PURPACTINS, NEW INHIBITORS OF ACYL-CoA:CHOLESTEROL ACYLTRANSFERASE PRODUCED BY *Penicillium purpurogenum*

III. CHEMICAL MODIFICATION OF PURPACTIN A

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Acylated drivatives of the C-1′ and/or C-11 hydroxy group(s) of penicillide were synthesized and their inhibitory activity against acyl-CoA:cholesterol acyltransferase (ACAT) was studied. Introduction of long acyl group into either or both hydroxy residue(s) decreased the inhibitory activity. A small acyl moiety such as acetyl or *n*-butyryl at the C-1′ hydroxy group is responsible for potent inhibitory activity against ACAT. The 1′-*O*-acetyl-11-*O*-tetrahydropyranyl derivative (11-*O*-2″-tetrahydropyranylpurpactin A) showed high selectivity (cytotoxic dose vs. effective dose) in a cell assay using J774 macrophages.

In the course of our screening, we have discovered the novel acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors, purpactins A, B and C, from the fermentation broth of *Penicillium purpurogenum* FO-608[1,2]. In this report, we describe the preparation of purpactin A derivatives and their ACAT inhibitory activity.

Synthesis

Purpactin A (**1**) was treated with 2,3-dihydropyran in CH_2Cl_2 containing 0.25% (w/v) *p*-toluensulfonic acid to give 11-*O*-2″-tetrahydropyranyl (THP) ether (**3**). The treatment of **3** with 1 M LiOH in tetrahydrofuran (THF) gave 1′-hydroxy-11-*O*-2″-THP ether (**4**). Compound **4** was treated with palmitoyl chloride or *n*-butyryl chloride in pyridine to give 1′-*O*-*n*-butyryl-11-*O*-2″-THP ether (**4a**) and 1′-*O*-palmitoryl-11-*O*-2″-THP ether (**4b**). By treating **4a** or **4b** with 1% *p*-toluensulfonic acid in MeOH, 1′-*O*-palmitoyl (**5**) and 1′-*O*-*n*-butyryl (**6**) substituents were obtained, respectively (Scheme 1).

The treatment of **1** with palmitoyl chloride or *n*-butyryl chloride in pyridine gave 11-*O*-palmitoyl (**7**) and 11-*O*-*n*-butyryl (**8**) substituents, respectively (Scheme 2).

By treating penicillide (**2**) with one equivalent of *n*-butyryl chloride or palmitoyl chloride in pyridine, 1′-hydroxy-11-*O*-*n*-butyryl (**11**) and 1′-hydroxy-11-*O*-palmitoyl (**9**) substituents were obtained, respectively (Scheme 3).

The treatment of **2** with excess *n*-butyryl chloride or palmitoryl chloride in pyridine gave 1′,11-*O*-di-*n*-butyryl (**12**) and 1′,11-*O*-dipalmitoyl (**10**) substituents, respectively (Scheme 3).

11-*O*-methyl purpactin A (**13**) was prepared by treating with diazomethane which was described in the previous paper[2].

Tetrahydropyranyl derivatives **3** and **4** may be a mixture of the diastereomer at the C-2″ position. In ^1H NMR spectra, the doublet signal of methoxy protons were observed.

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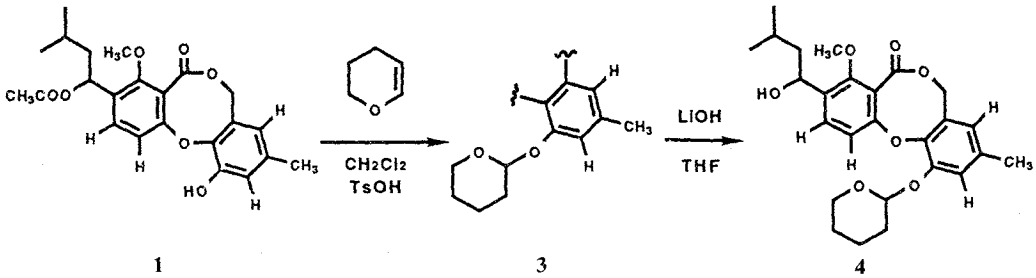
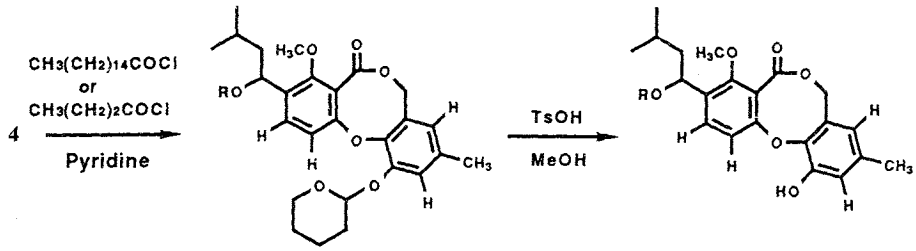
Scheme 1.



