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SYNTHESIS, PROPERTIES AND REACTIONS OF 3β-BENZOYLOXY-7α-15β-DICHLORO-5α-CHOLEST-8(14)-ENE

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Treatment of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (I) with gaseous HCl in chloroform at −40°C gave, in 87% yield, 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene (III). Reduction of the latter compound with lithium aluminum hydride in ether at room temperature for 20 min gave, in 86% yield, 7α-15β-dichloro-5α-cholest-8(14)-en-3β-ol (IV). The latter compound was fully characterized and assignments of the individual carbon peaks in the ^{13}C nuclear magnetic resonance spectra of this sterol have been completed. Reduction of III with excess lithium aluminum hydride in refluxing ether for 4 days gave, in 74% yield, 5α-cholesta-7,14-dien-3β-ol (VI). Reduction of the dichloro-steryl benzoate III with lithium triethylborohydride in tetrahydrofuran gave, in 88% yield, 5α-cholest-8(14)-en-3β-ol (VII). A similar reduction using lithium triethylborodeuteride led to the formation of [7β,15ξ-$^2H_2$]-VIIa. Treatment of III with concentrated HCl in a mixture of chloroform and methanol gave, in 79% yield, 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (II) which was characterized as such and as the corresponding free sterol.

I. Introduction

We have recently reported that a number of 15-oxygenated sterils are very potent inhibitors of sterol synthesis in animal cells in culture [1–5]. In addition, several of these 15-oxygenated sterils have been found to have significant hypocholesterolemic activity in animals (refs. 6–9 and A. Kisic et al., unpublished). An important intermediate in the chemical syntheses of a number of these 15-oxygenated sterols is 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (I) [10] whose structure has been unequivocally established by the results of X-ray crystallographic analysis of the corresponding 3β-*p*-bromobenzoate ester [11]. Examples of the importance of I in the syntheses of 15-oxygenated sterols are: (a) the formation of 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (II) upon treatment of (I) with acid [11]; (b) the formation of 5α-cholest-8(14)-en-3β,15α-diol upon reduction of the I with lithium aluminum hydride or lithium triethylborohydride [12–14];

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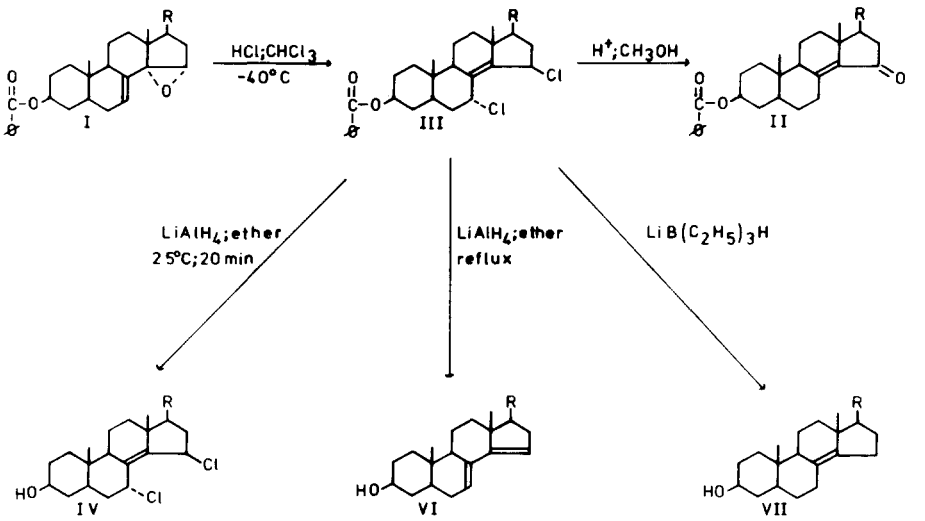

Fig. 1. Synthesis and reactions of 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene (III)

(c) the formation of 5α-cholestan-3β,7α,15α-triol upon treatment of the I with base (ref. 2 and M. Tsuda and G.J. Schroepfer, unpublished); and (d) the formation of 3β-benzoyloxy-5α,14β-cholest-7-en-15-one upon treatment of I with boron trifluoride-etherate [15–17].

We now wish to report that treatment of I with gaseous HCl in chloroform at –40°C gives, in high yield, 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene (III) (Fig. 1). In addition, we wish to report the properties and a number of novel reactions of III. 7α-15β-Dichloro-5α-cholest-8(14)-en-3β-ol (IV), derived from III, has been found to be a potent inhibitor of sterol synthesis in L cells in culture [18]. A preliminary account of portions of this research has been published [18].

II. Materials and methods

A. General

Procedures for the recording of melting points (m.p.) [2], optical rotations [19], infrared, mass spectra (MS) and combined gas-liquid chromatography-mass spectra [20] have been described previously. Ultraviolet spectra were measured on ethanol solutions of the sterols. Gas-liquid chromatographic (GLC) analyses were made using a Hewlett-Packard Model 402 unit equipped with dual flame ionization detectors. The columns (2 m × 0.6 cm, outside diam.) were packed with 3% OV-1 or 3% OV-17 on Gas-Chrom Q (100/120 mesh) and maintained at 270°C. High-resolution

