s)	1.65 (s)	1.66 (s)
12-Me	1.40 (s)	1.42 (s)	1.45 (s)	1.41 (s)	1.45 (s)
15-Me	1.07 (s)	1.17 (s)	1.26 (s)	1.10 (s)	1.26 (s)
R-C*H*<	4.13 (s)	5.23 (s)	5.54 (s)	—	5.50 (s)
	Me_3: 0.91 (9H, s)	OMe: 3.52 (3H, s)	C_6H_5: 7.50 (2H, m), 7.36 (3H, m)	C_6H_5: 7.58 (2H, dd, 1.5, 8.1), 7.33 (3H, m), Me: 1.71 (3H, s)	C_6H_4: 7.14~7.32 (4H, com.), Me: 2.32 (3H, s)

	13	**14**	**15**	**16**	**17**
2″	9.06 (br s)	9.02 (s)	9.07 (s)	9.02 (s)	9.08 (s)
6″	8.71 (br s)	8.71 (s)	8.71 (s)	8.70 (s)	8.73 (s)
4″	8.18 (br s)	8.14 (d, 8.2)	8.18 (d, 7.9)	8.13 (d, 8.2)	8.12 (dd, 5.3, 8.6)
5″	7.50 (s)	7.44 (m)	7.49 (m)	7.43 (dd, 4.8, 8.2)	7.48 (m)
5′	6.55 (s)	6.53 (s)	6.56 (s)	6.52 (s)	6.57 (s)
13	5.00 (d, 4.0)	5.00 (d, 4.3)	5.00 (d, 4.0)	5.00 (d, 4.3)	5.00 (d, 3.6)
7	3.83 (dd, 6.6, 9.9)	3.83 (dd, 6.9, 9.9)	3.83 (dd, 6.4, 9.9)	3.83 (dd, 6.3, 10.2)	3.84 (dd, 6.2, 8.9)
1	3.48 (m)	3.45 (m)	3.49 (m)	3.49 (m)	3.46 (m)
11	3.96 (d, 10.2)	3.93 (d, 10.2)	3.94 (d, 10.2)	3.94 (d, 10.2)	3.94 (d, 10.5)
11′	3.48 (m)	3.45 (m)	3.49 (m)	3.49 (m)	3.46 (m)
14-Me	1.66 (s)	1.66 (s)	1.66 (s)	1.66 (s)	1.66 (s)
12-Me	1.44 (s)	1.44 (s)	1.45 (s)	1.45 (s)	1.45 (s)
15-Me	1.25 (s)	1.25 (s)	1.27 (s)	1.24 (s)	1.24 (s)
R-C*H*<	5.68 (s)	5.50 (s)	5.86 (s)	5.52 (s)	5.52 (s)
	C_6H_4: 7.63 (1H, d, 4.9), 7.12~7.24 (3H, com.), Me: 2.35 (3H, s)	C_6H_4: 7.38 (2H, d, 7.9), 7.17 (2H, d, 7.9) ,Me: 2.34 (3H, s)	C_6H_4: 7.66 (1H, dt, 1.7, 7.3), 7.32 (1H, m), 7.19 (1H, m), 7.03 (1H, dd, 8.2, 10.2)	C_6H_4: 7.21~7.35 (3H, com.), 7.04 (1H, dt, 1.3, 7.3)	C_6H_4: 8.20 (1H, d, 7.9), 7.48 (1H, m), 7.10 (2H, dt, 8.6, 14.4)

Table 3-2. ^{1}H NMR data for **18**~**32**.

