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

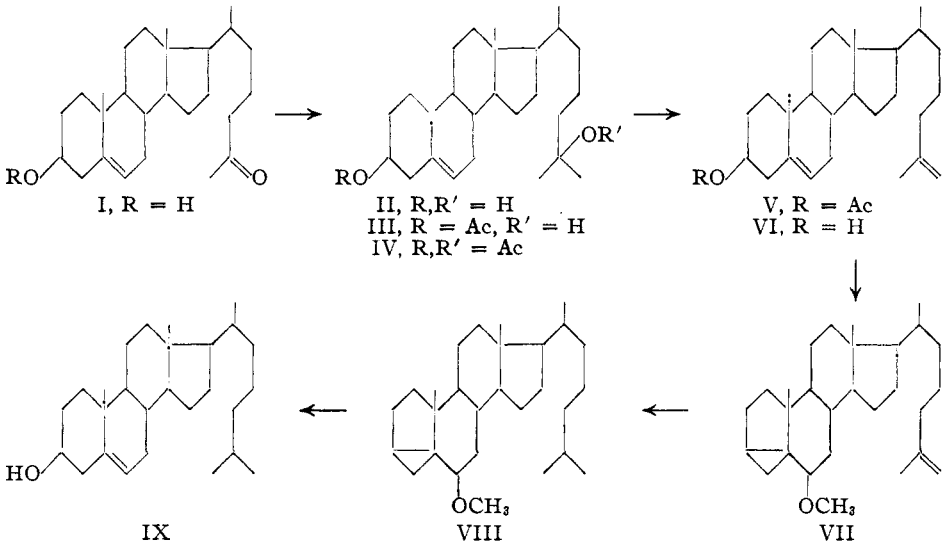
(1) This work was supported by a grant from the U. S. Public Health Service.

(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).



subsequently converted to a sirupy *i*-dehydrocholesteryl methyl ether (VII) in the usual manner.[6] The *i*-ether was then directly hydrogenated over platinum oxide in ethanol to give *i*-cholesteryl methyl ether (VIII) in an over-all yield of 61%. The infrared spectrum of this synthetic *i*-ether was identical with that of an authentic specimen. The product was subsequently converted to cholesterol (IX) by the method of McKennis.[7] The infrared spectrum of the synthetic cholesterol was identical with that of the natural material.

Acknowledgment.—We are indebted to the Schering Corporation for generous gifts of norcholestenolone and to Dr. Keith Freeman of Donner Laboratory for the infrared spectra.

Experimental[8]

Norcholestenol-25-one (I).—Norcholestenol-25-one acetate (25.0 g., 58.5 mmoles, m. p. 138.5–139.5°) was saponified in aqueous ethanolic potassium hydroxide and the product recrystallized from 500 ml. of ethanol; yield 22.3 g. (98.8%), m. p. 118.0–119.1°. Repeated recrystallization and high vacuum sublimation did not raise the melting point.[9]

25-Hydroxycholesterol (II).—A solution of 1.00 g. (2.85 mmoles) of norcholestenol-25-one in 50 ml. of benzene (previously dried by distillation of part of the solvent) was added dropwise with stirring to a solution of methylmagnesium iodide prepared from 1.42 g. (0.62 ml., 10.0 mmoles) of methyl iodide and 0.240 g. (10.0 mmoles) of magnesium turnings in 40 ml. of dry ether. The mixture was refluxed for four hours, kept at room temperature for twelve hours, decomposed with 25 ml. of water followed by 25 ml. of 50% acetic acid and finally steam distilled. The filtered and dried residual solid, m. p. 175–177°, weighed 1.07 g. (100%). Recrystallization from methanol gave an 84% yield of a powdery product, m. p. 177–179°, $[\alpha]^{25}D$ −38.6° (chloroform).

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.55; H, 11.51. Found: C, 80.98; H, 11.48.

25-Hydroxycholesteryl Acetate (III).—A solution of 13.5 g. (33.6 mmoles) of the diol in 120 ml. of dry pyridine and 30 ml. of acetic anhydride was warmed at 60–70° for one hour, allowed to stand overnight and then poured into water, filtered and washed thoroughly. The crude ester was dissolved in 400 ml. of acetone, filtered and the solution concentrated to 200 ml. The ester was obtained as needles; yield 12.7 g. (85.3%), m. p. 138.5–140.0°, $[\alpha]^{25}D$ −42.1° (chloroform).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.80; H, 10.56.

