Inhibitors of sterol synthesis. Chemical synthesis of 5β-cholest-8-ene-3β,15α-diol and its effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells

William K. Wilson, Frederick D. Pinkerton, Nanda Duhé Kirkpatrick, and George J. Schroepfer, Jr.[1]

Departments of Biochemistry and Chemistry, Rice University, P. O. Box 1892, Houston, TX 77251

Abstract 5β-Cholest-8-ene-3β,15α-diol, prepared by hydroboration of 5β-cholesta-8,14-dien-3β-ol, was determined to have the 14α-H,15α-OH configuration by comparisons of observed and calculated lanthanide-induced shifts for the 3-*tert*-butyldimethylsilyl derivative. The 3β,15α-diol was found to exist partially in a conformation in which ring B is a 5β, 6α-half chair and the axial-equatorial orientation of ring A substituents is reversed. This conformation has been observed previously for 3β-(p-bromobenzoyloxy)-5β-cholesta-8,14-diene and for some *cis*-decalin derivatives. 5β-Cholest-8-ene-3β,15α-diol was found to be highly active in the lowering of the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in Chinese hamster ovary cells and only slightly less active than the corresponding sterol (5α-cholest-8-ene-3β,15α-diol) with the *trans* A-B ring junction. — **Wilson, W. K., F. D. Pinkerton, N. D. Kirkpatrick, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Chemical synthesis of 5β-cholest-8-ene-3β,15α-diol and its effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells. *J. Lipid Res.* 1989. **30:** 919–928.

Supplementary key words Chinese hamster ovary cells • ^{1}H and ^{13}C NMR • lanthanide-induced shifts • conformational analysis • hydroboration

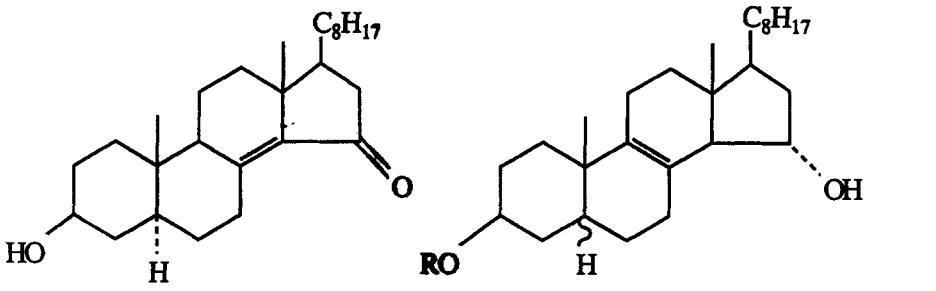
3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) (**Fig. 1**) and a number of other 15-oxygenated sterols have been shown to be potent inhibitors of sterol synthesis in cultured mammalian cells and to reduce the levels of activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (1–16). All of the 15-oxygenated sterols studied to date have had the *trans* orientation of the A–B ring junction. The isolation and characterization of 3β-benzoyloxy-5β-cholesta-8,14-diene as a byproduct in the acid-catalyzed isomerization of 7-dehydrocholesteryl benzoate (17, 18) provided the opportunity to prepare a 15-oxygenated sterol with the *cis* A–B ring junction. Reported here are the chemical synthesis of 5β-cholest-8-ene-3β,15α-diol (**III**) and its effects on HMG-CoA reductase activity in CHO-K1 cells.

MATERIALS AND METHODS

Melting points (uncorrected) were measured in evacuated capillary tubes. Ultraviolet (UV) spectra were recorded in absolute ethanol on an IBM 9430 spectrophotometer. Optical rotations were measured at 589 nm in chloroform solution on a JASCO DIP-4 digital polarimeter. Mass spectra (MS) were recorded on a Finnigan 6000 (70 eV) or Extrel ELQ-400 (20 eV) quadrupole instrument using electron impact. Infrared (IR) spectra were obtained on a Beckman 4230 spectrophotometer using KBr pellets. Thin-layer chromatography (TLC) was performed using precoated 0.25 mm silica gel G plates (Analtech; Newark, DE); substances were visualized by spraying with 5% ammonium molybdate in 10% H_2SO_4. TLC solvent systems were: SS-1, ethyl acetate–hexane 1:19; SS-2, ethyl acetate–hexane 1:3; SS-3, ethyl acetate–hexane 1:1. Gas–liquid chromatography (GLC) was performed with flame ionization detection using a 15–30 m DB-5 capillary column (0.25 mm ID, 0.10 μm film thickness, 5 psi N_2). High performance liquid chromatography (HPLC) was performed on a Spherisorb ODS-II column (C_{18}, 5 μm, 4.6 × 250 mm; Custom LC,

Abbreviations: CHO, Chinese hamster ovary; DEPT, distortionless enhancement by polarization transfer; GLC, gas–liquid chromatography; HETCOR, ^{1}H-^{13}C shift-correlated (spectroscopy); HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high performance liquid chromatography; IR, infrared; LIS, lanthanide-induced shifts; LIS_{calc}, calculated LIS; LIS_{obs}, observed LIS; LSR, lanthanide shift reagent; MPLC, medium pressure liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; TBDMS, *tert*-butyldimethylsilyl; TLC, thin-layer chromatography; UV, ultraviolet; $Yb(fod)_3$, (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium.

[1]To whom correspondence should be directed.



I

II 5α-H R = H
III 5β-H R = H
IV 5β-H R = C_6H_5CO
V 5β-H R = $(CH_3)_3C(CH_3)_2Si$

Fig. 1. Chemical structures of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), 5α-cholest-8-ene-3β,15α-diol (**II**), 5β-cholest-8-ene-3β,15α-diol (**III**), 3β-benzoyloxy-5β-cholest-8-en-15α-ol (**IV**), and 3-(*tert*-butyldimethylsilyloxy)-5β-cholest-8-en-15α-ol (**V**).

Houston, TX) using a Waters instrument (Model 6000 pump, U6K injector, and either a Model R401 refractive index detector or Model 490 UV detector; Milford, MA). Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on an IBM AF300 spectrometer. ^1H NMR spectra (300.1 MHz) were referenced to tetramethylsilane, and ^{13}C NMR spectra (75.5 MHz) were referenced to $CDCl_3$ at 77.0 ppm. Some ^{13}C NMR spectra were also recorded at 22.5 MHz on a JEOL FX-90Q instrument. ^1H and ^{13}C NMR assignments were made from DEPT (distortionless enhancement by polarization transfer), ^1H-^{13}C shift-correlated (HETCOR), and long-range HETCOR spectra, from lanthanide-induced shifts (LIS), and by comparison with spectra of similar sterols. DEPT, HETCOR (~50 increments, δ 0.6–2.6 window in the ^1H dimension), and long-range HETCOR (optimized for 10 Hz couplings, ~16 increments, δ 0.6–2.6 window in the ^1H dimension) experiments were carried out as described previously (19). 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) (1, 20, 21), 5α-cholest-8-ene-3β,15α-diol (**II**) (22), 5β-cholesta-8,14-dien-3β-ol (17), and 3β-benzoyloxy-5β-cholesta-8,14-diene (17) were prepared previously.

