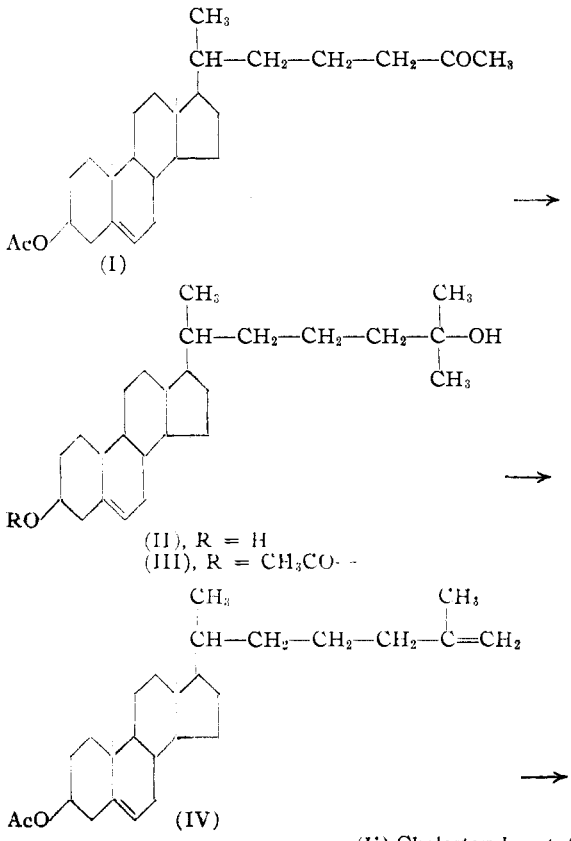
[CONTRIBUTION FROM THE CHEMICAL DEVELOPMENT DIVISION OF SCHERING CORPORATION]

The Preparation of Cholesterol from 25-Ketonorcholesterol

BY AUGUST I. RYER, WILLIAM H. GEBERT AND NATHANIEL M. MURRILL

The ketonic fraction from the oxidation of cholesteryl acetate dibromide contains 3β-acetoxy-5-cholestene-25-one (I) first described by Ruzicka and Fischer.[1] As a corollary to another project, we have converted this ketone to cholesterol in an over-all yield of better than 50%. This transformation is of interest since it offers the possibility of securing C_{26} labelled cholesterol. Cholesterol has also been prepared by the reduction of 24-ketocholesterol obtained from 3β-hydroxy-5-cholenic acid.[2]

The reaction of 3β-acetoxy-5-cholestene-25-one (I) with methylmagnesium iodide gave 25-hydroxycholesterol (II). The corresponding acetate (III) was dehydrated with phosphorus oxychloride in pyridine. Reduction of (IV) with palladium gave cholesteryl acetate (V). Better yields were obtained when the reduction product was saponified without isolation of the acetate (V) to give cholesterol (VI). This was probably due to ester exchange in the solvent ethyl alcohol resulting in a mixture of cholesteryl acetate and cholesterol.



(V) Cholesteryl acetate
(VI) Cholesterol

The side chain ethylenic bond in (IV) was shown to be terminal by the method of Bricker and Roberts.[3] The distillate from the Bricker reaction also gave a negative test for acetone with sodium nitroprusside.[4] The infrared spectrum of (IV) showed an absorption band at 11.30 microns. Barnes[5] and his co-workers place the band for terminal unsaturation of the type $R_2C{=}CH_2$ in the range 11.2–11.4 microns.

Experimental[6]

25-Hydroxycholesterol (5-Cholestene-3β,25-diol (II)).—A solution of 85.8 g. of 25-ketonorcholesteryl acetate (I) in 500 cc. of anhydrous thiophene-free benzene was added to a Grignard solution prepared from 24.3 g. of magnesium, 149 g. of freshly distilled methyl iodide, and 575 cc. of anhydrous ethyl ether. The mixture was refluxed for three hours and allowed to stand overnight. After cooling to 5°, the complex was decomposed by the slow addition of 200 cc. of ice water and 400 cc. of 50% acetic acid solution, and steam distilled until no more oil passed over. The product was filtered at 25°, washed with water until neutral and dried at 80°, yielding 82.5 g., m. p. 178.4–180.5°. The product was crystallized from methanol to give 70.0 g. of fine needles as the first crop, m. p. 179.6–181.2°. The analytical sample melted at 181.5–182.5°; $[\alpha]^{19}D$ −39.3° (2% in $CHCl_3$).

