

## Effects of 19-Oxygenated Lanosterol Derivatives on 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Activity and Lanosterol Demethylase Activity

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The effects of 19-oxygenated lanosterol derivatives on lanosterol 14 $\alpha$ -demethylase (P-450<sub>14DM</sub>) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity were studied. 3 $\beta$ -Hydroxylanost-9(11)-en-19-oic acid (**6**) was found to be an effective inhibitor (IC<sub>50</sub>: 0.5  $\mu$ M) of P-450<sub>14DM</sub> in the reconstituted system using purified pig liver P-450<sub>14DM</sub> and was most active in inhibiting HMG-CoA reductase activity (IC<sub>50</sub>: 1.0  $\mu$ M) in mouse L cells. In [2-<sup>14</sup>C]acetate incorporation studies using mouse L cells, **6** was found to reduce the incorporation of acetate into C<sub>27</sub>-sterol (desmosterol) with a concomitant increase in radiolabeled C<sub>30</sub>-sterols. These results demonstrate that **6** is a dual-action inhibitor of cholesterol biosynthesis.

**Key words** HMG-CoA reductase; cholesterol biosynthesis inhibitor; lanosterol demethylase; 19-oxygenated lanosterol derivative; P-450<sub>14DM</sub>

Cholesterol is an important constituent of lipids and is biosynthesized from acetate *via* mevalonic acid, squalene, and lanosterol.<sup>1)</sup> 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the formation of mevalonic acid, is considered to be the rate-limiting enzyme in the sterol biosynthetic pathway.<sup>1)</sup> Many oxygenated sterols have been found to be potent inhibitors of sterol synthesis in animal cells in culture, and the main mode of action is thought to be at the level of HMG-CoA reductase.<sup>2)</sup> However, mevalonic acid is a common precursor for all isoprenoids and, therefore, blocking mevalonic acid formation may induce undesirable side-effects in addition to inhibition of sterol synthesis. We have examined lanosterol 14-demethylation and the subsequent steps leading to cholesterol.

We have reported the effects of lanosterol analogs,<sup>3)</sup> cholesterol analogs,<sup>4)</sup> oxygenated lanosterol derivatives,<sup>5)</sup> and oxygenated cholesterol derivatives<sup>6)</sup> on cholesterol biosynthesis from lanosterol or 24,25-dihydrolanosterol (DHL). From these studies, it was clear that both the side-chain and skeleton of steroids are important for the inhibitory effects on cholesterol synthesis. Of the tested compounds, 7-oxolanost-8-en-3- $\beta$ -ol (7-oxo-DHL),<sup>5)</sup> 14 $\alpha$ -methylcholest-7-ene-3 $\beta$ ,15 $\alpha$ -diol,<sup>6)</sup> and lanost-7-ene-3 $\beta$ ,15- $\alpha$ -diol<sup>6)</sup> have been shown to be potent inhibitors of cholesterol biosynthesis from lanosterol or DHL in the hepatic subcellular fraction of rats, and the results have suggested that the target of inhibition is lanosterol 14-demethylase (P-450<sub>14DM</sub>).<sup>5,6)</sup> 7-Oxo-DHL exhibited inhibitory activity on partially purified P-450<sub>14DM</sub> from rat liver<sup>7)</sup> and purified P-450<sub>14DM</sub> from yeast.<sup>8)</sup> Furthermore, 7-oxo-DHL has been shown to exhibit hypolipidemic activity in rats.<sup>9)</sup> Recently, we reported the effects of 32-oxygenated lanosterol derivatives on HMG-CoA reductase and cholesterol biosynthesis from DHL.<sup>10)</sup> Among the tested compounds, 3 $\beta$ -hydroxylanost-7-en-32-oic acid ( $\Delta^7$ -14-COOH), an antineoplastic sterol,<sup>10)</sup> was the most active in suppressing HMG-CoA reductase (IC<sub>50</sub>: 0.7  $\mu$ M) and cholesterol biosynthesis (IC<sub>50</sub>: 0.4  $\mu$ M) from

