then dried over phosphorous pentoxide at 105° *in vacuo;* yield 3.4 g. (58%). This was chromatographically pure.

A portion of this product (0.5 g.) was dissolved in 70 ml. of water by adding sodium bicarbonate. The solution was treated with Norit on a steam-bath and filtered. The filtrate was reheated to 90° and acidified with acetic acid. The compound cystallized almost immediately. After standing overnight at room temperature the product was collected, washed with water, acetone and ether, then dried at 105° *in vacuo;* yield 0.35 g., R_f 0.57 (0.5% Na_2CO_3), R_f 0.45 (3% NH_4Cl); ultraviolet spectra in 0.1 *N* NaOH, λ_{max} 260 (ε 28,200), 280 (shoulder) (ε 17,800), 370 mμ (ε 7,500); 0.1 *N* HCl λ_{max} 241 (ε 21,400), 283 (ε 14,100), 336 mμ (ε 10,800).

Anal. Calcd. for $C_{20}H_{21}O_5N_8Br$: C, 45.0; H, 4.0; N, 21.0; Br, 15.0. Found: C, 44.5; H, 4.3; N, 20.6; Br, 15.0.

4-Amino-4-deoxy-3′-bromo-5′-chloro-N^{10}-methylpteroylglutamic Acid (3′-Bromo-5′-chloromethotrexate).—3′-Bromomethotrexate (12.7 g., 23.8 m*M*) was suspended and partially dissolved in 150 ml. of formamide. After cooling to 5° in an ice-bath, chlorine (12.7 g., 180 m*M*) was bubbled into the mixture during a period of 20 minutes causing complete solution of the starting material. After an additional 2 hours in the ice-bath, the solution was poured into 1200 ml. of water which was then adjusted to *p*H 3.0 to 3.5 with 15 g. of sodium acetate. A precipitate formed immediately. After cooling several hours, the product was collected, washed with water, acetone and ether and dried; yield 10.5 g. (78%). This product was dissolved in 350 ml. of water by adding 5.0 g. of sodium bicarbonate and then heated on the steam-bath to give a solution of *p*H 8. While hot, 3.7 g. of magnesium sulfate was added, the solution was treated with Norit, brought to boiling and filtered. The magnesium salt crystallized in lenticular crystals; yield 9.0 g.

This salt was converted to the free acid by dissolving in 600 ml. of hot water followed by acidification with 10 ml. of acetic acid. Upon reheating the resulting mixture the solid became crystalline; yield 6.8 g. (49%).

An analytical sample was prepared by recrystallizing a 100-mg. sample from 50 ml. of a 50% ethanol–water solution; clusters of needles, yield 60 mg., R_f 0.48 (0.5% Na_2CO_3), R_f 0.34 (3% NH_4Cl); ultraviolet spectra in 0.1 *N* NaOH, λ_{max} 257 (ε 26,700), 372 mμ (ε 7,900); 0.1 *N* HCl, λ_{max} 240 (ε 25,000), 332 mμ (ε 11,900).

Anal. Calcd. for $C_{20}H_{20}O_5N_8BrCl.H_2O$: C, 41.0; H, 3.9; N, 19.1; Cl, 6.1; Br, 13.7. Found C, 40.9; H, 3.8; N, 18.9; Cl, 6.40 Br, 13.8.

3′-Bromopteroylglutamic Acid (IX).—Pteroylglutamic acid (chem. assay 90%, 2.2 g., 4.5 m*M*) was dissolved in 20 ml. of concentrated hydrochloric acid which was then diluted with 20 ml. of water. This was cooled to 5° in an ice-bath and while stirring a solution of 0.72 g. (4.5 m*M*) of bromine in 12 ml. of acetic acid was added dropwise. A solid precipitated during the addition. After standing in the ice-bath for 2 hours the mixture was added to 250 ml. of water containing 15 g. of sodium chloride. This was warmed to give a clear solution. Upon cooling a crystalline product separated; yield 2.2 g. (85%).

