[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Preparation of 24-Keto and 24-Hydroxycholesterol and Certain Derivatives[1]

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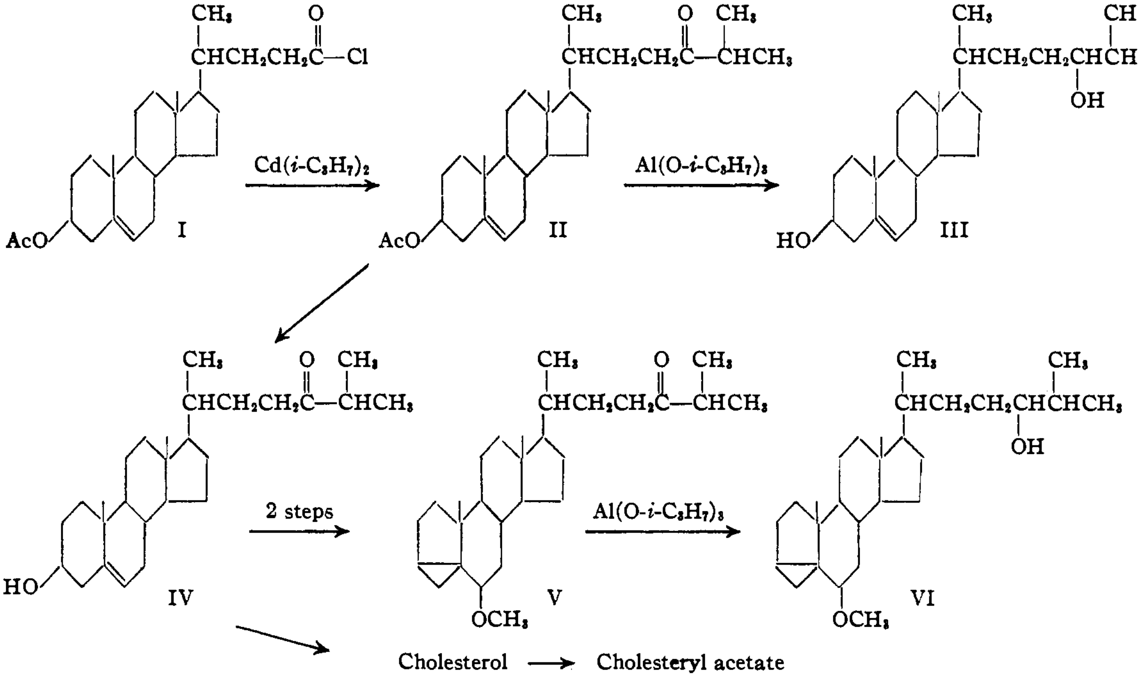
The structures of the side chains of sterols have been elucidated chiefly by degradative studies. Surprisingly little has been done to establish more definitely these structures by resyntheses from degraded products containing the 3-hydroxyl group and the 5,6-double bond.[2]

The carbon skeletons of naturally occurring sterols differ only in the substituents at the 24-position. Thus, a suitable intermediate for the preparation of sterols having various side chains would be 24-ketocholesterol (IV). This intermediate will also permit further study of the stereochemical contribution of the side chain to the optical rotation. An excellent method for the preparation of this compound involves the reaction of diisopropylcadmium with 3-acetoxy-5-cholenyl chloride[2d] (I). 24-Ketocholesterol melts at 137–138.5° and has a specific rotation of −37°. Its semicarbazone, acetate and the acetate oxime were prepared.



Cholesterol was obtained directly from 24-ketocholesterol by a modified Wolff–Kishner reduction of the carbonyl group. The Clemmensen and the ordinary Wolff–Kishner reductions gave tarry products. Reduction of the ketone (II) by means of aluminum isopropoxide gave 24-hydroxycholesterol (III). Intermediates from which the 24-hydroxyl group might be removed by dehydration were sought. Attempts to make the 3-monoacetate by partial acetylation gave only the diacetate or starting material.

Use was then made of the so-called *i*-ether configuration as a method for the specific protection of the 3-hydroxyl group. The tosylate of 24-ketocholesterol, when heated in a buffered methanol solution, gave 24-keto-*i*-cholesteryl methyl ether (V) which melted at 90.5–91.5° and gave a specific rotation of +52°. This ketone (V) in the presence of aluminum isopropoxide was reduced to 24-hydroxy-*i*-cholesteryl methyl ether. The conversion of some of these compounds to other naturally occurring sterols is now being studied.

