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In Vitro Effects of Oxygenated Lanosterol Derivatives on Cholesterol Biosynthesis from 24,25-Dihydrolanosterol

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The effects of oxygenated lanosterol derivatives (**1**—**27**, 5 μM) including 32-oxygenated lanosterol derivatives on cholesterol biosynthesis from [24,25-$^3H_2$]-24,25-dihydrolanosterol (18 μM) were tested in 10000 × *g* supernatant (S-10) fraction of rat liver homogenate. Among the derivatives, 7-oxolanost-8-en-3β-ol (7-oxo-DHL), 3β-acetoxylanost-8-en-7-one (7-oxo-DHL-3-OAc), and 7-oxolanosta-5,8,11-trien-3β-ol were highly active in depressing cholesterol biosynthesis from 24,25-dihydrolanosterol. The inhibitory activities of these derivatives on cholesterol synthesis are discussed on the basis of the position and stereochemistry of the oxygen functional groups on the sterol nucleus. The effect of aphidicolin on cholesterol synthesis was also compared with that of 7-oxo-DHL.

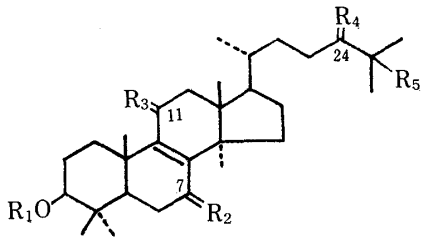
Keywords——cholesterol biosynthesis; [24,25-$^3H_2$]-24,25-dihydrolanosterol; oxygenated lanosterol derivative; inhibitory activity; cytochrome P-450; aphidicolin

Introduction

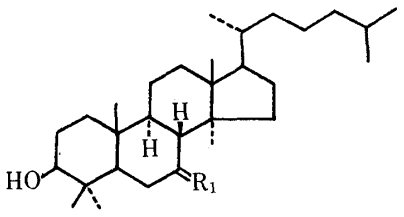
Cholesterol is an important constituent of lipids and is biosynthesized from acetate *via* mevalonic acid, squalene, and lanosterol.[1] There are several regulatory steps in cholesterol biosynthesis. Although 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is considered to be the major rate-limiting enzyme in the sterol biosynthetic pathway, conversion of lanosterol to C-27 sterols is also a natural regulatory step in a number of different tissues.[2] These facts suggest that inhibition of lanosterol metabolism could regulate cholesterol level *in vivo*.

Recently, we reported the effects of lanosterol analogs,[3] cholesterol analogs,[4] oxygenated lanosterol derivatives,[5] and oxygenated cholesterol derivatives[6] on cholesterol biosynthesis from lanosterol or 24,25-dihydrolanosterol. From these studies, it was clarified that both the side chain and the skeleton structures of steroids are important for the inhibitory effects on cholesterol synthesis. Of the tested compounds, 7-oxo-lanost-8-en-3β-ol (7-oxo-DHL), 14α-methylcholest-7-ene-3β,15α-diol, and lanost-7-ene-3β,15α-diol have been shown to be potent inhibitors of cholesterol biosynthesis from lanosterol or 24,25-dihydrolanosterol in rat hepatic subcellular fraction, and the results suggested that the site of inhibition is lanosterol 14-demethylation.[5] Further, 7-oxo-DHL[7] was shown to exhibit hypolipidemic activity in rats. In the hope of obtaining more potent derivatives of 7-oxo-DHL (**1**), 7-oxolanost-8-ene-3β,25-diol (**3**) and 7,24-dioxolanost-8-en-3β-ol (**4**) were synthesized and their inhibitory activities were determined. Further, the structure-inhibitory activity relationship of lanosterol derivatives (5—27, Fig. 1), including 32-oxygenated lanosterols, on cholesterol biosynthesis from 24,25-dihydrolanosterol was examined. The inhibitory potency of the lanosterol derivatives was proved to be influenced by the location of the hydroxyl or ketone function.

