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PREPARATION OF CHOLESTERILENE AND VARIOUS CHOLESTADIENES

by

Ralph Lawrence Van Peursem

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I. INTRODUCTION

The name cholesterilene refers to a compound prepared by the removal of one molecule of water from a molecule of cholesterol either directly by the use of various dehydrating reagents or indirectly from various cholesterol derivatives. Since the cholesterol molecule already contains one double bond, the introduction of a second double bond, by the removal of the hydroxyl group and a hydrogen atom from an adjacent carbon atom, should result in the formation of a cholestadiene. The double bond in cholesterol is considered to be in the 5,6-position (ring B) and, if no shift in its position takes place during dehydration, one of the double bonds in cholesterilene would occupy this same position. Likewise, the hydroxyl group in cholesterol is located on carbon atom number 3 (ring A) and it thus becomes evident that the second double bond might be formed in either the 2,3- or the 3,4-position. Disregarding rearrangements, cholesterilene should then be either 2,5- or 3,5-cholestadiene.

Since sterols and their derivatives are all optically active, their specific optical rotations are a property of unusual interest; this is especially true in the case of cholesterilene. However, the comparatively close agreement observed in the melting points of cholesterilene prepared by the different methods, is conspicuously absent in the specific rotations.

The questions which quite naturally arise in the mind of anyone reviewing this rather complicated situation, are (a) does the term

cholesterilene apply to a single compound of definite spacial configuration and (b) what is the structure of cholesterilene. Any explanation offered in answer to these questions must explain, among other things, the comparatively wide variation in the specific optical rotations of cholesterilene prepared by the various methods.

It was the purpose of this investigation to compare the physical properties of cholesterilene prepared by several different methods, under identical conditions. It was also desired to prepare several cholestadienes and to compare the physical properties of these cholestadienes with the physical properties of cholesterilene.

II. HISTORICAL

A. Nomenclature

This discussion is concerned with all hydrocarbons which have been obtained by the dehydration of cholesterol either directly by the action of various reagents on cholesterol or indirectly from various cholesterol derivatives. A number of products has been obtained by these methods and the description of them in the literature has been confused because of the variety of terms used in their designation. An obsolete name for a compound is indicated as cholesterilene ("α-cholesterilene") and an alternative name for a compound is given as pseudo-cholestene (coprostene).

1. Nomenclature of the dehydration products of cholesterol.

The names, a-cholesterilene, b-cholesterilene, and c-cholesterilene were given to the three compounds which were isolated from the reaction product, obtained by the action of sulfuric acid on cholesterol. The two products produced by the action of phosphoric acid on cholesterol were known as a-cholesteron and b-cholesteron. Cholesterilene (sometimes spelled "cholesterylene") is the name applied to the product which has been produced from cholesterol by three general types of reactions: (a) by the action of various dehydrating agents on cholesterol, (b) by the removal of hydrogen halide from a cholesteryl halide, and (c) by the

pyrolytic decomposition of various cholesteryl esters. Dicholesteryl ether is the ether obtained by the removal of one molecule of water from two molecules of cholesterol.

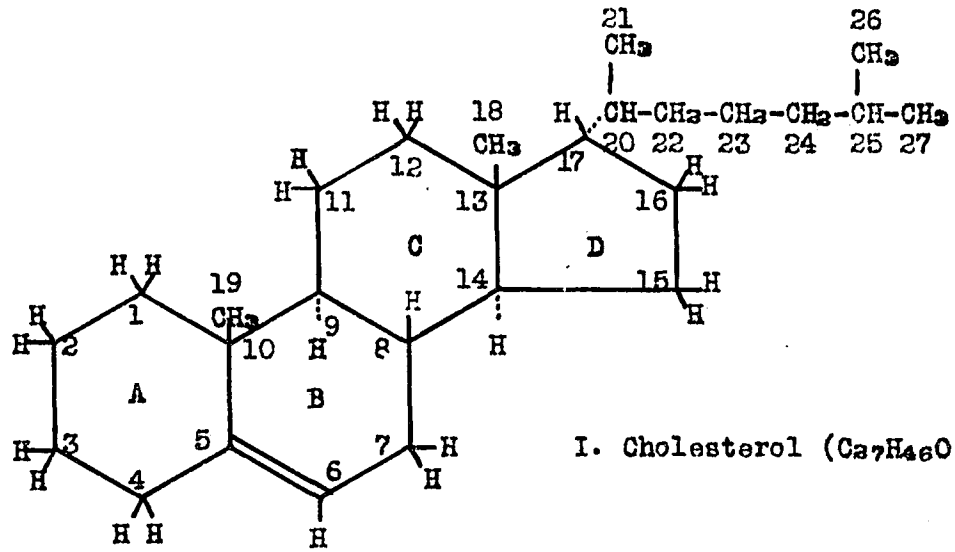
2. Nomenclature of cholesterol derivatives.

Cholesterol (I) is an unsaturated alcohol which is a derivative of the saturated hydrocarbon known as cholestane (II). Unsaturated hydrocarbon derivatives of cholestane which contained one double bond are known as cholestenes and those hydrocarbon derivatives which contain two double bonds are called cholestadienes. The prefix allo designates that rings A and B are trans to each other as in cholestane (79); allocholesterol (IV) is a misnomer (60). Epicholesterol is the epimer of cholesterol which has the hydroxyl group on carbon atom number 3 trans (60) to the methyl group attached to carbon atom number 10. The spacial structural configurations for cholesterol and typical cholesterol derivatives are shown in Figure 1.

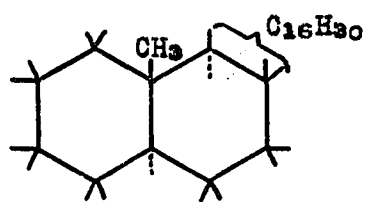
B. Methods of Preparation

1. a-, b-, and c-Cholesterilene.

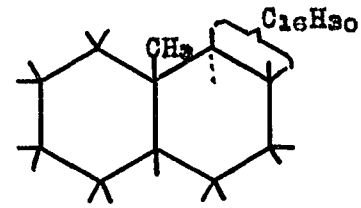
In 1848 Zwenger (84) treated cholesterol with sulfuric acid and obtained a mixture of three hydrocarbons, which he called respectively: a-cholesterilene, b-cholesterilene, and c-cholesterilene. Concentrated sulfuric acid, diluted with one-half volume of water, was allowed to react with cholesterol at a temperature of 60-70° until the cholesterol had lost its



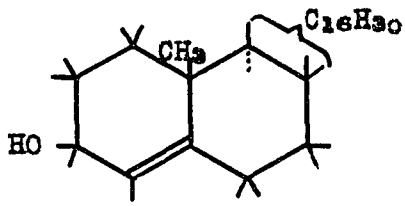
I. Cholesterol ($C_{27}H_{46}O$)



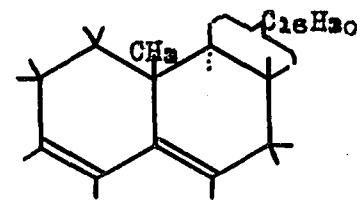
II. Cholestane



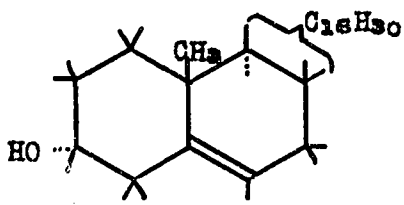
III. Coprostane



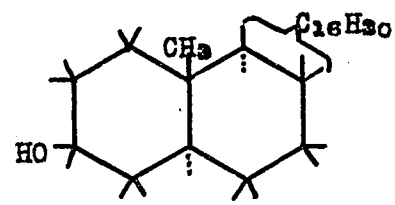
IV. Allocholesterol



V. 3,5-Cholestadiene



VI. Epicholesterol



VII. Dihydrocholesterol

FIGURE 1.

crystalline character. The red color of the product was destroyed by the addition of water and the residue left after extraction with ether was called a-cholesterilene. It crystallized in fine needles and melted at a temperature of about 240°. When alcohol was added to the ether extract, b-cholesterilene, m.p. 255°, and c-cholesterilene, m.p. 127°, were precipitated. These two compounds were separated by fractional crystallization from ether. The former crystallized in small plates while the latter could not be obtained in a definite crystalline form.