This was dissolved in 25 ml. of concentrated hydrochloric acid, treated with Norit and filtered. A solution of 15 g. of sodium chloride in 250 ml. of hot water (70°) was added to the filtrate. The product crystallized as needles; yield 1.9 g. (74%), R_f 0.65 (0.5% Na_2CO_3); ultraviolet spectra in 0.1 *N* NaOH, λ_{max} 256 (ε 26,200), 282 (ε 25,000), 366 mμ (ε 8,900); 0.1 *N* HCl, λ_{max} 296 mμ (ε 21,600).

Anal. Calcd. for $C_{19}H_{18}N_7O_6Br$: C, 43.9; H, 3.5; N, 18.8; Br, 15.4. Found: C, 43.5; H, 3.8; N, 18.7; Br, 15.4.

PEARL RIVER, N. Y.

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA AT LOS ANGELES AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF MICHIGAN]

Allylic Rearrangements. XLV.[1] The Reaction of Thionyl Chloride with 4β-Hydroxycholesteryl Benzoate

BY ROBERT E. IRELAND,[2] T. I. WRIGLEY AND W. G. YOUNG

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The product of the reaction of thionyl chloride with 4β-hydroxycholesteryl benzoate (I) has been shown to be 6β-chloro-3β-benzoyloxy-4-cholestene (II). Lithium aluminum hydride reduction of the chlorobenzoate (II) produces only cholestero- (IV). The stereospecificity of this reduction has been shown to be due to an intramolecular S$_N$2′ reaction sequence by using lithium aluminum deuteride reduction to introduce a 4β-deuterium substituent.

As an extension of our work on steroid allylic alcohols,[3] we undertook a study of the reaction of thionyl chloride with 4β-hydroxycholesteryl benzoate (I). Petrov, Rosenheim and Starling[4] first carried out such a reaction in pyridine–ether solution, and presented evidence which they construed as favoring the formation of a 4-chlorocholesteryl benzoate of unspecified stereochemistry. Spring and Swain[5] obtained the same chloro steroid by the thionyl chloride–pyridine dehydration of 6β-chloro-5-hydroxy-3β-cholestanyl benzoate (III). While the latter authors assigned the 6β-chloro-3β-benzoyloxy-4-cholestene structure (II) to their material, Petrov and co-workers preferred the 4-chlorocholesteryl benzoate structure formed by allylic rearrangement during dehydration.

It is apparent that allylic rearrangement has taken place during one of the above reactions, and in light of our recent work,[3] it seemed more reasonable to consider such a rearrangement to have occurred during the OH → Cl conversion rather than during dehydration. In particular, we found that 5-cholesten-4β-ol could be converted with rearrangement to 6β-chloro-4-cholestene by thionyl chloride in ether solution with or without the presence of amines. The same 6β-chloro-4-cholestene was formed in excellent yield without rearrangement on low temperature thionyl chloride–pyridine dehydration of 6β-chloro-5-cholestanol.

In order to investigate the position of the chlorine in the cholesteryl benzoate series, we repeated Petrov's preparation and obtained substantially the same results. We also found that the addition of excess chloride ion in the form of tri-*n*-butyl-

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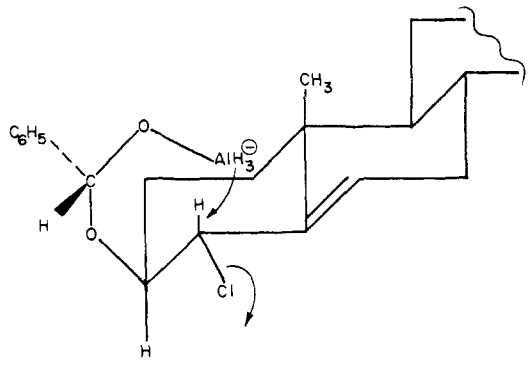
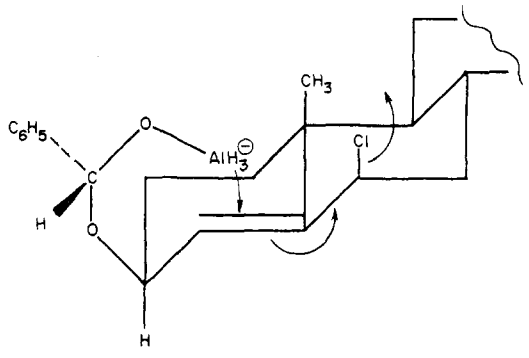
(3) R. E. Ireland, T. I. Wrigley and W. G. Young, THIS JOURNAL, **80**, 4604 (1958).

