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Syntheses of Epimeric 2- and 4-Deuteriocholesterols[1]

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In order to clarify the stereochemistry of hydrogen loss from C-2 and C-4 during microbial transformation of cholesterol into androsta-1,4-diene-3,17-dione, the stereospecific syntheses of epimeric 2- and 4-deuteriocholesterols (XIII, XVI, XIX, XXI) have been undertaken. The key intermediates leading to the required substrates, Δ^2- and Δ^3-5α-cholesten-5-ols (II, VIII), were prepared from cholesterol in several steps. These olefins were converted to the 2α- and 4α-deuterio-3α,5α-diols (XI, XVII) by treatment with deuteriodiborane. On the other hand *trans*-diaxial opening of the α-epoxides (III, Vb) with lithium aluminum deuteride provided the 2β- and 4β-deuterio-3,5α-diols (XIV, XX). Upon dehydration with thionyl chloride and pyridine these labeled products were led to the desired compounds.

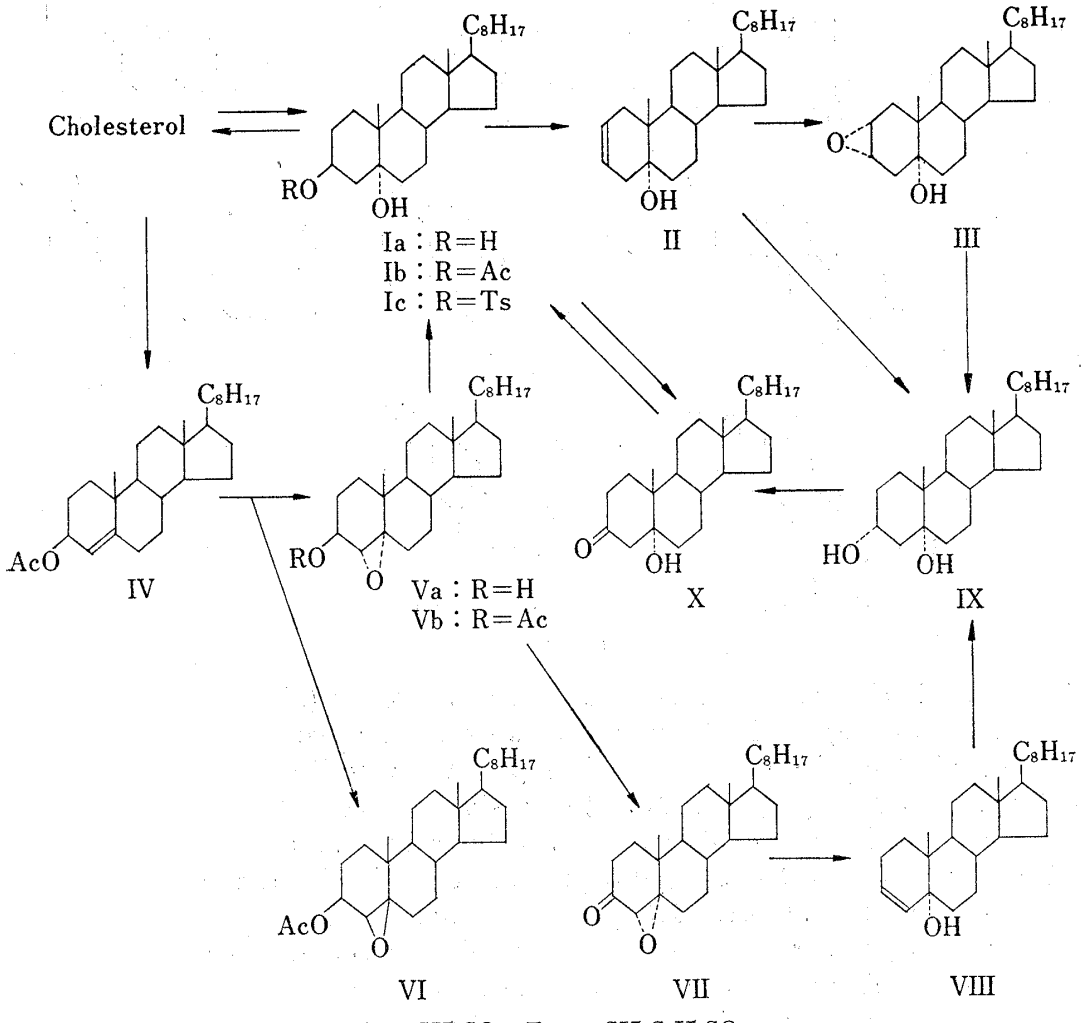
It has previously been shown that some microorganisms are capable of transforming cholesterol into androsta-1,4-diene-3,17-dione with elimination of the side chain.[3] This novel bioconversion to the C_{19} steroid is of particular interest from both biochemical and industrial points of view. Although the metabolic route has been disclosed to a certain extent, the mechanism of unsaturation in ring A still remains unclear. As a series of our studies on the biotransformation mechanism of steroids,[4] elucidation of the stereochemistry of hydrogen loss from C-2 and C-4 during enzymatic dehydrogenation has been undertaken. The design of the experiment required cholesterol labeled with the isotope stereospecifically at the C-2 and C-4 positions as a substrate for the microbial transformation. The present paper deals with the syntheses of two pairs of epimeric 2- and 4-deuteriocholesterols.