	18	**19**	**20**	**21**	**22**
2″	8.99 (s)	9.15 (br s)	9.05 (br s)	8.98 (d, 2.0)	8.98 (d, 1.7)
6″	8.67 (d, 4.0)	8.74 (br s)	8.71 (br s)	8.67 (dd, 1.5, 4.8)	8.68 (dd, 1.7, 4.6)
4″	8.09 (dt, 2.0, 8.3)	8.30 (d, 7.9)	8.20 (d, 8.3)	8.10 (dt 2.0, 8.9)	8.09 (dt, 2.0, 8.3)
5″	7.40 (m)	7.59 (m)	7.51 (m)	7.41 (dd, 5.0, 7.9)	7.40 (dd, 4.8, 8.1)
5′	6.49 (s)	6.64 (s)	6.54 (s)	6.50 (s)	6.49 (s)
13	4.98 (d, 4.0)	5.00 (d, 4.0)	4.98 (d, 4.0)	4.97 (d, 4.0)	4.96 (d, 4.3)
7	3.82 (m)	3.83 (m)	3.81 (m)	3.78 (m)	4.05 (m)
1	3.44 (m)	3.49 (m)	3.37 (m)	3.55 (m)	3.82 (dd, 5.1, 11.6)
11	3.91 (d, 10.6)	3.97 (d, 10.5)	3.58 (d, 10.5)	3.52 (d, 10.9)	4.17 (d, 9.9)
11′	3.41 (d, 10.6)	3.49 (m)	3.37 (m)	3.44 (d, 10.9)	4.01 (d, 9.2)
14-Me	1.71 (s)	1.66 (s)	1.64 (s)	1.64 (s)	1.66 (s)
12-Me	1.47 (s)	1.45 (s)	1.41 (s)	1.40 (s)	1.45 (s)
15-Me	1.13 (s)	1.23 (s)	1.13 (s)	1.10 (s)	1.13 (s)
R-C*H*<	5.47 (s)	5.61 (s)	—	—	—
	C_6H_4: 7.41 (2H, d, 8.6), 6.88 (2H, d, 8.9), OMe: 3.78 (3H, s)	C_6H_4: 7.69 (2H, d, 8.9), 8.30 (2H, d, 8.9)		1.8 (2H, m), 1.5 (6H, m), 1.4 (2H, m)	

	23	**24**	**25**	**26**	**27**
2″	8.99 (s)	8.99 (d, 1.7)	9.00 (d, 2.0)	9.02 (d, 2.0)	9.00 (d, 2.0)
6″	8.66 (d, 3.6)	8.66 (dd, 1.5, 4.8)	8.68 (dd, 1.5, 4.8)	8.69 (dd, 1.7, 5.3)	8.69 (dd, 1.7, 4.5)
4″	8.11 (dt, 2.0, 8.3)	8.10 (dt, 2.0, 8.2)	8.09 (dt, 2.0, 7.9)	8.11 (dt, 2.0, 8.3)	8.09 (dt, 2.0, 8.3)
5″	7.40 (dd, 5.0, 7.9)	7.39 (dd, 4.8, 8.2)	7.39 (dd, 4.8, 8.1)	7.41 (dd, 5.6, 8.9)	7.40 (dd, 4.8, 8.1)
5′	6.53 (s)	6.45 (s)	6.52 (s)	6.47 (s)	6.40 (s)
13	6.28 (s)	6.31 (s)	4.99 (s)	5.01 (d, 4.6)	4.99 (d, 4.6)
7	4.09 (t, 8.4)	5.24 (m)	4.99 (m)	5.03 (m)	5.01 (m)
1	3.48 (m)	3.51 (dd, 5.1, 11.4)	3.53 (dd, 3.8, 11.7)	3.51 (m)	3.54 (dd, 3.8, 11.7)
11	3.56 (d, 10.6)	3.49 (s)	3.47 (s)	3.88 (d, 10.6)	3.48 (s)
11′	3.45 (d, 10.9)	3.49 (s)	3.47 (s)	3.49 (d, 10.2)	3.48 (s)
14-Me	1.63 (s)	1.56 (s)	1.68 (s)	1.71 (s)	1.68 (s)
12-Me	1.53 (s)	1.43 (s)	1.43 (s)	1.47 (s)	1.44 (s)
15-Me	1.09 (s)	1.07 (s)	1.09 (s)	1.25 (s)	1.10 (s)
R-C*H*<	—	—	—	5.54 (s)	—
	Me_2: 1.43 (6H, s)	Me_2: 1.43 (6H, s), CH_3 $(CH_2)_3$: 0.97 (3H, t, 7.3), 2.42 (2H, dt, 1.3, 7.3)	Me_2: 1.42 (6H, s), Ac: 2.16 (3H, s)	C_6H_5: 7.3~7.5 (5H, m), Ac: 2.19 (3H, s)	Me_2: 1.43 (6H, s), CH_3 $(CH_2)_3$: 0.97 (3H, t, 7.3), 2.45 (2H, dt, 1.1, 7.3)