Mauthner and Suida (38) investigated the products obtained by Zwenger (84) by the action of sulfuric acid on cholesterol. They found that the c-cholesterilene constituted the major portion of the product, a-cholesterilene a much smaller portion, and b-cholesterilene was produced only in traces. They suggested the possibility that, beside a simple removal of water from cholesterol, a polymerization or rearrangement had taken place. Therefore, they determined the molecular weights of the three compounds. From these data, the authors concluded that c-cholesterilene was a dimer of cholesterilene. In order to study a-cholesterilene further, it was prepared by the action of sulfuric acid on cholesterilene, which had been prepared by the action of anhydrous copper sulfate on cholesterol. The principal product was c-cholesterilene in addition to a compound which was similar in properties to a-cholesterilene. No mention was made that b-cholesterilene was formed by the reaction. The following melting point behavior was described for a-cholesterilene: sintered at 210-20°, transparent at about 235°, and fluid at 260°.

Additional methods by which a-cholesterilene, m.p. 344° (bloc Macquenne), has been prepared (16) are recorded in Table I.

Table I.

Various Methods of Preparation of a-Cholesterilene (16).

Reagents	Temp. in °C.	Time
Cholesterol + sulfuric acid in acetic acid	85-90	3 hours
Cholesterilene + sulfuric acid in acetic acid	85-90	3 hours
Cholesteryl acetate + sulfuric acid in acetic acid	85-90	3 hours
Butyl cholesteryl ether + sulfuric acid in acetic acid	85-90	3 hours
Cholesterol + sulfuric acid + acetic anhydride + acetic acid	25	8 days
Cholesterol + sulfoacetic in acetic acid	85-90	3 hours
Cholesterol + zinc chloride in acetic acid	85-90	3 hours
Cholesterol + zinc chloride + acetyl chloride in acetic acid	85-90	3 hours
Cholesterilene + zinc chloride + acetyl chloride in acetic acid	85-90	3 hours
Cholesterilene + trichloroacetic acid	25	6 hours
Cholesterol + phosphorous pentoxide in benzene	80	2 hours
Cholesterilene + phosphorous pentoxide in benzene	80	2 hours

2. a- and b-Cholesteron.

Zwenger (85) obtained a mixture of two hydrocarbons by the action of six to eight parts of concentrated phosphoric acid on one part of cholesterol at a temperature near the melting point of cholesterol. The product was washed free from acid with water, the residue was extracted with hot

ethyl alcohol. On cooling, the alcohol extract yielded rhombic prisms, m.p. 68°, which the author called a-cholesteron. The alcohol insoluble residue was recrystallized from ether and yielded a compound, b-cholesteron, which crystallized in very fine needles, m.p. 175°. In this case, as in the case of a-, b-, and c-cholesterilene, very little evidence was produced concerning the constitution of the products except to show by analysis that they were hydrocarbons.

Mauthner and Suida (38) observed a melting point of 79.5-80.5° for a-cholesteron after repeated recrystallization from alcohol and a melting point of 192° (indefinite) for b-cholesteron. It was observed that the properties of a-cholesteron were identical with those of cholesterilene, ("cholestene"), prepared by Walitzky (72) by the action of sodium ethoxide on cholesteryl chloride. By a comparison of their physical properties, the possibility was suggested that b-cholesteron was identical with dicholesteryl ether (prepared by the action of copper sulfate on cholesterol). Stavely and Bergmann (61) considered that the compound, m.p. 79-80.5°, which was formed by the action of phosphoric acid on cholesterol, was cholesterilene.

3. Cholesterilene.

a. Direct dehydration of cholesterol and allocholesterol. A compound ("cholestene") m.p. 68°, was obtained (72) when cholesterol was treated with aqueous hydriodic acid (sp. g. =1.5) or with iodine and phosphorous. It was suggested that this was an impure sample of the hydrocarbon ("cholestene"), m.p. 80°, prepared by the action of sodium ethoxide on cholesteryl chloride.

Walitzky (73) heated cholesterol with sodium at 150-5° for a long time and obtained a slightly yellow powder ("cholestene"), which was insoluble in alcohol and completely soluble in ether. It resembled the c-cholesterilene obtained by Zwenger by the action of sulfuric acid on cholesterol and the compound ("cholestene") obtained by the action of hydriodic acid on cholesterol (72). All three compounds showed the same solubility behavior, softened at 68°, and were transformed at 100° into a thick, viscous resin. The compound, obtained by heating cholesterol with zinc chloride or with soda lime at 250° or with a mixture of calcium oxide and potassium hydroxide, also seemed to be identical with these three hydrocarbons.

Liebermann (28) obtained a compound, by the treatment of cholesterol with hydriodic acid (sp.g.=1.7) and phosphorous in a tube at 230°, which appeared to be identical with the compound ("cholestene"), m.p. 68°, obtained (72) by the action of hydriodic acid (sp.g.=1.5) on cholesterol.

In an attempt to originate a method of preparation by direct dehydration which would produce a larger yield of cholesterilene, Mauthner and Suida (38) studied the action of anhydrous copper sulfate on cholesterol. They heated dry cholesterol with an equal weight of anhydrous copper sulfate at 200° for fifteen minutes. The resulting mixture was extracted with benzene, the benzene solution was concentrated, and the residue was extracted with alcohol. The material which was insoluble in alcohol, was shown to be dicholesteryl ether (softened at 188° and melted at 195°). The alcohol extract yielded long needles which, when repeatedly recrystallized from alcohol and ether-alcohol, melted at 79-80° (60-70 per cent yield).

Steinkopf, Winternitz, Roederer, and Wolynski (64) heated an intimate mixture of cholesterol and kieselguhr in a retort under the vacuum of a water pump at 300° for 4-5 hours; 24 grams of distillate were obtained from 40 grams of cholesterol. This material was pressed on clay and was found to melt at 79°.

The use of fossil flour for the preparation of cholesterilene from cholesterol was applied by de Fazi (20). When cholesterol was heated with fossil flour in a vacuum at 300°, a small yield of cholesterilene, m.p. 78-9°, and some pseudo-cholestene (coprostene or cholestene-4) and pseudo-cholestane (coprostane) were obtained. Cholesterilene, pseudo-cholestene, and pseudo-cholestane were also obtained when cholesterol and copper sulfide were heated at 250°.

By distillation of cholesterol with eight parts of zinc dust in a stream of hydrogen above 200° and at 10 mm. pressure, a yield of 26-30 per cent of a product, which melted at 68°, was obtained by Fantl (19). It was repeatedly recrystallized from alcohol and ether-alcohol until it melted at 75°; the optical rotation, however, varied during the purification process.