As a preliminary experiment toward the final goal we started to establish a synthetic route to cholesterol by which the label could be unambiguosly introduced at the desired position.[5] An initial study was directed to the preparation of Δ^2- and Δ^3-5α-cholesten-5-ols, which would serve as a key intermediate leading to the desired compounds. First, 5α-cholestane-3β,5-diol 3-tosylate (Ic), readily obtainable from cholesterol, was subjected to elimination of the oxygen function at C-3. Being adsorbed on activated alumina, Ic was converted to 5α-cholest-2-en-5-ol (II) in a satisfactory yield. Subsequent reaction with diborane did take place at both sides of the Δ^2-double bond to give epimeric 5α-cholestane-3,5-diols (Ia, IX), whose separation was attained by preparative thin-layer chromatography (TLC). Of these two the 3α,5α-diol (IX) was led to the epimeric 3β,5α-diol (Ia) by oxidation with chromium trioxide-pyridine complex followed by reduction with sodium borohydride. When treated with thionyl chloride and pyridine under the mild conditions, the 3-monoacetate

1) This paper constitutes Part LXXV of the series entitled "Analytical Chemical Studies on Steroids"; Part LXXIV: T. Nambara, M. Takahashi, Y. Tsuchida, and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **22**, 2176 (1974).
2) Location: *Aobayama, Sendai.*
3) M. Nagasawa, M. Bae, G. Tamura, and K. Arima, *Agr. Biol. Chem.*, **33**, 1644 (1969).
4) T. Anjyo, M. Ito, H. Hosoda, and T. Nambara, *Chem. Ind.* (London), **1972**, 384; T. Nambara, T. Anjyo, M. Ito, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 1938 (1973); S. Ikegawa and T. Nambara, *Chem. Ind.* (London), **1973**, 230; T. Nambara, S. Ikegawa, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 2794 (1973).
5) T. Nambara, H. Hosoda, T. Anjyo, M. Yamauchi, and J. Mohri, *Chem. Pharm. Bull.* (Tokyo), **20**, 287 (1972); T. Nambara, H. Hosoda, T. Anjyo, and S. Ikegawa, *ibid.*, **20**, 2256 (1972).

(Ib) underwent dehydration in both directions to provide cholesterol together with the isomeric cholest-4-en-3β-ol in a ratio of *ca.* 2 to 1.

Treatment of II with *m*-chloroperbenzoic acid afforded solely the 2α,3α-epoxide (III). It is of interest that the attack of the reagent would be initiated preferentially from the less-hindered α-side of the molecule irrespective of the presence of the hydroxyl function at 5α. The α-epoxide (III) was then subjected to the reductive cleavage with lithium aluminum hydride. As was expected the *trans*-diaxial opening of the oxido ring proceeded to give the 3α,5α-diol (IX) in a reasonable yield.



Ac=CH_3CO–, Ts=*p*-$CH_3C_6H_4SO_2$–

Chart 1

A preliminary study for the stereospecific labeling of the isotope at C-4 was then carried out. An initial effort was focused to the preparation of a key intermediate, that is 5α-cholest-3-en-5-ol (VIII). Cholest-4-en-3β-ol acetate (IV), derivable from cholesterol in three steps, was treated with per acid to yield the 4α,5α-epoxide (Vb) accompanied with the epimeric β-epoxide (VI). Of these two Vb was oxidized to the epoxyketone (VII) with chromium trioxide-pyridine complex. The Huang-Minlon reduction provided the desired Δ^3-unsaturated compound (VIII) which was separated with success by means of multiple TLC. The Δ^3-olefin (VIII) and 4α,5α-epoxy-3β-ol acetate (Vb) were easily transformed into cholesterol employing hydroboration and metal hydride reduction, respectively.

