Oxolanosterol oximes: dual-action inhibitors of cholesterol biosynthesis

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Abstract A series of oxolanosterol oximes and oxime ethers have been prepared as potential dual-action inhibitors of cholesterol biosynthesis. The synthesis of these oximes along with the evaluation of their ability to inhibit lanosterol 14α-methyl demethylase ($P450_{DM}$) and to suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) activity is presented. 3β-Hydroxylanost-7-en-15-one 15-oxime **XIX** was found to be an effective inhibitor of $P450_{DM}$ in rat liver microsomal preparations. In [^{14}C]acetate incorporation studies using Chinese hamster ovary (CHO) cells, compound **XIX** was found to cause a dramatic reduction in the incorportion of acetate into C_{27} sterols with a concommitant increase in radiolabeled C_{30} sterols which is consistent with the inhibition of $P450_{DM}$. In addition, 15-oxime **XIX** was shown to suppress HMGR activity in both wild-type CHO and $P450_{DM}$-deficient (AR45) cells, indicating that suppression of HMGR is independent of any effects of this oxime on $P450_{DM}$. In both cell lines, parallel declines in HMGR activity and HMGR protein levels were observed suggesting that compound **XIX** suppresses HMGR activity by regulation of gene expression. These results demonstrate that, as predicted, 15-oxime **XIX** is indeed a dual-action inhibitor of cholesterol biosynthesis which causes both the inhibition of $P450_{DM}$ and a reduction in HMGR activity.—**Frye, L. L., K. P. Cusack, D. A. Leonard, and J. A. Anderson.** Oxolanosterol oximes: dual-action inhibitors of cholesterol biosynthesis. *J. Lipid Res.* 1994. **35:** 1333–1344.

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The rate-limiting step of overall cholesterol biosynthesis is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR). This enzyme catalyzes the reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to yield mevalonate (1–3). The activity of HMGR appears to be regulated through a multivalent feedback mechanism mediated by oxysterols along with another metabolite of mevalonate (4–8). The prototypical oxysterol, 25-hydroxycholesterol **I,** appears to regulate HMGR activity primarily via a transcriptional mechanism involving a 42 base-pair element in the 5' flanking region of the gene (9) (**Scheme 1**). However, recent studies indicate that some oxysterols, particularly oxylanosterols, may be involved in post-transcriptional regulation of HMGR synthesis (10, 11).

Of the numerous oxysterols that have been studied as suppressors of HMGR activity, a limited number were found to be more potent in the inhibition of overall cholesterol biosynthesis than could be explained by their ability to suppress HMGR activity. Two of these, 14α-ethylcholest-7-ene-3β,15α-diol **II** (12–14) and 3β-hydroxylanost-7-en-15-one **III** (15, 16), were found to inhibit the conversion of lanosterol **IV** (or dihydrolanosterol **XI**) (**Scheme 2**) to cholesterol, which suggests that they are also inhibitors of lanosterol 14α-methyl demethylase ($P450_{DM}$), the cytochrome P450 monooxygenase that catalyzes the first step in the conversion of lanosterol **IV** to cholesterol. A novel carboxylic acid analog of lanosterol (compound **V**) has also been reported to be a potent inhibitor of $P450_{DM}$ and a suppressor of HMGR activity (17). Recently, we reported the synthesis and evaluation of compounds **VIa&b** and **VII** (18, 19). As anticipated, these compounds were found both to inhibit $P450_{DM}$ and to suppress HMGR activity.[2]

$P450_{DM}$ catalyzes the oxidative removal of the 14α-methyl group of lanosterol **IV** via three NADPH-O_2-dependent steps (Scheme 2, eq. 1) (20). The methyl group is first oxidized to the hydroxymethyl moiety giving compound **VIII** followed by oxidation to the corresponding aldehyde **IX.** The third oxidative step, which results in the formation of 8,14-conjugated diene **X** with the loss of

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HMGR, HMG-CoA reductase; HPLC, high performance liquid chromatography; TLC, thin-layer chromatography; PPTS, pyridinium *p*-toluenesulfonate; rt, room temperature; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.

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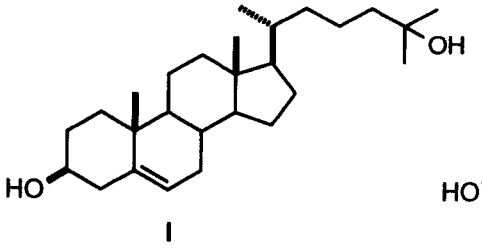
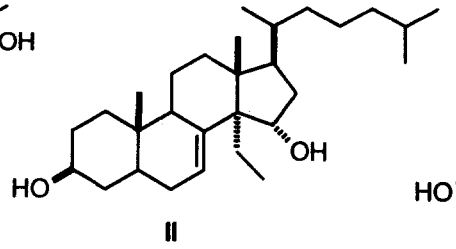
[2]It should be noted that a comparison of the potencies of compounds **VIa&b** and **VII** to those of other compounds reported to be inhibitors of $P450_{DM}$ and suppressors of HMGR activity is difficult as different cell lines and assay systems were used in the studies.



I



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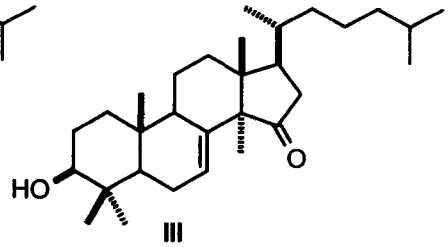
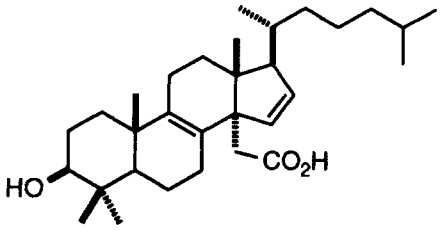
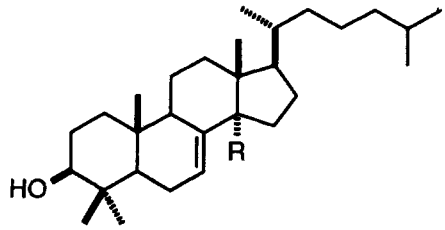


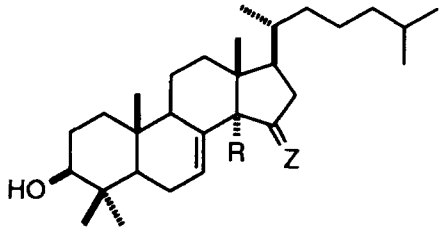
III



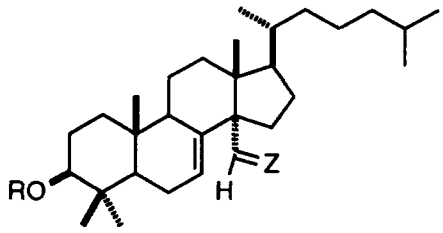
V



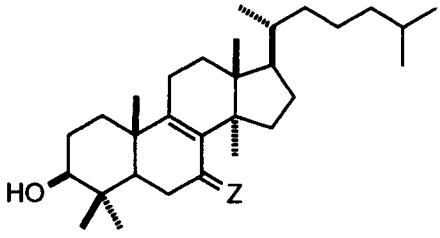
VIa R=$CH(CH_3)OH$ less polar diastereomer
VIb R=$CH(CH_3)OH$ more polar diastereomer
VII R=$COCH_3$