Supplies were obtained from the following sources: borane-tetrahydrofuran, *tert*-butyldimethylsilyl chloride, and tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium ($Yb(fod)_3$), Aldrich Chemical Co. (Milwaukee, WI); Chinese hamster ovary (CHO-K1) cells, American Type Culture Collection (Rockville, MD); (3RS)-[3-^{14}C]HMG-CoA (56 mCi per mmol) and (3RS)-[2-^3H]mevalonolactone (176 mCi per mmol), Amersham Corporation (Arlington Heights, IL); trypsin, Gibco Laboratories (Grand Island, NY); Lux tissue culture plasticware, Miles Scientific (Elkhart, IN); powdered Ham's F12 medium (23) and phosphate-buffered saline (PBS: KCl, 2.7 mM; KH_2PO_4, 1.2 mM; NaCl, 137 mM, and Na_2HPO_4, 8.1 mM), Irvine Scientific (Irvine, CA); fetal calf serum, Whittaker M. A. Bioproducts (Elkhart, IN). For cell culture studies, the sterols were added as ethanolic solutions to Ham's F12 medium supplemented with 5% delipidated (24) fetal calf serum (lipid-deficient medium) and allowed to equilibrate for at least 6 h at room temperature prior to storage at 4°C. Radioactivity was assayed in Packard 4640 and Beckman 9800 liquid scintillation counters using 2,5-diphenyloxazole (0.4%) in toluene or toluene–ethanol 2:1 as the scintillation fluid. Protein in detergent-solubilized extracts of cultured cells was assayed by the method of Lowry et al. (25) after precipitation with trichloroacetic acid.

Studies of the effects of **I**, **II**, and **III** on HMG-CoA reductase activity were made in CHO-K1 cells. The cells were maintained in Ham's F12 medium supplemented with 5% fetal calf serum (lipid-rich medium) in a humidified atmosphere of 5% CO_2–95% air at 37°C. Each experiment was initiated by inoculating 3.75×10^5 cells into 100-mm dishes containing lipid-rich medium (10 ml), followed by incubation for 48 h. The medium was aspirated and, after rinsing of the plates with PBS (10 ml), lipid-deficient medium (10 ml) was added to one set of plates. A separate set of plates received lipid-deficient medium containing the 15-oxygenated sterols. The cells were incubated for 18 h. The cells incubated in the absence of sterols then received fresh lipid-deficient medium containing the 15-oxygenated sterols and incubation continued for 4 h. Cells were harvested by scraping, and detergent-solubilized cell preparations were obtained for the assay of HMG-CoA reductase activity using the method of Brown, Dana, and Goldstein (26). Replicate assays (n = 3) were carried out as described by Pinkerton

et al. (10), except that the specific activity of the (3RS)-[3-^{14}C]HMG-CoA was 20,000 dpm per nmol.

5β-Cholest-8-ene-3β,15α-diol (III)

Hydroboration of 3.92 g of 5β-cholesta-8,14-dien-3β-ol, as described below for **IV**, gave a crude product containing **III** as the only major component detected by ^{13}C NMR. Recrystallization from toluene–chloroform gave 2.17 g of material, which was recrystallized repeatedly from methanol–water to give 680 mg of **III** of > 95% purity. An analytical sample (used for biological testing) was obtained by column chromatography (silica gel; elution with 20–50% ethyl acetate in hexane): mp 167.5–168°C; $[\alpha]_D^{27}$ +69.9° (c 0.5, chloroform); IR (KBr) 3350, 3000–2800, 1460, 1360, 1040 cm^{-1}; MS *m/z* (relative intensity) 402 (5, M^+), 387 (8, $M-CH_3$), 384 (18, $M-H_2O$), 369 (11, $M-CH_3-H_2O$), 351 (12, $M-CH_3-2H_2O$), 271 (31, $M-H_2O-C_8H_{17}$), 105 (67), 55 (100); TLC, single spots at R_f 0.01 (SS-1), R_f 0.10 (SS-2), R_f 0.38 (SS-3); HPLC, single peaks at 27.2 min (methanol–water, 7:1, 1.0 ml/min) and 5.5 min (methanol–water, 98:1, 1.0 ml/min); elemental analysis: Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.51. Found: C, 80.53; H, 11.62.

5β-Cholest-8-ene-3β,15α-diol, 3-benzoate (IV)

To a solution of 976 mg (2 mmol) of 3β-benzoyloxy-5β-cholesta-8,14-diene in 40 ml of ether at 0°C was added dropwise 3 ml of 1 M borane–tetrahydrofuran. The reaction was stirred at 20°C for 1 h and quenched with water (1 ml). The organic solvents were evaporated, the residue was dissolved in 40 ml of tetrahydrofuran and cooled to 0°C, and 7 ml of 30% H_2O_2 was added. A 10% NaOH solution (5 ml) was added over 30 min, and the solution was stirred at 0°C for an additional 50 min. The reaction mixture was extracted with dichloromethane (100 ml), and the organic layer was evaporated to a residue. The residue was dissolved in dichloromethane (40 ml), washed with 10% sodium sulfite solution (2 × 20 ml) and brine (2 × 40 ml), and evaporated to a white solid, which was fractionated by medium pressure liquid chromatography (MPLC) using a 1 m × 15 mm silica gel column (elution with 9% ethyl acetate in hexane; 25 ml fraction volumes). Early fractions contained the starting material and unidentified sterols (~150 mg, fraction 6) and material that apparently corresponded to epimer(s) of **IV** (~100 mg, fractions 12–18). The major component (480 mg, **IV**) was collected in fractions 23–32. Recrystallization from acetone–water afforded an analytical sample: mp 141–142°C; clearing at 150°C; $[\alpha]_D^{27}$ +79.1° (c 0.5, chloroform); UV (ethanol) $\lambda_{max}(\epsilon)$ 228 nm (14700); IR (KBr) 3500, 3060, 2980–2820, 1680, 1440, 1280, 700 cm^{-1}; MS *m/z* (relative intensity) 488 (3, $M-H_2O$), 384 (15, $M-C_6H_5CO_2H$), 369 (8, $M-CH_3-C_6H_5CO_2H$), 366 (12, $M-H_2O-C_6H_5CO_2H$), 351 (25, $M-CH_3-H_2O-C_6H_5CO_2H$), 312 (9, $M-C_6H_5CO_2H-C_4H_6$), 253 (19, $M-H_2O-C_6H_5CO_2H-C_8H_{17}$), 217 (19), 105 (100); TLC, single spots at R_f 0.06 (SS-1), R_f 0.59 (SS-2), R_f 0.81 (SS-3); HPLC, single peak at 12.3 min (methanol–water, 98:2, 1.0 ml/min); capillary GLC (280°C, 15 m DB-5 column), single peak at 18.48 min (39.74 methylene units); elemental analysis: Calcd for $C_{34}H_{50}O_3$: C, 80.58; H, 9.95. Found: C, 80.59; H. 9.94.

A sample of **IV** was saponified by treatment with 0.8 M KOH in ethanol for 1.5 h at 40°C. Chromatographic purification to remove the benzoic acid byproduct afforded a white solid, which was identical to **III** by ^{1}H and ^{13}C NMR.