DHL. In the hope of obtaining more potent derivatives of lanosterol, 19-oxygenated lanosterol derivatives (**3–6**, Chart 1) were synthesized and their inhibitory activities of HMG-CoA reductase activity using mouse L cells and lanosterol 14 $\alpha$ -demethylase activity using purified pig P-450<sub>14DM</sub> were determined. A recent study indicates that some oxylanosterols may be involved in post-transcriptional regulation of HMG-CoA reductase.<sup>11)</sup>

### MATERIALS AND METHODS

**Materials** 7-Oxo-24,25-dihydrolanosterol was prepared as described previously.<sup>12)</sup> The purity of compounds used in this study was >98%. Dithiothreitol (DTT) and NADPH were obtained from Boehringer Corporation (Mannheim, Germany). TLC was performed using Merck precoated Kiesel gel plates (0.25 mm thick). [2-<sup>14</sup>C]Acetate (1.85 GBq/nmol), DL-[3-<sup>14</sup>C]3-hydroxy-3-methylglutaryl CoA (1.92 GBq/mmol) and DL-[<sup>3</sup>H]mevalonic acid (925 MBq/mmol) were obtained from New England Nuclear Corp. (Wilmington, DE, U.S.A.). Brij 96 (detergent) was kindly supplied by Kao-Atras Co. (Japan). All other chemicals were obtained from Wako Pure Chemical Ind. Ltd. (Japan). Fetal bovine serum was purchased from GIBCO (Grand Island, NY, U.S.A.). Dulbecco's modified Eagle minimum essential medium (DMEM) was obtained from Nissui Co. (Japan).

**Synthesis of 19-Oxygenated Lanosterol Derivatives** All melting points were obtained on a micro-melting point determination apparatus and are uncorrected. <sup>1</sup>H-NMR spectra were obtained on a JEOL FX-270 NMR spectrometer using deuterated chloroform. Abbreviations used: s=singlet, br s=broad singlet, dd=double doublet, m=multiplet. MS were recorded on a JEOL D-100 spectrometer at an ionizing potential of 75 eV. The usual work-up refers to dilution of the reaction mixture with water, extraction with CH<sub>2</sub>Cl<sub>2</sub>, washing the extract with water, drying over Na<sub>2</sub>SO<sub>4</sub>, and concentration under reduced pressure.

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(i) Reaction of 3 $\beta$ -Acetoxy lanost-8-en-11 $\beta$ -ol (**1**) with Lead Tetraacetate: A suspension of lead tetraacetate (1.77 g, 4 mmol) and **1** (486 mg, 1 mmol) in abs. benzene (100 ml) was refluxed for 1 h. After the reaction mixture had cooled to room temperature, insoluble lead salts were filtered. The filtrate was washed with 10% Na<sub>2</sub>SO<sub>3</sub> solution and water, and dried over Na<sub>2</sub>SO<sub>4</sub>. The benzene was evaporated under reduced pressure and the residue chromatographed on silica-gel (50 g). Elution with methylene chloride afforded 11 $\beta$ ,19-epoxy lanost-8-en-3 $\beta$ -acetate (**2**, 100 mg), which was recrystallized from MeOH to give colorless needles, mp 183—185 °C. *Anal.* Calcd for C<sub>32</sub>H<sub>52</sub>O<sub>3</sub>: C, 79.28; H, 10.81. Found: C, 79.12; H, 11.26. MS *m/z*: 455 (M<sup>+</sup> - 29, base peak), 395 (M<sup>+</sup> - 29, AcOH). <sup>1</sup>H-NMR  $\delta$  (ppm): 0.81 (3H, s, 18-CH<sub>3</sub>), 0.86 (6H, d, 26, 27-CH<sub>3</sub>, *J* = 6.6 Hz), 0.85, 0.86, 0.91 (each s, 3 quaternary CH<sub>3</sub>), 2.04 (3H, s, 3-OCOCH<sub>3</sub>), 3.34, 3.98 (each 1H, each d, 19-CH<sub>2</sub>, *J* = 11 Hz), 4.55 (1H, dd, 3-H, *J* = 11, 5 Hz), 4.18 (1H, br s, 11-H).

