116.5–118° and 112.5–113°, giving no depression when mixed with the above product of sulfur dehydrogenation.

**8-Methyl-1,2-benzanthraquinone** was prepared by refluxing the pure hydrocarbon (0.4 g.) in glacial acetic acid (8 cc.) for eighteen minutes with potassium dichromate (0.49 g.) and diluting the solution with water. The crude quinone which crystallized was dried, dissolved in benzene, and put through a tower of alumina. The chromatogram was developed with benzene and the bright yellow zone was separated and eluted with acetone in a Soxhlet extractor. The yellow crystallizate obtained after concentration when crystallized once from acetone formed bright yellow needles, m. p. 196.5–197° (0.11 g.). Two more crystallizations from acetone and one from benzene–ligroin did not raise the melting point. The quinone gives a blood red vat with alkaline hydrosulfite.

*Anal.* Calcd. for $C_{19}H_{12}O_2$: C, 83.81; H, 4.44. Found: C, 84.15, 83.87;[10] H, 4.57, 4.56.[10]

(10) Analysis by the Arlington Laboratories.

**8-Methyl-1,2-benzanthrahydroquinone diacetate,** prepared by reductive acetylation in nearly quantitative yield and crystallized from acetic acid and from benzene–ligroin, formed colorless, felted needles, m. p. 202.5–203°.

*Anal.* Calcd. for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found:[10] C, 77.05; H, 5.24.

## Summary

A hydrocarbon regarded as8 -methyl-1,2-benzanthracene has been synthesized from 9,10-dihydrophenanthrene through the known 8-keto-3,4,5,6,7,8-hexahydro-1,2-benzanthracene of Burger and Mosettig. The hydrocarbon has a double melting point and both modifications melt considerably higher than the substance described by Cook and Robinson.

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[CONTRIBUTION FROM THE ANIMAL CHEMISTRY AND NUTRITION SUBSECTION OF IOWA STATE COLLEGE]

# The Preparation of 3,5- and 4,6-Cholestadienes and a Study of Cholesterilene and of "7-Dehydrocholestene Isomer"[1]

BY J. C. ECK, RALPH L. VAN PEURSEM[2] AND E. W. HOLLINGSWORTH

In connection with our research on the chemical antirachitic activation of sterols,[3] an investigation was made concerning the preparation of 3,5- and 4,6-cholestadienes. A study also was made of cholesterilene and of the hydrocarbon, $C_{27}H_{44}$, obtained by Dimroth and Trautmann[4] by the vacuum distillation of the benzoate of 7-hydroxycholestene at a higher temperature or under a less evacuated condition than is necessary for the preparation of 7-dehydrocholestene and also by the action of acetic anhydride on 7-hydroxycholestene. This hydrocarbon, the structure of which has not been determined, has been referred to as the "isomeric hydrocarbon" by Stavely and Bergmann[5] and as the "cholestadiene, m. p. 91°, prepared by Dimroth and Trautmann" by Heilbron, Shaw and Spring.[6] For the sake of clarity in distinguishing this hydrocarbon from other cholestadienes, it will be referred to simply as "7-dehydrocholestene isomer" so as to avoid any possible confusion.

The preparation of 3,5-cholestadiene, m. p. 78–79°, $(\alpha)^{21}D$ −63.75, by means of a Wolf–Kishner reduction of 7-ketocholesterilene has been reported[7] but a more convenient method of preparation was desired. This was accomplished by the removal of two molecules of hydrogen bromide from pseudo-cholestene dibromide (4,5-dibromocholestane) by the action of quinoline. 4,6-Cholestadiene was likewise prepared from $\beta$-cholestene dibromide (5,6-dibromocholestane); $\alpha$-cholestene dibromide also was used with equal success but the bromination product of cholestene consists chiefly of the $\beta$-isomer.

A halogen in the 5-position can be removed easily as hydrogen halide with a hydrogen atom from either the 4- or 6-position since alcoholic potassium acetate has been found to convert cholestene hydrochloride (5-chlorocholestane) to pseudo-cholestene[8] and to convert cholesterol hydrochloride (5-chlorocholestanol-3) to a mixture of cholesterol and allocholesterol.[9] It would be expected in the case of a dibromide of cholestane with one bromine atom in the 5-position and the other bromine atom in either the

(1) Journal Paper No. J579 of the Iowa Agricultural Experiment Station, Project No. 506.