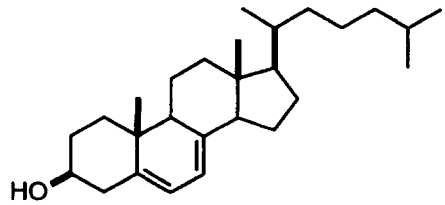
XIX	R=CH_3	Z=NOH
XX	R=CH_2OH	Z=NOH
XXI	R=CHO	Z=NOH
XXIII	R=CH_3	Z=$NOCH_3$
XXIV	R=CH_3	Z=$NOCH_2Ph$



XXVIII	R=THP	Z=O
XXIX	R=THP	Z=NOH
XXX	R=H	Z=NOH
XXXI	R=H	Z=$NOCH_3$

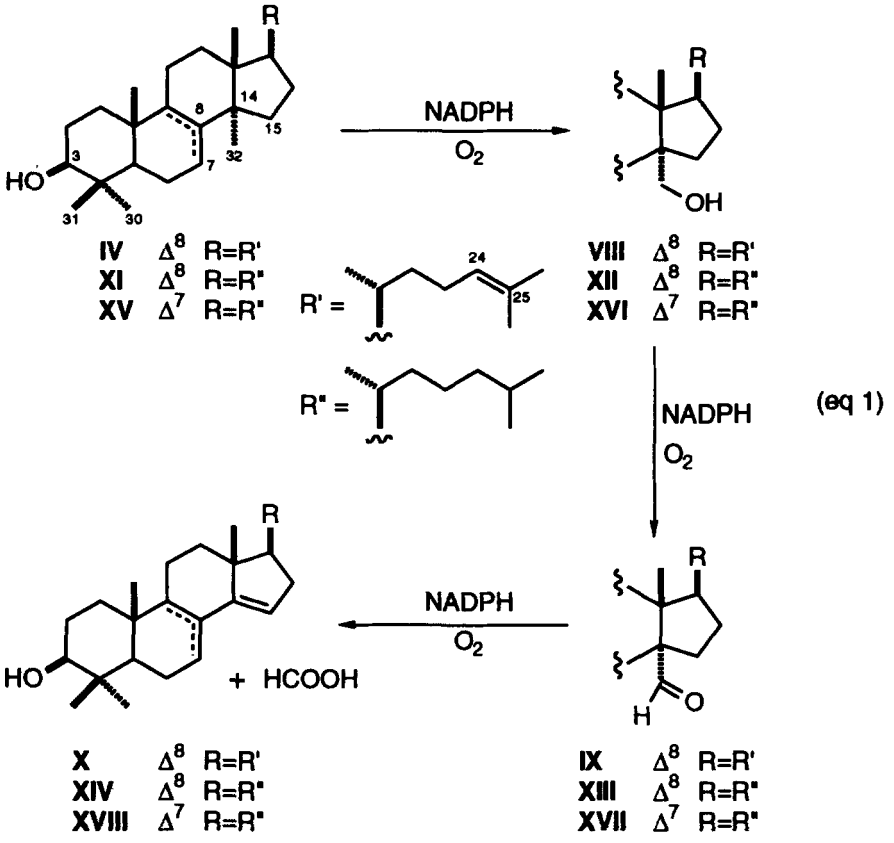


XXV	Z=O
XXVI	Z=NOH
XXVII	Z=$NOCH_3$



XXII

Scheme 1.



Scheme 2.

the 15α-proton and formic acid, appears to proceed via an enzyme-bound peroxyhemiacetal intermediate (21–23). Lanosterol **IV**, 24,25-dihydrolanosterol **XI**, and lanost-7-en-3β-ol **XV** appear to be substrates for $P450_{DM}$ (24–28).[3] Lanost-8-en-3β,32-diol **XII** and the corresponding Δ^7-isomer **XVI** have been shown to bind more tightly to mammalian $P450_{DM}$ than 24,25-dihydrolanosterol **XI** (K_m = 5.1 μM, 5.7 μM, and 32–35 μM for compounds **XII, XVI,** and **XI,** respectively) (27). The oxidative removal of C-32 is inhibited by aldehyde **XIII** in rat liver microsomes (29). In addition, 32-oxygenated lanosterol analogs generated during the removal of the 14α-methyl group by $P450_{DM}$ (compounds **XII** and **XIII**) and their Δ^7-isomers (compounds **XVI** and **XVII**) have been shown to decrease the activity of HMGR in Chinese hamster lung cells (30). Trzaskos et al. (31) have recently provided evidence that strongly suggests that aldehyde **XIII** is an endogenously generated regulator of HMGR activity.

A variety of oxygenated cholesterol and lanosterol analogs have been shown to be potent suppressors of HMGR activity. Based on the structures of these oxysterols, along with information concerning the mechanism of $P450_{DM}$, a series of oxolanosterol oximes and oxime ethers were designed as potential suppressors of both HMGR activity and inhibitors of $P450_{DM}$. We present the synthesis and evaluation of these potential dual-action inhibitors of cholesterol biosynthesis.

MATERIALS AND METHODS

General

^{1}H NMR spectra were obtained on either a Varian Unity-500 (500 MHz) or a Varian XL-200 (200 MHz) NMR spectrometer with tetramethylsilane as an internal standard. Infrared spectra were recorded on a Perkin Elmer 298 spectrometer. Direct insertion probe (DIP)

[3]It should be noted that there appears to be a controversy over whether or not Δ^7-lanosterols are substrates for $P450_{DM}$. Akhtar et al. (25, 26) claim that [9α-^{3}H]lanost-7-en-3β-ol is a substrate and is converted to the 7,14-diene in a cell-free preparation from rat liver. Bossard et al. (27) report a K_m for lanost-7-ene-3β,32-diol in a reconstituted system utilizing purified $P450_{DM}$. Sekigawa, Sonoda, and Sato (28), on the other hand, state that lanost-7-ene-3β,32-diol is not converted to 14-dehydroxymethylated products using a partially purified system.

chemical ionization mass spectral data were obtained from a Hewlett-Packard HP 5087 GC–MS system. High performance liquid chromatography (HPLC) was performed using a Waters 6000A pump with either a Waters 410 refractive index detector or a Waters Lambda-Max Model 481 variable wavelength UV detector. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratories, Eagle Harbor, MI.

The following reagents were purchased from Aldrich Chemical Company and were used as received: 7-dehydrocholesterol, hydroxylamine hydrochloride, methoxylamine hydrochloride, benzoxylamine hydrochloride, and benzyl bromide. Lanosterol was obtained from Sigma. 4-Chloro-1-naphthol was obtained from Bio-Rad. Silica gel (EM Science Silica Gel 60, 230–400 mesh) was used for all flash chromatography (32). DL-3-[Glutaryl-3-^{14}C]hydroxy-3-methylglutaryl coenzyme A (60 mCi/mmol), L-[3,4,5-^{3}H]leucine (176.5 Ci/mmol), RS-[5-^{3}H]mevalonolactone (24 Ci/mmol), and sodium [1-^{14}C]acetate (59 mCi/mmol) were obtained from DuPont-New England Nuclear. The HMGR activity experiments used Silica Gel Si-250 thin-layer chromatography (TLC) plates obtained from Baker. Nitrocellulose membranes (0.45 μ) were obtained from Schleicher and Schuell. Cab-O-Sil M-5 hydrated colloidal silica was from Kodak. McCoy's 5a medium was from Gibco and fetal bovine serum was from MA Bioproducts. ITS^{+} was from Collaborative Research. Digitonin (Fisher) was used as a 10 mg/ml stock solution in 50% ethanol. Pyridinium *p*-toluenesulfonate (PPTS) was prepared as described by Miyashita, Yoshikoshi, and Grieco (33). Anti(HMG-CoA reductase) IgG was a generous gift from Dr. Gene C. Ness, University of South Florida, Tampa, FL. The peroxidase-conjugated goat anti-rabbit IgG was obtained from Jackson Immunoresearch. AR45 cells were a generous gift from Dr. Harry W. Chen, DuPont Merck Pharmaceutical Company.