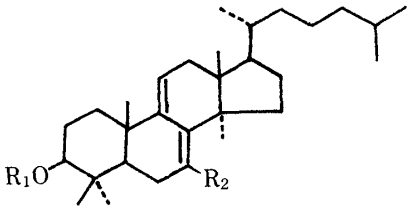
In addition, we used aphidicolin as a model sample in our experiment since aphidicolin is



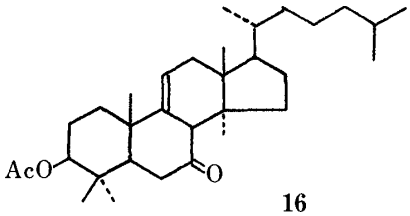
	R_1	R_2	R_3	R_4	R_5
1	H	O	H_2	H_2	H
2	Ac	O	H_2	H_2	H
3	H	O	H_2	H_2	OH
4	H	O	H_2	O	H
5	H	H, OH	H_2	H_2	H
6	H	O	O	H_2	H
7	H	H_2	O	H_2	H
8	H	H_2	H, OH	H_2	H
9	H	H_2	OH, H	H_2	H
10	H	H_2	H_2	H_2	OH
21	H	H_2	H_2	H_2	H



	R_1
11	O
12	H, OH



	R_1	R_2
13	H	Cl
14	Ac	OAc
15	H	OEt
20	H	H



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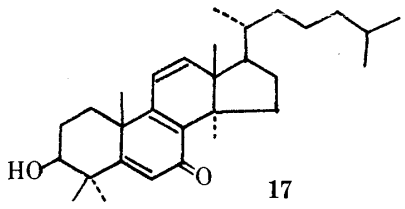

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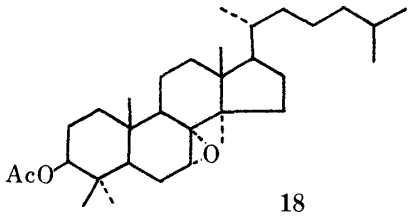

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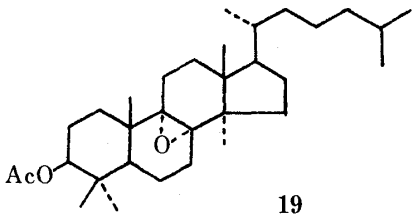

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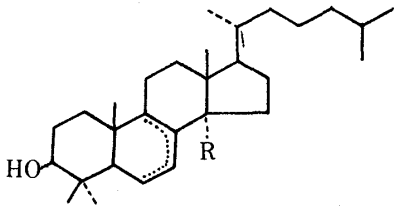

	R
22	CH_2OH (Δ^8)
23	CH_2OH (Δ^7)
24	CH_2OH (Δ^6)
25	CH_2OH
26	CHO (Δ^8)
27	CHO (Δ^7)

Fig. 1. The Structures of the Oxygenated Lanosterol Derivatives

known to inhibit the sterol synthesis from mevalonolactone by mouse liver homogenate *in vitro*. Moreover, aphidicolin is also known to inhibit deoxyribonucleic acid (DNA) polymerase α.[8)]

Materials and Methods

All melting points were obtained on a micro-melting point determination apparatus (type MM2, Shimadzu

Seisakusho Ltd.) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded at 270 MHz on a JEOL JNM-GX 270 FT NMR spectrometer in deuteriochloroform with tetramethylsilane as an internal standard. Abbreviations used: s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra (MS) were determined on a JEOL D-100 spectrometer at 75 eV ionizing potential. "The usual work-up" refers to dilution with water, extraction with methylene chloride, washing to neutrality with water, drying over anhydrous sodium sulfate, filtration, and evaporation under reduced pressure.