Hereupon these synthetic routes proved to be promising to introduce the deuterium label stereospecifically into the C-2 and C-4 positions. Hydroboration of the olefins (II, VIII) with deuteriodiborane, generated from lithium aluminum deuteride and boron trifluoride,

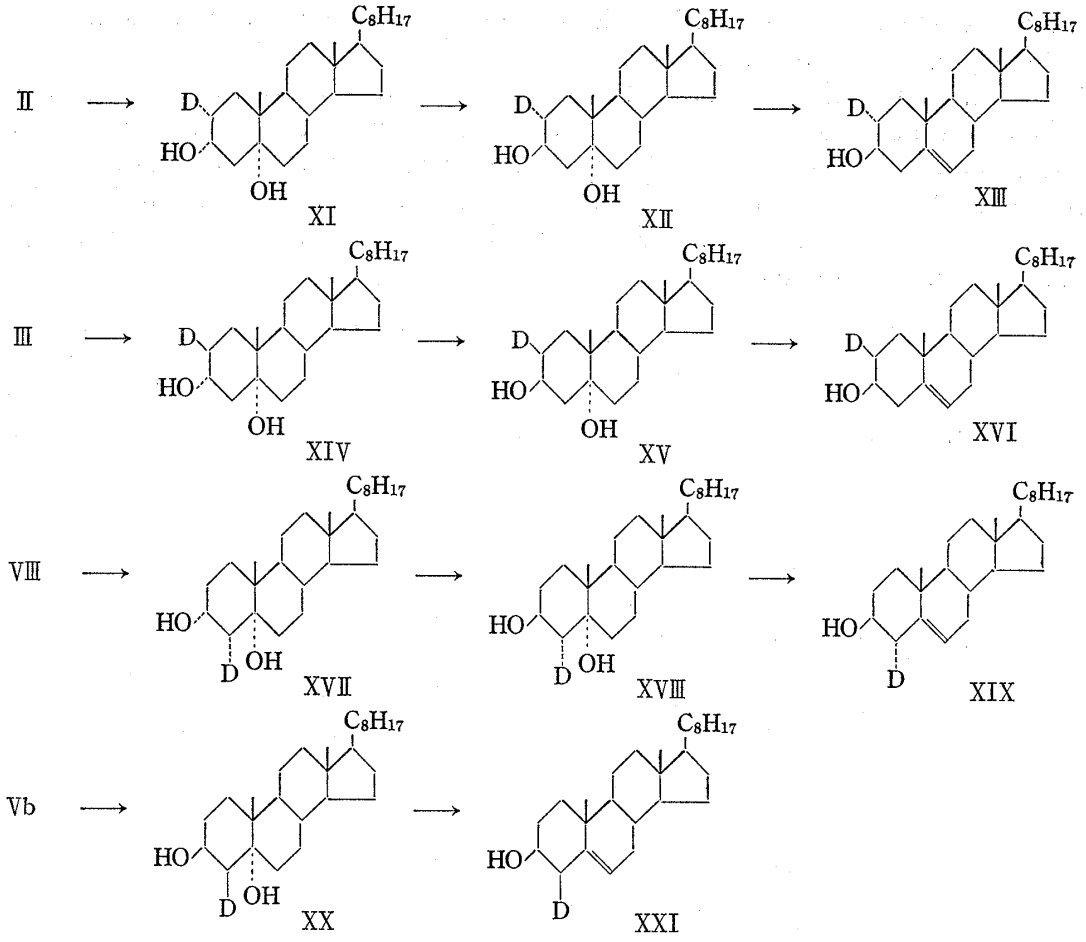


Chart 2

provided the *cis*-addition products, 2α- and 4α-deuterio-3α-ols (XI, XVII). On the other hand preparation of the 2β- and 4β-deuterated substrates was accomplished by utilizing the *trans*-diaxial opening reaction of the α-epoxides (III, Vb) with lithium aluminum deuteride. The deuterated 3α,5α-diols (XI, XIV, XVII) were similarly led to the 3β,5α-diols (XII, XV, XVIII) by chromium trioxide oxidation followed by sodium borohydride reduction. Subsequent dehydration of the 3β,5α-dihydroxyl compounds furnished the desired 2- and 4-deuterio-Δ^5-3β-ols (XIII, XVI, XIX, XXI) in a reasonable yield.