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.54; H, 11.20.

25-Hydroxycholesteryl Acetate.—The hydroxycholesterol (II) (26.5 g.) was acetylated by the usual procedure using acetic anhydride and absolute pyridine. The crude product (29.0 g.) melting at 136.0–139.0° was recrystallized from acetone to give 25.5 g. of fine needles, m. p. 140.2–141.2°. The analytical sample melted at 142.0–142.8°; $[\alpha]^{20}D$ −40.4° (2% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.15; H, 10.66.

25-Dehydrocholesteryl Acetate (IV).—The procedure used for the dehydration of (III) was a modification of that described by Koechlin and Reichstein.[7] The hydroxyacetate (III) (25.5 g.) was refluxed for one-half hour with 750 cc. of dry pyridine and 25.5 cc. of freshly distilled phosphorus oxychloride. The mixture was cooled to 20° and poured into ice water with agitation. The crystals were filtered, washed neutral with water and dried, yielding 24.0 g. The crude product was dissolved in hot benzene, treated with 20 g. of activated carbon and 20 g. of Adsorptive Magnesia Powder, No. 2642,[8] and filtered through a mat of diatomaceous earth. The filtrate was concentrated to a small volume and the benzene completely replaced with methanol by distillation. The methanol solution was concentrated to a thin slurry of crystals,

(1) Ruzicka and Fischer, *Helv. Chim. Acta*, **20**, 1291 (1937).
(2) Riegel and Kaye, THIS JOURNAL, **66**, 723 (1944).
(3) Bricker and Roberts, *Anal. Chem.*, **21**, 1331 (1949).
(4) F. Feigl, "Quantitative Analysis by Spot Tests," Nordemann Pub. Co., Inc., 1939, New York, N. Y., p. 288.
(5) Barnes, Gore, Stafford and Williams, *Anal. Ed.*, **20**, 402 (1948).
(6) All melting points are corrected. Carbon and hydrogen analyses by Edwin Conner and infrared measurements by William B. Tarpley, both from these laboratories.
(7) Koechlin and Reichstein, *Helv. Chim. Acta*, **27**, 549 (1944).
(8) Obtained from Westvaco Chlorine Products Corp., Newark, Calif.

cooled to 5° and filtered to give 21.5 g. of plates, m. p. 91.8–93.0°. The analytical sample melted at 93.5–94.0°; $[\alpha]^{20}D$ −43.6° (2% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.39; H, 11.08.

25-Dehydrocholesterol.—The diene acetate (IV) (21.5 g.) was hydrolyzed by the usual procedure using methanolic potassium hydroxide to give 20.0 g., m. p. 120.8–123.0°. The crude product was dissolved in hot acetone, concentrated to 100 cc. and 100 cc. of methanol was added. The solution was again concentrated to 100 cc., cooled to 5° and filtered to give 16.0 g. of very fine needles, m. p. 121.2–122.2°; $[\alpha]^{22.5}D$ −43.0° (2% in $CHCl_3$).

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.0; H, 11.30.

Cholesteryl Acetate.[9]—A solution of 7.0 g. (0.0164 mole) of the diene acetate (IV) dissolved in 500 cc. of absolute alcohol was hydrogenated at atmospheric pressure and room temperature using 3.5 g. of 5% palladium on charcoal. The reduction rate fell off sharply after 0.017 mole of hydrogen had been absorbed and was interrupted after 460 cc. of hydrogen had been consumed (20 minutes). The catalyst was removed by filtration and the volume reduced to approximately 60 cc. The solution was poured into water, the product filtered and dried to yield 6.5 g., m. p. 91.6–93.5°. Two crystallizations from methanol gave 2.6 g., m. p. 110.9–111.2°; $[\alpha]^{22}D$ −41.5° (2% in $CHCl_3$). A mixture with an authentic sample of cholesteryl acetate showed no depression in the melting point.