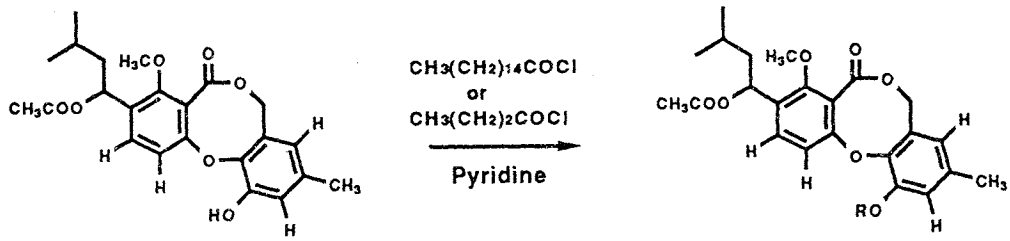
1 3 4



4a R = $CH_3(CH_2)_{14}CO$
4b R = $CH_3(CH_2)_2CO$

5 R = $CH_3(CH_2)_{14}CO$
6 R = $CH_3(CH_2)_2CO$

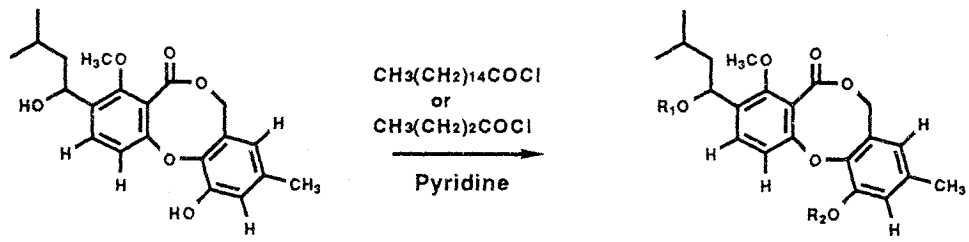
Scheme 2.



1

7 R = $CH_3(CH_2)_{14}CO$
8 R = $CH_3(CH_2)_2CO$

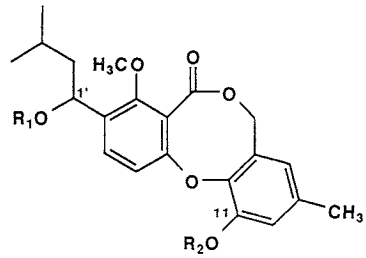
Scheme 3.



2

9 R_1 = H $R_2 = CH_3(CH_2)_{14}CO$
10 $R_1 = R_2 = CH_3(CH_2)_{14}CO$
11 R_1 = H $R_2 = CH_3(CH_2)_2CO$
12 $R_1 = R_2 = CH_3(CH_2)_2CO$

Table 1. Summary of ACAT inhibitory activity and cytotoxicity.