3β-(*tert*-Butyldimethylsilyloxy)-5β-cholest-8-en-15α-ol (V)

To a solution of 82 mg (0.54 mmol) of *tert*-butyldimethylsilyl (TBDMS) chloride and 193 mg (2.83 mmol) of imidazole in 2 ml of dry dimethylformamide was added 150 mg (0.37 mmol) of 5β-cholest-8-ene-3β,15α-diol. The resulting solution was heated at 75°C for 75 min, poured into water, and extracted with dichloromethane. The combined organic extracts were dried and evaporated to a residue, which was chromatographed (silica gel, 8 cm × 10 mm diameter column). Material (8 mg) tentatively identified as the disilylated ether of **III** was eluted with hexane immediately after the void volume; the 3-monosilyl ether **V** (83 mg) was eluted with SS-1; and recovered starting material (46 mg) was eluted with ethyl acetate. In addition to ^{1}H and ^{13}C NMR (see Tables 1 and 2), **V** was characterized as follows: MS *m/z* (relative intensity) 516 (3, M^+), 498 (38, $M-H_2O$), 459 (10, $M-C(CH_3)_3$), 441 (37, $M-C(CH_3)_3-H_2O$), 383 (7, $M-(CH_3)_3C(CH_3)_2SiOH-H$), 369 (19), 367 (34), 365 (59), 351 (100, $M-CH_3-(CH_3)_3C(CH_3)_2SiOH-H_2O$), 312 (6, $M-(CH_3)_3C(CH_3)_2SiOH-C_4H_6$), 253 (21, $M-(CH_3)_3C(CH_3)_2SiOH-H_2O-C_8H_{17}$), 238 (23), 213 (37); capillary GLC (200–280°C at 20°C/min, 30 m column), single peak at 7.88 min; and TLC, single spots at R_f 0.21 (SS-1), R_f 0.81 (SS-2).

Lanthanide-induced shift (LIS) measurements

LIS were determined by adding 1–64 μl of a ~0.15 mM solution of Yb(fod)$_3$ in $CDCl_3$ to a solution of 20–31 mg of **IV** or **V** in 0.4 ml of $CDCl_3$. Spectra were collected at 22°C for five molar ratios (0.005 to 0.07) of Yb(fod)$_3$ to **IV** and seven molar ratios (0.002 to 0.13) of Yb(fod)$_3$ to **V**. ^{1}H, ^{13}C, and HETCOR spectra (50 increments, ~8 min spectrometer time) were acquired for **V**, and induced shifts were measured to 0.001 ppm precision. No corrections were made for contact shifts. The relative LIS values were obtained by linear regression calculations (fixing the intercept at the origin (27)) on the plot of induced shifts

TABLE 1. ^{1}H NMR chemical shifts for sterols II–V[a]

Atom	Multiplicity; J	II	III	IV[b]	V[c]
3α-H	m	3.63	3.96	5.27	3.91
14-H	m	2.07	2.09	2.14	2.09
15β-H	dt; 3.9, 9.4 Hz	4.30	4.10	4.11	4.09
16-H_2	m	1.78, 1.95	1.78, 1.95	1.73, 1.95	1.77, 1.95
17-H	m	1.45	1.45	1.43	1.45
18-H_3	s	0.640	0.661	0.675	0.655
19-H_3	s	0.961	1.081	1.137	1.059
20-H	m	1.36	1.36	1.33	1.36
21-H_3	d; 6.3–6.6 Hz	0.909	0.912	0.921	0.910
24-H_2	m	1.12	1.12	1.12	1.11
25-H	m	1.52	1.52	1.52	1.51
26-H_3	d; 6.5–6.6 Hz	0.862	0.864	0.868	0.863
27-H_3	d; 6.5–6.6 Hz	0.867	0.867	0.871	0.867

[a]Spectra were recorded at 300 MHz in 0.01–0.1 M $CDCl_3$ solution. Chemical shifts of 14-H, 16-H_2, 17-H, 20-H, 24-H_2, and 25-H were determined from HETCOR spectra.
[b]Benzoate **IV** also showed aromatic signals at δ 8.06 (d, J = 7.1 Hz; ortho-H), 7.55 (t, J = 7.4 Hz; para-H), 7.44 (t, J = 7.3 Hz; meta-H).
[c]TBDMS ether **V** also showed signals at δ 0.888 (s; (C$\underline{H}_3$)$_3$C) and 0.032 and 0.029 (s; diastereotopic C$\underline{H}_3$-Si).

versus apparent lanthanide shift reagent (LSR) concentration, followed by normalization to the LIS of C-15. The apparent LSR concentrations, calculated for each LSR concentration from weighted ratios of all nonzero ^{1}H and ^{13}C induced shifts relative to those of 0.008 molar ratio data set, were similar to the LSR concentrations measured volumetrically. Atomic coordinates for LIS calculations were obtained from molecular mechanics (28) using PC Model (Serena Software, Bloomington, IN) on an IBM-compatible computer (Tandy 4000). In order to avoid calculating weighted averages of the numerous rotamers of the benzoate and TBDMS ether groups, coordinates for the underivatized diol **III** and its epimers were used to calculate the LIS for the candidate structures of **IV** and **V**. The sterol side chain was considered to exist only in its extended conformation. C-26, C-27, and their attached hydrogen atoms were placed at positions averaged for their two most populated rotamers. Average positions were calculated for each set of methyl hydrogen atoms and for the two hydrogen atoms at C-24. For each set of coordinates and each set of observed LIS values, the position of the coordinating Yb atom (single-site model) was adjusted to give a minimum value of the agreement factor R using the simplified McConnell-Robertson equation (29). Excluding C-19 and C-1 through C-6 from the calculation of R for **IV** and excluding C-5 for **V**, the position of Yb was optimized to locations ~2.5 Å from O-15. A further set of minimizations, which alternately optimized the position of Yb and the location of the principal magnetic axis, reduced R values by an additional 5–10% (relative). The R values are given in Tables 3 and 4. The minimizations were performed with identical sequences of optimization algorithms using a Microsoft QuickBASIC program on a Macintosh II computer. The minimization sequence contained numerous stochastic steps to avoid convergence to any local minimum that might be present. Repeated applications of the minimization sequence led to virtually identical (± 0.005%) values of R, albeit by a variety of pathways.

RESULTS AND DISCUSSION

Hydroboration of 5β-cholesta-8,14-dien-3β-ol resulted in a mixture of products, from which the major component (**III**, Fig. 1) was isolated by MPLC. ^{1}H and ^{13}C NMR chemical shifts and multiplicities (**Table 1** and **Table 2**) indicated that **III** retained the Δ^8 double bond and bore a hydroxyl group at C-15. Several of the ring A and B carbon peaks were markedly broadened at 75.5 MHz but of normal line width at 22.5 MHz. This pattern of broadened NMR peaks was virtually identical to that found for other 5β-Δ^8 sterols, such as 5β-cholesta-8,14-dien-3β-ol and its benzoate and *p*-bromobenzoate esters (18). We have recently reported crystallization of the latter derivative in conformation B (**Fig. 2**), which molecular mechanics calculations showed to be slightly favored over conformation A (18). In that work, the broadening of certain ^{13}C NMR peaks was attributed to the interconversion of conformers A and B at a moderately slow rate on the (75 MHz) NMR time scale. Based on the NMR line widths for **III** and on molecular mechanics calculations showing that conformers A and B of 5β-Δ^8-3β,15-diols are comparable in energy,[2] we concluded that a substan-

[2]Judging from energies calculated from molecular mechanics, conformer B of 3β-hydroxy-5β-Δ^8-steroids is usually slightly favored over conformer A. In the case of 5β-cholest-8-ene-3β,15α-diol and its 3-TBDMS ether, however, molecular mechanics calculations show conformer A to be favored by 0.1 and ~0.3 kcal/mol, respectively. Conformer B also appears to be a major conformer for 5β-steroids with a variety of other patterns of substitution and unsaturation (W. K. Wilson and G. J. Schroepfer, Jr., unpublished results).