(ii) 3 $\beta$ -Hydroxy lanost-8-en-19,11 $\beta$ -lactone (**3**): Jones reagent (0.5 ml) was added slowly (over 10 min) to a stirred solution of **2** (0.1 g) in acetone (30 ml) and stirring was continued for 2 h at room temperature. After the usual work-up, the extracted product was hydrolyzed with 10% methanolic KOH under reflux for 1 h. After the usual work-up, the residue was recrystallized from MeOH to give colorless needles of **3**, mp 261—263 °C. *Anal.* Calcd for C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>: C, 78.89; H, 10.59. Found: C, 78.93; H, 10.67. MS *m/z*: 456 (M<sup>+</sup>, base peak), 438 (M<sup>+</sup> - H<sub>2</sub>O). <sup>1</sup>H-NMR  $\delta$  (ppm): 0.79 (3H, s, 18-CH<sub>3</sub>), 0.86 (6H, d, 26, 27-CH<sub>3</sub>, *J* = 6.6 Hz), 0.84, 0.88, 0.94 (each 3H, each s, quaternary CH<sub>3</sub>), 3.23 (1H, dd, 3-H, *J* = 11, 5 Hz), 4.52 (1H, br s, 11-H).

(iii) 3 $\beta$ ,11 $\beta$ -Dihydroxy lanostan-19-oic-Acid (**4**): A solution of **3** (0.1 g) in EtOH (2 ml) was added to a solution of 2% NaOEt in EtOH (50 ml) and the reaction mixture was refluxed for 1 week, then poured into ice-water and neutralized with dilute HCl. The usual work-up afforded a yellow residue, which was column-chromatographed on Sephadex LH-20 (1 cm  $\times$  10 cm). Elution with methylene chloride (fr. 1) gave a solid (0.06 g) which was identified as **3**. Further elution with methylene chloride (fr. 5 and 6) gave a solid (0.03 g), which was recrystallized from MeOH to give **4** as colorless needles, mp 295—296 °C. *Anal.* Calcd for C<sub>30</sub>H<sub>52</sub>O<sub>4</sub>: C, 75.58; H, 11.00. Found: C, 75.29; H, 10.52. MS *m/z*: 458 (M<sup>+</sup> - H<sub>2</sub>O), 440 (M<sup>+</sup> - 2H<sub>2</sub>O), 414 (M<sup>+</sup> - H<sub>2</sub>O - CO<sub>2</sub>), 55 (base peak). <sup>1</sup>H-NMR  $\delta$  (ppm): 0.78 (3H, s, 18-CH<sub>3</sub>), 0.86 (6H, d, 26, 27-CH<sub>3</sub>, *J* = 6.6 Hz), 0.86, 0.88, 0.89 (each 3H, each s, quaternary CH<sub>3</sub>), 3.28 (1H, dd, 3-H, *J* = 11, 5 Hz), 4.00 (1H, br s, 11-H).

(iv) Methyl 3 $\beta$ -Hydroxy lanost-9(11)-en-19-oate (**5**): Compound **4** (0.1 g) was methylated using diazomethane in ether to give the methyl ester. This methyl ester was acetylated with Ac<sub>2</sub>O-pyridine for 2 h at room temperature to give the 3 $\beta$ -acetate. The crude product was dissolved in pyridine (1 ml), POCl<sub>3</sub> (0.5 ml) was added, and the mixture was refluxed for 1 h. The usual work-up afforded the residue, which was confirmed by <sup>1</sup>H-NMR to be methyl 3 $\beta$ -acetoxy lanost-9(11)-en-19-oate. This product was hydrolyzed with 10% methanolic KOH under reflux for

1 h. After the usual work-up, the residue was recrystallized from MeOH to give **5** as colorless needles, mp 98—100 °C. *Anal.* Calcd for C<sub>31</sub>H<sub>52</sub>O<sub>3</sub>: C, 78.76; H, 11.09. Found: C, 79.17; H, 10.78. MS *m/z*: 413 (M<sup>+</sup> - COOCH<sub>3</sub>), 395 (M<sup>+</sup> - COOCH<sub>3</sub>, 2H<sub>2</sub>O, base peak). <sup>1</sup>H-NMR  $\delta$  (ppm): 0.70 (3H, s, 18-CH<sub>3</sub>), 0.86 (6H, d, 26, 27-CH<sub>3</sub>, *J* = 6.6 Hz), 0.89, 0.90, 0.99 (each 3H, each s, quaternary CH<sub>3</sub>), 3.24 (1H, dd, 3-H, *J* = 11, 5 Hz), 3.63 (3H, s, COOCH<sub>3</sub>), 5.2 (1H, br s, 11-H).