Chemical synthesis, general procedure for the preparation of oximes

An oven-dried round-bottom flask with stirring bar was charged with the appropriate ketone or aldehyde, absolute ethanol, and dry pyridine. The required hydroxylamine hydrochloride ($NH_2OH \cdot HCl$, $NH_2OCH_3 \cdot HCl$, or $NH_2OCH_2C_6H_5 \cdot HCl$) was added as a solid. The flask was equipped with a condensor with a septum and a gas-needle inlet and flushed with argon. The mixture was stirred at reflux under argon for the given period of time and cooled to room temperature (rt). Water was added and the ethanol was removed in vacuo. The residue was extracted well with CH_2Cl_2. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2) with the indicated eluant.

3β-Hydroxylanost-7-en-15-one 15-oxime (XIX)

The general procedure was followed using 3β-hydroxylanost-7-en-15-one **III** (15) (90.0 mg, 0.2 mmol), absolute ethanol (7.0 ml), pyridine (1.2 ml, 14.7 mmol), and $NH_2OH \cdot HCl$ (250.0 mg, 3.6 mmol). The reaction was stirred at reflux for 48 h. The residue was purified by flash chromatography (SiO_2, hexanes–ethyl acetate 5:1) giving compound **XIX** (87.1 mg, 95%) as a white solid: mp 183–185°C, lit 185–186°C (35) (EtOH/H_2O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.7 (brs, 1H, NOH), 6.45 (m, 1H, C-7-H), 3.26 (dd, J = 4.3 Hz, J = 11.4 Hz, 1H, C-3-H), 2.77 (dd, J = 8.3 Hz, J = 20.0 Hz, 1H), 2.27 (dd, J = 7.8 Hz, J = 19.5 Hz, 1H), 2.10–1.21 (m, 34H), 1.209 (s, 3H, C-32-H), 0.987 (s 3H, C-30-H), 0.932 (d, J = 6.4 Hz, 3H, C-21-H), 0.69 (s, 3H, C-18-H),[4] lit (34,35)[5]; IR ($CHCl_3$) 3620 cm^{-1} (w), 2960 cm^{-1} (s); MS (CI, isobutane) *m/z* 458 (M+1, 56%), 440 (M+1-H_2O, 100%), 422 (M+1-2H_2O, 34%).

3β-Hydroxylanost-7-en-15-one 15-methyloxime (XXIII)

The general procedure was followed using 3β-hydroxylanost-7-en-15-one **III** (15) (60.0 mg, 0.14 mmol), absolute ethanol (4.0 ml), pyridine (0.17 ml, 2.1 mmol), and $NH_2OCH_3 \cdot HCl$ (40.0 mg, 0.5 mmol). The reaction was stirred at reflux for 48 h. The residue was purified by flash chromatography (SiO_2, 5:1 hexanes–ethyl acetate) giving compound **XXIII** (32 mg, 51%) as a white solid: mp 139–141°C (EtOH/H_2O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.60–6.50 (m, 1H, C-7-H), 3.87 (s, 3H, $NOCH_3$), 3.35–3.15 (m, 1H, C-3-H), 2.686 (dd, J = 8.3 Hz, J = 19.0 Hz, 1H), 2.197 (dd, J = 9.2 Hz, J = 19.1 Hz, 1H), 2.16–1.20 (m, 22H), 1.197 (s, 3H, C-32-H), 0.991 (s, 3H, C-30-H), 0.922 (d, J = 6.4 Hz, 3H, C-21-H), 0.898 (s, 3H, C-29-H), 0.894 (s, 3H, C-19-H), 0.867 (d, J = 6.8 Hz, 3H, C-27-H), 0.862 (d, J = 6.3 Hz, 3H, C-26-H) 0.686 (s, 3H, C-18-H)[4]; IR ($CHCl_3$) 3600 cm^{-1} (w), 2960 cm^{-1} (s); MS (DIP-CI, isobutane) *m/z* 472 (M+1, 54%), 455 (M+1-H_2O, 70%), 440 (M+1-CH_3OH, 100%). Anal. calcd for $C_{31}H_{53}NO_2$: C, 78.92; H, 11.32; N, 2.97. Found: C, 78.77; H, 11.44; N, 2.98.

[4]The assignment of methyl group resonances in the ^{1}H NMR spectra of the compounds described herein were based on those reported by Emmons, Wilson, and Schroepfer (55) for similar lanosterol analogs.

[5]We have found that the chemical shifts of the NOH protons in the ^{1}H NMR spectra vary with concentration; therefore, it is difficult to compare our chemical shift data for the oxime protons with those reported (34, 35). Our ^{1}H NMR spectral data for compound **XXX** is consistent with that reported by the DuPont group (35). Our assignment of the C-7 proton of compound **XIX** (δ 6.45) is in agreement with that reported by Dolle et al. (34). Gaylor and coworkers (35) report a resonance at this chemical shift (δ 6.44, 1H) but assign the C-7 proton to a resonance at δ 6.85. Our assignment of the C-3-H (δ 3.25–3.35) is consistent with that reported by Gaylor and coworkers (δ 3.26) (35). Dolle et al. (34) report the C-3-H for compound **XIX** at δ 3.45.

3β-Hydroxylanost-7-en-15-one 15-benzyloxime (XXIV)

Method A: The general procedure was followed using 3β-hydroxylanost-7-en-15-one **III** (15) (67.0 mg, 0.15 mmol), absolute ethanol (4.0 ml), pyridine (0.19 ml, 2.3 mmol), and $NH_2OCH_2C_6H_5 \cdot HCl$ (90.0 mg, 0.5 mmol). The reaction was stirred at reflux for 48 h. The residue was purified by flash chromatography (SiO_2, 5:1 hexanes:ethyl acetate) giving compound **XXIV** (74 mg, 90%) as a white solid: mp 149–152°C (CH_3CN); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.45–7.25 (m, 5H, Ar-H), 6.55–6.45 (m, 1H, C-7-H), 5.14–5.09 (m, 2H, $PhCH_2$), 3.30–3.15 (m, 1H, C-3-H), 2.728 (dd, J = 8.6 Hz, J = 19.3 Hz, 1H), 2.230 (dd, J = 9.0 Hz, J = 19.3 Hz, 1H), 2.15–1.20 (m, 22H), 1.196 (s, 3H, C-32-H), 0.992 (s, 3H, C-30-H), 0.916 (d, J = 6.4 Hz, 3H, C-21-H), 0.895 (s, 3H, C-29-H), 0.884 (s, 3H, C-19-H), 0.863 (d, J = 6.4 Hz, 3H, C-27-H), 0.858 (d, J = 6.9 Hz, 3H, C-26-H), 0.659 (s, 3H)[4]; IR ($CHCl_3$) 3600 cm^{-1} (w), 2950 cm^{-1} (s); MS (DIP-CI, isobutane) *m/z* 548 (M+1, 4%), 531 (M+1-H_2O, 8%), 440 (M+1-$OCH_2C_6H_5$, 29%), 107 ($OCH_2C_6H_5$, 100%). Anal. calcd for $C_{37}H_{57}NO_2$: C, 81.12; H, 10.49; N, 2.56. Found: C, 81.17; H, 10.69; N, 2.57.