Test Compounds——7-Oxolanost-8-en-3β-ol (**1**),[9] 3β-acetoxylanost-8-en-7-one (**2**),[9] lanost-8-ene-3β,7α-diol (**5**),[10] 7,11-dioxolanost-8-en-3β-ol (**6**),[11] 11-oxolanost-8-en-3β-ol (**7**),[12] lanost-8-ene-3β,11α-diol (**8**),[13] lanost-8-ene-3β,11β-diol (**9**),[13] lanost-8-ene-3β,25-diol (**10**),[14] 7-oxolanostan-3β-ol (**11**),[15] lanostane-3β,7α-diol (**12**),[15] 3β-acetoxylanost-9(11)-en-7-one (**16**),[10] 7-oxolanosta-5,8,11-trien-3β-ol (**17**),[16] 3β-acetoxy-7α,8α-epoxylanostane (**18**),[10] 3β-acetoxy-8α,9α-epoxylanostane (**19**),[17] lanostene-3β, 32-diols (**22**, **23**, and **24**),[18] and 3β-hydroxylanosten 32-als (**26** and **27**) were prepared as described previously. Aphidicolin was obtained from Wako Pure Chemical Industries, Ltd.

General Procedure for Oxidation of Position C-7 of Lanosterol Derivatives——Concentrated H_2SO_4 (0.6 ml) in AcOH (5 ml) was added to a solution of 3β-acetoxylanost-8-en-25-ol or 3β-acetoxylanost-8-en-24-one (1.0 g) in AcOH (140 ml) with stirring at 0 °C, then a solution of H_2O_2 (5 ml) in AcOH (5 ml) was added and the mixture was stirred for 17 h at room temperature. The reaction mixture was poured into H_2O and extracted with methylene chloride. The organic layer was washed with 10% $NaHSO_3$ and H_2O, dried and concentrated. The residue was hydrolyzed with 5% methanolic KOH under reflux for 1 h. After the usual work-up, the product was purified by chromatography on silica gel.

a) 7-Oxolanost-8-ene-3β,25-diol (3)——Starting from 3β-acetoxylanost-8-en-25-ol,[14] **3** was obtained in 55% yield. Recrystallization from MeOH gave **3** as colorless needles, mp 183—184 °C. *Anal.* Calcd for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.38; H, 10.99. MS *m/z*: 458 (M^+), 443 ($M^+ - CH_3$), 440 ($M^+ - H_2O$), 425 ($M^+ - CH_3$, H_2O, base peak). ^{1}H-NMR δ (ppm): 0.66 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 1.00 (3H, s, 4α-CH_3), 1.17 (3H, s, 14-CH_3), 1.22 (6H, s, 26,27-CH_3), 3.25 (1H, m, 3-H).

b) 7,24-Dioxolanost-8-en-3β-ol (4)——Starting from 3β-acetoxylanost-8-en-24-one (1.0 g), **4** was obtained in 60% yield. Recrystallization from MeOH gave **4** as colorless needles, mp 96—97 °C. *Anal.* Calcd for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 79.03; H, 11.09. MS *m/z*: 456 (M^+), 441 ($M^+ - CH_3$, base peak), 423 ($M^+ - CH_3$, H_2O). ^{1}H-NMR δ (ppm): 0.65 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 1.00 (3H, s, 4α-CH_3), 1.09 (6H, d, 26 and 27-CH_3, J = 6.6 Hz), 1.17 (3H, s, 14-CH_3), 2.60 (1H, septet, 25-H), 3.25 (1H, m, 3-H).

7-Chlorolanosta-7,9(11)-dien-3β-ol (13)——Thionyl chloride (1 ml) was added to a solution of 3β-acetoxylanost-8-en-7-one (0.2 g) in pyridine (5 ml) and the mixture was allowed to stand for 30 min at room temperature. After the usual work-up, the brown residue was column-chromatographed on silica gel (10 g). Elution with benzene gave a solid (0.12 g), which was hydrolyzed with 5% methanolic KOH to give **13**. Recrystallization from MeOH gave **13** as colorless needles, mp 153—154 °C. *Anal.* Calcd for $C_{30}H_{49}ClO$: C, 78.13; H, 10.71. Found: C, 78.09; H, 10.44. MS *m/z*: 460 (M^+ for ^{35}Cl-compound), 445 ($M^+ - CH_3$), 442 ($M^+ - H_2O$). ^{1}H-NMR δ (ppm): 0.67 (3H, s, 18-CH_3), 0.86 (6H, d, 26 and 27-CH_3, J = 6.6 Hz), 1.00 (3H, s, 19-CH_3), 3.15 (1H, m, 3-H), 5.37 (1H, m, 11-H).