(v) 3 $\beta$ -Hydroxy lanost-9(11)-en-19-oic Acid (**6**): Compound **5** (50 mg) in 2,4,6-collidine (5 ml) was heated under reflux with lithium iodide (0.25 g) for 3 h. The reaction mixture was cooled, poured into water, acidified with dilute HCl, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract was washed with water, dried, and evaporated to give a solid, which was column-chromatographed on silica-gel. Elution with CH<sub>2</sub>Cl<sub>2</sub> gave **6**, which was recrystallized from AcOEt-*n*-hexane to give colorless needles of mp 220—222 °C. *Anal.* Calcd for C<sub>30</sub>H<sub>50</sub>O<sub>3</sub>: C, 78.55; H, 10.99. Found: C, 78.21; H, 10.85. MS *m/z*: 458 (M<sup>+</sup>), 430 (M<sup>+</sup> - 28, base peak). <sup>1</sup>H-NMR (ppm): 0.70 (3H, s, 18-CH<sub>3</sub>), 0.86 (6H, d, 26, 27-CH<sub>3</sub>, *J* = 6.6 Hz), 0.89 (6H, s, quaternary CH<sub>3</sub>), 0.99 (3H, s, quaternary CH<sub>3</sub>), 3.24 (1H, dd, 3-H, *J* = 11, 5 Hz), 5.5 (1H, br s, 11-H).

**Assay for 14 $\alpha$ -Demethylation Activity Using the Reconstituted System** The substrate, DHL, was dispersed with dilauroylphosphatidyl choline (DLPC), as described previously.<sup>13</sup> The reaction mixture (final vol., 0.5 ml) consisted of 0.01 nmol P-450<sub>14DM</sub>, 1 unit NADPH-P-450 reductase, 23 nmol substrate dispersed in 130 nmol DLPC micelles, 100 mM potassium phosphate buffer (pH 7.4, containing 20% glycerol, 0.2  $\mu$ mol MgCl<sub>2</sub>, and 20  $\mu$ mol glucose 6-phosphate) and 0.2 units glucose 6-phosphate dehydrogenase. The reaction was started by adding 1  $\mu$ mol NADPH to this mixture, which was incubated at 37 °C for 30 min, with constant shaking, in air, then stopped by adding 1.5 ml 20% KOH and 2 ml methanol. After saponification of the mixture at 80 °C for 60 min, sterols were extracted with methylene chloride, the organic layer was dried over sodium sulfate and the solvent was evaporated. The extracted sterols were analyzed by high-performance liquid chromatography (HPLC) on a  $\mu$ -Bondapak C<sub>18</sub> reverse-phase column (3.9 mm  $\times$  30 cm), using acetonitrile-methanol-water (46 : 45 : 9, v/v/v) as the eluent (flow rate 1.5 ml/min) and detected at 248 nm. For the calculation of 14 $\alpha$ -demethylation activity, 1  $\mu$ g ergosterol was added as an internal standard prior to extraction of the sterols from the incubation mixture. The amount (nmol) of 14-demethylated product was calculated as follows. Peak area of the 14-demethylated product/peak area of ergosterol  $\times$  1000<sup>a</sup>  $\times$  1/5<sup>b</sup>  $\times$  1/412<sup>c</sup> (a: internal standard 1000 ng, b: peak of the 14-demethylated product was 5-fold larger than that of ergosterol at 248 nm in the case of equal weight injections, c: molecular weight of the 14-demethylated product). The test compound was added to the reaction mixture as a DLPC (50  $\mu$ g) suspension (5  $\mu$ l). A corresponding DLPC suspension without the test compound was added in the control experiment.