(4) V. A. Petrov, O. Rosenheim and W. W. Starling, *J. Chem. Soc.*, 135 (1943).

(5) F. S. Spring and G. Swain, *ibid.*, 1356 (1939).



XII



XIII

amine hydrochloride did not alter the product, thus making an SN2 displacement highly unlikely.[6] That no isomeric chloro compound was formed was indicated by the fact that the first-order ethanolysis rate constant of the crude chloride (K_1 = 1.53 ± 0.06 × 10^{-4} sec.$^{-1}$) was substantially the same as that of the pure compound (K_1 = 1.61 ± 0.03 × 10^{-4} sec.$^{-1}$) and neither showed any tendency to drift over the range 20–90% reaction.

Surprisingly, we found that lithium aluminum hydride reduction of the chlorobenzoate II afforded an 87% yield of pure cholesterol (IV, R = H). A similar reduction of 4β-chloro-5-cholestene led to a mixture of 4- and 5- cholestene, presumably *via* reduction of the C_{4-6} allyl cation initially formed by ionization of the chloride. A similar ionization hydride reduction process has been advanced to explain the formation of both 5-cholestene and 3,5-cyclocholestane on reduction of cholesteryl tosylate.[7] On this basis we might have expected the chlorobenzoate II to be converted to a mixture of cholesterol and 4-cholesten-3β-ol rather than pure cholesterol. While the possibility still exists that the hydride reduction is more cleanly SN2 in character in the chlorobenzoate II case, the absence of a mixture of products can better be explained by considering the reduction to take place first at the ester carbonyl. The initial reduction product then is particularly well suited for transferring a hydride ion to the C_4-position by a cyclic intramolecular process. For such a sequence to be operative the chlorobenzoate would have to have the chlorine atom in either the 4α (SN2 displacement by hydride) (XII) or the 6β (SN2′ displacement by hydride[8]) (XIII) position. The 4α-chlorocholesteryl benzoate representation is highly unlikely for two reasons: (a) the addition of tri-*n*-butylamine hydrochloride to the thionyl chloride reaction mixture did not alter the structure of the chlorobenzoate formed from 4β-hydroxylcholesteryl benzoate (I), and (b) even if allylic rearrangement had taken place during the thionyl chloride–pyridine dehydration of 6β-chloro-5-hydroxy-3β-cholestanyl benzoate (III), there is ample evidence[9] to support the view that the chlorine would remain on the β-side of the steroid molecule. The 6α-oriented chloro derivative may be excluded from consideration on this basis as well as on other obvious grounds.

In order to ascertain whether cholesterol (IV, R = H) was formed by an intramolecular SN2′ displacement mechanism from 6β-chloro-3β-benzoyloxy-4-cholestene (XIII) or by a specific SN2 displacement of 4β-chlorocholesteryl benzoate the reaction was carried out with lithium aluminum deuteride. In the former case the deuterium would be introduced in the 4β-position, whereas the latter sequence would introduce a 4α-deuterium atom.

The 4-deuteriocholesterol (IV, R = D) obtained was converted in high yield to 4-deuterio-5-cholestanol (VI) through 4-deuterio-5-cholestene (V) by well known methods[10], and for the purposes of comparison both the 4β-(VI) and 4α-deuterio (XI) isomers were prepared. The authentic 4β-deuterio-5-cholestanol (VI) was readily available by lithium aluminum deuteride reduction of 4α,5-oxidocholestane (VII), while the 4α-deuterio isomer XI was obtained by a hydride reduction of 4β-deuterio-4α,5-oxidocholestane (X). The 4β-deuterio-oxide X was prepared from the ketol VIII *via* lithium aluminum deuteride reduction to the 4α-deuterio-diol IX, followed by mesylation and treatment with base.

