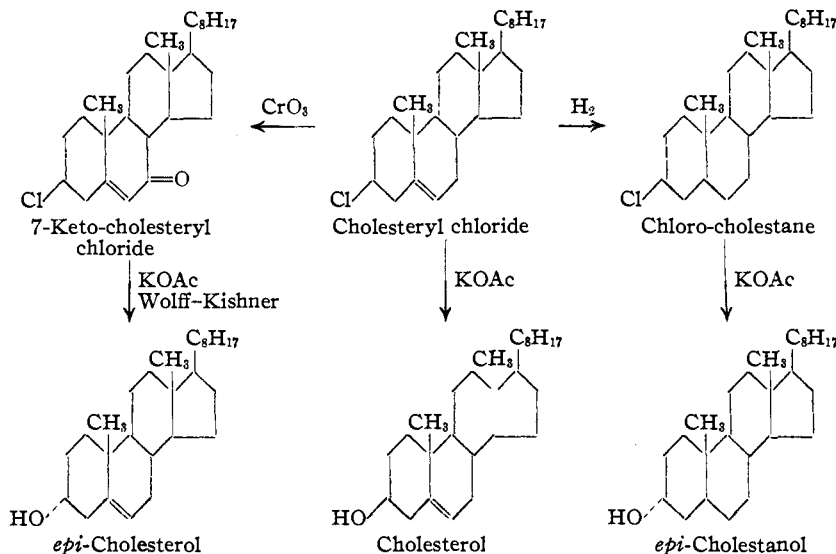


[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE AND THE PARKE, DAVIS & CO. RESEARCH LABORATORIES]

## Sterols. X. Cholesterol Derivatives

BY RUSSELL E. MARKER, OLIVER KAMM, GEORGE H. FLEMING, ALEXANDER H. POPKIN AND EUGENE L. WITTE

In our studies on sterols we have prepared a number of derivatives substituted in ring B. On the oxidation of cholesteryl chloride by chromic oxide in acetic acid, 7-keto-cholesteryl chloride can be isolated in yields of 25%. When this is treated with potassium acetate it gives a mixture of 7-keto-cholesterylene and the acetate of 7-keto-*epi*-cholesterol. The latter when reduced by the Wolff-Kishner method gives *epi*-cholesterol, which is identical with *epi*-cholesterol previously prepared by us.<sup>1</sup>



When cholesteryl chloride is hydrolyzed it gives a compound with the hydroxyl group in the normal (cholesterol) arrangement. However, if the double bond is first reduced to cholestyl chloride and this product is hydrolyzed a Walden inversion occurs leading to an epimeric hydroxyl group. The same is true when 7-keto-cholesteryl chloride is hydrolyzed, giving an epimeric -OH group. This is very unusual in view of the fact that the double bond in both compounds is in the same position. It was noticed that cholesteryl chloride is hydrolyzed readily with potassium acetate in boiling acetic acid whereas with the other two compounds it was necessary to heat to 180° for hydrolysis.

(1) Marker, Oakwood and Crooks, *THIS JOURNAL*, **58**, 481 (1936).

When 7-keto-cholesteryl chloride is reduced by platinum oxide in acetic acid it gives a mixture of 7-hydroxycholestyl chloride and  $\alpha$ -cholestyl chloride, the ketonic group of a portion of the material being reduced to a methylene group. Aluminum isopropylate and 7-keto-cholesteryl chloride give 7-hydroxycholesteryl chloride. When the latter compound is reduced by platinum oxide in acetic acid it also gives a mixture of 7-hydroxycholestyl chloride and  $\alpha$ -cholestyl chloride in about equal portions. 7-Hydroxycholestyl chloride, on reduction with sodium in amyl alcohol, gives 7-hydroxycholestane. The -OH group of both compounds may readily be oxidized to give the corresponding ketones.