**Cell Culture** Mouse L cells were grown as monolayers according to the method of Kaneko *et al.*<sup>14</sup> On day 0, 2.5  $\times$  10<sup>5</sup> cells were seeded in 60  $\times$  15 mm plastic dishes in

DMEM (5 ml) containing 5% (v/v) fetal bovine sera. On day 3 or 4, exponentially growing cells were treated with the test compound in 3  $\mu$ l EtOH for 24 h.

**[ $^{14}$ C]Acetate Incorporation into Nonsaponifiable Lipids** Exponentially growing cultures were labeled with [ $^{14}$ C]acetate (1 mM, 185 kBq). Each sterol was added at the same time with addition of [ $^{14}$ C]acetate as a solution in EtOH (3  $\mu$ l). Incorporation of the labeled acetate into the cell culture proceeded for 24 h. The cells were scrapped into 0.5 ml ice-cold saline and saponified at 80  $^{\circ}$ C for 1 h with the addition of 1.5 ml 20% KOH and 2 ml MeOH. Nonsaponifiable lipids were extracted with methylene chloride (10 ml  $\times$  2), washed with water, and dried under nitrogen. Extracts were analyzed by TLC (methylene chloride) and counted in a liquid scintillation counter.

**Assay of HMG-CoA Reductase Activity** HMG-CoA reductase activity was assayed by the method of Kaneko *et al.*<sup>14)</sup> Aliquots of each detergent-solubilized cell extract (40–50  $\mu$ g of soluble protein) were incubated for 60 min in a final volume of 80  $\mu$ l containing 0.1 M potassium phosphate buffer (pH 7.4), 2.5 mM NADPH, 4 mM DTT, and 35  $\mu$ M DL-[3- $^{14}$ C]HMG-CoA (1.92 GBq/mmol). The reaction was terminated by adding 10  $\mu$ l 5 M HCl. After adding DL-[ $^3$ H]mevalonate as an internal standard, the mixture was incubated at 37  $^{\circ}$ C for 30 min. The [ $^{14}$ C]mevalonolactone formed in each reaction was extracted with diethyl ether, isolated by TLC, and counted using an internal standard of [ $^3$ H]mevalonate to correct for incomplete recovery.

## RESULTS AND DISCUSSION

**Synthesis of Lanosterol Derivatives** This synthetic method (Chart 1) is similar to that of  $\Delta^7$ -14-COOH<sup>10)</sup> as reported previously. Hypiodite treatment of **1** afforded the 11,19-oxide (**2**) in 20% yield together with 3 $\beta$ -acetoxy-11-oxo-lanost-8-ene. Jones oxidation of **2** gave the lactone (3-OAc) in good yield. Treatment with aqueous alkali gave the lactone (**3**, 3-OH) without opening the lactone ring. Treatment of **3** with NaOEt in EtOH gave 11-OH,10-COOH (**4**) in high yield. Compound **4** was esterified with diazomethane to give 11-OH,19-COOMe, which was further reacted with pyridine-Ac<sub>2</sub>O, POCl<sub>3</sub>, and KOH to give  $\Delta^{9(11)}$ -10-COOMe (**5**). Finally, **5** was transformed to  $\Delta^{9(11)}$ -10-COOH (**6**) with LiI.

**Effects of Lanosterol Derivatives on Lanosterol 14 $\alpha$ -Demethylase** As described previously,<sup>13)</sup> DHL was incubated with the purified P-450<sub>14DM</sub> fraction of pig liver in the presence of 19-oxygenated lanosterol derivatives (40  $\mu$ M). The results are summarized in Table 1. Among the tested compounds,  $\Delta^{9(11)}$ -10-COOH (**6**) was the most potent inhibitor (99% inhibition), and the inhibition was similar to that of 7-oxo-DHL (98% inhibition) and  $\Delta^7$ -14-COOH (99% inhibition). However, 7,32-lactone (**3**) showed only a moderate effect (50% inhibition). Although 11-OH,10-COOH (**4**) also showed a strong inhibitory effect (87% inhibition),  $\Delta^{9(11)}$ -COOMe (**5**) had a weak effect (32% inhibition). These results suggest that in the interaction of lanosterol derivatives with P-450<sub>14DM</sub>, the carboxylic acid group makes an important contribution. In addition,  $\Delta^{9(11)}$ -10-COOH (**6**) exhibited a stronger