7-Ethoxylanosta-7,9(11)-dien-3β-ol (15)——Ethyl orthoformate (1 ml) and *p*-TsOH (10 mg) were added to a solution of 7-oxolanost-8-en-3β-ol (0.1 g) in dioxane (2 ml) and the mixture was allowed to stand for 20 h at room temperature. After usual work-up, the residue was column-chromatographed on silica gel (5 g). Elution with benzene gave a solid (0.5 g), which was recrystallized from MeOH to give **15** as colorless needles, mp 108—109 °C. *Anal.* Calcd for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81.58; H, 11.67. MS *m/z*: 470 (M^+), 455 ($M^+ - CH_3$), 442 ($M^+ - C_2H_4$), 427 ($M^+ - CH_3$, C_2H_4). ^{1}H-NMR δ (ppm): 0.64 (3H, s, 18-CH_3), 1.26 (3H, t, $OCH_2\underline{CH_3}$), 3.23 (1H, m, 3-H), 3.76 (2H, octet, $OC\underline{H_2}CH_3$), 5.17 (1H, m, 11-H).

Lanostane-3β, 32-diol (25)——A solution of lanost-6-ene-3β,-32-diol (15 mg) in AcOH (10 ml) was shaken under a stream of hydrogen in the presence of PtO_2 (30 mg) at room temperature for 3 h. After removal of the catalyst by filtration, the filtrate was poured into water and extracted with methylene chloride. The organic layer was washed with water, saturated $NaHCO_3$, and water, then dried (Na_2SO_4), and concentrated *in vacuo*. The residue was recrystallized from MeOH to give **25** as colorless needles, mp 237—238 °C. *Anal.* Calcd for $C_{30}H_{54}O_2$: C, 80.65; H, 12.18. Found: C, 80.70; H, 11.83. MS *m/z*: 446 (M^+), 428 ($M^+ - H_2O$), 415 ($M^+ - CH_2OH$), 397 ($M^+ - H_2O$, CH_2OH, base peak). ^{1}H-NMR δ (ppm): 0.80 (3H, s, 18-CH_3), 0.83 (3H, s, 4β-CH_3), 0.87 (6H, d, 26 and 27-CH_3, J = 6.6 Hz), 0.92 (3H, s, 19-CH_3), 0.97 (3H, s, 4α-CH_3), 3.20 (1H, m, 3-H), 3.57 and 3.88 (each 1H, d, 32-CH_2OH, J = 11 Hz).

Incubation Procedure and Assays for Effects of Lanosterol Derivatives on Cholesterol Biosynthesis from 24,25-Dihydrolanosterol——[24,25-$^3H_2$]-24,25-Dihydrolanosterol was prepared by catalytic tritiation of lanosteryl acetate in the presence of 5% Pd–C at the Radiochemical Centre, Amersham, England, followed by alkaline hydrolysis. Hepatic subcellular 10000 × *g* supernatant fractions (S-10) were prepared from Wistar male rats weighing 125—150 g as described by Gibbons *et al.*[19] The incubation mixture consisted of S-10 fraction (4 ml, 20.2—21.0 mg/ml), 1 mM ethylenediamine tetraacetic acid (EDTA), 30 mM nicotinamide, 10 mM glutathione (GSH), 2 mM nicotinamide adenine dinucleotide phosphate (NADP), 12 mM glucose-6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 1.3 mM