(2) Part of the experimental work included in this paper is from unpublished research conducted by Ralph L. Van Peursem as partial fulfilment for the degree of Doctor of Philosophy.

(3) Eck, Thomas and Yoder, *J. Biol. Chem.*, **117**, 655 (1937); Eck and Thomas, *ibid.*, **119**, 621, 631 (1937).

(4) Dimroth and Trautmann, *Ber.*, **69B**, 669 (1936).

(5) Stavely and Bergmann, *J. Org. Chem.*, **1**, 575 (1937).

(6) Heilbron, Shaw and Spring, *Rec. trav. chim.*, **57**, 529 (1938).

(7) Stavely and Bergmann, *J. Org. Chem.*, **1**, 567 (1937).

(8) Mauthner and Suida, *Monatsh.*, **28**, 1113 (1907).

(9) Schoenheimer and Evans, *J. Biol. Chem.*, **114**, 567 (1936).

4- or 6-position, that the halogen atom in the 5-position would be removed more easily as hydrogen halide with a hydrogen atom from the adjacent methylene group (6-position in the case of 4,5-dibromocholestane). The remaining halogen atom would be removed as hydrogen halide with a hydrogen atom from the adjacent methylene group (3-position in the case of 4,5-dibromocholestane or 7-position in the case of 5,6-dibromocholestane) to form a second double bond in conjugation with the double bond previously formed by the removal of the first molecule of hydrogen bromide.

Alcoholic potassium acetate was found to be ineffective for the complete removal of hydrogen bromide but quinoline, which has been used effectively for the conversion of cholesteryl chloride to cholesterilene,[10] was found to act satisfactorily for the conversion of both 4,5-dibromocholestane and 5,6-dibromocholestane into cholestadienes. The cholestadienes obtained would logically have one double bond in each of rings A and B because of the method of preparation and because both double bonds could not be formed in either ring A or B; they cannot be identical with either 2,4-cholestadiene[5] or 7-dehydrocholestene.[4] The cholestadiene obtained from 4,5-dibromocholestane was found to be levorotatory, which would indicate that one of the double bonds is in the 5,6-position;[5] the other double bond would be in conjugation in the 3,4-position. The cholestadiene obtained from 5,6-dibromocholestane was found to be dextrorotatory, which would indicate that one of the double bonds is in the 4,5-position; the other double bond would be in conjugation in the 6,7-position. The cholestadienes obtained also would be expected to have one double bond in each of rings A and B since they are not affected by the action of alcoholic hydrochloric acid. 2,4-Cholestadiene has been found[5] to be rearranged by the action of alcoholic hydrochloric acid with the formation of cholesterilene. Likewise *allo*- or *epi-allo*-cholesterol, which on dehydration without rearrangement would be expected to form 2,4-cholestadiene, was found to be dehydrated by the action of alcoholic hydrochloric acid[9] to form cholesterilene,[7] the absorption spectrum of which was found to be similar to that of cholesterilene obtained by the copper sulfate method and that of 3,5-cholestadiene prepared from 7-ketocholesterilene.[7]

(10) Mauthner and Suida, *Monatsh.*, **24**, 648 (1903).

The varied optical rotation of cholesterilene which has been prepared by numerous methods has confused the development of its definite characterization. Stavely and Bergmann[7] separated the cholesterilenes (cholesterilene prepared by some of the various methods reported in the literature) into two groups which have specific rotations between $-60$ and $-70°$ or greater than $-100°$ and suggested that both groups have the 3,5-conjugated system since a member of one group possessed a similar absorption spectrum to that shown by a representative of the other group.