	28	**29**	**30**	**31**	**32**
2″	9.00 (d, 2.0)	8.99 (d, 2.0)	9.02 (br s)	9.00 (s)	9.04 (br s)
6″	8.69 (dd, 1.7, 4.6)	8.68 (dd, 1.5, 4.8)	8.72 (br s)	8.68 (s)	8.71 (br s)
4″	8.10 (dt, 2.0, 8.6)	8.08 (dt, 2.0, 8.3)	8.29 (d, 8.3)	8.11 (dt, 1.9, 8.3)	8.20 (d, 8.3)
5″	7.42 (m)	7.40 (dd, 5.0, 7.9)	7.34 (m)	7.42 (dd, 5.1, 8.1)	7.53(m)
5′	6.42 (s)	6.39 (s)	6.47 (s)	6.40 (s)	6.54 (s)
13	5.01 (d, 4.0)	4.97 (d, 5.0)	5.02 (s)	4.98 (d, 4.0)	4.99 (d, 4.0)
7	5.04 (dd, 5.9, 11.2)	5.00 (dd, 5.9, 11.2)	5.05 (m)	4.99 (m)	5.03 (m)
1	3.50 (m)	3.26 (m)	3.77 (m)	3.57 (dd, 3.8, 11.7)	3.34 (m)
11	3.89 (d, 10.6)	3.69 (d, 10.6)	3.67 (s)	3.50 (d, 10.9)	3.52 (d, 10.5)
11′	3.49 (d, 10.2)	3.26 (d, 9.2)	3.67 (s)	3.44 (d, 10.6)	3.34 (m)
14-Me	1.70 (s)	1.67 (s)	1.70 (s)	1.67 (s)	1.68 (s)
12-Me	1.47 (s)	1.43 (s)	1.42 (s)	1.42 (s)	1.43 (s)
15-Me	1.25 (s)	1.11 (s)	1.10 (s)	1.10 (s)	1.12 (s)
R-C*H*<	5.54 (s)	4.72 (q, 5.0)	—	—	—
	C_6H_5: 7.3~7.5 (5H, m), CH_3 $(CH_2)_3$: 0.98 (3H, t, 7.3), 2.44 (2H, dt, 2, 7.3)	Me: 1.34 (3H, d, 5.0), $CH_3(CH_2)_3$: 0.96 (3H, t, 7.3), 2.42 (2H, dt, 2.0, 7.6)	C_6H_5: 7.3~7.6 (5H, m), Me: 1.72 (3H, s), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.44 (2H, m)	1.8 (2H, m), 1.5 (6H, m), 1.4 (2H, m), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.42 (2H, dt, 2, 7.3)	$CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.42 (2H, dt, 2, 7.6)

Table 3-3. ^{1}H NMR data for **33**~**47**.

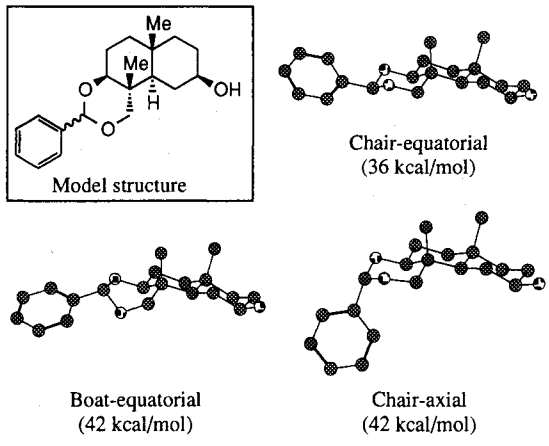
	33	**34**	**35**	**36**	**37**
2″	9.00 (d, 2.3)	9.00 (s)	9.00 (s)	9.00 (d, 2.0)	9.04 (s)
6″	8.70 (dd, 1.5, 6.3)	8.68 (dd, 1.5, 4.8)	8.69 (d, 4.0)	8.68 (dd, 1.3, 5.9)	8.72 (s)
4″	8.09 (dd, 2.0, 8.3)	8.11 (dt, 2.0, 8.3)	8.13 (dt, 2.0, 8.3)	8.11 (dd, 2.0, 8.3)	8.18 (d, 7.9)
5″	7.41 (dd, 4.8, 8.1)	7.40 (dd, 4.8, 8.1)	7.44 (dd, 5.0, 7.9)	7.43 (dd, 4.8, 7.8)	7.50 (br s)
5′	6.41 (s)	6.40 (s)	6.41 (s)	6.40 (s)	6.43 (s)
13	4.98 (d, 4.6)	4.98 (d, 4.0)	4.99 (d, 5.0)	4.98 (d, 3.6)	4.99 (d, 4.0)
7	5.00 (dd, 5.3, 11.6)	5.00 (dd, 5.9, 11.2)	5.00 (m)	5.00 (m)	5.01 (dd, 5.6, 11.5)
1	4.07 (m)	3.24 (m)	3.34 (m)	3.22 (m)	3.20 (m)
11	4.14 (d, 9.9)	3.71 (d, 10.2)	3.76 (d, 10.6)	3.72 (d, 10.2)	3.72 (d, 10.2)
11′	4.05 (d, 9.6)	3.25 (d, 9.6)	3.33 (d, 10.9)	3.22 (d, 10.6)	3.21 (d, 10.9)
14-Me	1.71 (s)	1.67 (s)	1.68 (s)	1.67 (s)	1.68 (s)
12-Me	1.47 (s)	1.43 (s)	1.43 (s)	1.42 (s)	1.43 (s)
15-Me	1.13 (s)	1.10 (s)	1.13 (s)	1.08 (s)	1.07 (s)
R-C*H*<	—	4.50 (t, 5.0)	4.99 (d, 5.0)	4.30 (d, 4.6)	4.14 (s)
	$CH_3(CH_2)_3$: 1.00 (3H, t, 7.3), 2.42 (2H, dt, 2.6, 7.6)	0.95 (3H, t, 7.3), 0.94 (3H, t, 7.6), 2.42 (2H, dt, 1.8, 7.3)	CH_2=C*H*–: 5.89 (1H, ddd, 5.0, 10.6, 17.2), C*H*$_2$=CH–: 5.48 (1H, d, 17.5), 5.32 (1H, d, 10.6)	Me_2CH: 0.94 (6H, d, 6.6), $CH_3(CH_2)_3$: 0.96 (3H, t, 7.3), 2.41 (2H, dt, 2.0, 7.3)	Me_3: 0.92 (9H, s), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.42 (2H, dt, 1.8, 7.3)