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Experimental[3]

24-Ketocholesterol (IV).—The 3-acetoxy-5-cholenyl chloride[2d] prepared from 25 g. of 3-acetoxy-5-cholenic acid[4] (m. p. 183–185°) was not isolated but was dissolved in dry benzene and then added to a diisopropylcadmium solution. The latter solution was made from 14.65 g. of magnesium, 125 g. of isopropyl bromide, anhydrous ether and 88 g. of

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[1] Original manuscript received August 10, 1943.

[2] (a) H. Wieland and R. Jacobi, *Ber.*, **59**, 2064 (1926); (b) F. Reindel and K. Niederländer, *Ann.*, **522**, 218 (1936); (c) E. Fernholz, *Ber.*, **69**, 1792 (1936); (d) S. Kuwada and M. Yogo, *J. Pharm. Soc.* (*Japan*), **57**, 963 (1937).

[3] All melting points are corrected. Analyses by Dr. T. S. Ma, University of Chicago.

[4] The authors wish to express their appreciation to Dr. Erwin Schwenk of the Schering Corporation for a generous quantity of this acid.

anhydrous cadmium bromide. The reaction mixture, after standing overnight, was worked up in the usual way and the product was saponified with methanolic potassium hydroxide. The ether soluble material from the saponification was crystallized three times from petroleum ether (Skellysolve C, b. p. 85–100°). A yield of 11.19 g. (46%) of large clumps of hard needles, melting at 134.5–135.5°, was obtained. More material was recovered from the mother liquors by forming the semicarbazone of the crude ketone. The semicarbazone was washed well with water, dried and decolorized by extraction with ether in a Soxhlet extractor. The white powdery product was hydrolyzed, dissolved in ether, and decolorized with Norit. Removal of the ether and crystallization from Skellysolve C gave 1.57 g. (7%) of material melting at 134–135.5°. After three more crystallizations of the ketone from aqueous methanol, it melted at 137–138.5°; $[\alpha]^{26}_D$ −37° (19.6 mg. made up to 2 ml. with chloroform, α^{26}_D −0.36°; *l*, 1 dm.).

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.87; H, 11.02.

24-Ketocholesteryl Acetate (II).—The interaction of the acid chloride with the cadmium Grignard reagent gave considerable esterification which is difficult to understand. However, from the reaction mixture the 24-ketocholesteryl acetate could be isolated by means of Girard's reagent-T. Even after two crystallizations from methanol and two more from 95% ethanol it gave a peculiar melting point. It melted at 127.5–128° but did not become fluid, then became turbid at 129–130° and finally melted forming a meniscus at 131°; $[\alpha]^{26}_D$ −41° (38.5 mg. made up to 2 ml. with chloroform, α^{26}_D −0.78°; *l*, 1 dm.).

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.48. Found: C, 78.75; H, 10.51.

Oxime of 24-Ketocholesteryl Acetate.—A solution of 50 mg. of the ketone acetate and 50 mg. of hydroxylamine hydrochloride in 8 drops of anhydrous pyridine and 8 drops of absolute ethanol was refluxed for two hours. Dilution with water precipitated the oxime which was removed by filtration. It was washed with water and crystallized twice from aqueous ethanol and once from methanol giving a product that softened at 155° and melted at 156–158.5°.

Anal. Calcd. for $C_{29}H_{47}O_3N$: N, 3.06. Found: N, 3.12.

Semicarbazone of 24-Ketocholesterol.—A small sample of the ketone was converted to the semicarbazone which was washed thoroughly with water and ether. Two crystallizations from dilute ethanol gave material melting at 166–168°.

Anal. Calcd. for $C_{28}H_{47}ON_3$: N, 9.52. Found: N, 9.67.

Reduction of 24-Ketocholesterol to Cholesterol.—A mixture of 250 mg. of the ketone, 0.45 g. of sodium, 15 ml. of absolute ethanol and 1.25 ml. of 85% hydrazine hydrate was heated in a sealed tube at 200° for twelve hours. The cooled reaction mixture was diluted with water and extracted with ether. After drying and removing the ether, the residue was crystallized three times from methanol, giving long white needles melting at 145.6–146.1°; $[\alpha]^{28.5}_D$ −36° (27.5 mg. made up to 2 ml. with chloroform, $\alpha^{28.5}_D$ −0.50°; *l*, 1 dm.). It gave a mixed m. p. of 144–145.8° when mixed with an authentic sample of cholesterol (m. p. 150–151°).