Method B: An oven-dried round-bottom flask with stirring bar was charged with potassium hydride (0.05 g, 35% dispersion in mineral oil, 0.44 mmol). The flask was equipped with a septum and a gas-needle inlet and flushed with argon. Dry dimethoxyethane (DME) (1 ml) was added via syringe and the slurry was cooled to 0°C. 3β-Hydroxylanost-7-en-15-one 15-oxime **XIX** (80.0 mg, 0.18 mmol) in dry DME (1 ml) was added dropwise and the mixture was stirred at 0°C for 15 min. Benzylbromide (52 μl, 0.44 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred for 25.5 h. The DME was removed in vacuo and the residue was dissolved in water and petroleum ether. The organic layer was removed and the aqueous layer was extracted well with petroleum ether. The combined organic layers were washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. Filtration, concentration, and purification by flash chromatography (SiO_2, 9:1 hexane:ethyl acetate) gave 15-benzyloxime **XXIV** (57 mg, 58%) with physical and spectroscopic characteristics identical to those given above.

3β-Hydroxylanost-8-en-7-one 7-oxime (XXVI)

The general procedure was followed using 3β-hydroxylanost-8-en-7-one **XXV** (36) (150.0 mg, 0.34 mmol), absolute ethanol (5.6 ml), pyridine (0.42 ml, 5.2 mmol), and $NH_2OH \cdot HCl$ (85.0 mg, 1.2 mmol). The reaction was stirred at reflux for 30 h. The residue was purified by flash chromatography (SiO_2, 5:1 hexanes-ethyl acetate) giving compound **XXVI** (93.2 mg, 60%) as a white solid: mp 176–178°C (EtOH); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.4 (brs, 1H, NOH), 3.260 (dd, J = 4.7 Hz, J = 11.5 Hz, 1H, C-3-H), 3.194 (dd, J = 3.9 Hz, J = 17.1 Hz, 1H), 2.30–0.89 (m, 39H), 0.868 (d, J = 6.8 Hz, 3H, C-27-H), 0.863 (d, J = 6.4 Hz, 3H, C-26-H), 0.710 (s, 3H, C-18-H)[4]; IR ($CHCl_3$) 3580 cm^{-1} (m), 3320 cm^{-1} (w), 2900 cm^{-1} (s), 1585 cm^{-1} (w); MS (CI, isobutane) *m/z* 458 (M+1, 87%), 440 (M+1-H_2O, 100%), 422 (M+1-$2H_2O$, 14%). Anal. calcd for $C_{30}H_{51}NO_2$: C, 78.72; H, 11.23; N, 3.06. Found: C, 78.94; H, 11.29; N, 3.14.

3β-Hydroxylanost-8-en-7-one 7-methyloxime (XXVII)

The general procedure was followed using 3β-hydroxylanost-8-en-7-one **XXV** (36) (112.0 mg, 0.25 mmol), absolute ethanol (5.0 ml), pyridine (0.31 ml, 3.8 mmol), and $NH_2OCH_3 \cdot HCl$ (75.0 mg, 0.9 mmol). The reaction was stirred at reflux for 48 h. The residue was purified by flash chromatography (SiO_2, 10:1 hexanes-ethyl acetate) giving compound **XXVII** (84.9 mg, 72%) as a white solid: mp 115–117°C (CH_3CN); ^1H NMR ($CDCl_3$, 500 MHz) δ 3.865 (s, 3H, $NOCH_3$), 3.237 (dd, J = 4.2 Hz, J = 11.6 Hz, 1H, C-3-H), 3.071 (dd, J = 3.9 Hz, J = 17.1 Hz, 1H), 2.30–0.92 (m, 42H), 0.911 (d, J = 6.0 Hz, 3H, C-21-H), 0.712 (s, 3H, C-18-H)[4]; IR ($CHCl_3$) 3450 cm^{-1} (w), 2950 cm^{-1} (s), 1580 cm^{-1} (w), 1030 cm^{-1} (m); MS (CI, isobutane) *m/z* 472 (M+1, 31%), 457 (M+1-CH_3, 31%), 440 (M+1-CH_3OH, 18%). Anal. calcd for $C_{31}H_{53}NO_2$: C, 78.92; H, 11.32; N, 2.97. Found: C, 78.99; H, 11.40; N, 2.99.

3β-Hydroxylanost-7-en-32-al 32-oxime (XXX)

The general procedure was followed using 3β-tetrahydropyranyloxylanost-7-en-32-al **XXVIII** (37–40) (50.0 mg, 0.095 mmol), absolute ethanol (3.0 ml), pyridine (0.12 ml, 1.5 mmol), and $NH_2OH \cdot HCl$ (24.0 mg, 0.34 mmol). The reaction was stirred at reflux for 4 h. The residue was purified by flash chromatography (SiO_2, 5:1 hexanes-ethyl acetate) giving two compounds: 3β-tetrahydropyranyloxylanost-7-en-32-al 32-oxime **XXIX** (11 mg, 22%) as a white solid: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.80–7.70 (m, 1H); 7.30–7.20 (m, 1H), 5.45–5.35 (m, 1H), 4.80–4.70 (m, 1/2H), 4.60–4.50 (m, 1/2H), 4.00–3.85 (m, 1H), 3.55–3.40 (m, 1H), 3.30–3.20 (m, 1/2H), 3.15–3.00 (m, 1/2H), 2.10–0.80 (m, 48H), 0.70 (s, 3H); and 3β-hydroxylanost-7-en-32-al 32-oxime **XXX** (34 mg, 77%) as a white solid: mp 209–211°C (CH_2Cl_2–hexane), lit 212.5–214.5°C (35) (EtOH/H_2O); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.767 (s, 1H, C-32-H); 7.264 (m, 1H, NOH), 5.45–5.35 (m, 1H, C-7-H), 3.245 (dd, J = 4.4 Hz, J = 11.3 Hz, 1H, C-3-H), 2.15–0.98 (m, 41H), 0.977 (s, 3H, C-30-H), 0.710 (s, 3H, C-18-H)[4]; lit (35)[5]; IR ($CHCl_3$) 3580 cm^{-1} (w), 2950 cm^{-1} (s); MS (DIP-CI, isobutane) *m/z* 458 (M+1, 63%), 440 (M+1-H_2O, 100%), 413 (M+1-H-CH_2NOH, 90%), 395 (M-CH_2NOH-

H_2O, 88%). Anal. calcd for $C_{31}H_{53}NO_2$: C, 78.72; H, 11.23; N, 3.06. Found: C, 78.54; H, 11.20; N, 3.19. The 3β-tetrahydropyranyloxylanost-7-en-32-al 32- oxime **XXIX** (11.3 mg, 0.02 mmol) was converted to 3β-hydroxylanost-7-en-32-al 32-oxime **XXX** by treatment with PPTS (10.0 mg, 0.04 mmol) in absolute ethanol (4 ml) at rt overnight. The ethanol was removed in vacuo. Water was added to the residue and the mixture was extracted well with CH_2Cl_2. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, 5:1 hexane–ethyl acetate) to give 3β-hydroxylanost-7-en-32-al 32-oxime **XXX** (9.5 mg, 0.02 mmol) with physical and spectroscopic characteristics identical to those given above. Total yield (0.043 g, 0.094 mmol) 99%.