reduced nicotinamide adenine dinucleotide (NADH), 0.8 mM nicotinamide adenine dinucleotide (NAD), 1.3 mM adenosine triphosphate (ATP), 4 mM $MgCl_2$ and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 5 ml. The control incubation was started by the addition of [24,25-$^3H_2$]-24,25-dihydrolanosterol (8.85×10^6 dpm; 42.6 μCi/μmol, 18 μM) in phosphate buffer emulsion (0.1 ml) containing Tween 80 (3 mg). In the inhibition experiments, [24,25-$^3H_2$]-24,25-dihydrolanosterol and lanosterol derivatives or aphidicolin were added at the same time in each experiment. Incubations were carried out at 37 °C for 3h, and MeOH and KOH were added to final concentrations of 50% and 10%, respectively. The mixture was heated at 70 °C for 1 h, and sterols were extracted with CH_2Cl_2. The extracts were washed with water, dried over sodium sulfate and concentrated to a few milliliters. After addition of carriers, 24,25-dihydrolanosterol (1 mg) and cholesterol (1 mg), to the solution, it was subjected to silica gel thin layer chromatography (TLC). As standard samples, 24,25-dihydrolanosterol and cholesterol were also applied to both edges of TLC plate to identify the zones of 4,4-dimethyl and 4,4-demethyl sterol fractions. After development with CH_2Cl_2 as the mobile phase, only the sterols of both edges were sprayed with concentrated H_2SO_4 and the plate was heated to develop color. Then the radioactive 4,4-dimethyl sterol fraction and 4,4-demethyl sterol fraction were collected separately. Appropriate amounts of 24,25-dihydrolanosterol were added to the eluate of the 4,4-dimethyl sterol fraction, and the mixture was recrystallized several times to a constant specific activity. The 4,4-demethyl sterol fraction obtained by silica gel TLC was isolated as the digitonin-precipitable sterols as described by Popjak[20] and counted with a liquid scintillation spectrometer. The amount of cholesterol biosynthesis was estimated from the radioactivity of the 4,4-demethyl sterol fraction (*i.e.*, cholesterol fraction). Results are expressed as the percentage inhibition as follows: Percent inhibition of cholesterol biosynthesis = [(percent yield of cholesterol in control − percent yield in run with test compound)/percent yield in control] × 100.

Results

Effects of Aphidicolin and 7-Oxo-DHL on Sterol Synthesis

Leonard and Chen[8] reported that the diterpene-type compound aphidicolin, a specific inhibitor of DNA polymerase α, was a rapid inhibitor of lanosterol metabolism at a proposed secondary regulatory site, 14-demethylation, in sterol biosynthesis. Further, they suggest that a naturally occurring compound may exist which can regulate both DNA replication and cholesterogenesis. Although 7-oxo-DHL is not a naturally occurring compound, it is the most potent triterpene-type inhibitor of lanosterol metabolism which we could obtain by simple

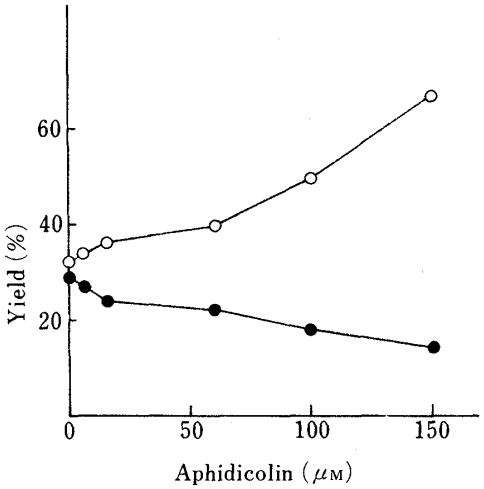

Fig. 2. Effect of Aphidicolin on Cholesterol Biosynthesis from 24,25-Dihydrolanosterol

S-10 fr. was incubated with [24,25-$^3H_2$]-24,25-dihydrolanosterol at various concentrations of aphidicolin. Closed circles (●) show the amount (%) of cholesterol biosynthesis from 24,25-dihydrolanosterol. Open circles (○) show the amount (%) of recovered 24,25-dihydrolanosterol. Points each represent the mean of duplicate determinations.