Cholesterol.—A solution of 5.0 g. of the diene acetate (IV) dissolved in 500 cc. of anhydrous ethanol was hydrogenated at atmospheric pressure and room temperature using 1.25 g. of 10% palladium on charcoal. The reduction was stopped when 340 cc. of hydrogen had been consumed (40 minutes). The catalyst was removed by filtration and the volume reduced to 60 cc. Potassium hydroxide (C. P., 2.5 g.) was added and the solution refluxed for two hours. The solution was poured into water, the product filtered and dried to give 4.8 g., m. p. 142.4–145.2°. The crude cholesterol was recrystallized from methanol and then from acetone to give 3.0 g. of pearly leaflets, m. p. 149.0–149.4°; $[\alpha]^{23}D$ −38.6° (2% in $CHCl_3$); $[\alpha]^{22}D$ −35.5° (2% in dioxane). A mixture with an authentic sample of cholesterol showed no depression in the melting point. The infrared spectrum was identical with that of a sample of authentic cholesterol.

(9) We are indebted to Dr. Eugene P. Oliveto of the Chemical Research Division for suggesting this reduction technique; compare, Bernstein and Wallis, *J. Org. Chem.*, **2**, 341 (1937).

Summary

Cholesterol has been prepared from norcholesteryl-25-one acetate in an over-all yield of better than 50%. This procedure offers a technique for obtaining a C_{26} labelled cholesterol.

BLOOMFIELD, NEW JERSEY RECEIVED FEBRUARY 20, 1950

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Preparation of Cholesterol from Δ^5-Norcholestene-3β-ol-25-one[1]

BY WILLIAM G. DAUBEN AND H. LEON BRADLOW

Recent studies on the physiological significance of cholesterol have made the availability of this sterol labeled with C^{14} highly desirable. For the purpose of studying *intact* cholesterol, a label located at the terminal positions of the side chain is most useful. One possible approach to this problem was suggested by the work of Riegel and Kaye[2] who prepared cholesterol from 3β-hydroxy-Δ^5-cholenic acid. This synthesis, however, did not seem adequate in view of the low over-all yield and the difficult availability of the required labeled intermediate. The use of Δ^5-norcholestene-3β-ol-25-one (I), a readily available steroid intermediate, appeared to offer more promise in view of its closer relationship to cholesterol and the requirement of only methyl iodide as the labeled starting material.[3]

When norcholestenolone (I) was allowed to react with methylmagnesium iodide, 25-hydroxycholesterol (II) was isolated in 84% yield. Acetylation of the diol with acetic anhydride in pyridine solution gave 25-hydroxycholesteryl acetate (III). When the diol was refluxed with acetic anhydride containing a trace of pyridine, the diacetate (IV) was obtained. The monoacetate upon treatment with phosphorus tribromide in benzene yielded 25-bromocholesteryl acetate, which was readily dehydrobrominated by collidine to 25-dehydrocholesteryl acetate (V) in an over-all yield of 72%. The infrared spectrum of (V) showed a strong absorption band at 11.30 μ which is indicative of terminal unsaturation.[4] The diacetate (IV) also could be converted to the dehydro compound (V) by the same sequence. Similar results (40–45%) were obtained when the diol was allowed to react with acetic anhydride and anhydrous formic acid. No pure product could be obtained, however, when the diol was treated with (1) acetic anhydride for 24–48 hours, (2) acetic anhydride and a trace of either zinc chloride or sulfuric acid or (3) acetic anhydride and 90% formic acid. When the 3-tosylate of (II) was allowed to react with phosphorus oxychloride and pyridine no definite product was obtained.[5]

Deacetylation of 25-dehydrocholesteryl acetate with alcoholic potassium hydroxide gave a 91% yield of 25-dehydrocholesterol (VI) which was

(1) This work was supported by a grant from the U. S. Public Health Service.

(2) Riegel and Kaye, THIS JOURNAL, **66**, 723 (1944).

(3) Dr. August Ryer of the Schering Corporation has informed us privately that he has also carried out a similar conversion and that a report on his work is now in press. For this reason we are reporting our results now without including the actual incorporation of C^{14} into the cholesterol molecule.

(4) Thompson and Whiffen, *J. Chem. Soc.*, 1412 (1948); Barnes, Gore, Stafford and Williams, *Anal. Chem.*, **20**, 402 (1948).

(5) Reich and Lardon, *Helv. Chim. Acta*, **29**, 671 (1946).