25-Dehydrocholesteryl Acetate (V).—A solution of 8.6 g. (19.4 mmoles) of the monoacetate in 300 ml. of dry benzene and 34.5 ml. of phosphorus tribromide was refluxed for five hours and then poured into ice and ether. The ether–benzene solution was washed with water, dilute sodium hydroxide solution and saturated sodium chloride solution, dried and concentrated at 50°. The solid product was used directly in the next step. A small sample was recrystallized from acetone to give shiny plates, m. p. 113.5–115.0. The solid bromo compound was dissolved in 75 ml. of collidine, the solution refluxed for three hours and then poured into cold, dilute hydrochloric acid with stirring. The crude product upon recrystallization from methanol yielded 6.2 g. (72%) of plates, m. p. 92.5–93.5°, $[\alpha]^{25}D$ −42.8° (chloroform).

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.64; H, 10.63.

The bromo compound dehydrohalogenated upon sublimation at 10^{-5} mm. to give crude dehydro ester, m. p. 86–88°, no depression upon admixture with an authentic sample.

25-Dehydrocholesterol (VI).—A solution of 5.0 g. (11.7 mmoles) of the ester (V) and 2.8 g. of potassium hydroxide in 75 ml. of methanol was refluxed for two hours and then poured into 600 ml. of water. The crude product was filtered and recrystallized from acetone–methanol to give 4.3 g. (91.5%) of leaflets, m. p. 120.5–121.5°, $[\alpha]^{25}D$ −40.2° (chloroform).

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.35; H, 11.48.

***i*-Δ^{25}-Dehydrocholesteryl Methyl Ether** (VII).—Tosyl chloride (1.5 g.) was added with swirling to a solution of 3.0 g. (7.8 mmoles) of dehydrocholesterol in 20 ml. of dry pyridine, and after standing twelve hours, the mixture was poured into ice and extracted with ether. The ethereal extract was washed with ice-water, cold dilute hydrochloric acid, cold dilute sodium bicarbonate solution and saturated sodium chloride solution, dried and concentrated at 50°. A sample of the solid residue was recrystallized from ether, m. p. 118.5–119.5°.

The remaining solid was refluxed for nine hours with 4.0 g. of freshly fused potassium acetate and 125 ml. of anhydrous methanol. After distillation of the bulk of the methanol, the residue was diluted with water and ether. The ethereal extract was washed, dried and concentrated under reduced pressure to give the product as a thick sirup which was used directly in the next step.

(6) Riegel and Meyer, THIS JOURNAL, **68**, 1097 (1947).

(7) McKennis, *J. Biol. Chem.*, **172**, 313 (1948).

(8) All analyses are by the Microanalytical Laboratory of the Department of Chemistry, University of California, Berkeley. All samples were either sublimed at 10^{-5} mm. or dried at 60° at the same pressure. All melting points are corrected.

(9) Ruzicka and Fischer, *Helv. Chim. Acta*, **20**, 1291 (1937), reported 126–127° with sintering at 114°, while Hatorri, *J. Pharm. Soc. Japan*, **58**, 548 (1938), reported 117–127° for an isolated sample and 127–129° for a synthetic sample.

i-**Cholesteryl Methyl Ether** (VIII).—The above sirup was dissolved in 60 ml. of absolute ethanol, 300 mg. of platinum oxide added and the hydrogenation carried out at atmospheric pressure. After 78% of the theoretical amount of hydrogen had been taken up, the reaction stopped and a small amount of white solid began to precipitate. The solution was warmed, filtered and concentrated to 15 ml. The solid product weighed 1.9 g. (61%) and melts at 73.0–74.5° and shows no depression on admixture with an authentic sample.

Cholesterol (IX).—The *i*-ether (VIII, 0.5 g., 1.20 mmoles) was converted to cholesterol following the procedure of McKennis.[5] Recrystallization from methanol yielded 0.42 g. (87%) of plates, m. p. 145.0–146.0°.

25-Acetoxycholesteryl Acetate (IV).—A solution of 0.20 g. (0.49 mmole) of the diol (II) in 10 ml. of acetic anhydride and 2 drops of pyridine was refluxed for one hour, cooled and poured into 250 ml. of cold water. The mixture was allowed to stand for several hours with occasional swirling until the product solidified. The solid was filtered and dried to give a quantitative yield of the crude diacetate which was recrystallized from methanol to give 0.17 g. (72%) of plates, m. p. 119.0–120.5°. $[\alpha]^{25}D$ −35.5° (chloroform).

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.35. Found: C, 76.96; H, 10.40.