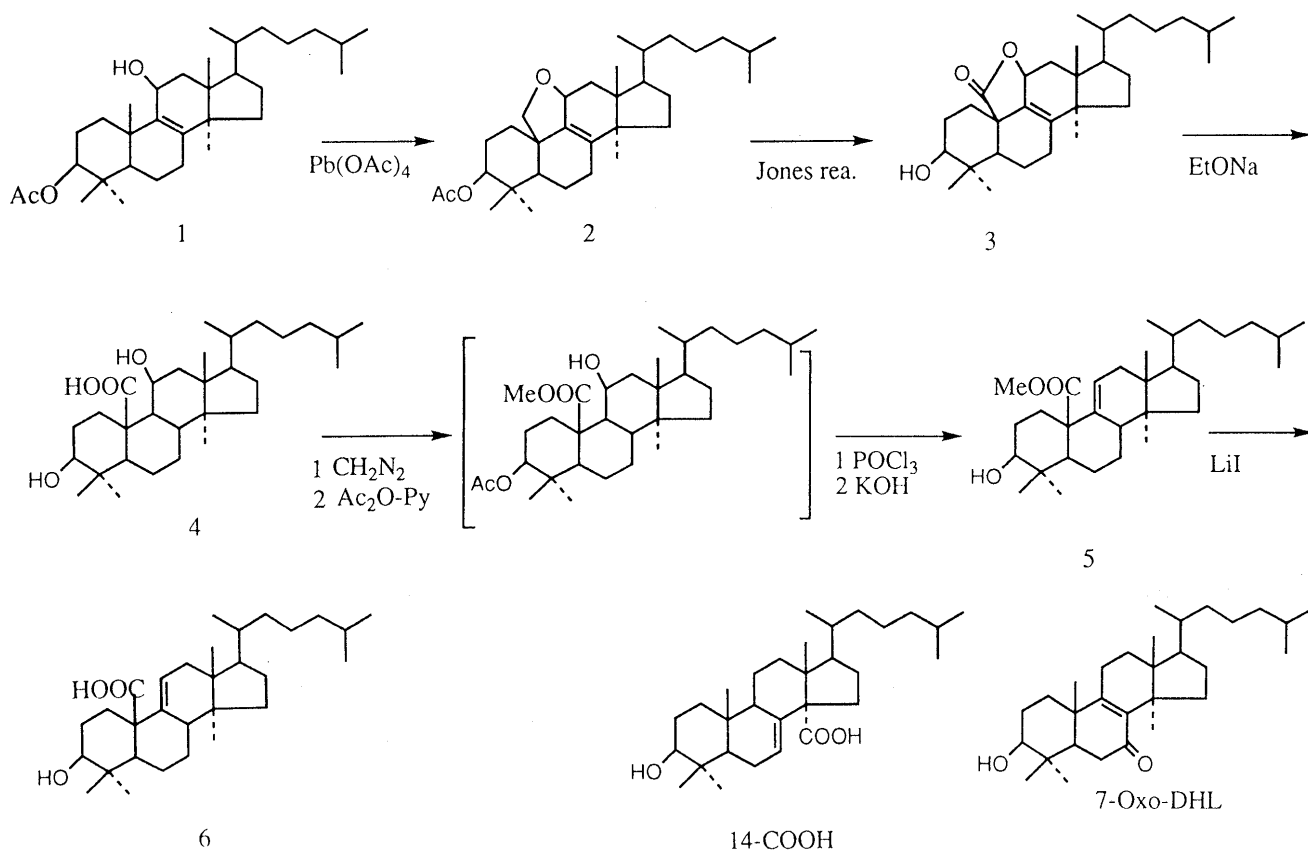
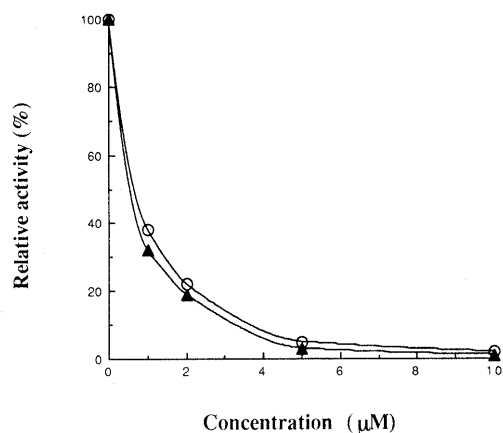