Infrared spectral analysis of the pure 4β- and 4α-deuterio-5-cholestanols showed significant differences in the carbon–deuterium stretching region. The axial 4β-deuterio compound VI had a single strong absorption peak at 2175 cm.$^{-1}$, while the equatorial 4α-deuterio isomer XI had a doublet at 2156 cm.$^{-1}$ (strong) and 2178 cm.$^{-1}$ (weak) (Fig. 1). These results are similar to those of Corey[7b] for the 3α- and 3β-deuteriocholestanes, which also showed small but significant differences in the C–D stretching region.

The infrared spectrum of the 4-deuterio alcohol obtained from the chlorobenzoate II showed a C–D stretching frequency of 2175 cm.$^{-1}$, indicat-

(6) For a discussion of the factors affecting the thionyl chloride reaction with allylic alcohols, see F. F. Caserio, G. E. Dennis, R. H. DeWolfe and W. G. Young, THIS JOURNAL, **77**, 4182 (1955).

(7) (a) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949); (b) E. J. Corey, M. G. Howell, A. Boston, R. L. Young and R. A. Sneen, THIS JOURNAL, **78**, 5036 (1956).

(8) The requisite *cis* relationship between entering and leaving groups in an SN2′ reaction is fulfilled by the 6β-chloro derivative; see G. Stork and W. N. White, *ibid.*, **78**, 4609 (1956).

(9) (a) H. L. Goering, T. D. Nevitt and E. F. Silversmith, *ibid.*, **77**, 4042, 5026 (1955); (b) E. J. Corey and R. A. Sneen, *ibid.*, **78**, 6269 (1956).

(10) (a) J. Mauthner and W. Suida, *Monatsh.*, **15**, 85 (1894); (b) A. J. Fudge, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 958 (1954).

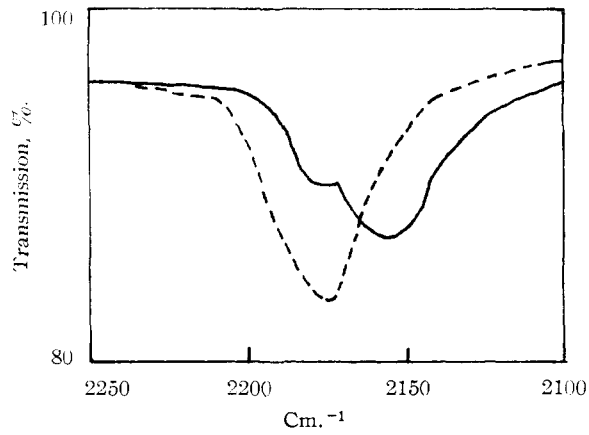
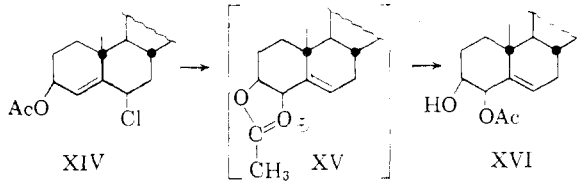


Fig. 1.—Infrared absorption in the C–D stretching region: ·····, 4β-deuterio-5-cholestanol; ———, 4α-deuterio-5-cholestanol.