Montignie (42) heated a mixture of cholesterol, phosphoric acid, and acetic acid for two hours. The liquid developed an amber color and a solid separated on cooling. The solid was removed by filtration and the filtrate was diluted by the addition of water. A pale yellow compound separated which melted at 79-80°; a yield of 0.40 grams was obtained from two grams of cholesterol. A mixed melting point of this compound with pure α-cholesterilene showed no depression. The solid which remained on

the filter was washed with water to remove the acid and dried at 80-90°. When recrystallized from absolute alcohol, it yielded a crystalline compound which melted at 112°. This compound was boiled for two hours with 10 per cent potassium hydroxide solution and produced a compound which melted at 94.5° and was called a cholesterol isomer. When this isomer of cholesterol was boiled with alcoholic potassium hydroxide, cholesterol was regenerated.

Montignie (43) also heated cholesterol at 150-60° for 25 minutes with phosphoric acid anhydride. The black reaction product was washed with water to remove all traces of acid and dissolved in alcohol. The alcohol was removed by evaporation on a water bath and the residue was treated with a mixture of equal parts of alcohol and benzene. Cholesterilene, m.p. 79-80°, remained undissolved. When allowed to evaporate at room temperature, the solution deposited fine needles, which melted at 66-7° and which formed a bromine addition compound, m.p. 61-2°.

Montignie (44) also investigated the action of perchloric acid on cholesterol. When a mixture of cholesterol and perchloric acid was maintained at a temperature of 100° for two hours, it yielded a mixture of cholesterilene, m.p. 79-80°, and a compound which was insoluble in ether and which Montignie maintained was dicholesteryl ether, m.p. 193°. This compound yielded a bromide, m.p. 164-6°.

Bose and Doran (5) showed that anhydrous sodium sulfate did not dehydrate cholesterol when heated together for fifteen minutes at 210-15° and that anhydrous potassium sulfate, under the same conditions, produced dicholesteryl ether in high yield. Cholesterilene was satisfactorily

prepared by the copper sulfate method (38) but a cleaner product was obtained by heating the reactants at 200° under reduced pressure (0.5 mm.). When Montignie (45) heated cholesterol with an equimolar quantity of mercuric iodide at 145-50° for one-half hour, he obtained cholesterilene, m.p. 79-80°, and dicholesteryl ether, m.p. 188-90°.

Cholesterilene and dicholesteryl ether were prepared by Fischer and Treibs (22) who passed a lively stream of hydrogen chloride into molten cholesterol, maintained at 180-90°, for one to two hours. Dicholesteryl ether was easily separated from the product whereas cholesterilene could hardly be separated from impurities. Dicholesteryl ether was obtained by recrystallization from ether and cholesterilene was obtained by the addition of alcohol to the ether solution.

The action of hydrochloric acid on cholesterol was studied by Minovici (41). An alcohol solution of cholesterol was refluxed for 16 hours, during which time concentrated hydrochloric acid was added in small portions. The yellow oil which separated was recrystallized from 80 per cent alcohol and yielded a compound, m.p. 74.5° which was called a cholesterol ether and which added bromine to form a viscous product. It was shown that this compound was not identical with the dicholesteryl ether which was produced by the action of copper sulfate on cholesterol (38) or by the action of zinc oxide on cholesteryl chloride (63). A compound m.p. 74.5°, which was similar to the compound obtained by the action of hydrochloric acid on cholesterol was separated from the reaction product obtained by heat treatment of a suspension of cholesterol in dilute sulfuric acid (41).

A mixture of cholesterol and epicholesterol was obtained by Marker, Kamm, Oakwood, and Laucius (31) who passed oxygen into an ether solution of cholesteryl magnesium chloride at 0°C. The epicholesterol was separated from cholesterol by the fractional crystallization of their benzoates. After saponification and recrystallization from alcohol, the epicholesterol melted at 141.5°. When it was refluxed with dilute alcoholic hydrochloric acid for sixteen hours, it yielded an insoluble oil. This oil was sublimed under a high vacuum and then recrystallized from alcohol which yielded cholesterilene, m.p. 76-7°. No depression in melting point was observed when it was mixed with cholesterilene, prepared by the action of quinoline on cholesteryl chloride.

Schoenheimer and Evans (60) reduced cholestenone with aluminum isopropoxide and obtained a mixture of allocholesterol and epiallocholesterol. The two sterols were obtained as a molecular compound, m.p. 141°. They were separated from each other by the addition of digitonin; the allocholesterol was precipitated. Epiallocholesterol, m.p. 84°, was recovered from the filtrate of the digitonin precipitation and produced an acetate, which melted at 82.5°. Each of these two sterols, when refluxed with dilute alcoholic hydrochloric acid, lost water readily to form cholesterilene, m.p. 79°. These investigators assumed that their compound was 2,4-cholestadiene, which compound would be the logical dehydration product of allocholesterol or epiallocholesterol.

b. Removal of hydrogen halide from cholesteryl halide. Cholesterilene, m.p. 79°, was obtained by distillation of cholesteryl chloride under the vacuum of a water pump (64). The fraction which boiled at 257-67° under

12 mm. pressure was collected and recrystallized.

Walitsky (72) found that it was not possible to convert cholesteryl chloride into the acetate by heating it with potassium acetate, sodium acetate, or silver acetate, either in alcohol or acetic acid solution under various conditions of temperature, pressure, and concentration. The chloride either remained unchanged or lost chlorine and yielded a resin, which was easily soluble in ether. It exhibited a similar behavior with alkali, sodium sulfite, potassium cyanate, potassium cyanide, mercuric cyanide, and silver cyanide. When cholesteryl chloride was heated with sodium ethoxide, a hydrocarbon ("cholestene") was formed, which was sparingly soluble in alcohol, more readily soluble in ether, and crystallized in needles, which melted at 80°.

Mauthner and Suida (38) experienced considerable difficulty in preparing cholesterilene by the action of sodium ethoxide on cholesteryl chloride, as described by Walitsky (72). It was found necessary to use an excess of sodium ethoxide and to heat the reactants in a pressure tube. Even then, the removal of hydrogen chloride was not complete. The melting point of the purified cholesterilene was 79-80°.

Mauthner and Suida (40) prepared cholesterilene from cholesteryl chloride by removing a molecule of hydrogen chloride. In one case, a mixture of cholesteryl chloride with eight parts of calcium oxide was distilled over a bare flame and the distillate, which soon solidified, was recrystallized from ether-alcohol to obtain cholesterilene, m.p. 79°. Cholesterilene was also prepared by the treatment of cholesteryl chloride with quinoline. Three grams of cholesteryl chloride and 20 cc. of quinoline were slowly

boiled for one hour. The mixture was cooled and shaken with dilute hydrochloric acid and ether. The ether solution was again shaken with hydrochloric acid, treated with bone black, and the ether was removed by evaporation. Further purification proceeded best by careful treatment of the ether solution with methyl alcohol. The substance was obtained in beautiful needles, m.p. 77°. The variation in the optical rotations of cholesterilene prepared by the different methods was explained by the suggestion that several isomeric compounds were produced by the various reactions.

Pirrone (49) refluxed cholesteryl chloride and ammonia in ethyl alcohol for three hours. Upon evaporation of the alcohol, cholesterilene, m.p. 79-80°, was obtained. Several crystalline "cholesterilenes", which melted between 82° and 256°, were obtained by Diels and Abderhalden (11) by the treatment of cholesteryl chloride with ammonia in ethyl or methyl alcohol.