mass spectral measurements were made on a Varian CH-5 spectrometer (courtesy of Professor C.C. Sweeley). Trimethylsilyl ether derivatives of the sterols (approx. 0.1 mg) were prepared by treatment with a mixture of hexamethyldisilazane, trichloromethylsilane, and pyridine (0.1 ml; 8 : 13 : 10) at 50°C for 10 min. Thin-layer chromatographic (TLC) analyses were made on plates of silica gel G (E. Merck, Darmstadt). Components on the plate were visualized after spraying with molybdic acid [21]. Medium pressure liquid chromatography (MPLC) was carried out on columns of silica gel (0.032 mm–0.63 mm; ICN Pharmaceuticals, Cleveland) at a flow rate of 4 ml/min using the appropriate solvent. Proton magnetic resonance (PMR) spectra were recorded in $CDCl_3$ solution on a Perkin-Elmer HR-12 spectrometer at 60 MHz and/or a Varian EM-390 spectrometer at 90 MHz using tetramethylsilane (TMS) as an internal standard. Peaks are reported as ppm (δ) downfield from the TMS standard. Proton chemical shifts for the C-18 and C-19 angular methyl resonances were calculated by the method of Zurcher [22]. The ^{13}C nuclear magnetic resonance spectra were recorded on a Varian XL-10015 spectrometer operating at 25.2 MHz in the Fourier transform mode using $CDCl_3$ solutions of the sterols. Data were accumulated with a maximum of 0.61 Hz per data point. A 5 mm ϕ sample tube was utilized and solvent-signal $CDCl_3$ was used as an internal standard. The chemical shifts (δ) are expressed in ppm relative to TMS and are estimated to be accurate to ±0.05 ppm ($\delta(TMS) = \delta(CDCl_3) + 76.9$ ppm). The probe temperature was approx. 30°C. Lanthanide-induced shift (LIS) experiments were performed using commercially available $Eu(fod)_3$. The ^{13}C NMR spectra (in $CDCl_3$) were first recorded in the proton noise-decoupling mode in order to measure the exact chemical shifts of all ^{13}C nuclei present. The degree of substitution of each carbon atom was determined by a second series of spectra in the single frequency off-resonance decoupling (SFORD) mode. Subsequently, an appropriate amount of $Eu(fod)_3$ was added to the $CDCl_3$ solution and the spectra data in the two modes were redetermined. The molar ratio of shift reagent to the sterol was 0.2.

3β-Benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (I) was prepared as described previously [10]. The preparation of 3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7α-ol (V) has been described elsewhere (M. Tsuda et al., unpublished).

B. 3β-Benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene (III)

Compound I (2.00; 3.96 mmol) was dissolved in $CHCl_3$, cooled to –40°C in a dry ice/acetone bath, and dry HCl gas was passed through the solution for 3 h. The reaction mixture was repeatedly washed with cold water and, after the washes were neutral to litmus, dried over $MgSO_4$. After evaporation of the solvent under reduced pressure at 30°C, the resulting residue was recrystallized 3 times from acetone/water to yield III (1.97 g; 87% yield) melting at 116–117°C (clearing at 138–139°C); infrared, ν_{max} 1720, 1600, 1582, 1272, 1117 and 714 cm^{-1}; PMR, 0.79 (s, 3H, C–19–CH_3), 1.12 (s, 3 H, C–18–CH_3) 4.95 (m, 2 H, C–3–H and C–7–H), 5.40 (m, 1 H,C–15–H), and 7.85 (m, 5 H, aromatic); MS, 524 and 522

(M–HCl; 2% and 5%) 509 and 507 (M–HCl–CH_3; 1% and 1%), 488 (M–2 Cl; 13%), 486 (M–2 HCl; 87%), 471 (M–2 HCl–CH_3; 4%), 411 and 409 (M–HCl–side chain; 13%), 401 (14%), 375 (M–2 Cl–side chain; 11%), 373 (M–2 HCl–side chain; 13%), 364 (M–2 HCl-benzonic acid; 60%), 349 (M–2 HCl–CH_3–benzoic acid; 100%), 251 (M–2 HCl–side chain; 30%), 236 (M–2 HCl–CH_3–side chain–benzoic acid; 8%), 209 (10%); high resolution MS on ion at *m/e* 522, 522.3275 (calc. for $C_{34}H_{47}O_2{}^{35}Cl$: 522.3264); elem. anal., calc. for $C_{34}H_{48}O_2Cl_2$: C, 72.97, H, 8.64; found: C, 72.95, H, 8.81; $[\alpha]_D$ −218.4° (*c*, 0.25). The compound showed a single component on TLC in 3 solvent systems.

C. 7α,15β-Dichloro-5α-cholest-8(14)-en-3β-ol (IV)

To III (1.00 g; 1.79 mmol) in ether (100 ml) was added $LiAlH_4$ (0.24 g; 6.32 mmol). After stirring at 25°C for 20 min, the mixture was cooled to 0°C, and ice was cautiously added to decompose the unreacted hydride. The mixture was poured into a saturated solution of NH_4Cl and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and evaporated to dryness. The resulting residue (0.72 g) was subjected to silica gel (60–200 mesh) column (40 cm × 3 cm) chromatography. Using 10% ether in benzene as the eluting solvent, fractions 20 ml in volume were collected. The contents of fractions 7 through 17 were pooled and, after evaporation of the solvent, recrystallized from acetone/water to yield IV (0.70 g; 86% yield) melting at 98–100°C; infrared, ν_{max} 3360, 1630, 1048, 678 cm^{-1}; PMR, 0.72 (s, 3 H,C–19–CH_3), 1.10 (s, 3 H, C–18–CH_3) 3.70 (m, 1 H, C–3–H), 4.96 (m, 1 H, C–7–H), and 5.32 (m, 1 H, C–15–H); $[\alpha]_D$ −246.1° (*c*, 0.38); MS, 420 and 418 (M–HCl; 4% and 10%), 405 and 403 (M–CH_3–HCl; 1% and 2%), 384 (M–2 Cl; 78%), 382 (M–2 HCl; 92%), 369 (M–2 Cl–CH_3; 20%), 367 (M–CH_3–2 HCl; 12%), 364 (M–H_2O–2 HCl; 12%), 349 (M–CH_3–H_2O–2 HCl; 100%), 307 and 305 (M–HCl–side chain, 1% and 2%), 297 (14%), 271 (M–2 Cl–side chain; 92%), 269 (M–2 HCl–side chain; 70%), 257 (28%), 255 (10%), 251 (M–2 HCl–H_2O–side chain; 24%), 236 (M–CH_3–H_2O–2 HCl–side chain; 8%), and 209 (16%); high resolution MS on ion at *m/e* 418, 418.3002 (calc. for $C_{27}H_{43}O^{35}Cl$: 418.3000); elem. anal., calc. for $C_{27}H_{44}OCl_2$: C, 71.19, H, 9.74; found, C, 71.22, H, 9.76. The compound showed a single component on TLC in 3 solvent systems. ^{13}C NMR assignments (based upon noise, SFORD, and LIS spectra and comparisons with recently completed analyses of the ^{13}C NMR spectra of 5α-cholest-8(14)-en-3β,7α,15α, triol (M. Tsuda et al., unpublished) and 5α-cholest-8(14)-en-3β-ol (Tsuda and Schroepfer, unpublished) are represented in Fig. 2 along with relative LIS values. The peaks due to the chlorine-substituted carbon atoms (C-7 and C-15) were easily identified (but not distinguished from each other) as those at 54.6 ppm (d, J_T = 60 Hz) and 57.7 ppm (d, J_T = 65 Hz) and distinguished from the methine carbon, C-17 (55.1 ppm ; J_T = 20 Hz), by their characteristically high residual coupling values. Definitive assignments of the peaks due to C-7 and C-15 were made on the basis of the