### Experimental

#### 7-Keto-cholesteryl Chloride.—

To a solution of 203 g. of cholesteryl chloride in 3500 cc. of acetic acid kept at 55° there was added with stirring over a period of one hour a solution of 150 g. of chromic oxide in 100 cc. of water and 100 cc. of glacial acetic acid. Stirring was continued during an additional two hours. Ethyl alcohol was added and the reaction mixture was concentrated under reduced pressure at 55° to a volume of 1.5 liters. To this was added 100 cc. of water and the mixture was cooled overnight. The crystalline material was filtered, washed with 600 cc. of alcohol followed by 300 cc. of acetone and finally was recrystallized from acetone. The yield was 50 g. of a product melting at 145°. The melting point of the semicarbazone was 176°.

*Anal.* Calcd. for  $\text{C}_{27}\text{H}_{48}\text{OCl}$ : C, 77.4; H, 10.3. Found: C, 77.8; H, 10.2. *Anal.* Calcd. for  $\text{C}_{28}\text{H}_{46}\text{OCIN}_2$ : C, 70.7; H, 9.7. Found: C, 70.5; H, 10.0.

*epi*-cholesterol from 7-Keto-cholesteryl Chloride.—To a solution of 5 g. of 7-keto-cholesteryl chloride in 25 cc. of valeric acid, 7 g. of fused potassium acetate was added and the mixture was refluxed for fourteen hours. The reaction mixture was extracted with ether, and the ether solution washed with sodium carbonate solution. The acetate obtained after evaporation of the ether was hydrolyzed by means of alcoholic potassium hydroxide. The resulting oily material was dried and treated with 2 g. of succinic

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anhydride in 15 cc. of pyridine, being heated four hours on the steam-bath. The reaction product was dissolved in ether and washed free of pyridine with hydrochloric acid. The succinate was then removed from ether by washing with sodium carbonate solution, the resulting solution being again washed with ether. [The ether solutions were worked up in a later experiment to give 7-keto-cholesteryl-ene.] The alkaline solution was acidified and extracted with ether to remove the succinate, which was then hydrolyzed by boiling with alcoholic potassium hydroxide. The resulting product was then converted to the semicarbazone by heating with alcoholic semicarbazide acetate. This crude product was added directly to 10 cc. of absolute alcohol and to this was added a solution of 0.2 g. of sodium in 10 cc. of absolute alcohol. This was heated at 170–180° for six hours after which water was added and the product extracted with ether. The product was then sublimed at 140° using a mercury vapor pump and the sublimate crystallized from alcohol. Its melting point was 141°. Mixed with cholesterol (m. p. 147°) it gave a depression in melting point of 9°. Mixed with known *epi*-cholesterol it gave no depression.

*Anal.* Calcd. for  $C_{27}H_{46}O$ : C, 83.9; H, 12.0. Found: C, 84.0; H, 11.8.

**7-Keto-cholesteryl-ene.**—To 1 g. of 7-keto-cholesteryl chloride dissolved in 30 cc. of alcohol was added 1.5 g. of potassium hydroxide in 2 cc. of water. The solution was refluxed two hours. Water was added and the product extracted with ether. After crystallization from alcohol it melted at 114°.

*Anal.* Calcd. for  $C_{27}H_{46}O$ : C, 84.8; H, 10.9. Found: C, 84.4; H, 10.8.

The same product was obtained as a by-product in the preparation of *epi*-cholesterol from 7-keto-cholesteryl chloride.

**Reduction of 7-Keto-cholesteryl Chloride with Platinum Oxide Catalyst.**—To a solution of 5 g. of 7-keto-cholesteryl chloride dissolved in 200 cc. of glacial acetic acid was added 1.5 g. of platinum oxide. The mixture was shaken with hydrogen under a pressure of 45 pounds (3 atm.) for six hours when the catalyst was filtered off and the acetic acid distilled under reduced pressure. The residue after crystallization from alcohol melted at 114.5°. Mixed with  $\alpha$ -cholestyl chloride (m. p. 114°) it gave no depression in melting point.

*Anal.* Calcd. for  $C_{27}H_{47}Cl$ : C, 79.6; H, 11.6. Found: C, 79.6; H, 11.6.

The mother liquors from the above reduction contained about 80% of a product which would not crystallize and which was not cholestyl chloride since that product is almost completely insoluble in cold alcohol. The subsequent experiments showed that these mother liquors contained 7-hydroxycholestyl chloride.