Conversion of IV to Dehydrocholesterol (VI).—The crude diacetate obtained by acetylating 0.50 g. (1.25 mmoles) of diol under the above conditions was refluxed for five hours with 25 ml. of dry benzene and 3 ml. of phosphorus tribromide, poured into ice and ether and the extract washed, dried and concentrated as described above. The residual material was refluxed with 5 ml. of collidine for three hours and processed as in the conversion of III to V. The solid residue was directly hydrolyzed by refluxing in 50 ml. of 5% ethanolic potassium hydroxide for two hours. The solution was concentrated, diluted with water, cooled and filtered to give 0.33 g. of crystals, m. p. 104–106°. After two further recrystallizations from ethanol 0.29 g. (60% over-all) of beautiful plates were obtained, m. p. 117–119°, undepressed upon admixture with an authentic sample.

The diacetate is not converted to the dehydro-ester by thermal cleavage in hot collidine and is recovered unchanged after refluxing in collidine solution for four hours.

The diacetate is converted, however, to the bromo compound by the action of phosphorus tribromide. A solution of 0.35 g. (0.79 mmole) of the diacetate in 20 ml. of dry benzene and 2 ml. of phosphorus tribromide was refluxed for five hours and then processed as described above. The twice recrystallized product, m. p. 114–116°, weighed 0.23 g. (58%). A mixed melting point between this product and the bromo compound previously obtained showed no depression. The compound also sublimed in the same characteristic slow fashion to give dehydrocholesteryl acetate, m. p. 85–89°, in good agreement with the value obtained for the sublimate from the first bromo compound. One recrystallization from methanol raised the m. p. to 89.5–90.5°. The diacetate, itself, sublimes without decomposition.

Infrared Spectra.—All spectra were taken in carbon disulfide solution using a narrow sodium chloride cell.

Summary

1. A procedure has been developed for the synthesis of cholesterol from Δ^5-norcholestene-3β-ol-25-one in 28.5% yield.

2. Norcholestenol-25-one was allowed to react with methylmagnesium iodide to give 25-hydroxycholesterol, which in turn was converted to the monoacetate. The monoacetate was dehydrated by the action of phosphorus tribromide and collidine to give Δ^{25}-dehydrocholesteryl acetate. The ester was hydrolyzed to the free sterol which was then converted to *i*-dehydrocholesteryl methyl ether. The *i*-ether was hydrogenated over platinum oxide to give *i*-cholesteryl methyl ether which was subsequently hydrolyzed to cholesterol.

BERKELEY 4, CALIF. RECEIVED APRIL 3, 1950

[CONTRIBUTION FROM THE LABORATORY OF RADIOCHEMISTRY, UNIVERSITY OF CINCINNATI]

The Structure of Fluorene[1]

BY JOHN H. WEISBURGER,[2] ELIZABETH K. WEISBURGER AND FRANCIS EARL RAY

Although the structure of fluorene would appear to be straightforward, there have been a number of conflicting opinions with regard to the spatial configuration of this hydrocarbon.

Cook and Iball[3] proposed a folded ring structure with the planes of the six-membered rings inclined at an angle of 20° to the plane of the five-membered ring. On the other hand Pinck and Hilbert[4] favor a uniplanar model.

A preliminary consideration of resonance energy, bond lengths, bond angles and absorption spectra provides evidence for one form.

According to theoretical organic chemistry resonance could occur throughout a planar structure but would be limited to the benzene rings in a folded fluorene molecule. The resonance energy given in the literature[5] for fluorene is appreciably higher (101 kcal./mole) than that corresponding to two benzene rings (82 kcal./mole) alone.

Moreover, from the bond lengths[4,6] in the five-membered ring, the partial double bond character of these bonds may be calculated with the following results: The bond joining the benzene rings has a 12.5% double bond character (like biphenyl), the bonds between the benzene rings and the 9 carbon a 15% and the bonds in the benzene rings a 37% double bond character. This could be interpreted as indicating that resonance takes place throughout the entire molecule.

(1) This work was supported by grant N7-onr-479 from the Office of Naval Research. Presented at the meeting of the Ohio Academy of Science, Granville, Ohio, April, 1949. A portion of the thesis submitted to the Graduate School, University of Cincinnati, by John H. Weisburger in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1949.

(2) National Cancer Institute, Bethesda 14, Maryland.

(3) Cook and Iball, *Chem. and Ind.*, **55**, 467 (1936).

(4) Pinck and Hilbert, THIS JOURNAL, **59**, 8 (1937).

(5) Wheland, "Theory of Resonance," John Wiley & Sons, Inc., New York, N. Y., 1944, p. 69.

(6) Iball, *Z. Krist.*, **94**, 397 (1936).