A compound, probably cholesterilene (whose bromine derivative had the same melting point as the tetrabromide of dicholesteryl ether), was obtained from one of the products which resulted by the action of phosphorous pentachloride on cholesterol. Pirrone (49) treated cholesterol with solid phosphorous pentachloride for 15 minutes and obtained cholesteryl chloride, "4-dichlorocholestene" (probably 3,3' -dichlorocholestene 4), and an unidentified yellow resin. "4-Dichlorocholestene" and sodium ethoxide were refluxed in absolute alcohol for two hours, the alcohol was evaporated, and the residue was recrystallized from ether-alcohol to obtain a compound, m.p. 78-9°, whose bromine derivative melted at 162-4°.

When cholesteryl chloride was heated with potassium cholesteroxide for twenty-four hours in a bomb at 120-40°, Steinkopf and Blümmer (63) obtained

cholesterilene which, when recrystallized from ether-alcohol, melted at 79-80°. These same authors also obtained cholesterilene, m.p. 79-80° when they heated cholesteryl chloride with zinc oxide at a temperature above 140°. When recrystallized from ether-alcohol and alcohol, it melted at 79-80°. A small amount of a compound, which melted above 200°, also resulted from this reaction. Cholesterilene was also obtained when cholesteryl chloride was heated with zinc dust.

Lindenmeyer (30) obtained a compound which melted at 71° by treatment of cholesteryl chloride with sodium cholesteroxide at 100°. A compound (63), m.p. 78°, originally considered (48) to be benzyl cholesteryl ether, was obtained by the action of benzyl chloride on sodium cholesteroxide.

de Fazi, Monforte, and Pirrone (21) obtained cholesterilene when they heated a dry mixture of cholesteryl chloride and silver chloride at 250-60° for one hour. Beside cholesterilene, a resin was recovered. When they heated a mixture of cholesteryl chloride and cuprous chloride for two hours at 240-60°, the authors obtained cholesterilene, a compound which melted at 124° which was not studied further, unchanged cholesteryl chloride, and a large quantity of a resinous substance.

Wagner-Jauregg and Werner (71) heated one mole of cholesteryl bromide with two moles of sodium iodide in acetone for sixteen hours in a pressure bomb at 100-25°. When the mixture was cooled, fine needles separated which, on repeated recrystallization from acetone, melted at 77-8° . When cholesteryl bromide and acetone were heated alone in a tube at 100-25° or refluxed with sodium iodide, no change took place but, when cholesteryl bromide, piperidine acetate, sodium iodide, and acetone were heated in a

pressure bomb for sixteen hours at 100-25°, cholesterilene, m.p. 77-80°, was obtained.

c. Pyrolytic decomposition of cholesteryl esters. Tschugaeff and Gasteff (68) prepared cholesterilene from cholesterol by application of the xanthogenate reaction. Cholesterol was treated with potassium tert-amoxide dissolved in toluene. The resulting potassium compound was treated with carbon disulfide and methyl iodide or dimethyl sulfate to obtain cholesteryl methyl xanthogenate, m.p. 126°, which crystallized from toluene in the form of almost colorless needles. When heated to about 200°, the ester was decomposed into methyl mercaptan, carbon oxysulfide, and a hydrocarbon ($C_{27}H_{44}$), m.p. 77°, which crystallized as colorless needles from ether-alcohol.

It was later reported (66,67) that, when cholesteryl methyl xanthogenate was heated in a vacuum at 200°, cholesterilene ("α-cholesterilene"), m.p. 77°, and a compound ("β-cholesterilene"), m.p. 59°, were obtained. These two "isomers" were separated by dissolving the residue left after heating the xanthogenate, which consisted of about two thirds "α-" and one third "β-cholesterilene", in ether and adding alcohol containing alkali. The "α-cholesterilene" crystallized first and the "β-cholesterilene" was recovered from the mother liquors. Addition of "β-cholesterilene" to the "α-cholesterilene" lowered its melting point sharply.

The decomposition of cholesteryl methyl xanthogenate was studied by Bose and Doran (5) who found that the so-called "β-cholesterilene" was not a definite compound and that, when care was taken to eliminate every trace of sulfur from the product, only the hydrocarbon, m.p. 79-80°, could be

isolated. When the directions of Tschugaeff and Gasteff (68) for the preparation of cholesteryl methyl xanthogenate were followed, low yields were repeatedly obtained and cholesterol was always recovered. Methyl amyl ether was invariably detected in the course of working up the final product, which indicated that the potassium amoxide had methylated during the second stage of the process. The authors described a method for preparing cholesteryl methyl xanthogenate which considerably shortened the time required for the reaction, eliminated the use of amyl alcohol, and produced high yields of pure colorless ester. In order to convert this ester to cholesterilene, it was heated to 200° under reduced pressure for at least one half hour.

Bloch (3) found that, when cholesteryl phenyl urethane was heated in a closed tube at 350°, it decomposed into aniline, cholesterol, and cholesterilene, needles, which melted at 75.5°. It was suggested that this cholesterilene was similar to, but probably not identical with, that prepared by the copper sulfate method (38), which Bloch found to melt at 74°.

Fischer and Treibs (22) prepared cholesterilene by the distillation of cholesteryl oleate under reduced pressure. Oleic acid distilled at 225-35° under 13 mm. pressure and the oil, which distilled at 260°, yielded needles, m.p. 79°, when recrystallized from ether-alcohol (50 per cent yield). When mixed with cholesterilene, prepared by the copper sulfate method, no depression of the melting point was observed.

Müller and Page (46) prepared monocholesterylphosphoric acid, m.p. 193-3.5°, according to the method of von Euler, Wolf, and Hellstrom (18). A solution of cholesterol in pyridine was added to a solution of an

equivalent amount of phosphorous oxychloride in acetone. They also prepared dicholesterylphosphoric acid, m.p. 203-3.5°, by a combination of the methods of von Euler and Bernton (17) and von Euler, Wolf, and Hellström (18). An equivalent amount of phosphorous oxychloride was added to a solution of cholesterol in pyridine. When monocholesterylphosphoric acid was melted, it decomposed and produced dicholesteryl ether, cholesterilene, and probably phosphoric acid. When dicholesterylphosphoric acid was heated for a few seconds at its melting point, cholesterilene and probably phosphoric acid were obtained, but no dicholesteryl ether was isolated. (When dicholesterylphosphoric acid was heated at 225° for ten minutes, the product was found to be antirachitic (15).) When monocholesterylphosphoric acid was saponified in propyl alcohol, which contained sulfuric acid (10 per cent by volume), a substance was obtained which melted at about 320°. The possibility was suggested that this substance was a dimeric cholesterilene. It was considered probable that it was formed from the propyl ether because, if the reaction was stoppered when the solution began to cloud, the propyl ether could be isolated. The longer the heating was continued after the appearance of this turbidity, the smaller was the amount of ether that could be recovered. The same compound was also obtained when cholesterol was heated in propyl alcohol which contained 10 per cent by volume of concentrated sulfuric acid. On analysis, it yielded 87.40 per cent of carbon and 12.12 per cent of hydrogen and two molecular weight determinations (Rast) yielded 771.4 and 736.7.