Samples of cholesterilene were prepared by representative reactions of the three general methods of preparing this compound which consist of (1) the direct dehydration of cholesterol, *allo*-cholesterol or their epimers, of (2) the removal of hydrogen halide from cholesteryl halides and of (3) the pyrolytic decomposition of cholesteryl esters. These samples were purified by adsorption of impurities on activated alumina, by treatment with decolorizing carbon in alcohol solution, by sublimation, by treatment with sodium and alcohol and by recrystallization from different solvents and mixed solvents such as alcohol, ether–alcohol, ether–methanol, acetone and acetone–methanol. The melting points, optical rotations and refractive indices of the various purified samples of cholesterilene and of 3,5-cholestadiene were compared under similar conditions. It was found that the melting point of 79.5–80° and the refractive index of 1.45974 (carbon tetrachloride) were obtained for 3,5-cholestadiene and for cholesterilene prepared by the action of copper sulfate on cholesterol, by the action of alcoholic hydrochloric acid on a mixture of *allo*- and *epi-allo*-cholesterol, by the action of quinoline on cholesteryl chloride and by the pyrolytic decomposition of cholesteryl methyl xanthogenate. No depression in melting point was obtained with a mixture of 3,5-cholestadiene and cholesterilene obtained by each of the above methods. The specific rotation of 3,5-cholestadiene was found to be $-103.24°$ and that of cholesterilene prepared by the various methods was $-100.33$, $-104.91$, $-123.23$ and $-123.23°$. The numerical agreement of the two highest levorotations observed, namely, those of the products obtained by the action of hydrochloric acid upon a mixture of *allo*- and *epi-allo*-cholesterol and by the pyrolysis of cholesteryl methyl

xanthogenate, leads to the indication that the products (including 3,5-cholestadiene) obtained by other methods contain impurities which could not be removed by the procedure employed, although the rotations observed are higher than previously reported.

Bromine titration indicated the presence of one double bond and titration with perbenzoic acid indicated two double bonds in both 3,5-cholestadiene and cholesterilene obtained by the copper sulfate method. Chromium trioxide oxidation of these two compounds yielded oxycholestenone (cholestene-4-dione-3,6) which was isolated as the monophenylhydrazone from both reaction products.

"7-Dehydrocholestene isomer" has been found[4] to have a slight positive rotation and not to form a normal maleic anhydride addition product. It was believed[5] to be identical with 4,6-cholestadiene on the basis that 4,6-cholestadiene should also have a positive rotation due to the 4,5-double bond. However, it is possible that the double bonds could be located in rings B and C or B and D as well as in rings A and B. This view has been suggested recently by Heilbron, Shaw and Spring.[6]

Bromine titration indicated one double bond and perbenzoic acid titration indicated two double bonds in both 4,6-cholestadiene and "7-dehydrocholestene isomer." Both compounds on catalytic hydrogenation were found to yield cholestane and coprostane although in different proportions and both were unaffected by sodium–alcohol. 4,6-Cholestadiene was found not to give a normal maleic anhydride addition product. The melting points and specific rotations were found to be different for 4,6-cholestadiene (m. p. 84–85°, $(\alpha)^{28}D$ +45.77) and a depression in melting point was found for a mixture (m. p. 76–79°) of the two compounds. 4,6-Cholestadiene is indicated not to be identical with "7-dehydrocholestene isomer" since the optical rotation of 4,6-cholestadiene continued to become more dextrorotatory to a constant value as the melting point was raised to a constant value during its purification and since the optical rotation of "7-dehydrocholestene isomer" is less dextrorotatory but has a higher melting point than 4,6-cholestadiene.

## Experimental

**3,5-Cholestadiene.**—A mixture of 11.7 g. of pseudocholestene dibromide[8] (m. p. 116–117°) and 100 cc. of quinoline[11] was refluxed slowly for two hours, during which time a deep red color developed. A solution of the reaction product dissolved in 200 cc. of ether was extracted with 3 *N* hydrochloric acid to remove the quinoline, washed with water until free from acid, dried over anhydrous sodium sulfate and concentrated *in vacuo*. An alcohol solution of the thick viscous residue was treated with decolorizing carbon and the 4.76 g. of fairly pure needles, which separated when the solution was cooled, on purification melted at 75.9–80°; $(\alpha)^{25}D$ −103.24 and $n^{25}D$ 1.45974 (*c*, 3.00 in $CCl_4$).[12] The melting point and optical rotation were unaffected by further purification. No depression in mixed melting point was obtained with 3,5-cholestadiene and cholesterilene obtained by each of the described methods. Anhydrous potassium acetate was found to be less efficient than quinoline for this reaction.