The acetate was prepared by heating 90 mg. of the above product with 3 ml. of acetic anhydride. After removing the acetic anhydride, the residue was crystallized once from methanol and twice from dilute ethanol. It melted at 113–114°, gave no depression in m. p. when mixed with an authentic sample of cholesteryl acetate and gave a specific rotation of −46° (6.8 mg. made up to 2 ml. with chloroform, α^{28}_D −0.15°; *l*, 1 dm.).

24-Hydroxycholesterol (III).—A solution of 0.5 g. of 24-ketocholesteryl acetate and 5 g. of aluminum isopropoxide in 75 ml. of anhydrous isopropyl alcohol was refluxed for three hours. About one-half of the solvent was removed by slow distillation, 75 ml. more of anhydrous isopropyl alcohol was added and again distilled slowly until removed. The reaction mixture was cooled and decomposed by adding 50 ml. of dilute hydrochloric acid. The product was removed by filtration and crystallized from dilute methanol giving 470 mg. (94%) of material melting at 166–169°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.55; H, 11.97.

Diacetate.—When the above diol was treated with acetic anhydride the diacetate was obtained readily. After two crystallizations from aqueous ethanol and two from methanol, it softened at 93° and melted at 95–96°.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.75; H, 10.44.

24-Ketocholesteryl *p*-Toluenesulfonate.—Following the method of Freudenberg and Hess,[5] 1 g. of 24-ketocholesterol gave 1.1 g. (82%) of tosylate. From low boiling petroleum ether, it formed small, hard crystals which softened at 115° and melted at 119–120° (dec.); $[\alpha]^{26}_D$ −35° (23.5 mg. made up to 2 [illegible] with chloroform, α^{26}_D −0.42°; *l*, 1 dm.).

Anal. Calcd. for $C_{34}H_{50}O_4S$: C, 73.60; H, 9.08. Found: C, 74.28; H, 9.34.

24-Keto-*i*-cholesteryl Methyl Ether (V).—A solution of 0.81 g. of the above tosylate and 1.2 g. of freshly fused potassium acetate in 40 ml. of anhydrous methanol was refluxed for four and one-half hours. A solution of the product in ether was washed with a sodium bicarbonate solution, dried, decolorized with Norit and evaporated to dryness. The residue, after two crystallizations from acetone, gave 0.32 g. (53%) of *i*-ether which melted at 90.5–91.5°; $[\alpha]^{26}_D$ +52° (24.7 mg. made up to 2 ml. with chloroform, α^{26}_D +0.64°; *l*, 1 dm.).

Anal. Calcd. for $C_{28}H_{46}O_2$: C, 81.10; H, 11.18. Found: C, 81.50; H, 11.28.

24-Hydroxy-*i*-cholesteryl Methyl Ether (VI).—By a procedure similar to that described above, 658 mg. of the keto *i*-ether was reduced with 2 g. of aluminum isopropoxide in 60 ml. of anhydrous isopropyl alcohol. The crude product was a brown oil which weighed 563 mg. It was purified by molecular distillation at a bath temperature of 106° and a pressure of 10^{-4} mm. The distillate, a viscous sirup, gave a specific rotation of +31° (133 mg. made up to 2 ml. with chloroform, $\alpha^{27.5}_D$ +2.07°; *l*, 1 dm.).

Anal. Calcd. for $C_{28}H_{48}O_2$: C, 80.71; H, 11.61. Found: C, 80.77; H, 11.66.

Summary

1. A bile acid, 3-hydroxy-5-cholenic acid, made from cholesterol was converted to 24-ketocholesterol which in turn was reduced to cholesterol.

2. 24-Ketocholesterol is a suitable intermediate for the preparation of many naturally occurring sterols.

3. Reduction of the 24-keto group, by the Meerwein–Ponndorff method, gave 24-hydroxycholesterol.

4. The 3-hydroxyl group of these compounds can be protected for some reactions by the excellent method of conversion to their *i*-ethers.

5. Several other derivatives of these steroids are reported.

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(5) K. Freudenberg and H. Hess, *Ann.*, **448**, 128 (1926).