Derivative	R_1 (C-1′)	R_2 (C-11)	ACAT inhibitory activity (IC_{50}, μM)		Cytotoxicity (CD_{50}, μM)	Specificity index
			Microsome	J774 (A)	B	B/A
1[a]	CH_3CO	H	120	1.2	9.7	8.1
2[b]	H	H	>269	>26.9	>26.9	—
3	CH_3CO	THP[c]	84	5.0	>25.1	>5.0
4	H	THP	182	28.5	>28.5	>1.0
5	$CH_3(CH_2)_{14}CO$	H	>164	>16.4	>16.4	—
6	$CH_3(CH_2)_2CO$	H	60	1.4	9.3	6.6
7	CH_3CO	$CH_3(CH_2)_{14}CO$	>153	>15.3	>15.3	—
8	CH_3CO	$CH_3(CH_2)_2CO$	81	2.5	>20.7	>8.3
9	H	$CH_3(CH_2)_{14}CO$	>164	>16.4	>16.4	—
10	$CH_3(CH_2)_{14}CO$	$CH_3(CH_2)_{14}CO$	>118	>11.8	>11.8	—
11	H	$CH_3(CH_2)_2CO$	60	>30.1	11.6	<2.6
12	$CH_3(CH_2)_2CO$	$CH_3(CH_2)_2CO$	88	>19.6	19.6	<1.0
13	CH_3CO	CH_3	224	11.7	9.3	0.8

[a] Purpactin A. [b] penicillide. [c] tetrahydropyranyl.

Chemical shift values for ^1H NMR and UV spectral data of all derivatives are described in the Experimental section.

ACAT Inhibitory Activity

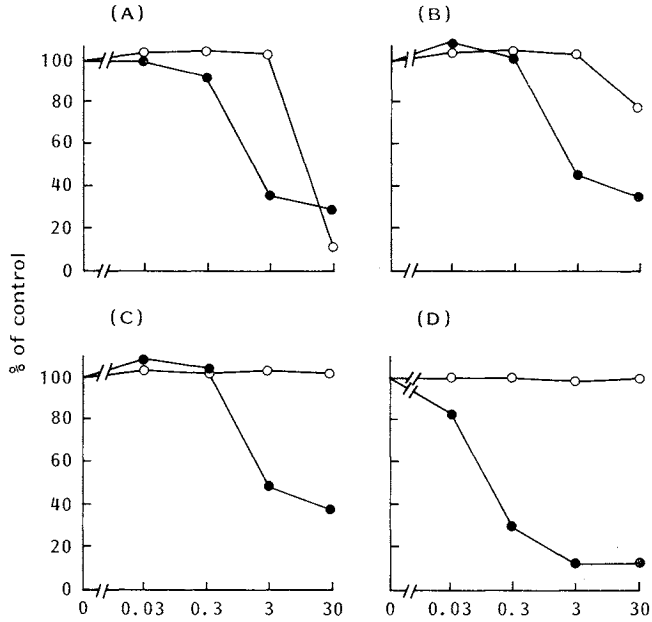
IC_{50} values of acyl and other derivatives against ACAT activity both in an enzyme assay using rat liver microsomes and in a cell assay using J774 macrophages are summarized in Tabel 1.

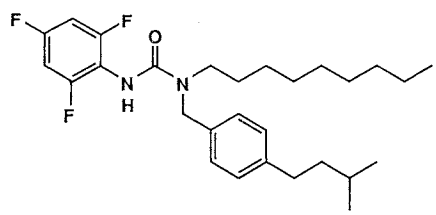
From the results of the enzyme assay, derivatives having a smaller hydrophobic residue at the C-1′ hydroxy group (**1** and **6**) exhibited potent inhibitory activity, though penincillide (**2**) and the derivative **5**, which have no substituent and palmitoyl residue at the hydroxy group, respectively, showed very weak inhibitory activity. Concerning the C-11 hydroxy group, all derivatives having a long acyl (palmitoyl) group (**7**, **9** and **10**) lost potent inhibitory activity.

The results of inhibitory potency against cholesterol ester formation in J774 cells were essentially similar to those in the enzyme assay. Derivatives having a smaller hydrophobic residue at the C-1′ and no or a smaller group at the C-11 hydroxy group (**1**, **3**, **6** and **8**) showed potent inhibitory activity. Cytotoxicity of these derivatives to J774 cells also tested and the drug concentration showing 50% cell damage (CD_{50}) after 12 hours incubation was measured by trypan blue exclusion method. Among them, derivatives **3** and **8** show the highest selectivity (Fig. 1 and Table 1). Under the same conditions, CL-283,546[3], a synthetic ACAT inhibitor, showed potent inhibitory activity against cholesterol ester formation (IC_{50}: 89 nM) and no cytocidal effect at 50 μM in the cell assay.