	38	**39**	**40**	**41**	**42**
2″	8.99 (d, 2.7)	9.02 (s)	9.02 (br s)	9.02 (s)	9.04 (br s)
6″	8.68 (dd, 2.7, 5.0)	8.70 (s)	8.71 (br s)	8.71 (s)	8.72 (br s)
4″	8.08 (dt, 2.0, 7.6)	8.17 (dd, 1.7, 8.3)	8.23 (br s)	8.15 (d, 8.3)	8.22 (d, 8.2)
5″	7.39 (dd, 4.7, 7.8)	7.48 (dd, 5.0, 7.9)	7.52 (br s)	7.48 (dd, 3.3, 7.9)	7.53 (m)
5′	6.39 (s)	6.44 (s)	6.45 (s)	6.43 (s)	6.45 (s)
13	4.97 (d, 4.0)	5.01 (d, 4.0)	5.00 (d, 4.3)	5.01 (d, 4.2)	5.00 (d, 3.9)
7	4.99 (dd, 5.6, 11.2)	5.03 (dd, 6.3, 11.2)	5.05 (m)	5.04 (m)	5.04 (dd, 5.9, 11.2)
1	3.35 (m)	3.51 (m)	3.50 (m)	3.48 (m)	3.52 (m)
11	3.75 (d, 10.6)	3.88 (d, 10.6)	3.88 (d, 10.6)	3.87 (d, 10.2)	3.88 (d, 10.6)
11′	3.39 (d, 9.9)	3.51 (m)	3.50 (m)	3.48 (m)	3.52 (m)
14-Me	1.68 (s)	1.70 (s)	1.70 (s)	1.70 (s)	1.70 (s)
12-Me	1.43 (s)	1.47 (s)	1.47 (s)	1.46 (s)	1.47 (s)
15-Me	1.14 (s)	1.25 (s)	1.24 (s)	1.24 (s)	1.26 (s)
R-C*H*<	5.21 (s)	5.68 (s)	5.50 (s)	5.50 (s)	5.96 (s)
	OMe: 3.51 (3H, s), $CH_3(CH_2)_3$: 0.96 (3H, t, 7.3), 2.41 (2H, dt, 1.6, 7.6)	C_6H_4: 7.12~7.24 (3H, com.), 7.63 (1H, dd, 2.0, 4.6), Me: 2.39 (3H, s), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.44 (2H, dt, 2, 7.3)	C_6H_4: 7.14~7.33 (4H, com.), Me: 2.36 (3H, s), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.43 (2H, dt, 2.0, 7.6)	C_6H_4: 7.17 (2H, d, 8.3), 7.38 (2H, d, 8.3), Me: 2.34 (3H, s), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.37 (2H, dt, 2.0, 7.6)	C_6H_4: 7.04~7.35 (3H, com.), 7.65 (1H, dt, 1.8, 7.2), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.2), 2.43 (2H, dt, 2.0, 7.2)