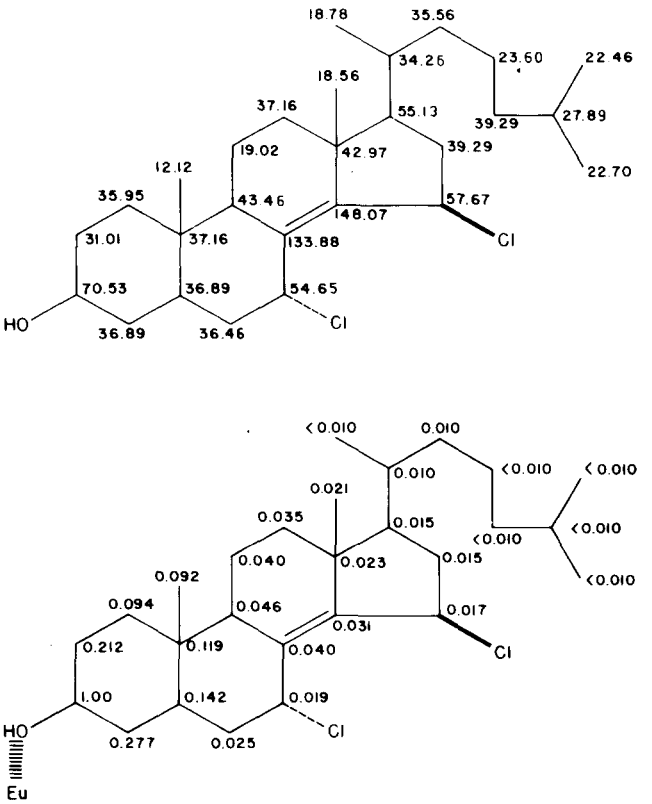

Fig. 2. ^{13}C nuclear magnetic resonance assignments (*above*) for 7α,15β-dichloro-5α-cholest-8(14)-en-3β-ol (VI) and the results of lanthanide-shift induced spectral studies (*below*) on the same compound (absolute value of LIS for C-3 was 11.62 ppm).

following considerations: Ernest [26] has derived the relationship between the residual coupling (J_r $^{13}C-^{1}H$) and the natural coupling constant (J_n $^{13}C-^{1}H$) which is given by the following expression:

$$J_r = J_n \Delta f \times 2\pi/H_2\gamma$$

or

$$\gamma H_2/2\pi = J_n f/J_r$$

At constant power, $\gamma H_2/2\pi$, the residual coupling (J_r) should vary linearly with Δf. the difference (in Hz) between the decoupling frequency (F_1; irradiation frequency used to obtain the SFORD spectrum) and correct frequency of the proton attached to the concerned carbon atom.

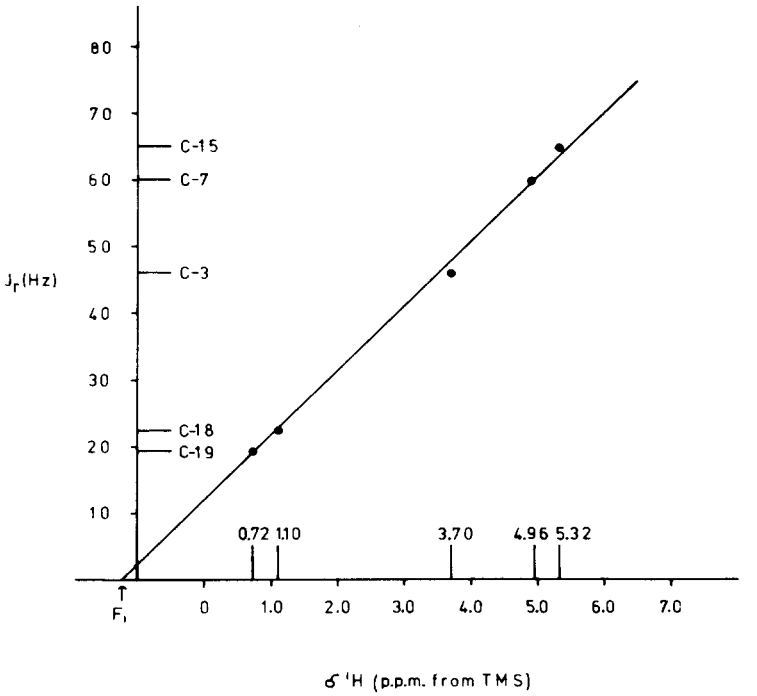

Fig. 3. Plot of J_T versus δ^1H for 7α,15β-dichloro-5α-cholest-8(14)-en-3β-ol (IV) (F_1 = irradiation frequency in the ^{13}C NMR analysis).

A plot of J_T (Hz) versus δ^1H is presented in Fig. 3. This plot shows good linearity if the carbon atom (54.6 ppm) with the J_T value of 60 Hz is assigned to C-7 and if the carbon atom (57.7 ppm) with the J_T value of 65 Hz is assigned to C-15.