Fig. 1. Effect of purpactin A derivatives and CL-283,546 on cholesterol ester formation and cytotoxicity in J774 cells.

(A) **1**, (B) **8**, (C) **3**, (D) CL-283,546. ● Cholesterol ester formation, ○the number of living cells.





CL-283,546

Discussion

Known synthetic ACAT inhibitors such as 57-118[4], CL-277,082[5] and CL-283,546[3] have a long alkyl or acyl moiety in their structures. On the view of this point, acyl or other derivatives at C-1′ and/or C-11 hydroxy group(s) of penicillide (**2**) were synthesized. Contrary to our expectation, introduction of a long acyl (palmitoyl) group into at least one of the two hydroxy groups resulted in a decline of the ACAT inhibitory activity. A smaller hydrophobic residue such as acetyl, *n*-butyryl and THP substituted at the C-1′ hydroxy moiety is responsible for potent inhibitory activity. In the cell assay, both ACAT inhibitory activity as inhibition of cholesterol ester formation (IC_{50}) and cytotoxicity (CD_{50}) were measured at the same time, and the specificity (CD_{50}/IC_{50}) of derivatives was calculated. Introduction of *n*-butyryl or THP group into C-11 resulted in a decline in exhibiting cytotoxicity, suggesting that the C-11 position to play an important role in cytotoxicity. Among derivatives 1′-*O*-acetyl-11-*O*-THP (**3**) and 1′-*O*-acetyl-11-*O*-*n*-butyryl (**8**) derivatives appeared to show the highest specificity (Table 1). It will be interesting to see *in vivo* efficacy of these derivatives.

Experimental

General Methods

NMR spectra were measured with Jeol FX90 and Varian XI-400 spectrometer in $CDCl_3$ solution.

MS was obtained with a Jeol model DX-300 spectrometer. UV spectrum was recorded on a Shimadzu UV-200S spectrophotometer. TLC was performed on pre-coated plates, Kieselgel 60 F_{254} (Merck) with $CHCl_3$ - MeOH (98 : 2) as solvent.

Preparation of Purpactin A (**1**) and Penicillide (**2**)

Purpactin A and penicillide were prepared as described in the previous paper[1].

Preparation of 3-1′-Acetyloxy-3′-methylbutyl-4-methoxy-9-methyl-11-2″-tetrahydropyranyloxy-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one (**3**)

To a solution of **1** (132.6 mg, 0.32 mmol) in CH_2Cl_2 containing 0.25% *p*-toluenesulfonic acid (3 ml), 2,3-dihydropyrane (400 μl) was added and stirred for 10 minutes at room temperature. The reaction mixture was applied to preparative HPLC (YMC pack AM-324 ODS) and eluted with 80% aq CH_3CN. The eluate was evaporated under reduced pressure to afford **3** as a colorless powder (129.5 mg, 81.2%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 498 $(M)^+$, 439 $(M-CH_3COO)^+$, 414 $(M-C_5H_8O)^+$, 354 $(M-CH_3COOH-C_5H_8O)^+$, 339 $(M-CH_3COOH-C_5H_8O_2)^+$, 315 $(M-C_2H_2O-C_5H_8O-C_4H_9)^+$, 297 $(M-CH_3COOH-C_5H_8O-C_4H_9)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^1H NMR (90 MHz, $CDCl_3$) δ 0.88 (6H, d, J=6.0 Hz), 1.20~1.80 (9H, m), 1.95 (3H, s), 2.15 (3H, s), 3.50~3.70 (2H, m), 3.95 (3H, d), 4.95 (1H, d, J=14.0 Hz), 5.10 (1H, d, J=14.0 Hz), 5.45 (1H, br s), 6.05 (1H, dd), 6.45 (1H, d, J=1.7 Hz), 6.90 (1H, d, J=8.5 Hz), 6.95 (1H, d, J=1.7 Hz), 7.40 (1H, d, J=8.5 Hz); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.61.