*Anal.* Calcd. for $C_{27}H_{44}$: C, 87.96; H, 12.04. Found: C, 87.47, 87.61; H, 12.06, 12.08.

**4,6-Cholestadiene.**—4,6-Cholestadiene was prepared in a similar manner by heating 6 g. of β-cholestene dibromide[13] (m. p. 105–106°) with 54 cc. of dry quinoline and also from α-cholestene dibromide (m. p. 140–141°) and was found to melt at 84–85°; $(\alpha)^{28}D$ +45.77 (*c*, 2.98 in carbon tetrachloride). Further recrystallization from acetone–methanol, ether–alcohol and ether–methanol did not affect the melting point or optical rotation.

*Anal.* Calcd. for $C_{27}H_{44}$: C, 87.96; H, 12.04. Found: C, 87.77, 87.54; H, 12.19, 12.21.

Cholesterilene was prepared by four methods. An intimate mixture of 25 g. of dry cholesterol and 25 g. of anhydrous copper sulfate (preheated until colorless) was heated at 200° for twenty minutes according to the method of Mauthner and Suida[14] and the 15 g. of product on purification melted at 79.5–80°; $(\alpha)^{25}D$ −104.91 and $n^{25}D$ 1.45974 (*c*, 3.00 in carbon tetrachloride). Without separation of the two isomers, a solution of 1 g. of a mixture of *allo*- and *epi-allo*-cholesterol dissolved in 60 cc. of 95% alcohol containing 8 drops of concentrated hydrochloric acid was refluxed for two hours according to the method of Schoenheimer and Evans[9] and the 0.8 g. of product on purification melted at 79.5–80°; $(\alpha)^{25}D$ −123.23 and $n^{25}D$ 1.45974 (*c*, 3.00 in carbon tetrachloride). A solution of 12 g. of cholesteryl chloride dissolved in 80 cc. of quinoline was refluxed slowly for two hours according to the method of Mauthner and Suida[10] and the 7.4 g. of product on purification melted at 79.5–80°; $(\alpha)^{25}D$ −100.33° and $n^{25}D$ 1.45974 (*c*, 2.99 in carbon tetrachloride). Ten grams of cholesteryl methyl xanthogenate was heated at 200–205° under the vacuum[15] of a water pump for forty-five minutes similar to the method of Tschugaeff and Gasteff[16] and the 6 g. of product on purification melted at 79.5–80°; $(\alpha)^{25}D$ −123.23° and $n^{25}D$ 1.45974 (*c*, 3.00 in carbon tetrachloride). The melting points and optical rotations were not affected by further purification.

(11) Dried over sodium hydroxide and redistilled.

(12) $n^{25}D$ 1.45625 for the carbon tetrachloride used.

(13) Mauthner and Suida, *Monatsh.*, **15**, 85 (1894).

(14) Mauthner and Suida, *ibid.*, **17**, 29 (1896).

(15) The pyrolytic decomposition of cholesteryl methyl xanthogenate gave a better product when carried out under reduced pressure.

(16) Tschugaeff and Gasteff, *Ber.*, **42**, 4631 (1909).

**"7-Dehydrocholestene Isomer."**—7-Hydroxycholestene was dehydrated by means or alcoholic hydrochloric acid according to the method used in dehydrating *allo-epi-allo*-cholesterol and the product (m. p. 91–92°, $(\alpha)^{24}D$ +4.27° (*c*, 3.6 in carbon tetrachloride)) gave no depression in mixed melting point with "7-dehydrocholestene isomer" obtained by the method of Dimroth and Trautmann.[4] A depression of melting point to 76–79° was obtained for a mixture of "7-dehydrocholestene isomer" and 4,6-cholestadiene.

*Anal.* Calcd. for $C_{27}H_{44}$: C, 87.96; H, 12.04. Found: C, 87.83, 87.63; H, 12.15, 12.12.