D. Conversion of 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene III to 5α-cholest-7,14-dien-3β-ol (VI)

To III (1.00 g; 1.79 mmol) in ether (150 ml) was added $LiAlH_4$ (2.00 g; 51.3 mmol). The stirred reaction mixture was heated under reflux for 4 days under nitrogen. After cooling to 0°C, ice was cautiously added to decompose the excess hydride. The resulting mixture was poured into a 2 N NaCl solution and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and evaporated to dryness. The resulting residue (0.68 g) was subjected to preparative TLC on silica gel PF impregnated with $AgNO_3$ (10%) using 35% ethyl acetate in $CHCl_3$ as the developing solvent. The major component (R_F 0.24) was extracted with warm $CHCl_3$ and recrystallized from acetone/water to yield VI (0.51 g; 74% yield) melting at 104.5–105.5°C (literature: 104–105°C [23]);

infrared, ν_{max} 3285, 1639, and 1045 cm^{-1} (identical with that of an authentic sample); PMR, 0.77 (s, 3 H, C–18–CH_3; calc., 0.78), 0.81 (s, 3 H, C–19–CH_3; calc., 0.81), 3.65 (m, 1 H, C–3–H), 5.59 (m, 1 H, C–7–H), and 5.85 (m, 1 H, C–15–H); MS, 384 (M; 100%), 369 (M–CH_3; 10%), 366 (M–H_2O; 2%), 271 (M–side chain; 39%), and 257 (10%); high resolution MS., 384.3385 (calc. for $C_{27}H_{44}O$: 384.3392); ultraviolet, 242 nm (ϵ 9400) (literature: 242 nm (ϵ 9440) [23]). The product showed a single component on TLC on silica gel G in 2 solvent systems and on silica gel PF-$AgNO_3$ (solvent, 35% ethyl acetate in $CHCl_3$ and on GLC (3% OV-1 and 3% OV-17) with the same chromatographic mobility as that of an authentic sample.

To acquire information regarding the mechanism of the above reaction the reduction of the dichloro-benzonate was repeated using lithium aluminum deuteride. To III (1.00 g; 1.79 mmol) in ether (75 ml) was added $LiAlD_4$ (1.00 g; 23.8 mmol). The stirred reaction mixture was heated under reflux for 8 days under nitrogen. After cooling to 0°C, ice was cautiously added to decompose the unreacted $LiAlD_4$. The mixture was poured into a 2 N NaCl solution and extracted thoroughly with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and evaporated to dryness to give a white residue (0.67 g). Analysis by TLC on silica gel G-$AgNO_3$ (10%) using 5% acetone in $CHCl_3$ as the developing solvent indicated that the major product had the same mobility (R_F 0.42) as that of authentic VI. A minor impurity has the mobility of a monounsaturated monohydroxysterol (R_F 0.53). The crude product was subjected to preparative TLC (in the same system noted above). The material of R_F 0.23 was extracted from the plate with warm $CHCl_3$ and recrystallized from methyl/water to give VI (0.24 g; 35%) melting at 104.0–105.5°C (literature: 104–105°C [24]); infrared, ν_{max} 3285, 1639, and 1045 cm^{-1} (identical with that of an authentic sample); PMR, 0.77 (s, 3 H, C–18–CH_3), 0.81 (s, 3 H, C–18–CH_3), 3.65 (m, 1 H,C–3–H), 5.59 (m, 1 H, C–7–H), 5.85 (m, 1 H, C–15–H); MS, no detectable incorporation of the isotopic hydrogen; 384 (M; 100%), 369 (M–CH_3; 9%), 366 (M–H_2O; 2%), 271 (M–side chain, 38%), and 257 (10%; ultraviolet, 242 nm (ϵ = 9400) (literature: 242 nm (ϵ = 9400) [24]). The compound showed a single component with the identical mobility as that of an authentic sample when analyzed by TLC and GLC.

E. Reduction of 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene (III) with lithium triethylborohydride: Formation of 5α-cholest-8(14)-en-3β-ol (VII)

Compound III (1.00 g; 1.79 mmol) was dissolved in a 1 M solution (10 ml) of lithium triethylborohydride in tetrahydrofuran and the resulting mixture was allowed to stand at 25°C for 24 h under nitrogen. The mixture was poured into water and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and, after evaporation of the solvent, recrystallized from acetone/water to give VII (0.61 g; 88% yield) melting at 120–121°C.

(literature: 120–121°C [25], 120°C [26], 121°C [27], and 121–122°C [14]); $[\alpha]_D$ +22.3°C (*c*, 0.58) (literature: +24.4°C [25], +23°C [27], and +22.9°C [14]); infrared, ν_{max} 3360 and 1049 cm^{-1} (identical with spectrum of an authentic sample); PMR, 0.70 (s, 3 H, C–19–CH_3; calc., 0.71), 0.87 (s, 3 H, C–18–CH_3; calc., 0.87), and 3.65 (m, 1 H, C–3–H); MS, 386 (M; 100%), 371 (M–CH_3; 17%), 368 (M–H_2O 2%), 353 (M–CH_3–H_2O; 2%), 273 (M–side chain; 17%), and 255 (M–H_2O–side chain; 16%); high resolution MS, 386.3543 (calc. for $C_{27}H_{46}O$: 386.3548). The compound showed a single component with the identical chromatographic behavior as an authentic sample on analysis by TLC on silica gel G or silica gel PF-$AgNO_3$ plates (solvent systems, 10% ether in benzene and 35% ethyl acetate in $CHCl_3$) and by GLC (3% OV-1 and 3% OV-17).

F. Reduction of 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene (III) with lithium triethylborodeuteride: Formation of [7β,15ξ-$^2H_2$]-5α-cholest-8(14)-en-3β-of (VIIa)