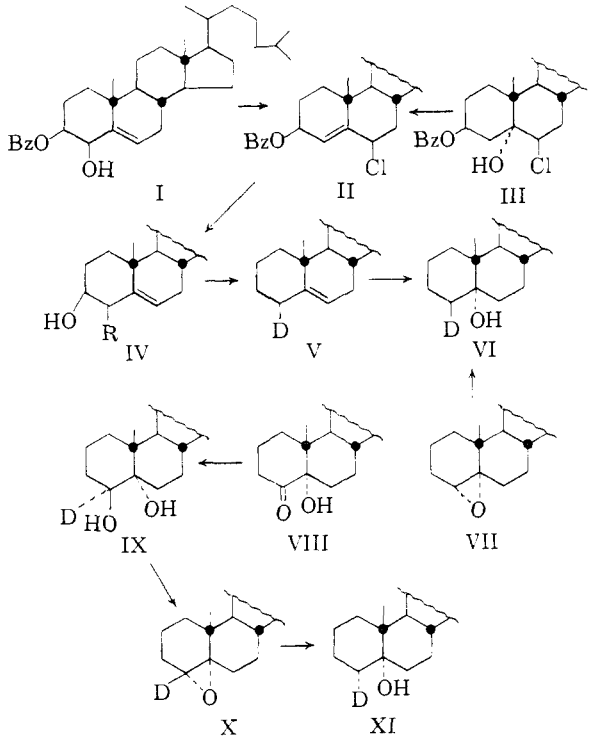
ing beyond reasonable doubt that the 4-deuterium atom is β-oriented. Thus we have been able to demonstrate by these data that the chlorobenzoate II is in reality the 6β-chloro-3β-benzoyloxy-4-cholestene and not a 4-chloro compound as Petrov believed.[11] The formation of this chloride from 4β-hydroxycholesteryl benzoate (I) with thionyl chloride must occur *via* in SN1′ process and corroborates our earlier observations[3] that in the thionyl chloride substitution of a quasiaxial allylic alcohol, the SN1′ reaction sequence takes precedence over all other possibilities. regardless of the reaction medium.

It is interesting to note the striking neighboring group effect of the 3β-benzoyloxy function in promoting the SN2′ displacement of the chlorine in the chlorobenzoate II. This also provides further support for the required *cis* relationship between the entering and leaving groups in an SN2′ reaction sequence.[8] It can be seen in the representation (XIII) for the cyclic process leading to the displacement of the chlorine that the required axial attack of the C_{4-5} double bond is readily attained.

It is intriguing to reconsider the data of Petrov[4] on the acetolysis of the corresponding chloroacetate XIV (now regarded to have the 6β-chloro structure as shown[11]) in light of the above mechanistic control exerted by a 3β-acyloxy grouping. An attractive hypothesis to explain the formation



of 4β-acetoxy-cholesterol (XVI) is that the reaction sequence involves the formation of an intermediate species[12] such as XV. Such an intermediate could easily be formed here by direct intramolecular SN2′ displacement of the chlorine by the 3β-acetyloxy or by ionization of the allylic chloride, followed by participation of this group. Hydrolysis of this intermediate would be expected to lead to the 4β-acetoxycholesterol (XVI), since it has been shown by Paige[13] that 4β-hydroxycholesteryl acetate readily undergoes acetyl migration to 4β-acetoxycholesterol (XVI) under the same reaction conditions and probably *via* a similar intermediate. However, on the basis of the meager yield of the 4β-acetate XVI isolated by Petrov, the direct substitution[11] of the C_{4-6} allyl cation cannot be ruled out as a possibility.



Experimental

Melting points were recorded on a Kofler block and are corrected. Rotations were determined in chloroform solutions. The petroleum ether referred to is the fraction with b.p. 67–68°. Infrared measurements were made on a Perkin–Elmer model 21 double beam spectrophotometer.

4β-Hydroxycholesteryl benzoate (I), m.p. 208–209° with softening at 206°, was prepared in 37% over-all yield from cholesterol by the method of Rosenheim and Starling.[14]

6β-Chloro-3β-benzoyloxy-4-cholestene (II).—A solution of 9.0 g. (0.04 mole) of tri-*n*-butylamine hydrochloride in 1000 ml. of anhydrous ether was prepared by treating the ethereal suspension of the amine hydrochloride with enough anhydrous hydrogen chloride to effect solution. To this solution was added 5.07 g. (0.01 mole) of 4β-hydroxycholesteryl benzoate (I), followed by 7.27 ml. (0.10 mole, 11.9 g.) of purified[15] thionyl chloride, and the system was flushed with nitrogen and sealed. After standing at room temperature for 21 hours, the ethereal solution was washed successively with water, dilute aqueous hydrochloric acid, dilute aqueous potassium bicarbonate, water and saturated salt solution. After drying, the solvent was removed, and the residue crystallized from petroleum ether, affording 4.38 g. (84%) of chlorobenzoate II, m.p. 122–128°. On recrystallization of a small sample from petroleum ether, the

(11) E. J. Corey and R. A. Sneen[9b] discuss the acetolysis of the corresponding chloroacetate ascribing to it the "6β-chloro-3β-acetoxy-Δ⁴-cholestene" structure. However, the only evidence provided by these authors for this structure is a reference to the work by Petrov, Rosenheim and Starling[4] where the 4-chlorocholesteryl acetate structure is advanced.