**7-Keto-cholestyl Chloride.**—The solvent from the mother liquors in the previous experiment was evaporated. To 2 g. of the residue was added 20 cc. of acetic acid and a solution of 0.7 g. of chromic oxide in 3.4 cc. of acetic acid. The reaction mass was kept at 50–60° for two hours, then allowed to stand overnight at room temperature. A crystalline product separated and was filtered off and recrystallized from alcohol. Its melting point was 139°.

*Anal.* Calcd. for  $C_{27}H_{46}OCl$ : C, 77.1; H, 10.7. Found: C, 77.1; H, 10.6.

**Cholestanol-7.**—To 6 g. of crude 7-hydroxycholestyl chloride in 175 cc. of dry boiling amyl alcohol was added 12.5 g. of sodium. When the reaction was complete water was added and the alcoholic layer was separated and evaporated under reduced pressure. The residue crystallized from alcohol in needles melting at 117.5°.

*Anal.* Calcd. for  $C_{27}H_{48}O$ : C, 83.5; H, 12.4. Found: C, 83.4; H, 12.3.

**Cholestanone-7.**—To a solution of 2 g. of 7-hydroxycholestane in 35 cc. of acetic acid was added 0.7 g. of chromic oxide in 4 cc. of acetic acid. The product was kept at 50° for four hours and allowed to stand at room temperature for an additional twenty-four hours. Water was then added and the oxidation product extracted with ether. The ether solution was washed with sodium carbonate solution, the ether evaporated and the residue crystallized from acetone. Its melting point was observed to be 117° which is the same as that of 7-hydroxycholestane; mixed with that product, however, it showed a depression of 10°.

*Anal.* Calcd. for  $C_{27}H_{46}O$ : C, 83.9; H, 12.0. Found: C, 84.3; H, 12.1.

**7-Hydroxycholesteryl Chloride.**—To a solution of 40 g. of 7-keto-cholesteryl chloride (m. p. 145°) in 510 cc. of dry isopropyl alcohol was added 30 g. of distilled aluminum isopropylate. The mixture was refluxed for five hours, after which 250 cc. of isopropyl alcohol was distilled off over a period of four hours. The residue was poured into 1300 cc. of 3% potassium hydroxide solution and shaken vigorously. The product was extracted with ether, the solvent concentrated to 100 cc. and 100 cc. of acetone added. Crystals separated on cooling which, recrystallized from acetone, melted at 142°. The yield was 31 g.

*Anal.* Calcd. for  $C_{27}H_{46}OCl$ : C, 77.1; H, 10.7. Found: C, 77.0; H, 10.8.

**Hydrogenation of 7-Hydroxycholesteryl Chloride.**—To a solution of 1.2 g. of 7-hydroxycholesteryl chloride in 120 cc. of acetic acid was added 400 mg. of platinum oxide. The mixture was shaken for four hours with hydrogen at 45 pounds (3 atm.) pressure after which the solution was filtered and concentrated. The residue was crystallized from acetone. It melted at 115° and mixed with cholestyl chloride it gave no depression in melting point.

*Anal.* Calcd. for  $C_{27}H_{47}Cl$ : C, 79.6; H, 11.6. Found: C, 79.7; H, 11.7.

The mother liquors from the above experiment were evaporated to dryness, the residue was dissolved in 15 cc. of glacial acetic acid at 50° and 0.2 g. of chromic oxide in 2 cc. of acetic acid added. After maintaining the solution at 50° for four hours it was kept at room temperature for an additional fifteen hours. Water was then added, the product extracted with ether, the ether evaporated and the residue crystallized from alcohol. Mixed with 7-keto-cholestyl chloride previously prepared, there was no depression in its melting point of 137°. This showed that the mother liquors contained 7-hydroxycholestyl chloride.

*Anal.* Calcd. for  $C_{27}H_{46}OCl$ : C, 77.1; H, 10.7. Found: C, 77.1; H, 10.9.