3β-Hydroxylanost-7-en-32-al 32-methyloxime (XXXI)

The general procedure was followed using 3β-tetrahydropyranyloxylanost-7-en-32-al **XXVIII** (37–40) (50.0 mg, 0.15 mmol), absolute ethanol (3.0 ml), pyridine (0.12 ml, 1.5 mmol), and $NH_2OCH_3 \cdot HCl$ (29.0 mg, 0.34 mmol). The reaction was stirred at reflux for 2 h. The residue was purified by flash chromatography (SiO_2, 15:1 hexanes–ethyl acetate) giving compound **XXXI** (38 mg, 84%) as a white solid: mp 124–126°C (CH_3CN); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.712 (s, 1H, C-32-H), 5.45–5.35 (m, 1H, C-7-H), 3.811 (s, 3H, $NOCH_3$), 3.30–3.15 (m, 1H, C-3-H), 2.10–0.80 (m, 41H), 0.970 (s, 3H, C-30-H), 0.698 (s, 3H, C-18-H)[4]; IR ($CHCl_3$) 3600 cm^{-1} (w), 2950 cm^{-1} (s); MS (DIP-CI, isobutane) *m/z* 472 (M+1, 84%), 454 (M+1-H_2O, 100%), 440 (M+1-CH_3OH, 50%), 413 (M+1-CH_2NOCH_3, 73%), 395 (M+1-H_2O-CH_2NOCH_3, 62%). Anal. calcd for $C_{31}H_{53}NO_2$: C, 78.92; H, 11.32; N, 2.97. Found: C, 78.77; H, 11.35; N, 3.09

Biochemical evaluation. Assay of lanosterol 14α-methyl demethylase ($P450_{DM}$) activity

The assay of $P450_{DM}$ developed by Trzaskos et al. (20) was used in these studies. Briefly, dihydrolanosterol **XI** (33 μM) and varying concentrations of the potential inhibitors (suspended with the aid of Tyloxapal) were incubated with rat liver microsomal protein, 0.2 mM NAD, 0.1 mM NADH, 0.3 mM NADP, 2.0 mM NADPH, 10 mM isocitrate, isocitrate dehydrogenase (0.25 units/ml final volume), 0.4 mM magnesium chloride, 50 μM AY-9944, and 1 mM sodium cyanide in 0.1 mM phosphate buffer (containing 1 mM glutathione, 0.1 mM EDTA, pH 7.4, 20% glycerol) at 37°C for 45 min. The reactions were stopped by the addition of 15% potassium hydroxide (w/v) in 95% methanol. The resultant mixtures were heated in boiling water for 30 min and the nonsaponifiable material was extracted with petroleum ether. The petroleum ether was blown off with nitrogen, the residue was dissolved in absolute ethanol, and the lanosta-8,14-dien-3β-ol **XIV** content was determined by high performance liquid chromatography using a UV detector (acetonitrile–methanol–water 45:45:10, 1.5 ml/min, Ultrasphere octyl column, λ = 254 nm).

Cell culture

Chinese hamster ovary (CHO-K1) cells were grown in a 5% CO_2 atmosphere in modified McCoy's 5a medium supplemented with 1% delipidated fetal bovine serum prepared by Cab-O-Sil treatment (41). Cultures were routinely passaged twice weekly by trypsinization. $P450_{DM}$-deficient (AR45) cells (42) were maintained in modified McCoy's 5A medium supplemented with 5% fetal bovine serum. Cells were refed with fresh medium containing 1% ITS$^+$ (a chemically defined serum-free medium supplement) 16 hr prior to assay. Test compounds were added as bovine serum albumin (BSA) suspensions from stock solutions in absolute ethanol. The final concentration of BSA was 0.5 mg/ml and the final ethanol concentration did not exceed 0.5%.

[^{14}C]Acetate incorporation into saponifiable and nonsaponifiable lipids

The [^{14}C]acetate incorporation studies were carried out as described previously (43). Briefly, exponentially growing cultures were labeled with [1-^{14}C]acetate, and harvested into ice-cold saline. An equal volume of 15% KOH in 90% methanol containing 100 μg/ml butylated hydroxytoluene was added and samples were saponified at 85°C for 30 min. Nonsaponifiable lipids were extracted with at least 30 volumes of petroleum ether, washed once with 3% Na_2CO_3 and twice with water, and dried under nitrogen. Extracts were analyzed by silica thin-layer chromatography (ethyl acetate–hexane 15:85). To measure incorporation into fatty acids, the aqueous phase was acidified, extracted with petroleum ether, and an aliquot was counted in a liquid scintillation counter.

HMG-CoA reductase enzyme activity

This assay has been described in detail previously (44). Briefly, cells were plated in 24-well cluster plates, grown for 2 days, and permeabilized by incubation for 5 min at room temperature with 30 μg/ml digitonin in cytoskeletal (CSK) buffer (0.3 M sucrose, 0.1 M KCl, 2.5 mM $MgCl_2$, 1 mM EGTA, 10 mM PIPES, pH 6.8). The digitonin solution was removed and the permeabilized cells were incubated for 20 min at 37°C in pre-incubation buffer (50 mM phosphate buffer, pH 7.4, 10 mM dithiothreitol, 1 mM EDTA). Alternatively, frozen cell pellets were sonicated in pre-incubation buffer and incubated at 37°C for 20 min. Reductase activity was determined by monitoring the conversion of [^{14}C]HMG-CoA to [^{14}C]mevalonolactone, using [^{3}H]mevalonolactone as an internal standard. The substrate and product were separated by thin-

layer chromatography on Silica Gel Si-250 plates, visualized with I_2 vapors, scraped, and counted. Protein concentrations were determined with Bio-Rad protein reagent.

Immunoblot analysis of HMGR protein

Quantitative immunoblot analysis of cells was performed as described previously (44). Cells were solubilized by scraping directly into sodium dodecyl sulfate (SDS)-urea sample buffer, heated at 90°C for 3 min, and frozen at −80°C. Proteins were separated by SDS-polyacrylamide gel electrophoresis on 10% slab gels. Proteins were electroblotted onto a 0.45 μ nitrocellulose membrane in 49.6 mM Tris, 384 mM glycine, 20% methanol, 0.0375% SDS at 0.4 A for 16 h. Membranes were blocked by washing in 5% nonfat dry milk in TBS/Tween (20 mM Tris, 500 mM NaCl, 0.05% Tween 20, pH 7.5) HMGR was detected using a polyclonal anti(HMGR) antibody, and visualized using a peroxidase-conjugated second antibody. Bands were visualized with 4-chloro-1-naphthol according to the manufacturer's instructions. Immunoblots were quantitated by scanning with a LKB laser densitometer and immunoreactive protein expressed as the area under the absorbance curve.