Preparation of 3-1′-Hydroxy-3′-methylbutyl-4-methoxy-9-methyl-11-2″-tetrahydropyranyloxy-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one (**4**)

To a solution of **3** (124.6 mg, 0.25 mmol) in THF (2 ml), 1 M LiOH aqueous solution (500 μl) was added and stirred for 6 hours at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (3 × 10 ml). The EtOAc layers were combined and dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative HPLC (YMC pack AM-324 ODS) and eluted with 80% aq CH_3CN. The eluate was evaporated under reduced pressure to afford **4** as a colorless powder (20.7 mg, 18.1%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 456 $(M)^+$, 439 $(M-H_2O)^+$, 399 $(M-C_4H_9)^+$, 372 $(M-C_5H_8O)^+$, 354 $(M-C_5H_8O-H_2O)^+$, 315 $(M-C_5H_8O-C_4H_9)^+$, 297 $(M-C_5H_8O-C_4H_9-H_2O)^+$, 219 $(C_{13}H_{15}O_3)^+$; ^1H NMR (90 MHz, $CDCl_3$) δ 0.95 (6H, d, J=6.0 Hz), 1.45~2.15 (9H, m), 2.25 (3H, s), 3.54~3.80 (2H, m), 4.00 (3H, s), 5.10 (3H, br s), 5.50 (1H, br t), 6.50 (1H, d, J=1.7 Hz), 6.95 (1H, d, J=8.5 Hz), 7.05 (1H, d, J=1.7 Hz), 7.60 (1H, d, J=8.5 Hz); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.35.

Preparation of 3-1′-Palmitoyloxy-3′-methylbutyl-4-methoxy-9-methyl-11-hydroxy-5*H*,7*H*-dibenzo-[b,g]-1,5-dioxocin-5-one (**5**)

To a solution of **4** (7.2 mg, 0.016 mmol) in pyridine (50 μl), palmitoyl chloride (10 μl, 0.036 mmol) was added and stirred for 10 minutes at room temperature. The reaction mixture was diluted with H_2O (10 ml) and extracted with EtOAc (10 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative HPLC (YMC pack AM-324 ODS, CH_3CN) to give a colorless powder (**4a**, 10.3 mg, 94.0%). To a solution of **4a** (10.3 mg, 0.017 mmol) in CH_2Cl_2 (50 μl), 0.1% *p*-toluenesulfonic acid in MeOH (200 μl) was added and stirred for 10 minutes at room temperature. The reaction mixture was applied to preparative HPLC (YMC pack AM-324 ODS) and eluted with CH_3CN. The eluate was evaporated under reduced pressure to afford **5** as a colorless powder (7.9 mg, 87.3%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 610 $(M)^+$, 371 $(M-C_{16}H_{31}O)^+$, 354 $(M-C_{16}H_{32}O_2)^+$, 298 $(M-C_{16}H_{32}O_2-C_4H_8)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^1H NMR (90 MHz, $CDCl_3$) δ 0.81~1.00 (9H, t), 1.00~2.00 (29H, m), 2.24 (3H, s), 2.30 (2H, t), 4.02 (3H, s), 4.97 (1H, d), 6.86 (1H, d), 7.42 (1H, d), 6.10 (1H, s, OH); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.59.

Preparation of 3-1′-Butyryloxy-3′-methylbutyl-4-methoxy-9-methyl-11-hydroxy-5*H*,7*H*-dibenzo-[b,g]-1,5-dioxocin-5-one (**6**)

To a solution of **4** (11.3 mg, 0.025 mmol) in pyridine (50 μl), *n*-butyryl chloride (5 μl, 0.047 mmol) was

added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (10 ml) and extracted with EtOAc (10 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative HPLC (YMC pack AM-324 ODS, CH_3CN) to give a colorless powder (**4b**, 12.6 mg, 90%). To a solution of **4b** (12.6 mg, 0.022 mmol) in CH_2Cl_2 (100 μl), 0.1%-toluenesulfonic acid in MeOH (1 ml) was added and stirred for 10 minutes at room temperature. The reaction mixture was evaporated and applied to preparative TLC ($CHCl_3$ - MeOH, 98 : 2) to afford **6** as a colorless powder (10.0 mg, 90%): UV λ_{max}^{EtOH} nm (ε) 280 (2,000); EI-MS (*m/z*) 442 $(M)^+$, 354 $(M-C_4H_8O_2)^+$, 298 $(M-C_4H_8O_2-C_4H_8)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.80 ~ 1.00 (9H, t), 1.00 ~ 2.00 (5H, m), 2.22 (3H, s), 2.28 (2H, t), 4.02 (3H, s), 4.98 (1H, d), 5.14 (1H, d), 6.10 (1H, m), 6.37 (1H, d), 6.82 (1H, d), 6.85 (1H, d), 7.41 (1H, d), 6.17 (1H, d); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.46.