(12) S. Winstein and R. E. Buckles, THIS JOURNAL, **65**, 613 (1943).

(13) M. F. C. Paige, *J. Chem. Soc.*, 437 (1943).

(14) O. Rosenheim and W. W. Starling, *ibid.*, 377 (1937).

(15) D. L. Cottle, THIS JOURNAL, **68**, 1380 (1946).

melting point was raised to 126–128°, $[\alpha]^{25}D$ −82.6° (*c* 2.5). The melting point of a mixture of this material and that by the method of Petrov, Rosenheim and Starling,[4] m.p. 126–128°, was 125–128°.

The ethanolysis rate of the chlorobenzoate II, m.p. 126–128°, was measured in absolute ethanol at 60° by titrating the evolved hydrochloric acid with 0.00493 *N* sodium methoxide in methanol to the bromophenol blue end-point. The average value for the first-order rate constant over three kinetic runs was found to be $1.61 \pm 0.03 \times 10^{-4}$ sec.$^{-1}$. Under the same conditions the crude chlorobenzoate showed an average rate constant of $1.53 \pm 0.06 \times 10^{-4}$ sec.$^{-1}$ over two kinetic runs. All solvolyses were followed from 20–90% reaction.

4β-Deuteriocholesterol (IV, R = D).—A solution of 0.856 g. (0.0016 mole) of the chlorobenzoate II and 0.100 g. (0.0024 mole) of lithium aluminum deuteride in 100 ml. of dry ether was stirred and refluxed for 2 hours. After the addition of 0.2 ml. of H_2O and 0.16 ml. of 10% aqueous sodium hydroxide, the mixture was stirred at room temperature for 2 hours and the precipitated salts removed by filtration. On evaporation of the ether, there was obtained 0.610 g. (98%) of 4β-deuteriocholesterol, m.p. 144–148°. Two recrystallizations from methanol afforded 0.546 g. (87%), m.p. 147–148°. The infrared spectrum in carbon tetrachloride solution showed a carbon–deuterium stretching band at 2140 cm.$^{-1}$.

4β-Deuterio-5-cholestene (V).—Treatment of 0.500 g. (0.0013 mole) of 4β-deuteriocholesterol (IV, R = D) with thionyl chloride, followed by chromatography of the crude product on 20 g. of alumina afford 0.500 g. (96%) of **4β-deuteriocholesteryl chloride,**[10a] m.p. 95–96°. The chloride, reduced according to the method of Mauthner and Suida,[9a] afforded 0.448 g. (98%) of 4β-deuterio-5-cholestene (V), m.p. 91.5–93°.

Catalytic hydrogenation of a sample of this olefin in cyclohexane–acetic acid solution over 10% palladium-on-carbon resulted in a quantitative yield of **4β-deuteriocholestane,** m.p. 82–83°. The infrared spectrum taken in carbon tetrachloride solution showed a single strong carbon–deuterium stretching bond at 2151 cm.$^{-1}$.

4β-Deuterio-5-cholestanol (VI). (a) **From 4β-Deuterio-5-cholestene (V).**—A solution of 0.304 g. (0.0008 mole) of the olefin in 10 ml. of benzene was treated at room temperature with 2 ml. of a benzene solution of perbenzoic acid (0.076 g./ml.). After 1.5 hours the reaction was worked up in the usual fashion, and the crude product chromatographed on 30 g. of alumina. Two factions were obtained: (a) 4β-deuterio-5-cholestene (V) (0.077 g., m.p. 90–93°) with 100% petroleum ether and (b) **4β-deuterio-5,6x-oxidocholestane** (10.214 g., m.p. 69–72°) with 5% ether–petroleum ether (reported[3] m.p. 74–75°).