**Titration with Bromine.**—A carbon tetrachloride solution of 0.1 g. of the compound was titrated with a 0.2186 *N* carbon tetrachloride solution of bromine. 3,5-Cholestadiene, 4,6-cholestadiene, cholesterilene (copper sulfate) and "7-dehydrocholestene isomer" required 2.50, 2.81, 2.73 and 2.72 cc. of the bromine solution which corresponds to 1.01, 1.13, 1.09 and 1.09 double bonds, respectively.

**Titration with Perbenzoic Acid.**—Seven cubic centimeters (7 cc. of a chloroform solution of perbenzoic acid containing approximately twice the theoretical amount of oxygen required by 0.1 g. of cholestadiene) was added to 0.1 g. of the compound in a 50-cc. Erlenmeyer flask. The samples together with blanks were kept at 0° for eight days and then the excess oxygen was titrated with 0.0940 *N* sodium thiosulfate after the addition of a solution of potassium iodide. 3,5-Cholestadiene, 4,6-cholestadiene, cholesterilene (copper sulfate) and "7-dehydrocholestene isomer" absorbed 8.26, 7.47, 8.17 and 8.35 mg. of oxygen which corresponds to 1.89, 1.71, 1.87 and 1.91 double bonds, respectively.

**Oxidation of Cholesterilene and 3,5-Cholestadiene.**—Chromium trioxide oxidation of 3,5-cholestadiene was carried out according to the method of Fantl[17] and the monophenylhydrazone of oxycholestenone obtained was recrystallized from chloroform–ethyl alcohol until it melted at 270–271°. The same procedure was followed for the oxidation of 3,5-cholestadiene and the phenylhydrazone of the neutral oxidation product was found to melt at 270–271° and to give no depression in mixed melting point with the monophenylhydrazone of oxycholestenone obtained from cholesterilene.

**Reduction of 4,6-Cholestadiene and "7-Dehydrocholestene Isomer."**—A solution of 0.43 g. of 4,6-cholestadiene dissolved in 40 cc. of ethyl acetate was shaken overnight with hydrogen under a pressure of 20 pounds (1.3 atm.) in the presence of 0.1 g. of palladium oxide catalyst. The reduction product was filtered and the solvent was removed under reduced pressure. The residue was dissolved in carbon tetrachloride and the solution was treated with acetic anhydride and sulfuric acid according to the method of Anderson and Nabenhauer[18] to remove traces of unreduced material. The solvent was removed under reduced pressure and the residue was treated with decolorizing carbon in alcohol solution. By means of repeated fractional crystallization cholestane, m. p. 79–80°, in the form of plates and coprostane, m. p. 68–69°, in the form of needles were obtained. The proportion of cholestane to coprostane isolated was 1.78 to 1. A solution of 0.75 g. of "7-dehydrocholestene isomer" dissolved in 50 cc. of ethyl acetate was hydrogenated catalytically and the reduction product was purified by the same procedure. The proportion of cholestane to coprostane isolated was 0.9 to 1.

4,6-Cholestadiene and "7-dehydrocholestene isomer" were recovered unchanged following the treatment of a solution of 0.3 g. of each compound dissolved in 40 cc. of *t*-amyl alcohol with 6 g. of sodium during a period of nine hours as shown by melting point, mixed melting point and optical rotation determinations. 4,6-Cholestadiene was recovered unchanged after refluxing a solution of 0.1 g. dissolved in 15 cc. of anhydrous benzene with 0.047 g. of maleic anhydride for twelve hours and subsequent hydrolysis. 3,5- and 4,6-Cholestadienes were also recovered unchanged after refluxing a solution of 0.1 g. dissolved in 15 cc. of 95% alcohol containing 0.2 cc. of concentrated hydrochloric acid for thirty hours.

## Summary

3,5- and 4,6-Cholestadienes were prepared by convenient methods and some of their physical and chemical properties were determined. Experimental evidence was obtained to indicate that 3,5-cholestadiene is identical with cholesterilene and that 4,6-cholestadiene is not identical with the cholestadiene, $C_{27}H_{44}$, m. p. 91°, obtained by Dimroth and Trautmann which was referred to as "7-dehydrocholestene isomer."

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(17) Fantl, *Monatsh.*, **47**, 251 (1926).

(18) Anderson and Nabenhauer, THIS JOURNAL, **46**, 1959 (1924).