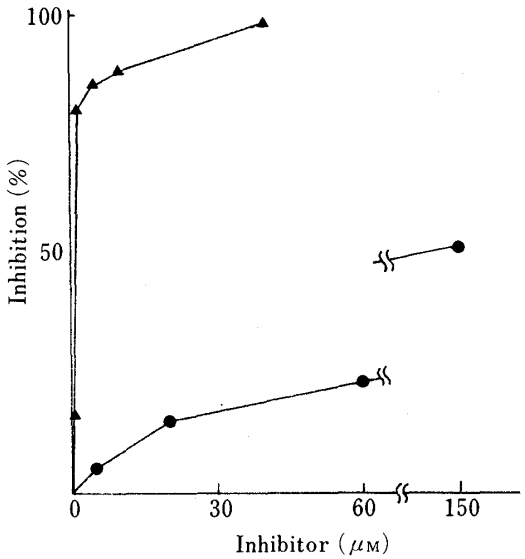

Fig. 3. Inhibitory Effects of Aphidicolin and 7-Oxo-DHL on Cholesterol Synthesis from 24, 25-Dihydrolanosterol

[24,25-$^3H_2$]-24,25-Dihydrolanosterol was incubated with various concentrations of aphidicolin (●) or 7-oxo-DHL (▲). Points each represent the mean of duplicate determinations.

chemical synthesis. Aphidicolin is commercially available. Therefore, we compared the inhibitory effects of 7-oxo-DHL and aphidicolin on cholesterol biosynthesis from 24,25-dihydrolanosterol.

Incubation of $10000 \times g$ supernatant of rat liver homogenates with [24,25-$^3H_2$]-24,25-dihydrolanosterol in the presence of increasing concentrations of aphidicolin resulted in a decrease in the incorporation of radioactivity into cholesterol and an increase in the recovery of the unmetabolized substrate. The results are shown in Fig. 2. 7-Oxo-DHL showed a very much higher inhibitory effect on cholesterol synthesis than that of aphidicolin (Fig. 3). The concentration of aphidicolin required for inhibiting *in vitro* cholesterol synthesis by 50% was as high as 144 μM, which corresponds to *ca.* 200-fold higher concentration than that in the case of 7-oxo-DHL. These results demonstrated that 7-oxo-DHL was a more potent inhibitor than aphidicolin in the *in vitro* 14-demethylation of 24,25-dihydrolanosterol.

Effects of Lanosterol Derivatives on Cholesterol Synthesis

The inhibitory effects of lanosterol derivatives were tested at a concentration of 5 μM and the results are shown in Table I. Among the tested compounds, 7-oxo-DHL (**1**), 7-oxo-DHL-3OAc (**2**), and the 7-oxo-triene compound (**17**) were highly potent inhibitors (85—87% inhibition) of cholesterol biosynthesis (Table I). Since 3β-acetoxylanost-8-ene is hydrolyzed to dihydrolanosterol and further metabolized to cholesterol by incubation with S-10 fr. of rat liver homogenate without cofactors, it is suggested that the 3β-acetoxy compound (**2**) was also hydrolyzed to the 3β-hydroxy compound (**1**) to cause the inhibition of cholesterol synthesis. 7-

TABLE I. Cholesterol Biosynthesis during Incubation of the S-10 Fraction of Rat Liver Homogenate with [24,25-$^3H_2$]-24,25-Dihydrolanosterol in the Presence of Lanosterol Derivatives