4. Cholestadienes.

a. 2,4-Cholestadiene. Stavely and Bergmann (62) prepared a hydrocarbon which they called 2,4-cholestadiene. Three parts of aluminum oxide and two parts of cholesterol were intimately mixed and then heated in a retort at a temperature of 200-20° for two hours at 1 mm. pressure. The temperature was then raised to 240-70° and the product was slowly distilled; a yield of 30 per cent was obtained. After repeated recrystallization from ether and acetone, the compound melted at 63°. Since the double bond in cholesterol is located at the 5,6-position, a shift in its position must occur in order to obtain 2,4-cholestadiene. If the heating at 200 was carried out for seven hours and the product distilled as before, a laevorotatory hydrocarbon, m.p. 72-4°, $(\alpha)_D^{23} = -56.5$, was obtained.

b. 3,5-Cholestadiene. Stavely and Bergmann (61) preapred a 3,5-cholestadiene by the following series of reactions. Cholesteryl acetate was oxidized with chromium trioxide to yield 7-ketocholesteryl acetate. This was refluxed with dilute alcoholic hydrochloric acid giving 7-keto-3,5-cholestadiene. This was then converted into the semicarbazone, which was heated in a sealed tube with sodium and absolute alcohol for eight hours at 200°. After repeated recrystallization from ether-alcohol or ether-acetone, a small yield of 3,5-cholestadiene, m.p. 78-9°, was obtained.

c. 5,7-Cholestadiene. Dimroth and Trautmann (12) reduced 7-keto-cholestene with aluminum isopropoxide and obtained 7-hydroxycholestene in the form of hydrated plates, which sintered at 83° and melted at 93-4°.

This 7-hydroxycholestene was then converted, by heating with acetic anhydride, into a hydrocarbon (7-dehydrocholestene isomer), m.p. 91°, which showed a maximum in its absorption curve at 238 mu and an extinction coefficient of 3.1 $mm.^{-1}$ in a 0.02 per cent solution in ether. When the benzoate of 7-hydroxycholestene was heated in small portions for forty hours at 120-5° in a high vacuum, it yielded 5,7-cholestadiene (7-dehydrocholestene), m.p. 88-9°. This compound was not identical with the hydrocarbon, m.p. 91°, since the mixture of the two depressed the melting point of 5,7-cholestadiene by six degrees. If the thermal decomposition of the benzoate of 7-hydroxycholestene was effected at a higher temperature, or in the vacuum of an oil or water pump, the hydrocarbon, m.p. 91°, was obtained, together with some unchanged benzoate.

C. Chemical and Physical Properties

1. a-, b-, and c-Cholesterilene.

The a-cholesterilene, which was obtained by the action of sulfuric acid on cholesterol (84), was decomposed by chlorine at ordinary temperature. Concentrated sulfuric acid combined with it to form a soft, brown-red resin. This combination was easily decomposed by ether or water and, if the sulfuric acid was not allowed to react for too long a time, the a-cholesterilene was almost completely recovered. It was attacked only slightly by ordinary nitric acid but more readily by fuming nitric acid. The end-product of this reaction seemed to be principally the acid derivative discovered by Redtenbacher (50). The behavior of b-cholesterilene

toward acids was similar to that of a-cholesterilene. Sulfuric acid converted it into a dark red, resinous, easily-decomposable mass and the sulfuric acid solution was colored Guignet's green (which later changed to dark green) by reflected light and a dark red color by transmitted light. a-Cholesterilene dissolved slightly in sulfuric acid, but the color of the sulfuric acid solution was a dirty, dark green by reflected light and a dark brown by transmitted light. c-Cholesterilene reacted with chlorine, nitric acid, and sulfuric acid in a manner similar to that exhibited by a- and b-cholesterilene.

2. a- and b-Cholesteron.

The a-cholesteron, which Zwenger (85) obtained by the action of phosphoric acid on cholesterol, was readily attacked by chlorine, which caused the liberation of hydrogen chloride. It was oxidized by nitric acid in a manner similar to a-cholesterilene and became red in color by the action of sulfuric acid. The b-cholesteron reacted with chlorine, nitric acid, and sulfuric acid in a manner similar to that described for a-cholesteron.

3. Cholesterilene.

a. Reduction. Windaus (76) treated a boiling absolute alcohol solution of cholesterilene with sodium. When the reaction mixture was worked up in the usual manner, only cholesterilene was recovered. The conclusion was reached that cholesterilene did not possess a conjugated system of double bonds. When sodium was added to a boiling amyl alcohol solution of cholesterilene (19) (prepared by the zinc dust distillation of cholesterol), the original material was recovered unchanged.

The hydrocarbon obtained by Schoenheimer and Evans (60) was also investigated by Stavely and Bergmann (61). It was recovered unchanged after it had been dissolved in boiling amyl alcohol and treated with sodium over a period of four hours. Catalytic hydrogenation with platinum yielded approximately 85 per cent cholestane (m.p. 76-8°, $(\alpha)_D^{20}$ + 25.5°) and 15 per cent pseudo-cholestane (m.p. 64-6°, $(\alpha)_D^{21}$ + 24.6°).

When "α-" and "β-cholesterilene" were subjected to catalytic hydrogenation with powdered platinum in ether solution (66,67), only cholestane, m.p. 79°, was recovered. The results obtained by Bose and Doran (5) indicated the identity of the hydrocarbons prepared by the heating of cholesterol with copper sulfate and by the decomposition of cholesteryl methyl xanthogenate because each yielded a mixture of cholestane and pseudo-cholestane when hydrogenated under exactly identical conditions.

Cholesterilene (b.p. 246-56°/11 mm., $(\alpha)_D^{15}$ 41.00°) was reduced by Nord (47) by hydrogen in acetone solution in the presence of (a) colloidal palladium and (b) colloidal platinum until no more of the gas was absorbed. Cholestane (b.p. 269-71°/12 mm., $(\alpha)_D^{15}$ + 43.32°) was the only reduction product recovered.

Windaus (78) showed that, when cholesterilene (prepared by the copper sulfate method) was catalytically reduced, it absorbed two moles of hydrogen. He showed further that pseudo-cholestane, as well as cholestane, was produced by this reduction. He suggested the possibility that cholesterilene consisted of several isomers, which only slightly influenced each other's melting points and formed mixed crystals with one another. He also pointed out that these isomers could not be "α-" and "β-cholesterilene",

since a mixed melting point of these two compounds caused a marked depression to result. He further pointed out that the variation in optical rotation could not result from a partial racemization, since the products of their catalytic hydrogenation possessed the proper optical rotations.

Steinkopf, Winternitz, Roederer, and Wolynaski (64) reduced cholesterilene (prepared by the distillation of cholesterol in the presence of kieselguhr) with hydrogen in the presence of platinum black and obtained pseudo-cholestane (m.p. 71°, $(\alpha)_D$ + 25.46 (c=3.283)($CHCl_3$) (50 mm. tube) which was identical with pseudo-cholestane, prepared by treating pseudo-cholestene with hydrogen in the presence of platinum black (36).

Cholesterilene (prepared by the zinc dust distillation of cholesterol) was reduced (19) by treatment in ether solution with hydrogen in the presence of palladium black. By repeated recrystallization of the product from alcohol, cholestane, obtained as plates which melted at 80°, and a compound, which melted at 61°, were isolated. When the latter compound was mixed with pseudo-cholestane, m.p. 68°, no depression of the melting point was observed.

Catalytic hydrogenation of cholesterilene (prepared by the copper sulfate method) in ethyl acetate (61) with platinum oxide yielded 80 per cent of cholestane (m.p. 78-9°, $(\alpha)_D^{28}$ + 22.5°) and 20 per cent of pseudo-cholestane (m.p. 58-60°, $(\alpha)_D^{28}$ + 25.9°).