Compound III (6.00 g; 10.7 mmol) was dissolved in a 1 M solution (60 ml) of lithium triethylborodeuteride in tetrahydrofuran and the resulting mixture was allowed to stand at 25°C for 48 h under nitrogen. The mixture was poured into water and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and, after evaporation of the solvent, recrystallized from acetone/water to give a white crystalline solid (4.25 g). Analyses by TLC on silica gel PF-$AgNO_3$ indicated the presence of 2 polar impurities (approx. 5% of total). Difficulties were encountered in attempts to purify the sterol by preparative silica gel PF-$AgNO_3$ TLC. Accordingly, purification via the benzoate ester was pursued. The crude sterol (4.00 g) was dissolved in a mixture of pyridine (60 ml) and benzoyl chloride (20 ml). After standing at 24°C for 24 h, the mixture was poured into water and extracted with ether containing CH_2Cl_2 (10%; 1000 ml). The ether extract was washed successively with water, cold 5% HCl, 0.5 N NaOH, and water, dried over $MgSO_4$, and, after evaporation of the solvent, recrystallized 3 times from acetone/water to give a white crystalline product (4.40 g). Analysis by TLC on silica gel PF-$AgNO_3$ indicated the presence of 2 polar impurities. Preparative TLC in the same system gave, after crystallization from acetone/water [7β,15ξ-$^2H_2$]-3β-benzoyloxy-5α-cholest-8(14)-ene (IX; 3.40 g; 64.5% yield) melting at 111–113°C; infrared, ν_{max} 2200 (C–D stretch), 1607, 1588, 1281, 1119, and 720 cm^{-1}; PMR, 0.73 (s, 3 H, C–19–CH_3), 0.89 (s, 3 H, C–18–CH_3), 5.10 (m, 1 H, C–3–H), and 7.85 (m, 5 H, aromatic); MS, 492 (M, 100%; d_2 = 87.2%, d_1 = 5.2%, d_0 = 7.6%), 477 (M–CH_3; 16%), 379 (M–side chain; 7%), 370 (M–benzoic acid; 20%); 355 (M–CH_3–benzoic acid; 15%), 257 (M–side chain–benzoic acid; 17%), 231 (17%), and 214 (14%); high resolution MS, 492.3960 (calc. for $C_{34}H_{48}D_2O_2$; 492.3952). The compound showed a single component on analyses by TLC on silica gel G and silica gel PF-$AgNO_3$ (10%) (solvent system, 25% hexane in benzene).

To the above benzoate (1.00 g; 2.00 mmol) in ether (100 ml) was added $LiAlH_4$

(2.00 g). After stirring at 25°C for 3 h, the mixture was cooled to 0°C and ice was added to decompose the unreacted hydride. The mixture was poured into a saturated solution of NH_4Cl and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined ether extracts were dried over $MgSO_4$ and, after evaporation of the solvent, recrystallized from acetone/water to give VIIa (0.70 g; 89% yield); infrared, ν_{max} 3320, 2200 (C–D stretch), 1049, and 951 cm^{-1}; PMR, 0.70 (s, 3 H, C–19–CH_3; calc., 0.71), 0.87 (s, 3 H, C–18–CH_3; calc., 0.87), 3.65 (m, 1 H, C–3–H); MS, 388 (M; 100%; d_2 = 86.2%, d_1 = 5.7%, d_0 = 8.0%), 373 (M–CH_3; 28%), 370 (M–H_2O; 2%), 355 (M–CH_3–H_2O; 4%), 275 (M–side chain; 14%), and 231 (14%); high resolution MS, 388.3677 (calc. for $C_{27}H_{40}D_2O$: 388.3672). The compound showed a single component on TLC analyses on silica gel G and silica gel PF-$AgNO_3$ (10%) plates (solvent systems, 10% ether in benzene and 35% ethyl acetate in $CHCl_3$) and on GLC analyses (3% OV-1 and 3% OV-17).

G. Chromic acid oxidation of [7β,15ξ-$^2H_2$]-3β-benzoyloxy-5α-cholest-8(14)-ene IX: Formation of [7β-$^2H_1$]-3β-benzoyloxy-5α-cholest-8(14)-en-15-one (IIa) and [15ξ-$^2H_1$]-3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7-one (VIIIa)

To IX (4.25 g; 8.60 mmol) in acetic acid (400 ml) was added sufficient benzene to dissolve the solid at room temperature. A solution of chromium trioxide (3.25 g) in 90% acetic acid (50 ml) was added dropwise with stirring. After standing for 24 h at 25°C, ethanol (13 ml) was added and the volume of the mixture was reduced to approx. 50 ml under reduced pressure. Water was added and the mixture was thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were washed with water, 1 N KOH, and water, dried over $MgSO_4$, and evaporated to dryness. The resulting residue was subjected to chromatography on a neutral alumina (Grade 1; ICN Pharmaceuticals, Inc.) column (70 cm × 3.0 cm) using the following solvent mixtures as the eluting solvents (hexane, 500 ml; 20% benzene in hexane, 500 ml; benzene, 500 ml; 50% ether in benzene, 500 ml; ether, 500 ml; 50% ether in ethanol, 500 ml). Fractions 100 ml in volume were collected. The contents of fractions 15 through 18 were pooled and, after evaporation of the solvent under reduced pressure, gave 0.24 g of material which was subjected to MPLC on a silica gel column (118 cm × 1.5 cm) using benzene as the eluting solvent. Fractions 20 ml in volume were collected. The contents of fractions 53 through 78 were pooled and, after evaporation of the solvent, recrystallized from acetone/water to give IIa (0.049 g; 1.1% yield) melting at 156°C (literature: 156–158°C [28], 157–158°C [10,29,30], 156°C [31]); infrared, ν_{max} 2230 (C–D stretch), 1710, 1612, 1580, 1112, and 710 cm^{-1}; PMR, 0.78 (s, 3 H, C–19–CH_3), 0.97 (s, 3 H, C–18–CH_3), 4.22 (m, 0.3 H, C–7b–H), 5.01 (m, 1 H, C–3–H), and 7.8 (m, 5 H, aromatic); ultraviolet, 258 nm (ϵ = 14 500) and 232 nm (ϵ = 15 300); MS, 505 (M; 100%; d_1 = 67%, d_0 = 33%), 490 (M–CH_3; 9%), 487 (M–H_2O; 5%), 392 (M–side chain; 8%), 383 (M–benzoic acid; 13%), 374 (M–H_2O–side chain; 8%), 368 (M–CH_3–benzoic acid; 43%), 350 (M–CH_3–benzoic acid–H_2O; 3%), 270 (M–side chain–

benzoic acid; 22%), and 252 (M–H_2O–side chain–benzoic acid; 24%); high resolution MS, 505.3661 (calc. for $C_{34}H_{47}DO_3$: 505.3666). Analyses by TLC on silica gel G plates in 3 solvent systems indicated a single component with the same chromatographic mobility as that of an authentic sample.