	43	**44**	**45**	**46**	**47**
2″	9.04 (s)	9.04 (s)	9.00 (s)	9.06 (s)	9.01 (br s)
6″	8.71 (d, 1.6)	8.71 (s)	8.68 (s)	8.72 (br s)	8.69 (br s)
4″	8.22 (d, 5.3)	8.22 (m)	8.11 (d, 8.3)	8.26 (br s)	8.09 (m)
5″	7.38 (m)	7.50 (m)	7.42 (m)	7.57 (br s)	7.41 (m)
5′	6.44 (s)	6.45 (s)	6.41 (s)	6.46 (s)	6.46 (s)
13	5.00 (d, 4.0)	5.00 (d, 3.9)	4.99 (d, 4.0)	5.01 (d, 3.3)	4.99 (d, 4.0)
7	5.05 (dd, 6.0, 11.2)	5.04 (dd, 5.8, 11.5)	5.01 (m)	5.04 (dd, 5.9, 11.6)	5.00 (dd, 5.5, 11.2)
1	3.50 (m)	3.49 (m)	3.47 (m)	3.53 (m)	3.67 (m)
11	3.88 (d, 10.6)	3.88 (d, 10.2)	3.86 (d, 10.6)	3.92 (d, 10.2)	3.65 (d, 10.6)
11′	3.50 (m)	3.49 (m)	3.46 (d, 10.6)	3.53 (m)	3.41 (d, 10.6)
14-Me	1.70 (s)	1.70 (s)	1.69 (s)	1.70 (s)	1.70 (s)
12-Me	1.47 (s)	1.47 (s)	1.46 (s)	1.47 (s)	1.42 (s)
15-Me	1.22 (s)	1.23 (s)	1.22 (s)	1.21 (s)	0.89 (s)
R-C*H*<	5.53 (s)	5.52 (s)	5.48 (s)	5.61 (s)	
	C_6H_4: 7.04 (1H, dd, 1.3, 7.4), 7.14~7.38 (2H, com.), 7.53 (1H, m), $CH_3(CH_2)_3$: 0.98 (3H, t, 7.3), 2.43 (2H, dt, 2.0, 7.6)	C_6H_4: 7.05 (2H, t, 8.6), 7.48 (2H, dd, 5.3, 8.6), Me: 2.34 (3H, s), $CH_3(CH_2)_3$: 0.98 (3H, t, 7.4), 2.44 (2H, dt, 1.3, 7.2)	C_6H_4: 6.88 (2H, d, 8.9), 7.42 (2H, d, 8.9), $CH_3(CH_2)_3$: 0.96 (3H, t, 7.4), 2.43 (2H, dt, 1.3, 7.9)	C_6H_5: 7.69 (2H, d, 8.9), 8.23 (2H, d, 8.9), $CH_3(CH_2)_3$: 0.97 (3H, t, 7.3), 2.44 (2H, dt, 2.0, 7.6)	

acetals, chair or boat forms. On the basis of the stability of the conformation, the chair form is preferable. To examine the conformation, an MM2 calculation was performed using a model structure for **10** (Fig. 1). The *trans*-decahydronaphthalene moiety was fixed to the chair-chair form, so the stability was predominantly dependent on the cyclic acetal moiety. The calculation for the three possible conformations, that is, chair form with an equatorial phenyl ring, chair form with an axial phenyl ring and boat form with an equatorial phenyl ring, was carried out using Chem3D on a Macintosh computer. The structure was fixed to a desired conformation and each local minimum total energy was adopted. The total energy of the chair form with an equatorial phenyl ring was approximately 6 kcal/mol less than that of other two forms. Furthermore, compound **28** was crystallized from ethyl acetate-methanol to carry out an X-ray analysis. The data support the chair form with the phenyl ring at the equatorial position (private data). These results suggest that other acetals take a chair form with the smaller group in the axial position.

Fig. 1. The model structure and possible conformations of the benzylidene acetal.



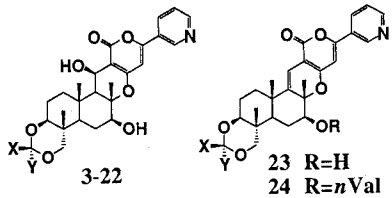
Biological activity

The *in vitro* ACAT activity was assayed by our established method using rat liver microsomes[6]. The structures and ACAT inhibitory activity (IC_{50} value) of 1,11-cyclic acetal derivatives of pyripyropenes are summarized in Tables 4 to 6.