The infrared (IR) spectra of non-labeled and epimeric 2- and 4-deuterated cholesterols were obviously distinguishable each other in the finger print region. The locality and quantity deuterium in these labeled steroids were determined by means of mass spectral technique. Inspection of the molecular ion peak, which appeared at *m/e* 387 with an increment of one mass unit, revealed that the isotopic purity of these labeled compounds was 93 to 98%.

The results on the stereochemistry of enzymatic dehydrogenation by microorganisms will be the subject of a future communication.

Experimental[6]

5α-Cholest-2-en-5-ol (II)——5α-Cholestane-3β,5-diol 3-*p*-toluenesulfonate (Ic) (1.6 g) was adsorbed on activated Al_2O_3 (100 g) overnight. Elution with hexane–benzene (4: 1) and recrystallization of the eluate

6) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on a Hitachi Model R-20 spectrometer at 60 MHz employing tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=duoblet, and m=multiplet. IR spectra were run on a JASCO Model IRA-1 spectrometer. Mass spectra were measured by a Hitachi Model RMU-7 spectrometer. For preparative TLC silica gel G (E. Merck AG, Darmstadt) was used as an adsorbent.

from acetone gave II (600 mg) as colorless plates. mp 94.5—95.5°. *Anal.* Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.79; H, 11.94. NMR (5% solution in $CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 0.85, 0.92 (12H, s, 19-, 21-, 26-, and 27-CH_3), 5.59 (2H, m, 2- and 3-H). Clayton, *et al.* prepared this compound by the different method (reported mp 93—95°).[7]

2α,3α-Epoxy-5α-cholestan-5-ol (III)——To a solution of II (480 mg) in benzene (15 ml) was added *m*-chloroperbenzoic acid (380 mg) and stirred at room temperature for 4.5 hr. The resulting solution was diluted with benzene, washed with 5% $NaHSO_3$, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from MeOH to give III (320 mg) as colorless plates. mp 142—143°. *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.04; H, 11.34. NMR (5% solution in $CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 0.80, 0.91 (12H, s, 19-, 21-, 26-, and 27-CH_3), 3.38 (2H, m, 2β- and 3β-H). Clayton, *et al.* prepared this compound by the different method (reported mp 144—145°).[7]

Reduction of III with Lithium Aluminum Hydride——To a solution of III (491 mg) in anhydrous tetrahydrofuran (THF) (40 ml) was added $LiAlH_4$ (340 mg) and refluxed for 1.5 hr. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with AcOEt. The organic layer was separated, washed with H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from MeOH to give 5α-cholestane-3α,5-diol (IX) (306 mg) as colorless leaflets. mp 198—200°. *Anal.* Calcd. for $C_{27}H_{48}O_2$: C, 80.14; H, 11.96. Found: C, 79.87; H, 11.86. NMR (5% solution in $CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 0.80, 0.91 (12H, s, 19-, 21-, 26-, and 27-CH_3), 4.00 (1H, m, $W1/2=6$ Hz, 3β-H). Henbest, *et al.* prepared this compound by the different method (reported mp 195—198°).[8]

Hydroboration of II——B_2H_6 gas, freshly prepared from $LiAlH_4$ (600 mg) and BF_3-etherate (5 g), was passed through a solution of II (500 mg) in anhydrous THF (50 ml) under ice-cooling and stirred for 3.5 hr. The resulting solution was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily residue was dissolved in THF (5 ml). To this solution were added dropwise 10% NaOH (3 ml) and then 30% H_2O_2 (3 ml) under ice-cooling and stirred for 1 hr. The resulting solution was diluted with ice-water and extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC using benzene–AcOEt (3: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.49) and recrystallization of the eluate from MeOH gave IX (112 mg) as colorless leaflets. mp 199—200°. Elution of the adsorbent corresponding to the spot (*Rf* 0.18) and recrystallization of the eluate from MeOH gave 5α-cholestane-3β,5-diol (Ia) (97 mg) as colorless needles. mp 218—219°. Mixed melting point on admixture with the authentic sample showed no depression, respectively.