TABLE 2. ^{13}C NMR chemical shifts for sterols II-V[a]

Atom	II	III	IV[b]	V[c]
C-1	35.05	30.62	31.28	30.86
C-2	31.56	30.79	27.40	31.24
C-3	71.14	67.07	71.28	67.56
C-4	38.18	35.73[d]	31.87[d]	36.26[d]
C-5	40.51	37.38[d]	37.03[d]	37.20[d,e]
C-6	25.41	24.23[d]	23.54[d]	24.24[d]
C-7	27.26	25.10[d]	24.15[d]	24.94[d]
C-8	126.71	127.74	128.14	127.45
C-9	136.19	132.69[d]	131.73[d]	132.96[d]
C-10	35.83	36.48	36.54	36.53
C-11	22.72	22.85	22.93	22.90
C-12	37.09	37.10	37.14	37.20[e]
C-13	43.22	43.09	43.20	43.16
C-14	59.62	59.50	59.50	59.59
C-15	72.08	71.95	72.06	72.08
C-16	40.49	40.81	40.72	40.77
C-17	52.92	52.64	52.76	52.68
C-18	12.50	12.49	12.50	12.49
C-19	17.71	26.83[d]	28.14[d]	27.31
C-20	35.89	35.85	35.89	35.89
C-21	18.46	18.42	18.46	18.45
C-22	35.99	35.97	35.97	36.00
C-23	23.78	23.78	23.77	23.79
C-24	39.43	39.38	39.41	39.42
C-25	27.99	27.94	27.97	27.98
C-26	22.54	22.51	22.54	22.54
C-27	22.80	22.76	22.79	22.79

[a]Spectra were recorded at 75.5 MHz in 0.02–0.1 M $CDCl_3$ solution and referenced to $CDCl_3$ (77.0 ppm).

[b]Benzoate **IV** also showed aromatic signals at δ 132.64 (para), 131.04 (quaternary), 129.45 (ortho), and 128.24 (meta). The larger than normal benzoylation shifts for ring B carbons were attributed to different populations of conformer B in **III** and **IV**.

[c]TBDMS ether **V** also showed signals at δ 25.88 (($\underline{C}H_3)_3C$), 18.17 ($(CH_3)_3\underline{C}$), and −4.76 and −4.79 (diastereotopic $\underline{C}H_3$-Si).

[d]Peaks 5–20 Hz wide at half height at 75.5 MHz; <3 Hz wide at half height at 22.5 MHz.

[e]Peaks separated by <0.02 ppm at 75.5 MHz; separated by 0.08 ppm at 22.5 MHz.

tial portion of **III** exists as conformer B. In conformation B, which is well known in the *cis*-decalin literature (30–32), in which it is often called the "nonsteroidal" conformation, the axial–equatorial orientation of A and B ring substituents (except those at C-8 and C-9 of steroids) are reversed from those of conformation A. Because ring B of steroids is anchored at C-8 and C-9, it becomes a twist boat in conformation B unless a double bond is present. In the case of Δ^8 steroids, ring B interconverts between a 5α,6β-half chair (conformation A) and a 5β,6α-half chair (conformation B), a process shown by the NMR line widths to occur readily at room temperature.

Based on the previously reported 14α-H, 15α-OH configurations for the major hydroboration products of 5α-$\Delta^{7,14}$, 5α-$\Delta^{8,14}$, and 5α-Δ^{14} sterols (22, 33–35) and on the similarity of the ^{13}C NMR chemical shifts of **II** and **III** ($\pm$ 0.3 ppm exclusive of C-19 and ring A and B carbons), we tentatively assigned **III** as the 14α-H, 15α-OH isomer. However, the 14β-H, 15β-OH configuration, which has been reported for similar hydroborations conducted at 50–60°C (36), could not be unequivocally ruled out since ^{13}C NMR data were unavailable for 14β-sterols related to **III**. For this reason and because **III** exists partially as conformer B, the reactivity of which has not been previously investigated, a more rigorous[3] structure assignment was considered desirable. Conformations A and B of four candidate stereoisomers were investigated: $\mathbf{III}_{\alpha\alpha}$ (14α-H, 15α-OH), $\mathbf{III}_{\beta\beta}$ (14β-H, 15β-OH), $\mathbf{III}_{\alpha\beta}$ (14α-H, 15β-OH), and $\mathbf{III}_{\beta\alpha}$ (14β-H, 15α-OH). Coupling constants $J_{15H\text{-}14H}$, $J_{15H\text{-}16\alpha H}$, and $J_{15H\text{-}16\beta H}$ calculated from an extended Karplus equation (37) using molecular mechanics structures were in reasonable agreement with the observed coupling constants only for $\mathbf{III}_{\alpha\alpha}$ (1.1, 1.0, and 0.1 Hz deviations) and $\mathbf{III}_{\beta\beta}$ (1.2, 2.0, and 1.2 Hz deviations). In order to discriminate between $\mathbf{III}_{\alpha\alpha}$ and $\mathbf{III}_{\beta\beta}$, we turned to lanthanide shift reagent techniques (29).

When an LSR is added to a sterol solution, reversible coordination of the LSR with the alcohol oxygen causes NMR signals of nearby atoms to be shifted in a systematic manner. Under suitable experimental conditions (38), these observed lanthanide-induced shifts (LIS_{obs}) may be closely approximated by the simplified McConnell-Robertson equation: $LIS_{calc} = k(3\cos^2\theta - 1)/r^3$ where k is a proportionality constant, r is the distance between the LSR and the substrate atom, and θ is the angle between the principal magnetic axis and the line joining the LSR and the substrate atom (**Fig. 3**). In order to apply the LIS technique, the LIS_{obs} are measured by adding LSR to a solution of the substrate and recording the induced shifts. Then, a set of LIS_{calc} is obtained by applying the simplified McConnell-Robertson equation using atomic coordinates from molecular mechanics calculations. For alcohols, the LSR is initially positioned ~2.5 Å from the coordinating oxygen atom along the C–O bond, and the magnetic axis is initially chosen parallel to this bond. The LSR position and magnetic axis direction are then systematically adjusted by an algorithm designed to minimize the agreement factor $R = \{\Sigma(LIS_{calc} - LIS_{obs})^2/\Sigma LIS_{obs}^2\}^{1/2}$. In our experience, use of the simplified McConnell-Robertson equation generally leads to $R<3\%$ for steroidal alcohols and ketones of known structure and $R>4\%$ for structures with incorrect

[3]Assignment of configuration by comparison of ^{13}C NMR chemical shifts is further complicated in this case because the spectra of conformers A and B may differ considerably, even in ring D. A ^{13}C NMR spectrum of 3β-benzoyloxy-5β-cholesta-8,14-diene in $CDCl_3$ at −60°C showed pairs of peaks of approximately equal intensity for nearly all the ring carbons. These peaks evidently arose from the "freezing out" of conformations A and B. The following chemical shift differences were observed at −60°C for ring D carbons: C-13, 0.1 ppm; C-14, 0.4 ppm; C-15, 0.9 ppm; C-16, unassigned; C-17, < 0.1 ppm; C-18, 0.5 ppm. These differences are substantially larger than the ring D chemical shift differences normally observed between 5α- and 5β-sterols.