As shown in Table 4, derivatives with a free hydroxy group at the 7-position showed no inhibitory activity.

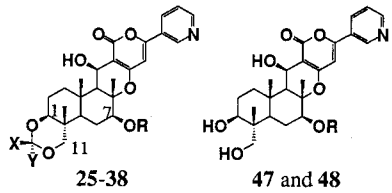
However, the 7-hydroxyl groups of **4** and **10** were acetylated to give **25** and **26** with potent inhibitory activity (IC_{50}: 1.2 and 0.12 μM), respectively (Table 5).

Table 4. Structure and *in vitro* ACAT inhibitory activity.



Compound	Structure X	Structure Y	IC_{50} (μM)
3	$-CH_3$	H	>10
4	$-CH_3$	$-CH_3$	>100
5	$-CH_2CH_3$	H	>100
6	$-CH=CH_2$	H	>100
7	$-CH(CH_3)_2$	H	>100
8	$-C(CH_3)_3$	H	>100
9	$-OCH_3$	H	>100
10	$-C_6H_5$	H	>100
11	$-C_6H_5$	$-CH_3$	>100
12	*o*-CH_3-C_6H_4	H	>100
13	*m*-CH_3-C_6H_4	H	>100
14	*p*-CH_3-C_6H_4	H	>100
15	*o*-F-C_6H_4	H	>100
16	*m*-F-C_6H_4	H	>100
17	*p*-F-C_6H_4	H	>100
18	*p*-OCH_3-C_6H_4	H	>100
19	*p*-NO_2-C_6H_4	H	>100
20	$-(CH_2)_4-$		>100
21	$-(CH_2)_5-$		>100
22	=O		>100
23	$-CH_3$	$-CH_3$	>100

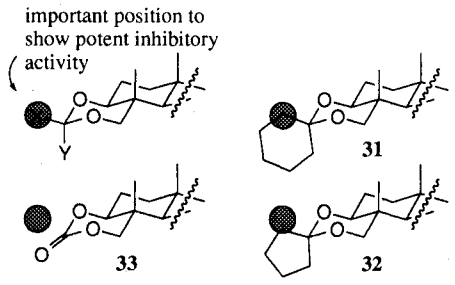
Table 5. Structure and *in vitro* ACAT inhibitory activity.



Compound	Structure X (eq.)	Structure Y (ax.)	R	IC_{50} (μM)
1	—	—	—	0.089
24	$-CH_3$	$-CH_3$	*n*Val	7.1
25	$-CH_3$	$-CH_3$	Ac	1.2
26	$-C_6H_5$	H	Ac	0.12
27	$-CH_3$	$-CH_3$	*n*Val	0.086
28	$-C_6H_5$	H	*n*Val	0.0056
29	$-CH_3$	H	*n*Val	0.025
30	$-C_6H_5$	$-CH_3$	*n*Val	0.15
31	$-(CH_2)_5-$		*n*Val	0.039
32	$-(CH_2)_4-$		*n*Val	3.0
33	=O		*n*Val	2.5
34	$-CH_2CH_3$	H	*n*Val	0.028
35	$-CH=CH_2$	H	*n*Val	0.13
36	$-CH(CH_3)_2$	H	*n*Val	0.17
37	$-C(CH_3)_3$	H	*n*Val	0.21
38	$-OCH_3$	H	*n*Val	0.091
47	—	—	Ac	100
48	—	—	*n*Val	80

*n*Val: $CO(CH_2)_3CH_3$

Fig. 2. The location for eliciting potent ACAT inhibition.



Next, **27**, which was *n*-valerylated at the C-7 hydroxy group of **4**, showed as potent inhibitory activity as pyripyropene A. It was thus found that the 1- and 11-*O*-acyl groups can be converted to a 1,11-cyclic acetal group with full retention of activity. And remarkably, *n*-valerylation at the C-7 hydroxy group of **10** gave **28** with an IC_{50} value of 5.6 nM, which is a 16 fold improvement in potency compared to **1**.

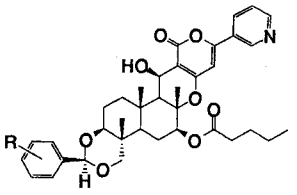
On the other hand, olefin analog **24** showed only weak inhibitory activity, even though the 7-hydroxyl was *n*-valerylated. Removal of the ketal from **25** and **27** to give, 1,11-dihydroxyl derivatives **47** and **48** resulted in loss of the inhibitory activity. These results are consistent with our previous reports, and suggest that the 1,11-cyclic acetal and 1,11-*O*-acyl moieties interact at the same site of the enzyme and also that substituent groups at both the 1 and 11 positions are necessary.