The contents of fractions 19 through 26 from the alumina column chromatography were pooled and, after evaporation of the solvent yielded 2.06 g of material which was subjected to MPLC on a silica gel column (118 cm × 1.5 cm) using 2.5% ether in benzene as the eluting solvent. Fractions 20 ml in volume were collected. The contents of fractions 21 through 50 were pooled and, after evaporation of the solvent, recrystallized from tetrahydrofuran/hexane at –40°C to give VIIIa (0.69 g; 15% yield) melting at 152.0–153.5°C; infrared, ν_{max} 2230 (C–D stretch), 1710, 1595, 1578, 1112, and 715 cm^{-1}; PMR, 1.00 (s, 3 H, C–18–CH_3), 1.05 (s, 3 H, C–19–CH_3), 5.1 (m, 1 H, C–3–H), and 8.1 (m, 5 H, aromatic); MS, 521 (M; 17%; d_1 = 68%, d_0 = 32%), 506 (M–CH_3; 16%), 505 (M–O; 31%), 502 (M–HDO; 18%), 488 (M–H_2O–CH_3; 7%), 408 (M–side chain; 44%), 399 (M–benzoic acid; 5%), 389 (M–HDO–side chain; 100%), 380 (M–HDO–benzoic acid; 30%), 339 (23%), 314 (23%), 267 (M–HDO–side chain–benzoic acid; 16%), and 221 (23%); high resolution MS, 521.3624 (calc. for $C_{34}H_{47}DO_4$: 521.3615). The compound showed a single component of TLC in 3 solvent systems with the same mobility as that of an authentic sample of VIII prepared by chromic acid oxidation of 3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7α-ol (V) (see below).

H. 3β-Benzoyloxy-8α,14α-epoxy-5α-cholestan-7-one (VIII)

To 3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7α-ol (V; 1.00 g; 1.9 mmol) in glacial acetic acid (20 ml) was added chromium trioxide (0.35 g) in 80% acetic acid (30 ml). After stirring at 25°C overnight, water was added and the resulting precipitate was collected and subjected to silica gel (60–200 mesh; 40 g) column (50 cm × 1.5 cm) chromatography using 7% ether in benzene as the eluting solvent at a flow rate of 5 ml per min. Fractions 20 ml in volume were collected. The contents of fractions 17 through 25 were pooled and, after evaporation of the solvent, recrystallized from acetone/water to give VIII (0.75 g; 75% yield) melting at 152.0–153.5°C; infrared, ν_{max} 1710, 1595, 1578, 1111, and 714 cm^{-1}; PMR, 1.00 (s, 3 H, C–18–CH_3), 1.05 (s, 3 H, C–19–CH_3), 5.1 (M, 1 H, C–3–H), and 8.1 (m, 5 H, aromatic); MS, 520 (M; 25%), 505 (M–CH_3; 27%), 504 (M–O; 47%), 502 (M–H_2O; 27%), 487 (M–CH_3–H_2O; 6%), 407 (M–side chain; 57%), 398 (M–benzoic acid; 7%), 389 (M–H_2O–side chain; 100%), 380 (M–H_2O–benzoic acid; 21%), 339 (28%), 314 (30%), 285 (M–side chain–benzoic acid; 20%), 267 (M–H_2O–side chain–benzoic acid; 32%), and 220 (32%); high resolution MS, 520.3548 (calc. for $C_{34}H_{48}O_4$: 520.3552). The compound showed a single component on TLC in 3 solvent systems.

I. Conversion of 3β-benzoyloxy-7α,15β-dichloro-5α-cholest-8(14)-ene III to 3β-benzoyloxy-5α-cholest-8(140)-en-15-one (II)

To III (1.00 g; 1.8 mmol) in chloroform (15 ml) was added a mixture of methanol (25 ml) and concentrated HCl (2 ml). After heating the mixture under reflux for 2 h, the volume was reduced to approx. 1/3 of its initial value under reduced pressure. The mixture was diluted with water and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined ether extracts were washed with water, dried over $MgSO_4$, and evaporated to dryness to yield a light yellow residue which was subjected to silica gel (60–200 mesh; 50 g) column (60 cm × 2.0 cm) chromatography using benzene as the eluting solvent at a flow rate of 5.0 ml/min. Fractions of 20 ml were collected. The contents of fractions 21 through 41 were pooled and, after evaporation of the solvent, recrystallized from acetone/water to give II (0.71 g; 79% yield) melting at 157–158°C [literature: 157–158°C [10], 156°C [31], and 156–158°C [28]]; infrared, ν_{max} 1710, 1612, 1580, 1112, and 710 cm^{-1}; PMR, 0.78 (s, 3 H, C–19–CH_3), 0.97 (s, 3 H, C–18–CH_3), 4.22 (m, 1 H, C–7β–H), 5.01 (m, 1 H, C–3–H), and 7.8 (m, 5 H, aromatic); ultraviolet, 258 nm (ϵ = 14 500); MS, 504 (M; 100%), 489 (M–CH_3; 10%), 391 (M–side chain; 9%), 382 (M–benzoic acid; 15%), 373 (11%), 367 (M–side chain–CH_3; 53%), 276 (M–CH_3–side chain; 5%), 269 (M–side chain–benzoic acid; 24%), 261 (7%), 251 (30%), and 213 (10%). The compound showed single component on TLC in 2 solvent systems with the same mobility as an authentic sample.

The product was further characterized by conversion to the free sterol. Compound II (0.50 g; 1.00 mmol) was heated under reflux for 18 h with a mixture of ethanol (175 ml), water (10 ml), and concentration H_2SO_4 (30 ml). The cooled mixture was poured into a saturated solution (1000 ml) of NaCl and, after standing for several hours in an ice-water bath, filtered to collect the precipitate which formed. The solid was recrystallized from acetone/methanol/water to give 5α-cholest-8(14)-en-3β-ol-15-one (0.34 g; 86% yield) melting at 147.5–149.0°C (literature: 147.5–149.0°C [2]); infrared, ν_{max} 3350, 1704, and 1620 cm^{-1}; PMR, 0.71 (s, 3 H, C–19–CH_3), 0.97 (s, 3 H, C–18–CH_3), 3.66 (m, 1 H, C–3–H), and 4.18 (m, 1 H, C–7β–H); ultraviolet, λ_{max} 258 nm (ϵ = 13 600); MS, 400 (M; 100%), 385 (M–CH_3; 16%), 393 (M–H_2O; 10%), 368 (M–CH_3–H_2O; 24%), 287 (M–side chain; 15%), 269 (M–H_2O–side chain; 45%), 261 (12%), 259 (10%), and 251 (13%). The compound showed a single component on TLC in 2 solvent systems and on GLC (3% OV-1 and 3% OV-17), with the same chromatographic behavior as that of an authentic sample.