Doree and Petrow (13) heated cholesterilene with selenium for 155 hours at 230-50°. A yield of 25 per cent of cholestane (m.p. 80°, $(\alpha)_D^{18}$ + 30.2°) was obtained but no pseudo-cholestane could be recovered from the mother liquors.

b. Reaction with bromine and iodine. Walitzky (73) stated that the c-cholesterilene obtained by Zwenger by the action of sulfuric acid on cholesterol and the compound ("cholestene") obtained by the action of hydriodic acid (sp. g. = 1.6) on cholesterol as well as the compound (cholesterilene or "cholestene") obtained by the action of sodium on cholesterol all yielded the same bromine compound, $C_{26}H_{34}Br_8$, and at the same time evolved hydrogen bromide, when treated with an excess of bromine.

Mauthner and Suida (38) believed that cholesterilene possessed one double bond since it added only one mole of bromine. However, Windaus (76) considered it likely that cholesterilene contained two double bonds which absorbed bromine by 1,4-addition and left a double bond which was inactive to the addition of more bromine.

When cholesterilene (prepared by the copper sulfate method) was dissolved in chloroform or carbon disulfide and treated with bromine dissolved in the same solvent, the bromine solution was immediately decolorized (39). In spite of repeated attempts, it was not possible to recover a crystalline bromine addition product. Quantitative tests showed that one mole of cholesterilene absorbed one mole of bromine. Upon the addition of more bromine, decolorization no longer took place and hydrogen bromide was produced. It was, likewise, shown (68) that cholesterilene (prepared by the pyrolytic decomposition of cholesteryl methyl xanthogenate), when dissolved in carbon tetrachloride, absorbed bromine. Montignie (42) also reported that cholesterilene (prepared by the treatment of cholesterol with acetic and phosphoric acids) added bromine in chloroform solution.

The cholesterilene (prepared by the distillation of cholesteryl oleate

(under reduced pressure) was shown to add bromine but it was not found possible to isolate a crystalline bromide (22). Both "α-" and "β-cholesterilenes" (prepared from cholesteryl methyl xanthogenate) were found to discolor a bromine solution (66,67).

Mention has been made (31) of a tetrabromocholestane, which was obtained by the addition of bromine to cholesterilene (prepared by the action of alcoholic hydrochloric acid on epicholesterol). The properties of the compound, however, were not described and none of the details of its preparation were given except that the bromine derivative was different from the tetrabromocholestane, m.p. 110°, obtained by the action of bromine on epicholesteryl acetate.

When a solution of bromine in glacial acetic acid was added to a solution of cholesterilene (prepared by the copper sulfate method) in ether (61), decolorization took place until exactly one mole of bromine had been absorbed but a crystalline addition product could not be isolated. When two moles of bromine were added in a similar manner, a brown liquid was obtained, which did not yield crystalline bromine derivatives, even when allowed to stand for a long time at low temperatures. When attempts were made to recover cholesterilene from the bromides by treatment with sodium iodide, only a black, tarry material was obtained.

The iodine values: 70.9, 70.0, and 72.2, obtained for cholesterilene (prepared by the copper sulfate method) nearly correspond to that required for one double bond per molecule (38). Cholesterilene, prepared from monocholesterylphosphoric acid, gave an iodine number of 73.36 and cholesterilene, prepared from dicholesterylphosphoric acid, gave an iodine

number of 77.87 (46).

c. Reaction with maleic anhydride. Wagner-Jauregg and Werner (71) reported that they were not able to produce a maleic anhydride addition compound from their cholesterilene (cholestadiene)(prepared from cholesteryl bromide and sodium iodide) in boiling toluene. Similar results were obtained (61) when cholesterilene (prepared by the copper sulfate method) and maleic anhydride were refluxed in benzene for several hours. When the mixture was dissolved in xylene and heated in a sealed tube at 135° for 12 hours, an amorphous material was obtained, which decomposed at 240-5° and which gave the proper analysis for $C_{31}H_{48}O_4$. When the hydrocarbon (obtained by refluxing a mixture of allocholesterol and epiallocholesterol with alcoholic hydrochloric acid) was treated with maleic anhydride in xylene in the manner just described, an acid resulted which decomposed at 240-5° and which, in all respects, behaved similarly to the acid obtained from cholesterilene and maleic anhydride.

d. Oxidation. Both "α-cholesterilene" and "β-cholesterilene" (produced from cholesteryl methyl xanthogenate) reacted with potassium permanganate (66,67). When cholesterilene was oxidized with chromic acid (39), it yielded acids with 27 carbon atoms. Alkaline permanganate was slightly reduced when it was boiled with cholesterilene (39).

Windaus (76) reported that, when cholesterilene was oxidized with chromic acid, a yield of 5 per cent of oxycholestenone (cholestene-4, dione-3,6) was obtained. It was identified by means of its melting point and by the preparation of its phenylhydrazone. To explain this behavior, it was suggested that cholesterilene, under the influence of the sulfuric

acid in the chromic acid mixture, was transformed back into cholesterol and that the cholesterol, by oxidation, yielded oxycholestenone. It was shown, however, that when a chloroform solution of cholesterilene was shaken with 10 per cent sulfuric acid for six days, it remained unchanged and did not take up any water.

A benzene-acetic acid solution of cholesterilene (prepared by the zinc dust distillation of cholesterol) was oxidized (19) by shaking, for six hours, with a solution of chromic acid in dilute sulfuric acid. The oxidation products were separated into an acid and a neutral fraction. The acid fraction was dissolved in ammonia and, after removal of the excess ammonia, was treated with copper sulfate. It yielded a dark green, amorphous copper salt which gave the analysis: C,65.5; H,9.17; and Cu, 8.05 per cent. When it was treated with phenylhydrazine, the neutral fraction yielded a compound which melted at 271°. A mixed melting point with the phenylhydrazone of cholestene-4,dione-3,6 showed no depression.

Titration of the compound (obtained from a mixture of allo- and epi-allocholesterol by the action of alcoholic hydrochloric acid) with perbenzoic acid (60) required 1.39 and 1.49 moles after 24 hours; 1.56 and 1.66 moles after 48 hours; and 1.63 and 1.74 moles after 82 hours. A similar titration of cholesterilene (prepared by the copper sulfate method) with perbenzoic acid (61) gave 1.97 moles after 48 hours.

e. Color reactions. Both "α-cholesterilene" and "β-cholesterilene" (prepared from cholesteryl methyl xanthogenate) gave positive reactions to the characteristic color tests for cholesterol (66,67). The cholesterilene (obtained by heating cholesterol with mercuric iodide) was found to disply

a positive Liebermann reaction (45).

Mauthner and Suida (38) observed the behavior of cholesterilene (prepared by the copper sulfate method) toward various reagents. Concentrated sulfuric acid produced a brownish-yellow solution, with a strong green fluorescence. Nitric acid colored the crystals at first a slight rose color, which later changed to a cherry red color. This red color was destroyed by the addition of sodium nitrite to produce a yellow resin.

Bloch (3) showed that cholesterilene (prepared by the pyrolytic decomposition of cholesteryl phenyl urethane) displayed a citron-yellow color with sulfuric acid and exhibited intense Hesse (27), Salkowski (57), and Liebermann (28) color reactions.