Chart 1

Table 1. Effect of 19-Oxygenated Lanosterol Derivatives on DHL 14-Demethylase Activity<sup>a)</sup>

| Compound (40 $\mu$ M)          | DHL 14-demethylase activity (nmol/min/mg) | Inhibition (%) |
|--------------------------------|---|----------------|
| None                           | 20.0                                      | —              |
| Lactone (3)                    | 14.6                                      | 27             |
| 11-OH,10-COOH (4)              | 2.6                                       | 87             |
| $\Delta^{9(11)}$ -10-COOMe (5) | 10.0                                      | 50             |
| $\Delta^{9(11)}$ -10-COOH (6)  | 0.2                                       | 99             |
| $\Delta^7$ -14-COOH            | 0.2                                       | 99             |
| 7-Oxo-DHL                      | 0.4                                       | 98             |

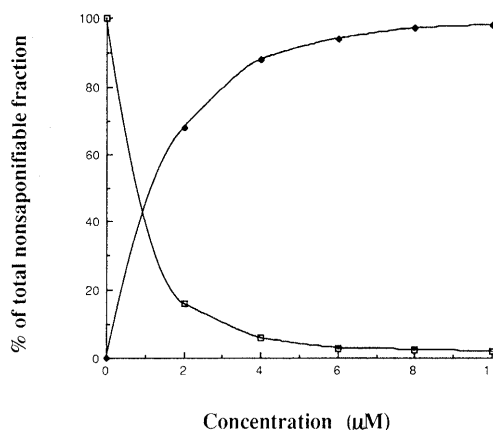
a) Assays were performed using purified pig liver P-450<sub>14DM</sub>. Methods for analysis of the incubation products are described in Materials and Methods. Each value represents the mean of triplicate determinations, and the S.E. of each value is less than 5% of the mean.

Fig. 1. Effects of  $\Delta^7$ -14-COOH and  $\Delta^{9(11)}$ -10-COOH (6) on DHL 14 $\alpha$ -Demethylation Activity

DHL was incubated with various concentrations of  $\Delta^7$ -14-COOH (○) and  $\Delta^{9(11)}$ -10-COOH (6, ▲). Data points represent the mean of triplicate determinations and the S.E. of each point is less than 5% of the mean.

inhibitory effect (99% inhibition) than 11-OH,10-COOH (4) (87% inhibition). This result suggests that the 9(11)-double bond structure is more suitable than a saturated skeleton with the 11-hydroxy group for producing the inhibitory activity. As  $\Delta^7$ -14-COOH and  $\Delta^{9(11)}$ -10-COOH (6) exhibited the most potent inhibitory effects, these compounds were tested at lower concentrations (1, 5, 10 and 20  $\mu$ M) and the results are shown in Fig. 1. The concentrations required for 50% inhibition ( $IC_{50}$ ) of these compounds were 0.7 and 0.5  $\mu$ M for  $\Delta^7$ -14-COOH and  $\Delta^{9(11)}$ -10-COOH (6), respectively.

Evidence for the inhibition of P-450<sub>14DM</sub> in intact cells by  $\Delta^{9(11)}$ -10-COOH (6) was provided by [<sup>14</sup>C]acetate incorporation studies. Exponentially growing cells were treated with increasing concentrations of 6, and the <sup>14</sup>C-radiolabeled nonsaponifiable lipids were analyzed by TLC. The amount of incorporation into nonsaponifiable lipids decreased with increasing concentrations of 6. At a concentration of 10  $\mu$ M, incorporation decreased to 15% of the control experiment. Compound 6 caused a reduction in the incorporation of [<sup>14</sup>C]acetate into C<sub>27</sub> sterols (corresponding to desmosterol in the case of L cells) as shown in Fig. 2. This decrease was accompanied by an increase in radioactivity in the C<sub>30</sub>-sterols (corresponding to lanosterol and DHL). These results confirmed the inhibition of P-450<sub>14DM</sub> by 6 in intact cells.