The epoxide was reduced with lithium aluminum hydride in ether, and the resulting oil chromatographed on 10 g. of alumina. Elution with 10% ether–petroleum ether afforded 0.140 g., m.p. 104–106°, of 4β-deuterio-5-cholestanol. After recrystallization from methanol the melting point was raised to 106–107° (0.108 g., $[\alpha]^{26}D$ +11° (*c* 2.1), 34% over-all yield; reported[10b] m.p. 107–109°, $[\alpha]D$ +12.5° (*c* 2.1)). The infrared spectrum taken in carbon tetrachloride solution had a carbon–deuterium stretching band at 2175 cm.$^{-1}$.

(b) **From 4α,5-Oxidocholestane (VII).**—A solution of 0.300 g. (0.00078 mole) of 4α,5-oxidocholestane[3] in 20 ml. of dry ether was reduced with 0.200 g. (0.0048 mole) of lithium aluminum deuteride. After the usual work-up, the crude alcohol was crystallized from methanol, affording 0.260 g. (84%), m.p. 106–107°, $[\alpha]^{27}D$ +10.6° (*c* 1.9). The mixture melting point of this material with 4α,5-oxidocholestane was depressed to 93–100°, and the infrared spectrum taken in carbon tetrachloride solution had a carbon–deuterium stretching band at 2175 cm.$^{-1}$.

4α-Deuterio-4β,5-cholestanediol (IX).—A solution of 0.900 g. (0.0022 mole) of 5-hydroxy-4-cholestaneone (VIII)[16] in 40 ml. of dry ether was reduced with 0.420 g. (0.01 mole) of lithium aluminum deuteride. After the usual work-up and crystallization of the residue from acetone–water, there was obtained 0.850 g. (94%) of the diol IX, m.p. 176.5–177.5°, $[\alpha]^{25}D$ +25° (*c* 1.9) (reported[16] m.p. 171–172°, $[\alpha]D$ +27°).

4β-Deuterio-4α,5-oxidocholestane (X).—A solution of 0.77 g. (0.002 mole) of 4α-deuterio-4β,5-cholestanediol (IX) and 0.16 ml. (0.00021 mole) of methanesulfonyl chloride in 10 ml. of dry pyridine was allowed to stand 3 days at 0°. After the customary work-up the crude solid obtained was added to a solution of 0.16 g. (0.004 mole) of potassium in 30 ml. of dry *t*-butyl alcohol, and the mixture stirred and refluxed for 1 hour. Most of the *t*-butyl alcohol was removed at reduced pressure, and the residue treated with water and ether. The ethereal solution was separated, washed with water and dried (Na_2SO_4). The residue obtained after removal of the ether was chromatographed on 80 g. of alumina, affording 0.483 g. (67%) of the oxide X, m.p. 106–107.5° (reported[3] m.p. 101–103°), eluted with 5% ether–petroleum ether.

4α-Deuterio-5-cholestanol (XI).—A solution of 0.483 g. (0.0013 mole) of 4β-deuterio-4α,5-oxidocholestane (X) in 25 ml. of dry ether was reduced with 0.250 g. (0.0066 mole) of lithium aluminum hydride. After the usual work-up and crystallization from ether, there was obtained 0.425 g. (85%) of 4α-deuterio-5-cholestanol, m.p. 106–107°, $[\alpha]^{27}D$ +11° (*c* 2.0). The infrared spectrum taken in carbon tetrachloride solution showed a doublet in the carbon–deuterium stretching region at 2156 cm.$^{-1}$ (strong) and 2178 cm.$^{-1}$ (weak).

Lithium Aluminum Hydride Reduction of 4β-Chloro-5-cholestene.—A solution of 0.300 g. (0.0007 mole) 4β-chloro-5-cholestene in 25 ml. of dry ether was reduced with 0.200 g. (0.0052 mole) of lithium aluminum hydride. After the usual work-up, evaporation of the ether left a clear oil that could not be induced to crystallize. This material gave a negative Beilstein test and had a specific rotation (25°) of +8.6° (*c* 8.1). The rotation corresponds to 53% 4-cholestene (+65°) and 47% 5-cholestene (−56°).

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(16) D. N. Jones, J. R. Lewis, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 2876 (1955).