Compound	24,25-Dihydrolanosterol fr. (%)	Cholesterol fr. (%)	Inhibition (%)
None (control)	32.9	29.0	—
7-Oxolanost-8-en-3β-ol (**1**)	84.5	4.3	85
3β-Acetoxylanost-8-en-7-one (**2**)	86.4	3.8	87
7-Oxolanost-8-ene-3β,25-diol (**3**)	74.8	6.0	79
7,24-Dioxolanost-8-en-3β-ol (**4**)	61.9	8.9	69
Lanost-8-ene-3β,7α-diol (**5**)	51.0	15.4	47
7,11-Dioxolanost-8-en-3β-ol (**6**)	47.4	17.4	40
11-Oxolanost-8-en-3β-ol (**7**)	45.0	19.9	31
Lanost-8-ene-3β,11α-diol (**8**)	63.5	11.4	61
Lanost-8-ene-3β,11β-diol (**9**)	31.9	29.5	0
Lanost-8-ene-3β,25-diol (**10**)	40.7	20.8	28
7-Oxolanostan-3β-ol (**11**)	30.9	27.8	4
Lanostane-3β,7α-diol (**12**)	34.6	24.6	15
7-Chlorolanosta-7,9(11)-dien-3β-ol (**13**)	34.8	25.1	13
3β,7-Diacetoxylanosta-7,9(11)-diene (**14**)	30.6	33.1	0
7-Ethoxylanosta-7,9(11)-dien-3β-ol (**15**)	63.6	13.3	55
3β-Acetoxylanost-9(11)-en-7-one (**16**)	29.0	31.4	0
7-Oxolanosta-5,8,11-trien-3β-ol (**17**)	84.0	4.0	86
3β-Acetoxy-7α,8α-epoxylanostane (**18**)	39.7	24.6	15
3β-Acetoxy-8α,9α-epoxylanostane (**19**)	34.5	26.4	9
Lanosta-7,9(11)-dien-3β-ol (**20**)	33.1	25.8	11
Lanost-8-en-3β-ol (**21**)	34.0	25.0	14

[24,25-$^3H_2$]-24,25-Dihydrolanosterol (8.85×10^6 dpm; 42.6 μCi/μmol, 18 μM) was incubated with rat liver S-10 fraction at 37 °C for 3 h. The incubation mixture contained, in a total volume of 5 ml, 4 ml of S-10 fraction and cofactors. Incubation was started by the addition of the substrate and the test compound as an emulsion (0.1 ml) with Tween 80 (3 mg). Analytical methods for incubation products and calculation of the inhibition were described in Materials and Methods. Each incubation was carried out in triplicate and the relative standard deviation of each value listed was less than 5 percent.

Oxolanost-8-ene-3β,25-diol (**3**) and 7,24-dioxolanost-8-en-3β-ol (**4**) also showed high inhibitory effects (79 and 69% inhibition, respectively). In connection with the O-functional group at C-11, the order of inhibitory activity was 11α-hydroxy compound (**8**) > 11-oxo compound (**7**) > 11β-hydroxy compound (**9**). Further, concerning the position C-7, the order of inhibitory activity was 7-oxo compound (**1**) > 7α-hydroxy compound (**5**) > 7,11-dioxo compound (**6**). These results suggest that for interaction of lanosterol derivatives with the enzyme concerned there are some steric and electrostatic requirements in the region of C-7, C-8, C-9 and C-11 of the steroid nucleus. 3β-Acetoxylanost-9(11)-en-7-one (**16**), 7-oxolanostan-3β-ol (**11**) and lanostane-3β, 7α-diol (**12**) showed no inhibitory effect even though these compounds have a 7-oxo or 7-hydroxy group. 7,8-Epoxy and 8,9-epoxy compounds (**18** and **19**) showed slightly inhibitory effects, and the 7-chloro compound (**13**) also showed an equally low effect. These results clearly demonstrate that the 8,9-double bond and O-functional groups at C-7 in lanosterol derivatives make an important contribution in exhibiting the inhibitory effect. The 7-acetoxy compound (**14**) showed no activity, but the 7-ethoxy compound (**15**) had a moderate inhibitory effect.

The 25-hydroxy compound (**10**), which has a hydroxy group in the side chain, showed a weak inhibitory effect at the concentration of 5μM.[21] Compounds **20** and **21** having no additional oxygen functional group showed slight inhibitory effects (11 and 14%).