RESULTS AND DISCUSSION

During our pursuit of the synthesis of various 15-substituted lanosterol derivatives as potential cholesterol-lowering agents, we prepared 3β-hydroxylanost-7-en-15-one 15-oxime **XIX** (34, 35).[5,6] This oxime lacks the 15α-proton that is required for the removal of the 14α-methyl group by $P450_{DM}$ and is therefore a substrate analog that is incapable of being completely metabolized to the corresponding diene. If 15-oxime **XIX** is accepted as a substrate by $P450_{DM}$, 14α-hydroxymethyl and 14α-formyl compounds (i.e., compounds **XX** and **XXI**) would be formed. These compounds may be effective inhibitors of $P450_{DM}$ since, as mentioned earlier, alcohol **XII** and aldehyde **XIII** (eq. 1 in Scheme 2) have been shown to inhibit the loss of C-32 in rat liver microsomes. Furthermore, 15-oxime **XIX** is similar in structure to ketone **III** which is a potent suppressor of HMGR activity (15)[7] and appears to be an inhibitor of $P450_{DM}$ (16).[8] Therefore, 15-oxime **XIX** was evaluated as an inhibitor of $P450_{DM}$ and as a suppressor of HMGR activity. It should be noted that during our evaluation of compound **XIX**, Dolle, Allandeen, and Kruse (34) reported the synthesis of this compound as an intermediate in the preparation of aza-D-homosterols. In addition, Gaylor and coworkers (35) at DuPont have independently studied compound **XIX** as an inhibitor of cholesterol biosynthesis.[6]

15-Oxime **XIX** was prepared by oximation of ketone **III** which is available from 7-dehydrocholesterol **XXII** in 9 steps (15, 45). The 15-position of ketone **III** is quite hindered and a large excess of hydroxylamine hydrochloride in the presence of pyridine at an elevated temperature for 2 days was required for the reaction to proceed to completion.[9] The inhibition of $P450_{DM}$ by 15-oxime **XIX** was assessed using a modification of the assay developed by Trzaskos and coworkers (20). Briefly, this assay utilizes 24,25-dihydrolanosterol **XI** as the substrate and quantitates the build-up of diene **XIV** by UV-HPLC. As rat liver microsomal preparations are used, the pathways by which diene **XIV** is degraded must be inhibited. The reduction of the Δ^{14}-double bond of diene **XIV**, the next step in the cholesterol biosynthetic pathway, is inhibited by AY-9944 and the removal of the methyl groups at C-4 is blocked by the addition of cyanide. A plot of turnover^{-1} versus inhibitor concentration obtained by varying the concentration of inhibitor at constant substrate concentration (24,25-dihydrolanosterol **XI**, 33 μM) was used to determine the IC_{50} value for the inhibitor. An IC_{50} value of 3.0 μM was obtained for 15-oxime **XIX** in this manner (**Table 1**). This result suggests that 15-oxime **XIX** is an effective inhibitor of $P450_{DM}$ (K_m of 24,25-dihydrolanosterol **XI** is 33 μM under the assay conditions) and is similar in potency to ketone **III** (IC_{50} = 7.1 μM, Table 1). It should be noted that 15-oxime **XIX**, and all of the other oximes that we have studied, are stable for extended periods of time in phosphate buffer at 37°C and to the saponification conditions utilized in the rat liver $P450_{DM}$ assays (KOH/methanol at 100°C for 30 min). Evidence for the inhibition of $P450_{DM}$ in intact cells by 15-oxime

[6]Ko and coworkers at DuPont have independently studied 15-oxime **XIX** as an inhibitor of cholesterol biosynthesis. Their results are described in their recent patent (35) (date of patent: August 20, 1991). They report an IC_{50} value for suppression HMGR of 0.2 μM and an IC_{50} value for inhibition of $P450_{DM}$ of 55 μM. We presented the preliminary results on the inhibition of $P450_{DM}$ and suppression of HMGR activity by 3β-hydroxylanost-7-en-15-one 15-oxime (compound **XIX**) at the 201st ACS meeting on April 16, 1991. The IC_{50} value we obtained for inhibition of $P450_{DM}$ is different from the value reported by the DuPont group. This difference may be due to differences in the assay conditions. We have found our IC_{50} value to be reproducable (5 ×) and it is consistent with the data we obtained in CHO cells.

[7]Schroepfer et al. (15) report an IC_{50} value for 15-ketone **III** of 0.8 μM in mouse L cell cultures which is in close agreement with our results in CHO cell cultures (0.5 μM).

[8]Morisaki et al. (16) report 15-ketone **III** as an inhibitor of the conversion of dihydrolanosterol **XI** to cholesterol in the S-10 fraction of a rat liver homogenate. An IC_{50} value was not provided.

[9]Each of the oximes and oxime ethers described herein appears to be one stereoisomer. All of the oximes exhibit a single peak for the oxime hydrogen and the oxime methyl ethers exhibit singlets for the methyl group in their ^{1}H NMR spectra. In addition, the C-18 methyl group is a singlet in all cases.

TABLE 1. IC_{50} values for inhibition of $P450_{DM}$ and HMGR activity by oxylanosterols and oxolanosterol oximes

	IC_{50} (μM)			
	$P450_{DM}$		HMGR	
Compound	In vitro[a]	CHO[b]	CHO[b]	AR45[c]
III	7.1[d]	0.7	0.5[e]	1.8
XIX[f]	3.0	2.0	0.6	1.5
XXIII	77	>20	15.0	>20
XXIV	101	>20	15.0	nd[g]
XXV	0.57[h]	>20	0.9	8
XXVI	21	>20	6.7	19
XXVII	28	>20	>20	nd[g]
XXX[i]	8.9	1.0	1.0	2
XXXI	102	>20	10.0	>20

[a]Assays performed using rat liver microsomal preparations.
[b]Chinese hamster ovary cells.
[c]P-450_{DM}-deficient cell line.
[d]See footnote 8.
[e]See footnote 7.
[f]See footnote 6.
[g]Not determined.
[h]See footnote 10.
[i]See footnote 11.

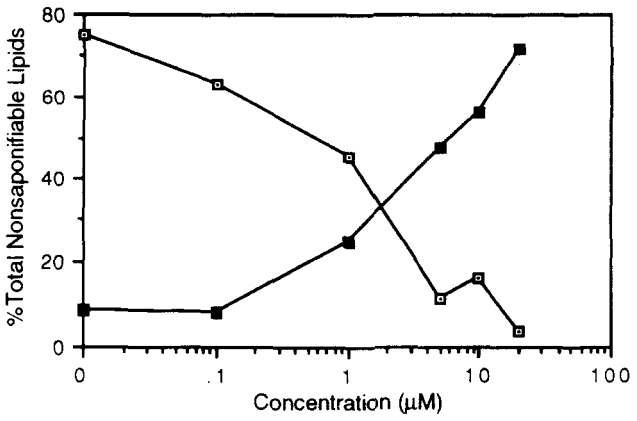


Fig. 1. Changes in sterol labeling profiles in CHO cells treated with 15-oxime **XIX.** Cells were treated for 2 h with the indicated concentration of 15-oxime **XIX** and then labeled for an additional 2 h with 1 μCi/ml [1-^{14}C]acetate. Nonsaponifiable lipids were extracted and fractionated by TLC as described in the text. Incorporation of [1-^{14}C]acetate into C_{30} (■) and C_{27} (□) sterols is expressed as a percentage of the total incorporation into nonsaponifiable lipids. Values for incorporation into total nonsaponifiable lipids decreased with increasing sterol concentration. Values were 28.1 dpm/μg cell protein in control cultures and 8.1 dpm/μg protein at a sterol concentration of 20 μM.

XIX was provided by [^{14}C]acetate incorporation studies (43). Exponentially growing cells were treated with increasing concentrations of inhibitor, pulsed with [1-^{14}C]acetate, and the ^{14}C-radiolabeled nonsaponifiable lipids were analyzed by thin-layer chromatography. Results from a representative experiment are shown in **Figure 1.** 15-Oxime **XIX** caused a reduction in the incorporation of [^{14}C]acetate into material with the chromatographic behavior of C_{27} monohydroxysterols. This decrease was accompanied by an increase in radioactivity in the region of the chromatogram corresponding to the migration of authentic lanosterol (C_{30}). The reduction of incorporation of radiolabel into C_{27} sterols with the concommitant increase in radiolabeled C_{30} sterols is consistent with the inhibition of $P450_{DM}$ by 15-oxime **XIX.** Plots of the data from this study (Fig. 1) were used to calculate the concentration of inhibitor that resulted in an equal incorporation of ^{14}C radioactivity into C_{27} and C_{30} sterols. The average value obtained from two independent experiments is given in Table 1. Compound **XIX** at concentrations up to 20 μM had no effect on the incorporation of [^{14}C]acetate into fatty acids.