Preparation of 3-1′-Acetoxy-3′-methylbutyl-4-methoxy-9-methyl-11-palmitoyloxy-5*H*,7*H*-dibenzo-[b,g]-1,5-dioxocin-5-one (**7**)

To a solution of **1** (7.0 mg, 0.017 mmol) in pyridine (50 μl), palmitoyl chloride (10 μl, 0.036 mmol) was added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (5 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative TLC ($CHCl_3$ - MeOH, 99 : 1) to afford **7** as a colorless powder (7.8 mg, 70%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 652 $(M)^+$, 592 $(M-C_2H_4O_2)^+$, 414 $(M-C_{16}H_{30}O)^+$, 354 $(M-C_2H_4O_2-C_{16}H_{30}O)^+$, 298 $(M-C_2H_4O_2-C_{16}H_{30}O-C_4H_8)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.90 ~ 1.00 (9H, m), 1.00 ~ 1.90 (29H, m), 2.05 (3H, s), 2.27 (3H, s), 2.62 (2H, t), 4.00 (3H, s), 4.85 (1H, d, J = 14 Hz), 5.12 (1H, d, J = 14 Hz), 6.10 (1H, m), 6.75 (1H, d, J = 1.7 Hz), 6.90 (1H, d, J = 1.7 Hz), 6.95 (1H, d, J = 8.5 Hz), 7.43 (1H, d, J = 8.5 Hz); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.66.

Preparation of 3-1′-Acetoxy-3′-methylbutyl-4-methoxy-9-methyl-11-*n*-butyryloxy-5*H*,7*H*-dibenzo-[b,g]-1,5-dioxocin-5-one (**8**)

To a solution of **1** (10.0 mg, 0.024 mmol) in pyridine (50 μl), *n*-butyryl chlorid (10 μl, 0.094 mmol) was added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (5 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative TLC ($CHCl_3$ - MeOH, 99 : 1) to afford **8** as a colorless powder (8.2 mg, 70%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 484 $(M)^+$, 424 $(M-C_2H_4O_2)^+$, 414 $(M-C_4H_6O)^+$, 354 $(M-C_2H_4O_2-C_4H_6O)^+$, 298 $(M-C_2H_4O_2-C_4H_6O-C_4H_8)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.94 (6H, d, J = 6.0 Hz), 1.04 (3H, t), 1.00 ~ 2.00 (5H, m), 2.02 (3H, s), 2.26 (3H, s), 2.60 (2H, t), 4.02 (3H, s), 4.95 (1H, d), 5.14 (1H, d), 6.10 (1H, m), 6.75 (1H, d), 6.90 (1H, d), 6.95 (1H, d), 7.45 (1H, d); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.61.

Preparation of 3-1′-Hydroxy-3′-methylbutyl-4-methoxy-9-methyl-11-palmitoyloxy-5*H*,7*H*-dibenzo-[b,g]-1,5-dioxocin-5-one (**9**)

To a solution of **2** (10.0 mg, 0.027 mmol) in pyridine (100 μl), palmitoyl chloride (6.8 μl, 0.025 mmol) was added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (5 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative TLC ($CHCl_3$ - MeOH, 99 : 1) to afford **9** as a colorless powder (6.7 mg, 41%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 610 $(M)^+$, 592 $(M-H_2O)^+$, 553 $(M-H_2O-C_4H_9)^+$, 372 $(M-C_{16}H_{32}O^+$, 354 $(M-C_{16}H_{32}O_2)^+$, 315 $(M-C_{16}H_{32}O-C_4H_9)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.80 ~ 1.00 (9H, m), 1.00 ~ 2.00 (29H, m), 2.27 (3H, s), 2.63 (2H, t), 3.96 (3H, s), 5.06 (3H, m), 6.74 (1H, d), 6.92 (1H, d), 7.54 (1H, d); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.46.