Positive reactions were reported (68) for cholesterilene (prepared by the pyrolytic decomposition of cholesteryl methyl xanthogenate) when subjected to the following cholesterol color reactions: a blood-red color with sulfuric acid in chloroform solution (Salkowski reaction (57)), a red to violet to blue color with sulfuric acid and acetic anhydride in chloroform solution (Liebermann-Burchard reaction (6)), and a rose-red color with a beautiful green fluorescence when heated with acetyl chloride and zinc chloride in acetic acid solution (Tschugajew reaction (69)).

Montignie (42) reported that cholesterilene (prepared by the action of acetic and phosphoric acids on cholesterol) gave a cherry-red color with nitric acid and a yellow solution with a green fluorescence with sulfuric acid.

Schoenheimer and Evans (60) reported that their hydrocarbon (obtained

by the treatment of allo- and epiallocholesterol with alcoholic hydrochloric acid), which they called 2,4-cholestadiene but which was later shown (61) to be cholesterilene, gave an immediate and intense red color with 90 per cent trichloroacetic acid (Rosenheim reaction)(52). They obtained the same red color with cholesterilene (prepared by the action of anhydrous copper sulfate on cholesterol). After they had tested a number of sterols and sterol derivatives, they came to the conclusion that the Rosenheim reaction was given by those sterols possessing a conjugated double bond system or by those capable of forming such a system by the action of the reagent. These investigators also obtained a positive Salkowski reaction with their compound.

It has been observed (15) that an ether solution of cholesterilene displayed a yellow to light brown to brownish red color with a green fluorescence when it was treated with hydrogen chloride.

Cholesterilene (m.p. 78-9°, $(\alpha)_D^{20} = 102.1°$) gave positive reactions to the following sterol color tests (82): Salkowski (56), Rosenheim and Drummond (53), Carr and Price (9), Liebermann (28), Lifschütz (29), Rosenheim (51), Whitby A and B (74), Tschugajew (69), and Steinle and Kahlenburg (65). When cholesterilene was irradiated until its characteristic absorption bands disappeared and then subjected to these color tests, very little difference in its behavior was observed. Whitby (74) suggested that, in the typical color tests for cholesterol, cholesterilene was first formed and that it then coupled with a second substance, for example, formaldehyde, to give the colored product. However, Wokes (82) was not able to obtain any colors by the treatment of cholesterilene with formaldehyde

alone (either as formalin solution or in the nascent condition).

f. Miscellaneous reactions. The unsaturated nature of "α-cholesterilene" and "β-cholesterilene" was demonstrated by the reaction with tetranitromethane (66,67). Both compounds produced an intense brown-red color, whereas cholesterol and cholestene gave only a slight yellow color under the same conditions.

Cholesterilene (prepared by the zinc dust distillation of cholesterol) was dissolved in benzene and shaken with a mixture of acetic acid, water, and concentrated sulfuric acid for six hours (19). After removal of the benzene, an alcoholic solution of the product was treated with a similar solution of digitonin and yielded a digitonide, which decomposed above 240°. The authors concluded that water had added to the cholesterilene and that cholesterol had been formed in small quantities. It was likewise found (42) that when cholesterilene was boiled for three hours with alcholic sulfuric acid, it yielded a compound, m.p. 145°, which was identified as cholesterol by means of its melting point, optical rotation, and bromine derivative.

The thermal decomposition of cholesterilene (prepared by the copper sulfate method), in the presence of aluminum chloride, was studied by Zelinski and Semiganovski (83). When heated with aluminum chloride at atmospheric pressure, cholesterilene yielded fractions corresponding to those obtained from cholesterol. When the same reaction was carried out at a pressure of 16 mm., the distillate was freed from the lighter fractions and compounds were obtained, all of which were optically active, practically non-fluorescent and, as shown by the iodine numbers, almost completely saturated.

Cholesterilene was found to be converted into an antirachitic product when it was treated with various reagents but not by ultra-violet irradiation (14,15).

Windaus and Kuhr (80) studied the action of sulfuric acid and acetic anhydride on cholesterilene. Cholesterilene was added to a mixture of acetic anhydride and concentrated sulfuric acid, cooled in ice, and the mixture was allowed to stand at 20° for four hours. When dilute methyl alcohol was added to the filtered reaction product and the mixture concentrated in vacuo, a product was obtained which was soluble in water as well as in ether. This material was esterified with diazo-methane in ether solution and yielded the methyl ester of cholesterilene sulfonic acid, m.p. 175-6°. Upon saponification of this compound in methyl alcohol solutions of potassium, sodium, and lithium hydroxides, the corresponding alkali salts were precipitated. The lithium salt turned brown at 220°.

g. Optical rotations. The methods of preparation and physical properties of the hydrocarbons, which have been obtained by the dehydration of cholesterol either directly by the action of various reagents on cholesterol or indirectly from various cholesterol derivatives, are summarized in Table II.

The physical properties of a number of derivatives, some of which have been mentioned in this discussion, are listed in Table III. It is of interest to note that those derivatives, which possess a double bond located at the 4,5-position exhibit a positive optical rotation while those, which possess a double bond located at the 5,6-position, exhibit a negative optical rotation. In this connection, Mauthner (34) determined the effect

Physical Properties of Cholesterilene and Cholestadienes

Compound	Method of Preparation	M.P. in °C	Optical Rotation $(\alpha)_D$	Temp. in C	c or (p)	Solvent	Reference	Remarks
a-Cholesterilene	Cholesterol + H_2SO_4	240					84	Formula: $C_{27}H_{42}$
"	"	210-60					38	Mol. wt: 482
"	see Table I	344*					16	*bloc Macquenne
b-Cholesterilene	Cholesterol + H_2SO_4	255					84	Formula: $C_{27}H_{42}$
c-Cholesterilene	"	127					84	Formula: $C_{27}H_{42}$
"	"						38	Mol. wt: 772
a-Cholesteron	Cholesterol + H_3PO_4	68					85	
"	"	79-5-80.5					38	(Considered identical with cholesterilene (61))
b-Cholesteron	"	175					85	
"	"	192					38	Suggested identical with dicholesteryl ether
Cholesterilene	Cholesterol + HI	68					72	("Cholestene")
"	Cholesterol + Na	68					73	("Cholestene")
"	Cholesterol + HI + P_4	68					28	("Cholestene")
"	Cholesterol + $CuSO_4$	79-80					38	
"	"	79-80	-81.63				40	
"	"	74	-71.88	18	0.626	C_6H_6	3	
"	"	78	-102.1	20	4.000	$CHCl_3$	24	
"	"	78-9	-97.5	20	0.9	$CHCl_3$	61	
"	Cholesterol + kieselguhr	79	-46.6		4.504	$C_6H_5CH_3$	64	
"	Cholesterol + fossil flour	78-9					20	
"	Cholesterol + zinc dust	68	+1.45	26	2.75		19	Constants during purification
		73	-53.37	22	2.08			
		75	-4.49	20	2.34			
"	Cholesterol + H_3PO_4 in HOAc	79-80					42	
"	Cholesterol + P_2O_5	79-80					43	
"	Cholesterol + $HClO_4$	79-80					44	
"	Cholesterol + HgI_2	79-80					45	
"	Cholesterol + HCl	74.5					41	
"	Epicholesterol + HCl	76-7	-78.3	30	(1)	C_6H_6	31	
"	Allocholesterol + HCl	79	-112.5	23	(2)	C_6H_6	60	
"	Epiallocholesterol + HCl	79					60	
"	Cholesteryl chloride distilled	79	-47.04				64	
"	Cholesteryl chloride + CaO	79	-61.55					