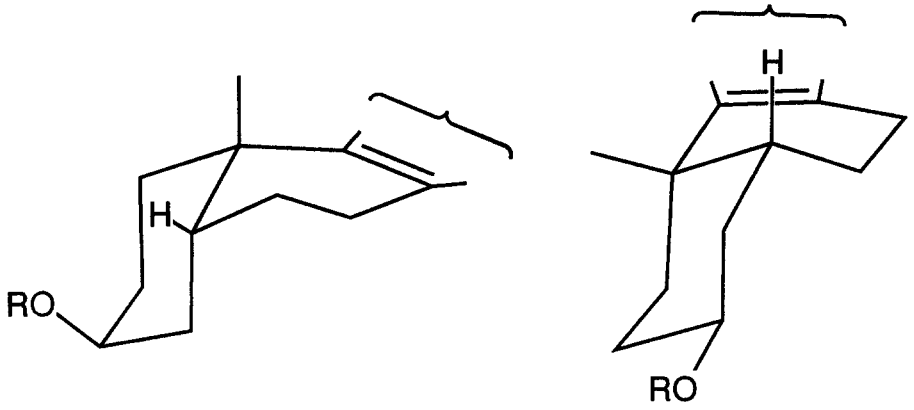


Fig. 2. Conformations of rings A and B of 5β-Δ^8 sterols.

stereochemistry in the vicinity of the coordinating functional group. Thus, by comparing the agreement between the experimental and calculated LIS, one can discriminate between two (or more) structures under consideration. When both agreement factors are reasonably good, statistical tests for significance, such as the jackknife test (39, 40) or the Hamilton test (40, 41), can be applied to estimate the probability that the structure with the higher R value is incorrect.

In order to block coordination of the LSR to the functional group at C-3 (42), we prepared the 3-TBDMS ether **V** by treating diol **III** with 1.5 equivalents of TBDMS chloride. As much data as feasible was collected by measuring LIS_{obs} for all methine and methyl protons of **V** and for all carbon atoms (except H-5 and C-5, which were broadened and/or obscured by other peaks). The LIS_{obs} for **V** are shown in **Table 3** along with the LIS_{calc} for conformers A and B of structures $\mathbf{III}_{\alpha\alpha}$ and $\mathbf{III}_{\beta\beta}$. Because **V**, like **III**, exists in solution as a mixture of both conformers, averages of the two sets of LIS_{calc} for $\mathbf{III}_{\alpha\alpha}$ and $\mathbf{III}_{\beta\beta}$ were also computed. The resulting R values were lower than those of either individual conformer. **Table 4** summarizes the R values for conformer A, conformer B, and the averaged form for each of the four candidate structures. Table 4 also shows that the probabilities that structure $\mathbf{III}_{\beta\beta}$ is incorrect are $>99.95\%$ for the Hamilton test for all forms, $\geq 99.95\%$ for the jackknife test for conformer B and the averaged form, and 98% for the jackknife test for conformer A. We judged this relatively low probability of 98% to be misleading because *1*) it rises to 99.9% when atoms of rings A and B are excluded and *2*) the probabilities for the averaged form best reflect the actual conformations in solution. Although these significance tests are somewhat controversial (40) and have seldom been applied in recent structure determinations based on LIS, they are a useful complement to the researcher's intuition and to the rule of thumb that $R \sim 3\%$ divides the correct from the incorrect struc-

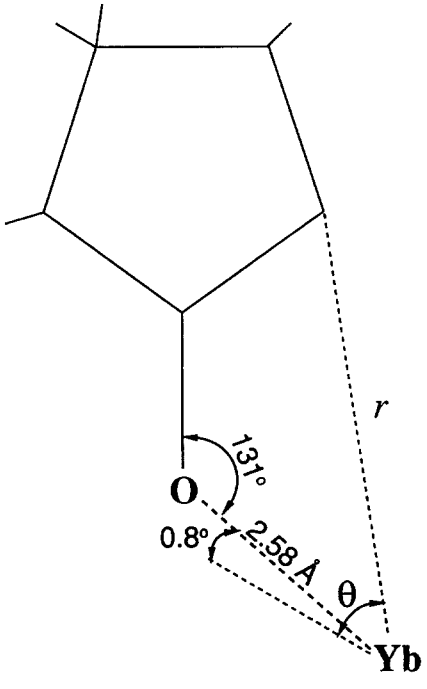


Fig. 3. Coordination of Yb to a 15-hydroxysterol, illustrating r and θ for computing LIS_{calc} of C-16. The Yb–O distance, the Yb–O-C15 angle, and the deviation of the magnetic axis from the Yb–O vector are given for conformer B of $\mathbf{III}_{\alpha\alpha}$.

TABLE 3. Observed LIS (normalized to the LIS for C-15) for 3-(*tert*-butyldimethylsilyloxy)-5β-cholest-8-en-15α-ol (**V**) and calculated LIS for conformer A (A) and conformer B (B) of structures $\mathbf{III}_{\alpha\alpha}$ and $\mathbf{III}_{\beta\beta}$

Atom	Observed LIS **V**	Calculated LIS			
		$\mathbf{III}_{\alpha\alpha}$ (A)	$\mathbf{III}_{\alpha\alpha}$ (B)	$\mathbf{III}_{\beta\beta}$ (A)	$\mathbf{III}_{\beta\beta}$ (B)
C-1	50	49	57	48	58
C-2	40	47	30	45	27
C-3	28	35	19	26	14
C-4	30	50	18	40	17
C-6	65	60	76	53	68
C-7	255	245	254	249	245
C-8	268	270	270	289	288
C-9	152	140	139	148	150
C-10	74	73	74	75	78
C-11	110	111	110	116	118
C-12	135	140	140	151	154
C-13	220	227	226	228	229
C-14	446	454	451	462	465
C-15	1000	1000	1000	1000	1000
C-16	437	437	443	452	454
C-17	224	219	220	213	213
C-18	136	143	139	133	132
C-19	44	50	47	51	51
C-20	97	95	93	98	96
C-21	63	65	64	64	63
C-22	48	51	51	51	47
C-23	22	16	15	17	13
C-24	−6	−4	−5	−7	−10
C-25	−10	−7	−8	−8	−10
C-26	−13	−11	−12	−11	−14
C-27	−14	−6	−7	−8	−10
3-H	23	28	18	14	6
14-H	435	434	433	414	417
15-H	628	629	626	625	628
17-H	211	197	202	169	170
18-H_3	116	119	114	113	111
19-H_3	43	43	41	45	45
20-H	74	75	69	86	83
21-H_3	58	59	58	57	56
24-H_2	−10	−9	−10	−14	−18
25-H	−8	−8	−10	−7	−10
26-H_3	−12	−12	−13	−12	−15
27-H_3	−12	−6	−6	−8	−10
R (%)		2.45	2.11	4.24	4.58
Yb-O distance (Å)		2.47	2.54	2.28	2.30
Yb-O-C15 angle		136°	133°	160°	162°
Yb-O-C15-C14 torsion angle		−160°	−161°	−176°	−169°
Magnetic axis deviation from Yb-O		0.8°	0.3°	1.7°	1.9°

tures. The LIS analysis for **V**, thus, firmly establishes the configuration of **III** as 14α-H, 15α-OH.[4]

The effects of 5β-cholest-8-ene-3β,15α-diol (**III**) on the levels of activity of HMG-CoA reductase in CHO-K1 cells are presented in **Table 5**. Also presented are data on the effects of the corresponding sterol with the *trans* A-B ring junction, 5α-cholest-8-ene-3β,15α-diol (**II**), and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**). The 5β-Δ^8-3β,15α-dihydroxysterol (**III**) was highly active not only in the suppression of the rise of HMG-CoA reductase activity induced by transfer of the cells to lipid-deficient media but also in the suppression of elevated levels of HMG-CoA reductase activity induced by transfer of the cells to lipid-deficient media. In the former case, **III** caused a 50% inhibition at $\sim 5 \times 10^{-8}$ M. A 50% reduction in levels of elevated reductase activity by **III** was observed at 1×10^{-7} M. The effects of the 5β-Δ^8-3β,15α-diol (**III**)