Fig. 2. Changes in Sterol Labeling Profiles in Mouse L Cells Treated with  $\Delta^{9(11)}$ -10-COOH (6)

Cells were labeled for 24 h with 37 kBq/ml [<sup>14</sup>C]acetate, with or without 6. Nonsaponifiable lipids were extracted and fractionated by TLC as described in the Materials and Methods. Incorporation of [<sup>14</sup>C]acetate into C<sub>30</sub> (■) and C<sub>27</sub> (□) sterols is expressed as a percentage of the total incorporation into nonsaponifiable lipids.

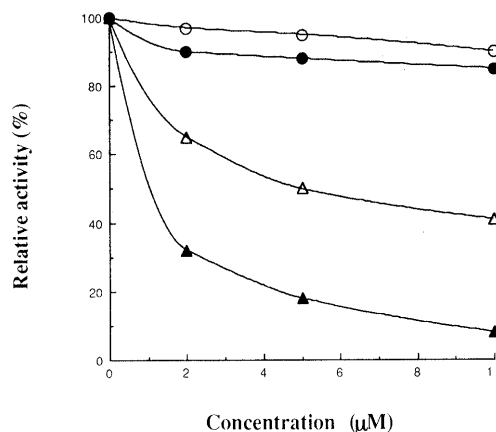


Fig. 3. Effects of 19-Oxygenated Sterols on HMG-CoA Reductase Activity

Mouse L cells were incubated with 19-oxygenated sterols for 24 h; lactone (3, ○),  $\Delta^{9(11)}$ -COOMe (5, ●), 11-OH, 10-COOH (4, △), and  $\Delta^{9(11)}$ -10-COOH (6, ▲) at various concentrations. Data points represent the mean of triplicate determinations and the S.E. of each point is less than 5% of the mean.

**Effects of 19-Oxygenated Lanosterol Derivatives on HMG-CoA Reductase Activity in Mouse L Cells** Since 19-oxygenated lanosterol derivatives (3–6; 40  $\mu$ M) exhibited various degrees of inhibition (27–99%) on P-450<sub>14DM</sub>, it was of interest to determine the effects of these compounds on HMG-CoA reductase activity. These compounds inhibited HMG-CoA reductase activity upon treatment of mouse L cells for 24 h at various concentrations. Figure 3 shows the effects of 3–6. The inhibitory activities of these compounds were concentration-dependent.

$\Delta^{9(11)}$ -10-COOH (6) was found to be the most effective inhibitor of P-450<sub>14DM</sub> in both purified P-450<sub>14DM</sub> and mouse L cells. Furthermore, 6 was shown to inhibit HMG-CoA reductase activity. These results show that 6 is a dual-action inhibitor of cholesterol biosynthesis. These inhibitory activities of 6 were very similar to those of  $\Delta^7$ -14-COOH.<sup>10)</sup>

Mayer *et al.*<sup>15)</sup> reported that 3 $\beta$ -hydroxylanosta-8,15-diene-32-oic acid was a dual-action inhibitor of P-450<sub>14DM</sub>

and HMG-CoA reductase activity. Frye *et al.*<sup>16)</sup> reported that some 32-oxygenated lanosterol derivatives and 3 $\beta$ -hydroxylanost-7-en-15-one 15-oxime were inhibitors of P-450<sub>14DM</sub> (IC<sub>50</sub>; 3.0  $\mu$ M) and suppressors of HMG-CoA reductase activity (IC<sub>50</sub>; 0.6  $\mu$ M). The inhibitory activities were similar to those of **6**. Furthermore, they compared the reduction of HMG-CoA reductase activity *versus* HMG-CoA reductase protein levels and found that both showed a parallel decline. They suggested that 15-oxygenated lanosterol derivatives regulate gene expression. Although we did not quantify the protein and mRNA levels of HMG-CoA reductase, a similar mechanism might operate in the case of 19-oxygenated lanosterol derivatives.

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