III. Discussion

Treatment of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (I) with gaseous HCl in chloroform at –40°C gave, in 87% yield, 3β-benzoyloxy-7α,15β-dichloro-

5α-cholest-8(14)-ene (III; Fig. 1). The results of infrared, PMR, ^{13}C NMR, low- and high-resolution mass spectral analyses and the results of conventional elemental analysis were compatible with the assigned structure. Unequivocal establishment of structure and, in particular, the stereochemical orientation of the two chlorine atoms were based upon X-ray analysis of the corresponding 3β-*p*-bromobenzoate ester (ref. 18 and M.E. Newcomer et al., unpublished). The precise mechanism of the formation of III from I is not clear. One possible reaction course would include initial opening of the epoxide to give a Δ^7-14α-hydroxy-15β-chloro derivative which, upon attack by chloride ion at the C-7α-position, could initiate an S_N2' rearrangement leading to the formation of the 7α-15α-dichloro-$\Delta^{8(14)}$-system. Alternatively, the overall reaction could be envisioned as proceeding via an initial introduction of chlorine at carbon atom 7 in the α-configuration *n* followed by ring opening of the 14α,15α-epoxide function. If the reaction follows an S_N2' course analogous to that observed previously for the same α,β-unsaturated epoxide upon reaction with hydroxide ion to give 5α-cholestan-3β,7α,15α-triol (M. Tsuda et al., unpublished), the formation of the 7α-chloro-$\Delta^{8(14)}$-15α-hydroxy derivative would be anticipated. A stereospecific displacement of the 15α-hydroxyl function by chloride could thereupon yield the 7α,15β-dichloro-$\Delta^{8(14)}$-derivative. In the course of our studies to date we have been unable to isolate any intermediates in the overall conversion of I to III.

Reduction of III with lithium aluminum hydride in ether at 25°C for 20 min gave 7α,15β-dichloro-5α-cholest-8(14)-en-3β-ol (IV) in 86% yield (Fig. 1). The product was characterized by its melting point, optical rotation, and elemental analysis and by the results of infrared, PMR, ^{13}C NMR, and low- and high-resolution mass spectral analyses. When the reaction of III with lithium aluminum hydride was carried out for an extended period in refluxing ether, the major product isolated was 5α-cholesta-7,14-dien-3β-ol (VI; Fig. 1) which was obtained in pure form in 74% yield. The product was characterized by its melting point and chromatographic properties and by the results of infrared, ultraviolet, PMR, high- and low-resolution mass spectral analyses and by comparisons with authentic VI prepared by an independent approach. When III was reacted with lithium aluminum deuteride over an extended period of time in refluxing ether, VI was recovered in pure form in 35% yield and fully characterized. No incorporation of the isotopic hydrogen was observed. The overall conversion of III to VI under the conditions described above can be envisioned as a hydride-induced retrodiene rearrangement. Compatible with this postulation was the lack of the incorporation of deuterium into VI when III was reduced with lithium aluminum deuteride. A similar reaction course has been postulated in the lithium aluminum hydride induced retro-1,4-cyclization of the adduct of ergosteryl acetate and 4-phenyl-1,2,4-triazolin-3,5-dione (M.E. Newcomer et al., unpublished). The high recovery (74% yield) of pure VI upon reaction of III with lithium aluminum hydride in refluxing ether is noteworthy. The other alternative approach to the synthesis of pure $\Delta^{7,14}$-sterol dienes is via pyrolysis of the 7α-acetoxy or benzoyloxy derivatives of $\Delta^{8(14)}$-sterols [33, 34].

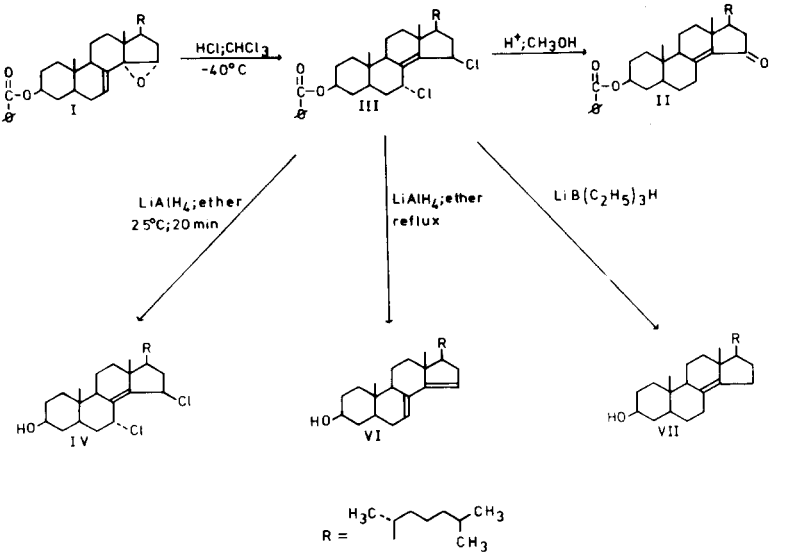

Fig. 4. Synthesis and reactions of [7β,15ξ-$^2H_2$]-3β-benzoyloxy-5α-cholest-8(14)-ene (IX).

Reduction of III with lithium triethylborohydride, a reagent noted for its nucleophilicity [35], in tetrahydrofuran at 25°C for 24 h gave, in 88% yield, VIII (Fig.1). The compound was characterized by its melting point and chromatographic properties and by the results of infrared, PMR, and high- and low-resolution mass spectral analyses and by comparisons with authentic VII prepared by an independent approach.