Preparation of 3-1′,11-Dipalmitoyloxy-3′-methylbutyl-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one (**10**)

To a solution of **2** (3.4 mg, 0.009 mmol) in pyridine (50 μl), palmitoyl chloride (10 μl, 0.036 mmol) was

added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (5 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative TLC ($CHCl_3$ - MeOH, 99 : 1) to afford **10** as a colorless powder (6.0 mg, 77%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 848 $(M)^+$, 610 $(M-C_{16}H_{30}O)^+$, 592 $(M-C_{16}H_{32}O_2)^+$, 354 $(M-C_{16}H_{32}O_2-C_{16}H_{30}O)^+$, 298 $(M-C_{16}H_{32}O_2-C_{16}H_{30}O-C_4H_8)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^1H NMR (90 MHz, $CDCl_3$) δ 0.75~1.00 (12H, m), 1.00~2.00 (58H, m), 2.21 (2H, t), 2.27 (3H, s), 2.62 (2H, t), 4.01 (3H, s), 4.97 (1H, d), 6.94 (1H, d), 7.42 (1H, d); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.75.

Preparation of 3-1′-Hydroxy-3′-methylbutyl-4-methoxy-9-methyl-11-*n*-butyryloxy-5*H*,7*H*-dibenzo-[b,g]-1,5-dioxocin-5-one (**11**)

To a solution of **2** (5.0 mg, 0.013 mmol) in pyridine (50 μl), *n*-butyryl chloride (1.4 μl, 0.013 mmol) was added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (5 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative TLC ($CHCl_3$ - MeOH, 99 : 1) to afford **11** as a colorless powder (1.7 mg, 29%): UV λ_{max}^{EtOH} nm (ε) 280 (2,000); EI-MS (*m/z*) 442 $(M)^+$; ^1H NMR (90 MHz, $CDCl_3$) δ 0.80~1.00 (9H, t), 1.00~2.00 (5H, m), 2.26 (3H, s), 2.62 (2H, t), 3.97 (3H, s), 5.04 (3H, m), 6.74 (1H, d), 7.55 (1H, d); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.35.

Preparation of 3-1′,11-Di-*n*-butyryl-3′-methylbutyl-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one (**12**)

To a solution of **2** (5.0 mg, 0.013 mmol) in pyridine (50 μl), *n*-butyryl chloride (7.2 μl, 0.068 mmol) was added and stirred for 30 minutes at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with EtOAc (5 ml). The EtOAc layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was applied to preparative TLC ($CHCl_3$ - MeOH, 99 : 1) to afford **12** as a colorless powder (5.0 mg, 72%): UV λ_{max}^{EtOH} nm (ε) 280 (1,900); EI-MS (*m/z*) 512 $(M)^+$, 442 $(M-C_4H_6O)^+$, 424 $(M-C_4H_8O_2)^+$, 354 $(M-C_4H_8O_2-C_4H_6O)^+$, 298 $(M-C_4H_8O_2-C_4H_6O-C_4H_8)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; ^1H NMR (90 MHz, $CDCl_3$) δ 0.80~1.10 (12H, m), 1.00~2.00 (10H, m), 2.25 (2H, t), 5.14 (1H, d), 6.10 (1H, m), 6.74 (1H, d), 6.90 (1H, d), 6.95 (1H, d), 7.45 (1H, d); Rf value ($CHCl_3$ - MeOH, 98 : 2) 0.64.

Preparation of 3-1′-Acetyloxy-3′-methylbutyl-4-methoxy-11-methoxy-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one (**13**)

The preparation of **13** was described in previous paper.

Assay for ACAT Using Rat Liver Microsomes

ACAT activity was assayed as described in the preceding paper[1].

Assay for Cholesterol Ester Formation in J774 Macrophages

The method for cholesterol ester formation in J774 macrophages and cytotoxicity was described in the preceding paper[1].

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