[4]An LIS analysis (^{13}C data only) had initially been performed on the 3-benzoyl derivative **IV**, which was prepared by hydroboration of 3β-benzoyloxy-5β-cholesta-8,14-diene. The LSR coordinated primarily to O-15, but a secondary coordination to the benzoate carbonyl affected the LIS_{calc} values for C-19 and C-1 through C-6. After elimination of these carbons from the calculations, poor agreement factors *R* were obtained, as anticipated, for $\mathbf{III}_{\alpha\beta}$ (4.62,4.65%; conformer A, conformer B) and $\mathbf{III}_{\beta\alpha}$ (4.67, 4.70%). Surprisingly, reasonable *R* values were found for both $\mathbf{III}_{\alpha\alpha}$ (1.30, 1.39%) and $\mathbf{III}_{\beta\beta}$ (1.91, 1.89%). Although the Hamilton test ($\sim$15 degrees of freedom) ruled against $\mathbf{III}_{\beta\beta}$ with 99.6–99.9% probability, the more demanding jackknife test gave probabilities of only 89–95%. These probabilities are substantially lower than those obtained for **V**, for which almost twice as many usable LIS_{obs} were available.

TABLE 4. Agreement factors and results of significance tests by comparison of LIS observed for 3-(*tert*-butyldimethylsilyloxy)-5β-cholest-8-en-15α-ol (**V**) with LIS calculated for structures $\mathbf{III}_{\alpha\alpha}$, $\mathbf{III}_{\alpha\beta}$, $\mathbf{III}_{\beta\alpha}$, and $\mathbf{III}_{\beta\beta}$[a,b,c]

		Agreement Factor (R)			Confidence Levels (Jackknife Test)[d]		
	Configuration	A	B	Avg	A	B	Avg
$\mathbf{III}_{\alpha\alpha}$	14α-H,15α-OH	2.46	2.11	1.70	—	—	—
$\mathbf{III}_{\alpha\beta}$	14α-H,15β-OH	14.15	13.62	13.72	99.95	99.95	99.95
$\mathbf{III}_{\beta\alpha}$	14β-H,15α-OH	16.93	14.90	15.71	99.95	99.95	99.95
$\mathbf{III}_{\beta\beta}$	14β-H,15β-OH	4.24	4.58	4.23	98	99.95	99.95
Calculations excluding atoms of rings A and B[e]							
$\mathbf{III}_{\alpha\alpha}$	14α-H,15α-OH	1.57	1.35	1.40	—	—	—
$\mathbf{III}_{\beta\beta}$	14β-H,15β-OH	3.93	4.02	3.95	99.9	99.95	99.95

[a]A, conformer A; B, conformer B; avg, a 1:1 mixture of conformers A and B (R was computed after averaging each LIS_{calc} for the two conformers). The lowest R values for mixtures of conformers A and B of $\mathbf{III}_{\alpha\alpha}$ and $\mathbf{III}_{\beta\beta}$ were 1.68% (58% conformer B) and 4.18% (27% conformer B), respectively.

[b]In the Hamilton test, the confidence levels relative to structure $\mathbf{III}_{\alpha\alpha}$ were >99.95% for all comparisons. The estimated degrees of freedom was 32, although the confidence levels were >99.95% even when degrees of freedom were estimated at only 12.

[c]The following relative energies were calculated by molecular mechanics for the free sterols (conformer A, conformer B): $\mathbf{III}_{\alpha\alpha}$, 2.9, 3.0; $\mathbf{III}_{\beta\beta}$, 0.7, 0.7; $\mathbf{III}_{\alpha\beta}$, 4.9, 4.0; $\mathbf{III}_{\beta\alpha}$, 0.4, 0.0 kcal/mol (reproducibility, ~ ± 0.1 kcal/mol).

[d]For rejection of $\mathbf{III}_{\alpha\beta}$, $\mathbf{III}_{\beta\alpha}$, or $\mathbf{III}_{\beta\beta}$ relative to structure $\mathbf{III}_{\alpha\alpha}$.

[e]Calculations made by excluding C-19, 3-H, and all carbon atoms of rings A and B after determination of the LIS_{calc}.

were similar to those observed with the 5α-Δ^8-3β,15α-diol (**II**) and with the 15-ketosterol (**I**). The 5α-Δ^8-3β,15α-diol appeared to be slightly more active than **III** with regard to the blockage of the increase in HMG-CoA reductase activity. Similar findings were observed with regard to the effects of **II** and **III** in the reduction of the levels of elevated HMG-CoA reductase activity.

The high activity of **III** in the reduction of the levels of HMG-CoA reductase activity was unanticipated. Estimates of the potency of oxygenated sterols with respect to HMG-CoA reductase are the resultant of multiple factors including, but not limited to, the rates of uptake of the oxygenated sterols (43), rates of metabolism of a given oxygenated sterol to species of higher, lower, or unchanged activity, additional sites of action other than the suppression of the level of HMG-CoA reductase activity (10, 44), and the nature of the interaction of a given oxygenated sterol with a specific oxysterol-binding protein thought to

TABLE 5. Effects of 5β-cholest-8-ene-3β,15α-diol (**III**), 5α-cholest-8-ene-3β,15α-diol (**II**), and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on levels of HMG-CoA reductase activity in CHO-K1 cells

	HMG-CoA Reductase Activity (% of Control Activity)[a]					
	Suppression of Rise Induced by Transfer of Cells to Lipid-Deficient Media			Suppression of Elevated Levels Induced by Transfer of Cells to Lipid-Deficient Media		
Sterol Concentration	**II**	**III**	**I**	**II**	**III**	**I**
nM						
0	100.0[b]	100.0[c]	100.0[d]	100.0[e]	100.0[f]	100.0[g]
10	90.3 ± 17.5	100.3 ± 0.7	72.6 ± 1.3	77.5 ± 1.7	79.4 ± 2.3	85.2 ± 2.0
25	56.4 ± 4.3	70.5 ± 2.7	56.8 ± 1.9	61.5 ± 0.7	75.3 ± 0.7	94.6 ± 8.2
50	42.8 ± 3.0	49.6 ± 2.1	50.0 ± 0.5	40.4 ± 2.3	81.0 ± 2.0	90.2 ± 0.7
100	17.1 ± 3.4	34.3 ± 0.7	42.6 ± 1.4	36.3 ± 0.8	51.4 ± 0.7	48.7 ± 2.8
500	26.4 ± 2.5	26.6 ± 0.9	26.4 ± 0.7	28.7 ± 2.6	25.0 ± 1.3	45.7 ± 4.2
1000	32.0 ± 0.7	25.6 ± 1.6	23.4 ± 0.8	29.1 ± 3.6	22.7 ± 1.0	34.4 ± 0.9
2500	24.8 ± 1.3	24.0 ± 2.4	20.1 ± 1.8	24.3 ± 4.4	20.4 ± 0.8	30.2 ± 0.8
3500		27.9 ± 0.8	22.4 ± 2.0		20.2 ± 1.8	25.2 ± 1.0
5000		23.9 ± 2.6	18.0 ± 1.7		17.0 ± 0.5	28.4 ± 1.1

[a]Variation is expressed as ± SD of replicate (n = 3) assays for the experimental values.