Epoxidation of Cholest-4-en-3β-ol Acetate (IV)——To a solution of IV (186 mg) in benzene (22 ml) was added *m*-chloroperbenzoic acid (125 mg) and allowed to stand at room temperature overnight. The resulting solution was diluted with AcOEt, washed with 5% $NaHSO_3$, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to column chromatography on silica gel. Elution with hexane–benzene (4: 1) and recrystallization of the eluate from MeOH gave 4α,5α-epoxycholestan-3β-ol acetate (Vb) (76 mg) as colorless plates. mp 120—121°. *Anal.* Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.46; H, 10.80. NMR (5% solution in $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.80, 0.91 (9H, s, 21-, 26-, and 27-CH_3), 1.11 (3H, s, 19-CH_3), 2.08 (3H, s, -OCOCH_3), 2.87 (1H, s, 4β-H), 4.90 (1H, m, 3α-H). Henbest, *et al.* prepared this compound by the different method (reported mp 117—119°).[9] Elution with hexane–benzene (2: 1) and recrystallization of the eluate from MeOH gave 4β,5β-epoxycholestan-3β-ol acetate (VI) (50 mg) as colorless needles. mp 89—90°. *Anal.* Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.02; H, 10.94. NMR (5% solution in $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.80, 0.91 (9H, s, 21-, 26-, and 27-CH_3), 1.04 (3H, s, 19-CH_3), 2.08 (3H, s, -OCOCH_3), 3.13 (1H, d, $J=3$ Hz, 4α-H), 5.07 (1H, m, 3α-H). Coxon, *et al.* prepared this compound by the different method (reported mp 97—98°).[10]

4α,5α-Epoxycholestan-3β-ol (Va)——To a solution of Vb (4.38 g) in dioxane (50 ml) was added 30% KOH (5 ml) and stirred at room temperature for 3 hr. The resulting solution was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from MeOH to give Va (2.8 g) as colorless plates. mp 123—125°. *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.58; H, 11.53. NMR (5% solution in $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.81, 0.91 (9H, s, 21-, 26- and 27-CH_3), 1.11 (3H, s, 19-CH_3), 2.88 (1H, broad s, 4β-H), 3.95 (1H, m, 3α-H). Collins prepared this compound by the different method (reported mp 136—137°).[11]

7) R.B. Clayton, H.B. Henbest, and M. Smith, *J. Chem. Soc.*, **1957**, 1982.
8) H.B. Henbest and J. McEntee, *J. Chem. Soc.*, **1961**, 4478.
9) H.B. Henbest and R.A.L. Willson, *J. Chem. Soc.*, **1957**, 1958.
10) J.M. Coxon, M.P. Hartshorn, and D.N. Kirk, *Tetrahedron*, **20**, 2547 (1964).
11) D.J. Collins, *J. Chem. Soc.*, **1959**, 3919.

4α,5α-Epoxycholestan-3-one (VII)——To a solution of Va (2.8 g) in pyridine (34 ml) was added CrO_3–pyridine complex (70 ml) and stirred at room temperature overnight. The reaction mixture was diluted with ether, washed with 10% AcOH, 10% Na_2CO_3, and H_2O, successively, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from MeOH to give VII (1.75 g) as colorless plates. mp 121—123°. *Anal.* Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.87; H, 10.96. NMR (5% solution in $CDCl_3$) δ: 0.69 (3H, s, 18-CH_3), 0.80, 0.90 (9H, s, 21-, 26- and 27-CH_3), 1.04 (3H, s, 19-CH_3), 3.00 (1H, s, 4β-H). Collins prepared this compound by the different method (reported mp 123—124.5°).[11]

5α-Cholest-3-en-5-ol (VIII)——VII (106 mg) was heated with $NH_2NH_2 \cdot H_2O$ (1 ml) at 90° for 5 min and then at 135° for 20 min. The resulting solution was diluted with ice-water and extracted with ether. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from aq. MeOH to give VIII (82 mg) as colorless plates, mp 75—76°. NMR (5% solution in $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.83, 0.94 (12H, s, 19-, 21-, 26- and 27-CH_3), 5.60 (2H, s, 3- and 4-H). Glotter, *et al.* prepared this compound by the different method (reported mp 75—76°).[12]

Hydroboration of VIII——VIII (106 mg) was treated with B_2H_6 gas in the manner as described in II. The crude product was submitted to preparative TLC using benzene–AcOEt (3: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.49) and recrystallization of the eluate from MeOH gave IX (10 mg) as colorless leaflets. mp 196—197°. Elution of the adsorbent corresponding to the spot (*Rf* 0.18) and recrystallization of the eluate from MeOH gave Ia (32 mg) as colorless needles. mp 217—219°. Mixed melting point on admixture with the authentic sample showed no depression, respectively.