To acquire information relative to the mechanism of the reaction, III was reduced with lithium triethylborodeuteride (Fig. 4). The resulting impure $\Delta^{8(14)}$-sterol was converted to the benzoate ester by treatment with benzoyl chloride in pyridine and the pure benzoate (IX) was obtained in approx. 65% yield (after purification by chromatography and recrystallization) and characterized by melting point and the results of infrared, PMR, and high- and low-resolution mass spectral analyses. The latter studies indicated the presence of two deuterium atoms (87% d_2). The benzoate ester was converted to the free sterol (VIIa) by reduction with lithium aluminum hydride and characterized as such as described above. The results of mass spectral studies indicated the presence of two deuterium atoms (approx. 86% d_2). Chromic acid oxidation of IX [36] gave a complex mixture from which [7β-$^2H_1$]-3β-benzoyloxy-5α-cholest-8(14)-en-15-one (IIa) and [15ξ-$^2H_1$]-3β-benzoyloxy-8α-14α-epoxy-5α-cholestan-7-one (VIIIa) were isolated in pure form by repeated chromatography and recrystallization. The results of mass spectral analyses of the IIa indicated that approx. 67% of the molecules contained one atom of deuterium and that approx. 33% of the molecules contained no deuterium. Determination of

the location and stereochemical orientation of the deuterium atom in IIa was based upon the results of PMR studies. The PMR spectra of 5α-cholest-8(14)-en-3,15-dione and 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (II) contain a deshielded 7β-hydrogen at 4.22 ppm while the 7α-hydrogen is not deshielded by the 15-ketone function and is not distinguishable in the PMR spectra of these compounds [37,38]. Similar deshielding of the 7β-hydrogen by a ketone function at C-15 has also been observed in the case of 5α-androstan-15-one [39]. Compound II with no deuterium in the 7β-position should therefore show one proton of 4.22 ppm. Compound IIa showed a resonance peak at 4.22 ppm which, upon integration, corresponded to approx. 0.3 H. The latter finding indicates the presence of 0.7 atom of deuterium in the C-7β position of IIa, a finding which corresponds to the isotopic content of IIa (approx. 67% d_1) as indicated by the results of mass spectral analyses. These combined results indicate that IIa contained species composed of predominantly monodeuterated molecules (67% d_1) and that the deuterium atom was located in the 7β-position. The results further suggest that one of the deuterium atoms in IX and in VIIa was located at C-7 and had the β-configuration.

The results of mass spectral analyses of VIIIa indicated that approx. 68% of the molecules contained one atom of deuterium and that approx. 32% of the molecules contained no deuterium. Since one of the two deuterium atoms of IX was located at C-7 in the β-configuration and since one of the deuterium atoms of IX was lost upon conversion to the 15-keto derivative (VIIIa), the combined results indicate location of one atom of deuterium in IX at the C-15 position. The location of the absorption due to the C–D stretch mode in the infrared spectrum of a sterol with a pseudo-axially-oriented deuterium at C-15 should occur at a different frequency than that with a pseudoequatorially-substituted deuterium at the same position. However, since samples of authentic 3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7-one labeled stereospecifically with deuterium in the 15α- and 15β-positions were not available, the absorption at 2230 cm^{-1} in the infrared spectrum of VIIIa does not permit assignment of the configuration of the deuterium atom at C-15. Similarly, the results of PMR spectral studies did not permit assignment of the configuration of the deuterium at C-15. This situation arises from the fact that neither of the hydrogens at C-15 of VIII were significantly deshielded by the 7-ketone function. In view of this situation the configuration of the deuterium at C-15 in VIIIa could not be specified. Since IX and VIIa contained 86–87% dideuterated species, the finding that chromic acid oxidation of IX gave IIa containing approx. 67% monodeuterated species and VIIIa containing approx. 68% monodeuterated species suggests some loss of isotopic hydrogen upon conversion of IX to IIa and VIIa under the conditions employed.

In the course of these studies an authentic sample of unlabeled 3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7-one (VIII) was prepared by chromic acid oxidation of the corresponding 7α-hydroxysteryl benzoate (V). Apart from the results of the infrared and MS analyses noted above, VIIIa was identical in all properties with those of VIII.

Treatment of III with concentrated hydrochloric acid in a mixture of methanol and chloroform gave, in 79% yield, 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (II) which was fully characterized as such and, after acid hydrolysis, as the corresponding free sterol, 5α-cholest-8(14)-en-3β-ol-15-one. The latter compound has been found to be a potent inhibitor of sterol synthesis and to reduce the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in both L cells and primary cultures of fetal mouse liver cells [1,2]. In addition, the same compound and several of its derivatives have been shown to significantly lower serum cholesterol levels of rats upon oral or subcutaneous administration [6–9].

7α,15β-Dichloro-5α-cholest-8(14)-en-3β-ol (IV) was found to be a potent inhibitor of sterol synthesis in L cells in culture. When assayed, as described previously [2], III was found to cause a 50% inhibition of the synthesis of digitoninprecipitable sterols from labeled acetate at 2×10^{-6} M [18]. This inhibitor activity appeared to be specific for sterol synthesis and not due to an effect on the general metabolism of acetate since no significant inhibition of the metabolism of acetate to fatty acids was observed. The site of action of this new inhibitor of sterol synthesis appears to be at the level of mevalonate formation in much as the compound caused a 50% reduction of the level of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in the cells at a concentration of 6×10^{-7} M [18]. It is noteworthy that, of a very large number of sterols and sterol derivatives found to have significant inhibitory activity on sterol synthesis in the L cells, all have either been dioxygenated or trioxygenated sterols [1–5,40–42]. 3,7- and 3,15-Dioxygenated [1–5,43–45] and 3,7,15-trioxygenated [2] sterols have previously been shown to be potent inhibitors of sterol synthesis in these cells. The case of IV constitutes the first example of an inhibitor of sterol biosynthesis at the level of mevalonate formation of this type in these cells (without affecting fatty acid synthesis) in which no oxygen substitution is present other than that at carbon atom 3.

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