An initial screening of the effects of 15-oxime **XIX** on HMGR activity in CHO cells indicated that it is a potent suppressor of HMGR activity. Exponentially growing cells were treated for 4 h with compound **XIX** and HMGR activity was determined in permeabilized cells or cell sonicates by monitoring the conversion of [^{14}C]HMG-CoA to [^{14}C]mevalonate, using [^{3}H]mevalonolactone as an internal standard (44). The results of concentration studies of 15-oxime **XIX** versus HMGR activity in both normal CHO and $P450_{DM}$-deficient (AR45) cells (42) are shown in **Figure 2** and Table 1. The concentration of inhibitor required to reduce HMGR activity to 50% of control in CHO cells was found to be 600 nM. The ability of compound **XIX** to suppress HMGR activity is similar in both CHO cells and in AR45 cells. These results suggest that the suppression of HMGR by compound **XIX** is independent of its effects on $P450_{DM}$. Compounds that are competitive inhibitors of $P450_{DM}$ can

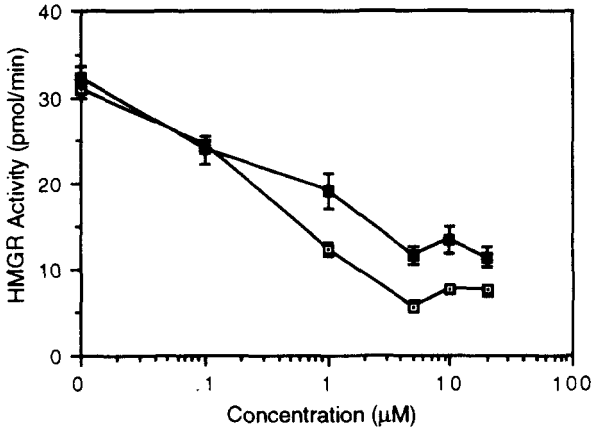


Fig. 2. HMGR activity in CHO and AR45 cells treated with 15-oxime **XIX.** Wild type (CHO) and lanosterol 14α-methyl demethylase ($P450_{DM}$)-deficient (AR45) cells were treated for 4 h with increasing concentrations of 15-oxime **XIX.** HMGR activity was then assayed in permeabilized cells as described in Materials and Methods. Data represent the mean and standard error of four determinations: (□) CHO; (■) AR45.

cause the build-up of the natural intermediates **XII** and **XIII,** which in turn suppress HMGR activity. If compound **XIX** was acting by this mechanism, it would not be expected to suppress HMGR activity in AR45 cells. The addition of 15-oxime **XIX** to CHO cell sonicates at concentrations up to 5 μM had no effect on HMGR activity, demonstrating that this compound is not a direct inhibitor of HMGR. The effect of compound **XIX** on HMGR protein concentration was determined by quantitative immunoblot analysis (44). Comparisons of the reduction of HMGR activity versus HMGR protein levels with increasing 15-oxime **XIX** concentration in CHO and AR45 cells are shown in **Figure 3.** The parallel decline in HMGR activity and protein levels suggests that compound **XIX** suppresses HMGR activity in both cell lines by regulating gene expression. The suppression of HMGR protein levels by 15-oxime **XIX** is not due to nonspecific inhibition of cellular protein synthesis as the treatment of cells with the oxime at concentrations up to 5 μM had no effect on the incorporation of [^{3}H]leucine into total acid-precipitable proteins. These results demonstrate that, as predicted, 15-oxime **XIX** is indeed a dual-action inhibitor of cholesterol biosynthesis that causes both the inhibition of $P450_{DM}$ and a reduction in HMGR activity and protein concentration.[6] This is in contrast to the results reported by Schroepfer et al. (46) for the oxime of 3β-hydroxy-5α-cholest-8(14)-en-15-one. The IC_{50} value for suppression of HMGR activity in mouse L cells was reported to be 0.2 μM for this compound. However, this oxime does not appear to suppress other enzymes in the cholesterol biosynthetic pathway as it exhibited comparable effects on the incorporation of [^{14}C]acetate into digitonin-precipitable sterols and on HMGR activity. Studies are underway to determine the mechanism by which 15-oxime **XIX** regulates HMGR gene expression.

In an attempt to assess whether or not the oxime hydrogen is required for the inhibitory properties of compound **XIX,** the corresponding 15-methyl- and 15-benzyloximes (compounds **XXIII** and **XXIV**) were studied. 15-Methyloxime **XXIII** was prepared from ketone **III** in a manner analogous to that discussed previously for compound **XIX** using methoxylamine hydrochloride instead of hydroxylamine hydrochloride (for examples of preparation of oxime ethers by treatment of a ketone or aldehyde with alkyloxylamine hydrochlorides or treatment of oximates with an alkylating agent, see references 47 and 48). 15-Benzyloxime **XXIV** was prepared either in the same manner using benzoxylamine hydrochloride or by treatment of 15-oxime **XIX** with potassium hydride followed by benzyl bromide (48). The 15-methyl- and 15-benzyloximes were evaluated for inhibition of $P450_{DM}$ and suppression of HMGR activity. The results of these studies are summarized in Table 1. Both the 15-methyl- and 15-benzyloximes, compounds **XXIII** and **XXIV,** were significantly less active than 15-oxime **XIX** in their ability to suppress HMGR and $P450_{DM}$ activities in cultured cells. In the in vitro assay for $P450_{DM}$ activity, 15-methyloxime **XXIII** was considerably less active than 15-oxime **XIX;** 15-benzyloxime **XXIV** was the least active of the three. These results suggest either that the oxime hydrogen is important for the inhibitory properties of compound **XIX** or that the larger methyl and benzyl groups are inhibiting the binding of the oxime to the active site of $P450_{DM}$ as well as interfering with the ability of these compounds to regulate HMGR gene expression. It is also

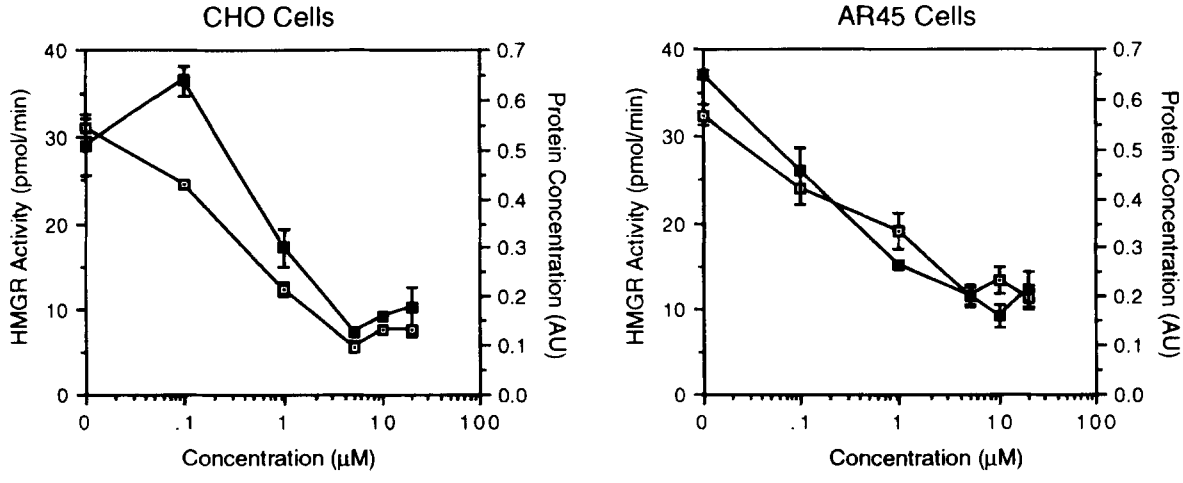


Fig. 3. Concentration-dependent suppression of HMGR activity and protein in cells treated with 15-oxime **XIX.** Wild type (CHO) and lanosterol 14α-methyl demethylase ($P450_{DM}$)-deficient (AR45) cells were treated for 4 h with increasing concentrations of 15-oxime **XIX.** HMGR activity was determined in permeabilized cells as described in Fig. 2. HMGR protein levels were determined by quantitative immunoblot analysis as described in Materials and Methods, with data representing the average and range of duplicate determinations; (□) activity; (■) immunoreactive protein.