5α-Hydroxycholestan-3-one (X)——To a solution of IX (300 mg) in pyridine (5 ml) was added CrO_3–pyridine complex (10 ml) and stirred at room temperature for 23 hr. The reaction mixture was diluted with ether, washed with 10% AcOH, 10% Na_2CO_3, and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from acetone to give X (179 mg) as colorless leaflets. mp 227—228°. *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.56; H, 11.51. NMR (5% solution in $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.80, 0.90 (9H, s, 21-, 26- and 27-CH_3), 1.16 (3H, s, 19-CH_3). Eastham, *et al.* prepared this compound by the different method (reported mp 222—223°).[13]

Reduction of X with $NaBH_4$——To a solution of X (10 mg) in MeOH (4 ml) was added $NaBH_4$ (40 mg) dissolved in H_2O (0.2 ml) under ice-cooling and stirred for 5 min. After addition of AcOH to decompose the excess reagent the resulting solution was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was submitted to preparative TLC using benzene–AcOEt (3: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.49) and recrystallization of the eluate from MeOH gave IX (2 mg) as colorless leaflets. mp 197—199.5°. Elution of the adsorbent corresponding to the spot (*Rf* 0.18) and recrystallization of the eluate from MeOH gave Ia (5 mg) as colorless needles. mp 218—219°. Mixed melting point on admixture with the authentic sample showed no depression, respectively.

5α-Cholestane-3β,5-diol 3-Acetate (Ib)——Treatment of Ia with Ac_2O and pyridine in the usual manner followed by recrystallization from MeOH gave Ib as colorless leaflets. mp 186—187°. *Anal.* Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found: C, 77.98; H, 11.29. NMR (5% solution in $CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 0.81, 0.92 (9H, s, 21-, 26- and 27-CH_3), 1.00 (3H, s, 19-CH_3), 2.02 (3H, s, -OCOCH_3), 5.05 (1H, m, 3α-H). Henbest, *et al.* prepared this compound by the different method (reported mp 184—186°).[14]

Dehydration of Ib——To a solution of Ib (87 mg) in pyridine (1.5 ml) was added dropwise $SOCl_2$ (0.2 ml) dissolved in $CHCl_3$ (1 ml) under ice-cooling and stirred for 5 min. The resulting solution was diluted with moist AcOEt, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was dissolved in dioxane (3 ml)–30% KOH (1 ml) and stirred overnight at room temperature. The resulting solution was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. Evaporation of solvent gave a crystalline product, which in turn was submitted to preparative TLC using benznee–AcOEt (5: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.40) and recrystallization of the eluate from MeOH gave cholesterol (33 mg) as colorless leaflets. mp 145—147°. Elution of the adsorbent corresponding to the spot (*Rf* 0.47) and recrystallization of the eluate from MeOH gave cholest-4-en-3β-ol (20 mg) as colorless needles. mp 125—127°. Mixed melting point on admixture with the authentic sample showed no depression, respectively.

2α-Deuterio-5α-cholestane-3β,5-diol (XII)——B_2D_6 gas, freshly prepared from $LiAlD_4$ (1.2 g) and BF_3-etherate (16 g), was passed through a stirred solution of II (1.46 g) in anhydrous THF (60 ml) for 4 hr. The resulting solution was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. To a solution of an oily residue dissolved in THF (60 ml) were added dropwise 10% NaOH (30 ml) and then 30% H_2O_2 (23 ml) under ice-cooling and stirred for 2 hr,

12) E. Glotter, S. Greenfield, and D. Lavie, *Tetrahedron Letters*, **1967**, 5261.
13) J.F. Eastham, G.B. Miles, and C.A. Krauth, *J. Am. Chem. Soc.*, **81**, 3114 (1959).
14) H.B. Henbest and B.J. Lovell, *J. Chem. Soc.*, **1957**, 1965.

The resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to column chromatography on silica gel. Elution with hexane–benzene (1: 3) gave 2α-deuterio-5α-cholestane-3α,5-diol (XI) (260 mg) as colorless amorphous substance. Eluate with benzene was submitted to preparative TLC using benzene–AcOEt (3: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.49) gave additional 77 mg of XI. To a solution of XI (337 mg) in pyridine (5 ml) was added CrO_3–pyridine complex (8.5 ml) and allowed to stand at room temperature overnight. The reaction mixture was diluted with ether, washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, successively, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave 2α-deuterio-5α-hydroxycholestan-3-one as colorless amorphous substance. To a solution of the 3-ketone in MeOH (20 ml) was added $NaBH_4$ (600 mg) dissolved in H_2O (1 ml) under ice-cooling and stirred for 2 hr. The resulting solution was acidified with 10% AcOH to decompose the excess reagent and then extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC using benzene–AcOEt (3: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.18) gave XII (80 mg) as colorless amorphous substance.

2α-Deuteriocholesterol (XIII)——Treatment of XII (80 mg) with Ac_2O and pyridine in the usual manner gave the 3-acetate (88 mg) as colorless amorphous substance. To a solution of the 3-acetate in pyridine (1 ml) was added dropwise $SOCl_2$ (0.2 ml) dissolved in $CHCl_3$ (1 ml) under ice-cooling and stirred for 5 min. The resulting solution was diluted with moist AcOEt, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was dissolved in 3% methanolic KOH and stirred overnight at 37°. The solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was submitted to preparative TLC using benzene–AcOEt (5: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.40) and recrystallization of the eluate from MeOH gave XIII (33 mg) as colorless leaflets. mp 148—149.5°. Mixed melting point on admixture with the authentic sample showed no depression. Mass Spectrum *m/e*: 387 (M^+) (93.0% d_1).

2β-Deuterio-5α-cholestane-3β,5-diol (XV)——To a solution of III (1 g) in anhydrous THF (30 ml) was added $LiAlD_4$ (800 mg) and refluxed for 40 min. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. Evaporation of solvent gave 2β-deuterio-5α-cholestane-3α,5-diol (XIV) (1.022 g) as colorless amorphous substance. XIV was dissolved in pyridine (14 ml) and treated with CrO_3–pyridine complex (20 ml) in the manner as described in XII to give 2β-deuterio-5α-hydroxycholestan-3-one (600 mg) as colorless amorphous substance. The 3-ketone was treated with $NaBH_4$ (2 g) in MeOH (40 ml) in the usual manner. The crude product obtained was purified by preparative TLC to give XV (380 mg) as colorless amorphous substance.

2β-Deuteriocholesterol (XVI)——Treatment of XV (380 mg) in the manner as described in XIII gave XVI (62 mg) as colorless leaflets. mp 147.5—148°. Mixed melting point on admixture with the authentic sample showed no depression. Mass Spectrum *m/e*: 387 (M^+) (97.5% d_1).

4α-Deuterio-5α-cholestane-3β,5-diol (XVIII)——VIII (2 g) was submitted to hydroboration with B_2D_6 gas freshly prepared from $LiAlD_4$ (2.5 g) and BF_3-etherate (10.5 g). The crude product obtained was treated in the manner as described in XII to give XVIII (20 mg) as colorless amorphous substance.

4α-Deuteriocholesterol (XIX)——Treatment of XVIII (20 mg) in the manner as described in XIII gave XIX (7 mg) as colorless leaflets. mp 148—149°. Mixed melting point on admixture with the authentic sample showed no depression. Mass Spectrum *m/e*: 387 (M^+) (96.5% d_1).

4β-Deuterio-5α-cholestane-3β,5-diol (XX)——Vb (500 mg) was treated with $LiAlD_4$ (750 mg) in the manner as described in XIV to give XX (435 mg) as colorless amorphous substance.

4β-Deuteriocholesterol (XXI)——Treatment of XX (435 mg) in the manner as described in XIII gave XXI (181 mg) as colorless leaflets. mp 147.5—148°. Mixed melting point on admixture with the authentic sample showed no depression. Mass Spectrum *m/e*: 387 (M^+) (98.4% d_1).

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