Regarding groups at the X and Y positions of 1,11-cyclic acetal derivatives, **28** and **29** were much more potent than **27** and **30**. The order of ACAT inhibitory activity is H > methyl at the axial Y position, and phenyl > methyl at the equatorial X position. Although the cyclohexylidene derivative (**31**) showed strong inhibitory activity, the cyclopentylidene (**32**) and cyclic carbonate derivatives (**33**) showed less potent inhibitory activity. These result suggest that occupying the X position of the acetal is important for potent inhibitory activity (Fig. 2).

Furthermore, propylidene analog **34** was as potent as ethylidene analog **29**, the derivatives with ethylene (**35**), *i*-propyl (**36**), *t*-butyl (**37**) and methoxy (**38**) at the X position were prepared. These four compounds showed similar IC_{50} values but were less potent than **34** and **29**.

As the benzylidene analog (**28**) showed the most potent inhibitory activity, further modification of the phenyl ring was carry out (Table 6). Among the three methyl benzylidene analogs (**39**, **40** and **41**), the *o*-methyl (**39**) showed the best inhibitory effect, and the *m*-methyl (**40**) and the *p*-methyl (**41**) were equivalent. However, among the fluoride benzylidenes (**42**, **43** and **44**), the activity order of the inhibition was *m*-fluoro (**43**) > *o*-fluoro (**42**) > *p*-fluoro (**44**). Incorporation of a methyl or fluoro group in the *p*-position of the phenyl ring decreased the ACAT inhibitory activity (**41**, **44**), and the *p*-methoxy (**45**) and *p*-nitro (**46**) benzylidene derivatives were also less potent.

Table 6. Structure and *in vitro* ACAT inhibitory activity.



Compound	R	IC_{50} (μM)
39	-*o*-CH_3	0.038
40	-*m*-CH_3	0.19
41	-*p*-CH_3	0.18
42	-*o*-F	0.085
43	-*m*-F	0.035
44	-*p*-F	0.14
45	-*p*-OCH_3	0.35
46	-*p*-NO_2	5.9

In conclusion, several 7-*O*-*n*-valeryl-1,11-cyclic acetal analogs had comparable or improved ACAT inhibitory activity relative to **1**. For example, isopropylidene (**27**), methoxymethylene acetal (**38**) and *o*-fluorobenzylidene (**42**) analogs showed similar inhibitory activity to **1**. Moreover, ethylidene (**29**), cyclohexylidene (**31**), propylidene (**34**), *o*-methylbenzylidene (**39**), and *m*-fluorobenzylidene (**43**) analogs were much more potent than 1. Especially notable is the benzylidene analog (**28**), which is 16 times more potent than **1**.

Experimental

Reagents were obtained from commerical suppliers and were used without purification. Column chromatography was carried out on silica gel (Merck, Kieselgel 60, 230~400 mesh). For preparative TLC (PTLC), Kiesel gel 60 F-254 (Merck) was used. Mass spectra were obtained with a JEOL model DX-300 mass spectrometer. ^1H NMR (270 MHz) and ^{13}C NMR (76.5 MHz) spectra were acquired on a JEOL-EX270 spectrophotometer. Chemical shifts are given in ppm with solvent peak ($CDCl_3$: 7.26 ppm) as the standard, and coupling constants (J) are given as Hz. Abbreviations of ^1H NMR signal patterns are following: s = singlet, d = doublet, dd = doublet of doublets, ddd = double doublet of

doublets, t=triplet, dt=double triplet, q=quartet, m=multiplet, br s=broad singlet. IR spectra were taken with a Horiba model FT-210 spectrophotometer.

General Method of 1,11-Cyclic Acetalization

1,11-Isopropylidene Derivative (4) and its Eliminated Compound (23)

To a solution of **2** (24 mg) in dry DMF (0.5 ml) was added isopropenyl methyl ether (50 μl) and pyridinium hydrochloride (4 mg), and the solution was stirred at room temperature for 4 days. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to afford a pale yellow solid (26 mg) that was purified by PTLC (0.25 mm, 20×20 cm, CH_2Cl_2 : MeOH = 10 : 1) to obtain **4** (16.8 mg, 64%) as colorless solid and **23** (5 mg, 20%) as yellow solid. Analytical data for **23**: $C_{28}H_{33}O_6N$; HR FAB-MS 480.2368 (M+H) Calcd: 480.2386 (for $C_{28}H_{34}O_6N$); IR (KBr) cm^{-1} 1710.