[b-g]Mean values for controls were 3750, 6840, 8080, 2280, 6070, and 5790 pmol/min per mg protein, respectively.

be intimately involved in the repression of HMG-CoA reductase (11, 45, 46). With a few exceptions, the apparent binding affinities of the oxysterol-binding protein for individual oxygenated sterols in in vitro assays correlate closely with potencies of the oxygenated sterols in lowering the levels of HMG-CoA reductase activity in cultured mammalian cells (11).

To our knowledge, none of the oxygenated sterols studied in the binding assays or with respect to their effects on HMG-CoA reductase have had a *cis* orientation of the A/B ring junction. Such 5β-sterols generally adopt conformation A (Fig. 2), which differs markedly from the usual conformation of Δ^5- and 5α-sterols in shape and in orientation of the 3-hydroxyl group, both of which factors may have a strong bearing on the biological activity of a sterol. For example, in the 5α-series, 3β-hydroxy-5α-cholest-8(14)-en-15-one (equatorial 3β-hydroxyl) has been shown to be considerably more potent in lowering HMG-CoA reductase activity (6, 11) and to have a higher binding affinity for the oxysterol-binding protein (11) than 3α-hydroxy-5α-cholest-8(14)-en-15-one (axial 3α-hydroxyl). Given these considerations and our initial assumption that **III** exists only in conformation A with its axially oriented 3β-hydroxyl group and its marked change in geometry relative to the 5α analog, we initially anticipated that **III** would be much less potent than **II** in its effects on HMG-CoA reductase activity. The finding that a significant portion of **III** exists in conformation B and that the conformers A and B readily interconvert at room temperature suggests that the activity of **III** might be due to its ability to form significant amounts of conformer B, which, like **II**, has an equatorial 3β-hydroxyl group. However, although conformer B of **III** more closely resembles the 5α-sterol **II** than does conformer A of **III**, when conformer B of **III** is overlaid with **II** so that ring A atoms are matched, the D rings and the 15α-hydroxyl groups of the two molecules are not closely matched.[5] Thus, the extent to which conformation B contributes to the high biological activity of **III** remains an open question.

The cell culture and NMR studies and the synthetic work were supported by grants HL-15376 and HL-22532, respectively, from the National Institutes of Health. The support of the Ralph and Dorothy Looney Endowment Fund and the American Cyanamid Company is gratefully acknowledged. The Arco Foundation provided funds for the puchase of the NMR instrument.

Manuscript received 4 November 1988 and in revised form 19 December 1988.

[5]The angles between the least squares plane of ring A and the least squares plane containing atoms of rings B, C, and D are 17° for **II**, 75° for **III** (conformer A), and 47° for **III** (conformer B).

REFERENCES

1. Schroepfer, G. J., Jr., E. J. Parish, H. W. Chen, and A. A. Kandutsch. 1977. Inhibition of sterol biosynthesis in L cells and mouse liver cells by 15-oxygenated sterols. *J. Biol. Chem.* **252:** 8975–8980.
2. Schroepfer, G. J., Jr., E. J. Parish, and A. A. Kandutsch. 1977. 14α-Ethyl-5α-cholest-7-en-3β,15α-diol, an extraordinarily potent inhibitor of sterol synthesis in animal cells. *J. Am. Chem. Soc.* **99:** 5494–5496.
3. Schroepfer, G. J., Jr., D. L. Raulston, and A. A. Kandutsch. 1977. Inhibition of sterol synthesis in L cells by 14α-ethyl-5α-cholest-7-en-15α-ol-3-one at nanomolar concentrations. *Biochem. Biophys. Res. Commun.* **79:** 406–410.
4. Schroepfer, G. J., Jr., E. J. Parish, M. Tsuda, D. L. Raulston, and A. A. Kandutsch. 1979. Inhibition of sterol biosynthesis in animal cells by 14α-alkyl substituted 15-oxygenated sterols. *J. Lipid Res.* **20:** 994–998.
5. Schroepfer, G. J., Jr., E. J. Parish, M. Tsuda, and A. A. Kandutsch. 1979. Inhibition of sterol biosynthesis by 9α-fluoro and 9α-hydroxy derivatives of 5α-cholest-8 (14)-en-3β-ol-15-one. *Biochem. Biophys. Res. Commun.* **91:** 606–613.
6. Schroepfer, G. J., Jr., E. J. Parish, and A. A. Kandutsch. 1979. Further studies on the inhibition of sterol biosynthesis in animal cells by 15-oxygenated sterols. *Chem. Phys. Lipids.* **25:** 265–285.
7. Schroepfer, G. J., Jr., R. A. Pascal, Jr., and A. A. Kandutsch. 1979. Inhibition of sterol synthesis in animal cells by 15-oxygenated sterols with the unnatural *cis*-C-D ring junction:5α,14β-cholest-7-en-15α-ol-3-one and 5α,14β-cholest-7-en-15β-ol-3-one. *Biochem. Pharmacol.* **28:** 249–252.
8. Schroepfer, G. J., Jr., E. J. Parish, R. A. Pascal, Jr., and A. A. Kandutsch. 1980. Inhibition of sterol biosynthesis in animal cells by 14α-hydroxymethyl sterols. *J. Lipid Res.* **21:** 571–584.
9. Miller, L. R., F. D. Pinkerton, and G. J. Schroepfer, Jr. 1980. 5α-Cholest-8(14)-en-15-one, a potent inhibitor of sterol synthesis, reduces the levels of activity of enzymes in volved in the synthesis and reduction of 3-hydroxy-3-methylglutaryl coenzyme A in CHO-K1 cells. *Biochem. Int.* **1:** 223–228.
10. Pinkerton, F. D., A. Izumi, C. M. Anderson, L. R. Miller, A. Kisic, and G. J. Schroepfer, Jr. 1982. 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol, a potent inhibitor of sterol biosynthesis, has two sites of action in cultured mammalian cells. *J. Biol. Chem.* **259:** 12382–12387.
11. Taylor, F. R., S. E. Saucier, E. P. Shown, E. J. Parish, and A. A. Kandutsch. 1984. Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase. *J. Biol. Chem.* **259:** 12382–12387.
12. Schroepfer, G. J., Jr., E. J. Parish, A. A. Kandutsch, S. T. Bowen, and F. A. Quiocho. 1987. 15β-Methyl-5α,14β cholest-7-ene-3β, 15α-diol, synthesis, structure, and inhibition of sterol synthesis in animal cells. *Biochem. Int.* **15:** 403–408.
13. Schroepfer, G. J., Jr., H-S. Kim, J. L. Vermilion, T. W. Stephens, F. D. Pinkerton, D. H. Needleman, W. K. Wilson, and J. St. Pyrek. 1988. Enzymatic formation and chemical synthesis of an active metabolite of 3β-hydroxy-5α-cholest-8(14)-en-15-one, a potent regulator of cholesterol metabolism. *Biochem. Biophys. Res. Commun.* **151:** 130–136.