**Benzoate of 7-Hydroxycholesteryl Chloride.**—A solution of 22.6 g. of 7-hydroxycholesteryl chloride in 150 cc. of dry pyridine was cooled in ice and 50 cc. of benzoyl chloride was added in small portions with shaking. The solution was allowed to stand at room temperature for two days after which it was poured into 4 liters of water and allowed to stand overnight. The water was decanted and the residue dissolved in ether, the ether solution being washed with sodium carbonate solution after which it was concentrated to 100 cc. and 200 cc. of methyl alcohol added. After standing overnight, crystals formed which were recrystallized from acetone. The yield was 11 g. and the melting point 119°.

*Anal.* Calcd. for  $C_{34}H_{48}O_2Cl$ : C, 77.9; H, 9.4. Found: C, 78.2; H, 9.6.

### Summary

*epi*-Cholesterol was prepared from the hydrolysis of 7-keto-cholesteryl chloride with potassium acetate followed by the Wolff-Kishner method. Catalytic reduction by platinum oxide in acetic acid of either 7-keto-cholesteryl chloride or 7-hydroxycholesteryl chloride gave a mixture of cholesteryl chloride and 7-hydroxycholesteryl chloride. Derivatives of the various products were prepared.

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## Physical Studies of Non-aqueous Solvates. II. The Vapor Pressure of Magnesium Bromide-Diethyl Ether Solutions

BY H. H. ROWLEY<sup>1</sup>

The vapor pressure of an ethyl ether solution saturated with magnesium bromide was reported by Evans and Rowley<sup>2</sup> to be 410 mm. at +25°. Scherer and Newton,<sup>3</sup> using the vapor pressure values of magnesium bromide-ethyl ether systems to calculate the free energy of formation of magnesium bromide, also reported a value of 410 mm. at +25° for a solution of ether saturated with magnesium bromide. It is a well established fact<sup>2,4</sup> that magnesium bromide with excess ethyl ether forms two conjugate liquid phases at +25°, the upper layer consisting of ether saturated with magnesium bromide (3.23 g./100 g.  $Et_2O$ ), the bottom layer containing about 39% magnesium bromide. Re-examination of the data of Evans and Rowley indicated that under the conditions of the experiment the only liquid present was the oily bottom layer containing about 39% magnesium bromide and absolutely none of the light upper layer. Thus, the vapor pressure reported was not for an ethyl ether solution saturated with magnesium bromide.

In order to prove this, the vapor pressure of the two conjugate liquid phases has now been measured over a temperature range from 0 to +25°. The system also has been studied in more detail

at +25° varying the ratio of  $MgBr_2:Et_2O$  from  $1/4$  to 1.

### Experimental

**Apparatus.**—The vapor pressures were measured by the static method as shown in Fig. 1. The samples were placed in a flat-bottomed flask (A), about 4 cm. in diameter and 5 cm. in height, sealed to a Pyrex spiral (B) about 4 cm. in diameter and 16 cm. in length, connected to a mercury U-manometer (C). The flexible glass spiral together with the relatively large surface of the sample, allowed the

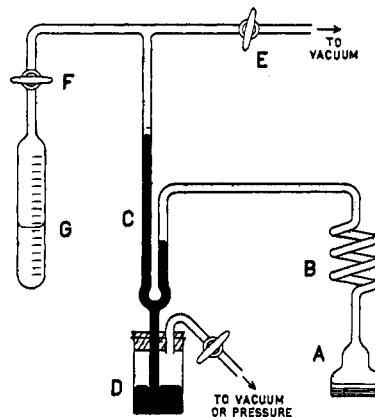


Fig. 1.

sample to be agitated vigorously by gently tapping the flask (A). Thus, thorough mixing and equilibrium conditions were attained rapidly. The mercury in the manometer (C) could be raised or lowered by adjusting the pressure in reservoir (D) and served also to seal the sample from the rest of the system and protect it from stopcock grease. When it was necessary to evacuate the space above the sample, the mercury was lowered and the sample

(1) Department of Chemistry, State University of Iowa, Iowa City, Iowa.

(2) W. V. Evans and H. H. Rowley, *THIS JOURNAL*, **52**, 3523 (1930).

(3) Geo. A. Scherer and Roy F. Newton, *ibid.*, **56**, 18 (1934).

(4) (a) H. H. Rowley, *ibid.*, **58**, 1337 (1936); (b) B. N. Menshutkin, *Z. anorg. Chem.*, **49**, 34 (1906).