Trideacetyl-1,11-cyclic Carbonate Pyripyropene A (22)

To a suspension of **2** (40 mg) in dry THF (2 ml) was added *N,N*-carbonyldiimidazole (43 mg), and the mixture was refluxed for 1 hour. The reaction mixture was workup as for **4** to afford **22** (12 mg, 27%) as colorless solid. $C_{26}H_{29}O_8N$; HR FAB-MS 484.1972 (M+H) Calcd: 484.1971 (for $C_{26}H_{30}O_8N$); IR (KBr) cm^{-1} 1750, 1690.

General Method of 7-*O*-Acylation

Trideacetyl-1,11-isopropylidene-7-*O*-*n*-valeryl 13-dehydroxy-5,13-dehydro Pyripyropene A (24)

To a solution of **23** (7.2 mg) in dry CH_2Cl_2 (1 ml) was added *n*-valeric anhydride (5 μl), Et_3N (10 μl) and DMAP (2 mg), and the solution was stirred at room temperature. The reaction mixture was washed with water and the organic layer dried (Na_2SO_4), concentrated *in vacuo*, and the residure purified by column chromatography (0.5×4 cm, CH_2Cl_2 : MeOH = 25 : 1) to give **24** (8.5 mg, 100%) as yellow powder.

Compounds **4** and **10** were acetylated with acetic anhydride to afford **25** and **26**, respectively.

Hydrolysis of Isopropylidene Analogs

Trideacetyl-7-*O*-*n*-valeryl Pyripyropene A (48)

Compound **24** (13 mg) was dissolved in 60% aq. AcOH (2 ml) and stirred at room temperature for 4 hours. The reaction mixture was extracted with EtOAc. The organic layer was treated in a similar manner to **24** to obtain **48** (9.9 mg, 82%) as colorless solid. $C_{30}H_{39}O_8N$; HR EI-MS 541.2675 (M+) Calcd: 541.2675; IR (KBr) cm^{-1} 1730, 1700; ^1H NMR ($CDCl_3$) δ 0.90 (3H, s), 0.97 (3H, t, J=7.3 Hz), 1.42 (3H, s), 1.70(3H, s), 2.39 (2H, dt, J=2.3, 7.6 Hz), 3.42 (H, d, J=10.2 Hz), 3.67 (1H, d, J=10.6 Hz), 3.69 (1H, m), 4.99 (1H, d, J=5.0 Hz), 5.01 (1H, dd, J=5.3, 11.2 Hz), 6.41 (1H, s), 7.41 (1H, dd, J=5.6, 8.3 Hz), 8.09 (1H, dt, J=2.0, 8.3 Hz), 8.69 (1H, dd, J=1.5, 4.8 Hz), 9.00 (1H, d, J=1.7).

Analytical data for **47**: $C_{27}H_{33}O_8N$; HR FAB-MS 500.2278 (M+H) Calcd: 500.2284 (for $C_{27}H_{34}O_8N$); IR (KBr) cm^{-1} 1700.

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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) OBATA, R.; T. SUNAZUKA, L. ZHUORONG, H. TOMODA & S. ŌMURA: Structure-activity relationships of pyripyropenes fungal acyl-CoA: cholesterol acyltransferase inhibitors. J. Antibiotics 48: 749~750, 1995
3) OBATA, R.; T. SUNAZUKA, H. TOMODA, Y. HARIGAYA & S. ŌMURA: Chemical modification and structure-activity relationships of pyripyropenes; Potent, bioavailable inhibitor of acyl-CoA: cholesterol acyltransferase. Bioorg. & Med. Chem. Lett. 5: 2683~2688, 1995
4) NARASAKA, K.: Trends in synthetic carbohydrate chemistry. *In* ACS symposium series 386. *Ed.*, D. HORTON *et al.*, p. 290, American Chemical Society, Washington DC, 1989
5) LEWBART, M. L. & J. J. SCHNEIDER: Preparation and properties of steroidal 17, 20-acetonides epimeric at C-20. I. Derivatives of 5β-pregnan-3α-ol. J. Org. Chem. 34: 3505~3512, 1969
6) TOMODA, H.; H. NISHIDA, R. MASUMA, J. CAO, S. OKUDA & S. ŌMURA: Purpactins, new inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Penicillium purpurogenum*. J. Antibiotics 44: 136~143, 1991