14. Schroepfer, G. J., Jr., E. J. Parish, M. Tsuda, and A. A. Kandutsch. 1988. Inhibitors of sterol synthesis. Chemical syntheses, properties, and the effects of 4,4-dimethyl-15-oxygenated sterols on sterol synthesis and on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured mammalian cells. *Chem. Phys. Lipids.* **47:** 187–207.
15. Pajewski, T. N., F. D. Pinkerton, L. R. Miller, and G. J. Schroepfer, Jr. 1988. Inhibitors of sterol synthesis. Studies of the metabolism of 5α-cholest-8(14)-en-3β-ol-15-one in Chinese hamster ovary cells and its effects on activities of early enzymes in cholesterol biosynthesis. *Chem. Phys. Lipids.* **48:** 153–168.
16. Kim, H-S., W. K. Wilson, D. H. Needleman, F. D. Pinkerton, D. K. Wilson, F. A. Quiocho, and G. J. Schroepfer, Jr. 1989. Inhibitors of sterol synthesis. Chemical synthesis, structure, and biological activities of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one, a metabolite of 3β-hydroxy-5α-cholest-8(14)-en-15-one. *J. Lipid Res.* **30:** 247–261.
17. Wilson, W. K., and G. J. Schroepfer, Jr. 1988. Acid-catalyzed isomerization of 7-dehydrocholesteryl benzoate. A revised mechanism and improved synthetic procedure. *J. Org. Chem.* **53:** 1713–1719.
18. Wilson, D. K., W. K. Wilson, F. A. Quiocho, and G. J. Schroepfer, Jr. 1988. Concerning the structure of 3β-benzoyloxy-5β-cholesta-8,14-diene, a major byproduct in the chemical synthesis of 5α-cholest-8(14)-en-3β-ol-15-one. *Chem. Phys. Lipids.* **47:** 273–282.
19. Emmons, G. T., W. K. Wilson, and G. J. Schroepfer, Jr. 1989. 24,25-Epoxysterols. Differentiation of 24R and 24S epimers by ^{13}C nuclear magnetic resonance spectroscopy. *J. Lipid Res.* **30:** 133–138.
20. Parish, E. J., T. E. Spike, and G. J. Schroepfer, Jr. 1977. Sterol synthesis. Chemical synthesis of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene. A key intermediate in the synthesis of 15-oxygenated sterols. *Chem. Phys. Lipids.* **18:** 233–239.
21. Wilson, W. K., K-S. Wang, A. Kisic, and G. J. Schroepfer, Jr. 1988. Concerning the chemical synthesis of 3β-hydroxy-5α-cholest-8(14)-en-15-one, a novel regulator of cholesterol metabolism. *Chem. Phys. Lipids.* **48:** 7–17.
22. Parish, E. J., and G. J. Schroepfer, Jr. 1979. Chemical synthesis of 5α-cholesta-6,8(14)-dien-3β-ol-15-one and related 15-oxygenated sterols. *Chem. Phys. Lipids.* **25:** 381–394.
23. Ham, R. G. 1965. Clonal growth of mammalian cells in a chemically defined, synthetic medium. *Proc. Natl. Acad. Sci. USA.* **53:** 288–293.
24. Cham, B. E., and B. R. Knowles. 1976. A solvent system for delipidation of plasma or serum without protein precipitation. *J. Lipid Res.* **17:** 176–181.
25. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
26. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1974. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human fibroblasts. *J. Biol. Chem.* **249:** 789–796.
27. Myers, R. H. 1986. Classical and Modern Regression with Applications. Duxbury Press, Boston, MA. 23–26.
28. Burkert, U., and N. L. Allinger. 1982. Molecular Mechanics. ACS Monograph 177. American Chemical Society, Washington, DC.
29. Wenzel, T. 1987. NMR Shift Reagents. CRC Press, Boca Raton, FL.
30. Fringuelli, F., E. W. Hagaman, L. N. Moreno, A. Taticchi, and E. Wenkert. 1977. Conformational analysis of cyclopropanodecalin derivatives by carbon-13 nuclear magnetic resonance spectroscopy. *J. Org. Chem.* **42:** 3168–3173.
31. Huffman, J. W., and W. H. Balke. 1988. Enolization of 2-decalones. *J. Org. Chem.* **53:** 3828–3831.
32. Dalling, D. K., D. M. Grant, and L. F. Johnson. 1971. Conformational inversion rates in the dimethylcyclohexanes and in some *cis*-decalins. *J. Am. Chem. Soc.* **93:** 3678–3682.
33. Nussim, M., Y. Mazur, and F. Sondheimer. 1964. The hydration of unsaturated steroids by the Brown hydroboration reaction. I. Monounsaturated steroids. *J. Org. Chem.* **29:** 1120–1133.
34. Partridge, L. G., I. Midgley, and C. Djerassi. 1977. Mass spectrometry in structural and stereochemical problems. 249. Elucidation of the course of the characteristic ring D fragmentation of unsaturated steroids. *J. Am. Chem. Soc.* **99:** 7686–7695.
35. Taylor, E. C., and C. Djerassi. 1977. Synthesis of cholest-5-ene-3β,11α,15β-triol-7-one. A model for the steroid nucleus of oogoniol, a sex hormone of the water mold *Achlya*. *J. Org. Chem.* **42:** 3571–3579.
36. Anastasia, M., P. Allevi, A. Fiecchi, A. Oleotti, and A. Scala. 1986. Stereochemical course of the hydroboration of highly hindered steroidal olefins, a ready synthesis of 14β- and 8α,14β-steroids. *Steroids.* **47:** 131–141.
37. Haasnoot, C. A. G., F. A. A. de Leeuw, and C. Altona. 1980. A relationship between proton-proton NMR coupling constants and substituent electronegativities—I. An empirical generalization of the Karplus equation. *Tetrahedron.* **36:** 2783–2792.
38. Schneider, H-J., U. Buchheit, and P. K. Agrawal. 1984. Topological, stereochemical and NMR line assignments in alcohols and ketones by normalized NMR shifts induced by ytterbium. *Tetrahedron.* **40:** 1017–1023.
39. Richardson, M. F., S. M. Rothstein, and W-K. Li. 1979. Significance testing of lanthanide shift reagent data. *J. Magn. Reson.* **36:** 69–79.
40. Ribo, J. M., and G. Valera. 1987. Significance testing of lanthanide shift reagent data. *Magn. Reson. Chem.* **25:** 293–296.
41. Davis, R. E., and M. R. Wilcott, III. 1972. Interpretation of the pseudocontact model for nuclear magnetic resonance shift reagents. II. Significance testing on the agreement factor R. *J. Am. Chem. Soc.* **94:** 1744–1745.
42. Raber, D. J., and G. J. Propeck. 1982. Structure elucidation with lanthanide-induced shifts. 15. Blocking groups for polyfunctional compounds. *J. Org. Chem.* **47:** 3324–3325.
43. Kandutsch, A. A., and H. W. Chen. 1975. Regulation of sterol synthesis in cultured cells by oxygenated derivatives of cholesterol. *J. Cell. Physiol.* **85:** 415–424.
44. Raulston, D. L., T. N. Pajewski, L. R. Miller, B. W. Phillip, D. J. Shapiro, and G. J. Schroepfer, Jr. 1980. Inhibition of cholesterol biosynthesis in cell-free preparations of rat liver by 14α-ethyl-5α-cholest-7-ene-3β,15α-diol. *Biochem. Int.* **1:** 113–119.
45. Kandutsch, A. A., and E. B. Thompson. 1980. Cytosolic proteins that bind oxygenated sterols. *J. Biol. Chem.* **255:** 10813–10826.
46. Kandutsch, A. A., and E. P. Shown. 1981. Assay of oxysterol-binding protein in a mouse fibroblast, cell-free system. *J. Biol. Chem.* **256:** 13068–13073.