32-Oxygenated dihydrolanosterols (**22**—**27**) were tested at concentrations of 5 and 40 μM, and results are shown in Table II. 14-Hydroxymethyl compounds (**22**, **23** and **24**) and 14-aldehydes (**26** and **27**), which are suggested to be the important intermediates of cholesterol synthesis from lanosterol, showed slight inhibitory effects at a concentration of 5 μM on [^{3}H]-24,25-dihydrolanosterol transformation into [^{3}H]-cholesterol by incubation with S-10 fraction. At a concentration of 40 μM, however, these oxygenated sterols showed strong inhibitory effects (65—76%). These results are consistent with those of Gibbons *et al.*[22] However, the 14-hydroxymethyl compound (**25**) having no double bond in the skeleton had not appreciable inhibitory effect at concentrations of 5 and 40 μM. These results are consistent with the view that an oxygen functional group and double bond are indispensable for the inhibitory interaction with the enzyme. Recently, Aoyama *et al.*[23] demonstrated that lanost-8-ene-3β,32-diol is an intermediate of the 14α-demethylation of 24,25-dihydrolanosterol by purified cytochrome P-450$_{14DM}$ of *Saccharomyces cerevisiae*. Further, Trzaskos *et al.*[24] reported

TABLE II. Cholesterol Biosynthesis during S-10 Fraction of Rat Liver Homogenate with [24,25-$^3H_2$]-24,25-Dihydrolanosterol in the Presence of 32-Oxygenated Lanosterols

Compound	Concentration (μM)	24,25-Dihydrolanosterol fr. (%)	Cholesterol fr. (%)	Inhibition (%)
None (control)	—	30.5	30.2	—
Lanost-8-ene-3β,32-diol (**22**)	5	53.2	17.5	42
	40	78.9	7.3	76
Lanost-7-ene-3β,32-diol (**23**)	5	52.1	18.5	39
	40	70.1	9.2	70
Lanost-6-ene-3β-32-diol (**24**)	5	51.5	19.7	35
	40	68.0	10.8	64
Lanostane-3β,32-diol (**25**)	5	32.3	29.1	4
	40	35.3	27.2	10
3β-Hydroxylanost-8-en-32-al (**26**)	5	45.2	18.8	38
	40	71.2	8.2	73
3β-Hydroxylanost-7-en-32-al (**27**)	5	44.3	19.7	35
	40	70.9	8.8	71

Incubation and assays were carried out by the same methods as described in Table I.

conditions which promote the accumulation of oxysterol intermediates (lanost-8-ene-3β,32-diol and 3β-hydroxylanost-8-en-32-al) during the demethylation process with intact hepatic microsomes. These and our present results support the view that the oxygenated sterols produced as intermediates during cholesterol biosynthesis may possibly act as regulators of cholesterol biosynthesis.

Discussion

In the presence of active inhibitors, the recovery yield of the substrate ([24,25-$^3H_2$]-24,25-dihydrolanosterol) increased in parallel with the extent of inhibition. The results suggest that a potent inhibitor may inhibit 14-demethylation of 24,25-dihydrolanosterol, which is the first step of transformation of 24,25-dihydrolanosterol to cholesterol, as described in the preceding paper.[5] Further, the enzyme involved in the initial step of the 14-demethylation was thought to be a cytochrome P-450, and oxygenated lanosterol derivatives with high inhibitory activity are suggested to interact with an active center of cytochrome P-450, as in the case of 7-oxo-DHL.

Lanosterol derivatives which show insufficient interaction with cytochrome P-450 may have only a slight inhibitory effect. It is important that the 7-oxygenated 8,9-saturated compounds (**11** and **12**) are not inhibitory, suggesting the importance of the presence of the double bond in active inhibitors. The structural requirements of lanosterol derivatives for interaction with cytochrome P-450 are proposed to be as follows: (1) an O-functional group near the center of the skeleton (C-8—C-9). (2) a double bond in the same neighborhood.

On the other hand, lanosterol derivatives having a β-hydroxy group with axial stereochemistry could not interact with cytochrome P-450, even though this compound has the structural features mentioned above. The 7α, 8α- and 8α, 9α-epoxy compounds (**18** and **19**) have the B/C-*cis* structure which is very different from the lanost-8-ene skeleton; this may account for their negligible effect.

In summary, the present studies on the effects of many lanosterol derivatives on cholesterol synthesis indicate that endogenous intermediates in sterol synthesis are noteworthy candidates as regulators of biosynthesis.

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