possible that there is a difference in the stereochemistry about the C-N bond for the oxime versus the oxime ethers.[9]

In light of the results with 15-oxime **XIX,** oxolanosterol oximes at positions other than C-15 were pursued. Sato and coworkers (49–52) have reported that 3β-hydroxylanost-8-en-7-one **XXV** is an inhibitor of $P450_{DM}$. Based on this report, 3β-hydroxylanost-8-en-7-one 7-oxime **XXVI** was prepared by treatment of ketone **XXV** with hydroxylamine hydrochloride. Ketone **XXV** was prepared as described by Pinhey and coworkers (36) in five steps from lanosterol **IV.** The effects of 7-oxime **XXVI** on $P450_{DM}$ and HMGR activities are summarized in Table 1. This oxime exhibits an IC_{50} value of 21 μM for inhibition of $P450_{DM}$ in the rat liver microsomal preparation assay, suggesting that it is not as effective an inhibitor of $P450_{DM}$ as 15-oxime **XIX.** In accordance with the results reported by Sato and coworkers (49–52),[10] we found 7-ketone **XXV** to be a potent inhibitor of $P450_{DM}$ in the same assay system (IC_{50} = 0.57 μM). However, both the 7-oxime **XXVI** and the 7-ketone **XXV** at concentrations up to 20 μM had little effect on the incorporation of [^{14}C]acetate into C_{27} sterols and C_{30} sterols in CHO cells. This is not surprising in regard to the 7-oxime **XXVI** as its inhibitory activity in the in vitro (rat liver microsomal preparations) assay was limited. However, the discrepancy between the inhibition of $P450_{DM}$ by 7-ketone **XXV** in the in vitro versus the cultured cells studies suggests that this ketone may be metabolized to a compound that is incapable of inhibiting $P450_{DM}$. As can be seen in Table 1, both the 7-ketone and the 7-oxime cause the suppression of HMGR activity with IC_{50} values in CHO cells of 0.9 and 6.7 μM, respectively. Quantitative immunoblot analysis similar to that shown in Fig. 3 revealed that suppression of HMGR by compounds **XXV** and **XXVI** was occurring at the level of gene expression (data not shown). To our knowledge, this is the first report of 7-substituted lanosterol analogs acting as suppressors of HMGR activity. The potency of 7-ketone **XXV** as a suppressor of HMGR activity is similar to that of the 15-oxime **XIX** and the 15-ketone **III** discussed previously, suggesting that if 7-ketone **XXV** is being metabolized by the cells, the metabolite is acting as a suppressor of HMGR activity. This putative metabolite may be similar in structure to 7-ketocholesterol or 4,4-dimethyl-3β-hydroxycholest-5-en-7-one (53, 54), known suppressors of HMGR activity. Reported IC_{50} values for suppression of HMGR activity by these compounds in mouse L cells were 3.7 μM and 1.5 μM, respectively (53, 54). However, suppression of HMGR activity by 7-ketone **XXV** in the demethylase-deficient AR45 cell line suggests that this compound can suppress HMGR activity in the absence of active $P450_{DM}$. Experiments to investigate the metabolism of 7-oxylanosterols in CHO cells are in progress. 7-Methyloxime **XXVII** was prepared in the same manner as 15-methyloxime **XXIII.** As can be seen in Table 1, 7-methyloxime **XXVII** is not very effective in causing the inhibition of $P450_{DM}$ or the suppression of HMGR activity. This decrease in potency when the oxime hydrogen is replaced with a methyl group is consistent with the results for the 15-substituted compounds **XIX** and **XXIII.**

32-Oxime **XXX**[11] is similar in structure to both the alcohol and aldehyde intermediates generated during the removal of the 14α-methyl group (i.e., compounds **XII** and **XIII**) which are known inhibitors of $P450_{DM}$ (29) and suppressors of HMGR activity (30). Treatment of 3β-tetrahydropyranyloxylanost-7-en-32-al **XXVIII** (37–40) with hydroxylamine hydrochloride in the presence of pyridine gave a mixture of 3β-tetrahydropyranyloxylanost-7-en-32-al 32-oxime **XXIX** and the desired 3-deprotected 32-oxime **XXX.** The 3β-tetrahydropyranyloxylanost-7-en-32-al 32-oxime **XXIX** was easily deprotected to give 32-oxime **XXX** by treatment with pyridinium *p*-toluenesulfonate (PPTS) in ethanol (33). 32-Oxime **XXX** was found to be an inhibitor of $P450_{DM}$ exhibiting an IC_{50} value of 8.9 μM in vitro and 1.0 μM in cultured cells (Table 1). It is also a potent suppressor of HMGR activity with an IC_{50} value of 1.0 μM in CHO cells and 2.0 μM in $P450_{DM}$-deficient (AR45) cells. 32-Methyloxime **XXXI** was also prepared in a manner analogous to the preparation of compound **XXIII** (Table 1). Once again, the replacement of the hydrogen of the oxime moiety with a methyl group greatly diminished the activity of the oxime for both inhibition of $P450_{DM}$ and suppression of HMGR activity.

CONCLUSION

A series of oxolanosterol oximes and oxime ethers have been prepared as potential dual-action inhibitors of cholesterol biosynthesis. 3β-Hydroxylanost-7-en-15-one 15-oxime **XIX** was found to be an effective inhibitor of $P450_{DM}$ in both rat liver microsomal preparations and in CHO cells. In addition, 15-oxime **XIX** was shown to suppress HMGR activity by regulation of gene expression. These results demonstrate that, as predicted,

[10]We have included our data for the inhibition of $P450_{DM}$ by 3β-hydroxylanost-8-en-7-one **XXV** in Table 1 since Sato and coworkers (49–52) do not report an IC_{50} value or a K_i for this compound in rat liver microsomal preparations. They report an IC_{50} value of 0.2–0.3 μM for inhibition of $P450_{DM}$ by 3β-hydroxylanost-8-en-7-one **XXV** in *Saccharomyces cerevisiae.*

[11]This compound is included in DuPont's patent (35). They report an IC_{50} value for suppression of HMGR of 1.4 μM and an IC_{50} value for inhibition of $P450_{DM}$ of 3.0 μM.

15-oxime **XIX** is indeed a dual-action inhibitor of cholesterol biosynthesis that causes both the inhibition of $P450_{DM}$ and a reduction in HMGR activity. Oximes at C-7 and C-32 also exhibited inhibition of both $P450_{DM}$ and HMGR activity. In all cases, replacement of the oxime hydrogen with a methyl group greatly reduced the activity of the oxime for both inhibition of $P450_{DM}$ and suppression of HMGR activity.

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