"	:Cholesterol + HgI_2	:79-80	:	:	:	:	45	:
"	:Cholesterol + HCl	: 74.5	:	:	:	:	41	:
"	:Epicholesterol + HCl	:76-7	: -78.3	: 30	: (1)	: C_6H_6	: 31	:
"	:Allocholesterol + HCl	: 79	:-112.5	: 23	: (2)	: C_6H_6	: 60	:
"	:Epiallocholesterol + HCl	: 79	:	:	:	:	: 60	:
"	:Cholesteryl chloride : distilled	: 79	: -47.04	:	:	:	: 64	:
"	:Cholesteryl chloride + CaO	: 79	: -61.55	:	:	:	:	:
"	:Cholesteryl chloride + : quinoline	: 77	: -86.09	:	:	:	:	:
"	:Cholesteryl chloride + : $NaOC_2H_5$	: 80	: -65.87	:	:	:	: 40	:("Cholestene")
"	:Cholesteryl chloride + po- :tassium cholesteroxide	:79-80	:	:	:	:	: 63	:
"	:Cholesteryl chloride + :sodium cholesteroxide	: 71	:	:	:	:	: 30	:
"	:Benzyl chloride + sodium :cholesteroxide	: 78	:	:	:	:	: 63	:Originally considered :benzyl cholesteryl :ether
"	:Cholesteryl chloride + ZnO	:79-80	:-116.2	:	: 2.641	: $CHCl_3$	: 63	:
"	:"4-Dichlorocholestene" + : $NaOC_2H_5$	:78-9	:	:	:	:	: 49	:
"	:Cholesteryl bromide + NaI	:77-8	: -65.4	: 19	:	:$C_6H_4CH_3$	: 71	:
"	:Cholesteryl bromide + :piperidine acetate + NaI	:77-80	:-103.0	:	:	:	: 71	:
"	:Cholesteryl methyl : xanthogenate	: 77	:-104	:	: 11	:$C_6H_4CH_3$	: 68	:("α-Cholesterilene")
"	: "	: 77	:-109.3	: 20	:(4.503)	:$C_6H_4CH_3$	:66,67	:("α-Cholesterilene")
"	: "	:79-80	:	:	:	:	: 5	:("α-Cholesterilene")
"	: "	: 59	: -76.68	: 20	:(3.785)	:$C_6H_4CH_3$	:66,67	:("β-Cholesterilene")
"	: "	:79-80	:	:	:	:	: 5	:("β-Cholesterilene") :Identical with :"α-cholesterilene"
"	:Cholesteryl phenylurethane	:75.5	:-100.25	:	: 1.04	: C_6H_6	: 3	:
"	:Cholesteryl oleate	: 79	:	:	:	:	: 22	:
"	:Monocholesterylphosphoric : acid	:76-8	: -68.99	: 20	:	:	: 46	:
"	:Dicholesterylphosphoric : acid	:78.2	: -77.53	: 18	:	:	: 46	:
2,4-Cholestadiene	:Cholesterol + Al_2O_3	: 63	:+114.0	: 27	: 1.523	: $CHCl_3$	: 62	:
3,5-Cholestadiene	:Reduction of 7-keto-3,5- : cholestadiene	:78-9	: -63.75	: 21	: 1.026	: $CHCl_3$	: 61	:
5,7-Cholestadiene	:Dehydration of 7-hydroxy- : cholestene	:88-9	:	:	:	:	: 12	:
7-Dehydrocholes-	:7-Hydroxycholestene + : Ac_2O	: 91	:	:	:	:	: 12	:(Suggested iden- :tical with 4,6-

Table III

Physical Constants of Various Cholesterol Derivatives.

Derivative	M.P. °C.	Optical Rotation $(\alpha)_D$	Temp. °C.	c or (p)	Solvent	Reference
Cholestane	80.5	+23.5		(2)	$CHCl_3$	23
Pseudo-cholestane (coprostane)	69-70	+25.56	22	3.012	$CHCl_3$	36
Neocholestene (cholestene-2)	69	+64.07	20	3.02	$CHCl_3$	36
Pseudo-cholestene (cholestene-4)	78-9	+64.86	23	3.176	$CHCl_3$	35
Cholestene (cholestene-5)	88-90	-55.5		(4)	$CHCl_3$	23
Allocholesterol (cholesten-4-ol-3)	132	+43.7	23	(1)	C_5H_5	60
Epiallocholesterol (epicholesten-4-ol-3)	84	+120.8	24	(2)	C_5H_5	60
Allo- epiallocholesterol	141	+84.1	21	(2)	C_5H_5	60
Cholesterol (cholesten-5-ol-3)	145-6	-37.02	15	(2)	$CHCl_3$	26
Epicholesterol (epicholesten-5-ol-3)	141.5	-35.0	30	(1)	C_5H_5OH	31
γ-Cholestenol (cholesten-7-ol-3)	122-3	0			$CHCl_3$	58
α-Cholestenol (cholesten-8-ol-3)	119-20	+20.36	21	1.08	$CHCl_3$	58
β-Cholestenol (cholesten-14-ol-3)	130-1	+34	22	1.235	$CHCl_3$	58
7-Dehydrocholesterol (cholestadiene-5, 7-ol-3)	142-3.5	-113.6	20	2.455	$CHCl_3$	81
Dehydrocholesterol-B_3 (cholestadiene-7, 14-ol-3)	117-8	-145.5	23	1.58	$CHCl_3$	58
Cholestanone (cholestanone-3)	127-8	+43.7*		(5)	$CHCl_3$	70
Coprostanone (coprostenone-3)	61-2	+36		(3.2)	C_5H_5	55
Cholestenone (cholesten-4-one-3)	79-80	+90.5	18	2.54	$CHCl_3$	4
Δ^5-Cholestenone (cholesten-5-one-3)	127	-4.2	20	0.47	$CHCl_3$	7
Cholesteryl methyl xanthogenate	127	-51.1	25	6.4	$C_6H_4CH_3$	5
Dicholesterylphosphoric acid	203-3.5	-34.5	23		$CHCl_3$	46
Monocholesterylphosphoric acid	193-3.5	-35.64	20		$CHCl_3$	46
Dicholesteryl ether	203-9	-40.8	20	(2)	CCl_4	2

*wave length = 578

of the substitution of various groups and atoms for one another in cholesterol derivatives. As a result of his studies, he reached the following conclusions: Replacement of a hydroxyl group with an acetoxy- group or a chlorine atom produced very little effect on the optical rotation. Addition of chlorine to a double bond produced only a very slight effect. In the case of cholesterol, it produced no change; in the case of the acetate and chloride, a slight increase in negative rotation was observed. Addition of bromine to a double bond produced a very noticeable effect. An increase in negative rotation was observed in each case except for the α-dibromide of cholestene, in which case a high positive rotation resulted. Addition of hydrogen chloride produced a profound effect. Compounds which possessed negative optical rotations were converted into compounds with positive rotations. Addition of oxygen, accompanied by the rupture of a double bond, caused a sharp decrease in the optical rotation to result.

Callow and Young (8) studied the effect of various structural factors on the optical rotatory power of sterols and closely related compounds. In fifteen out of eighteen cases, an increase in dextrorotatory power was produced by the inversion of the hydroxyl group on carbon atom number 3 from the cis to the trans position relative to the methyl group on carbon atom number 10. The effect of inversion of the hydrogen atom on carbon atom number 5 was small and irregular. Inversion of the acetyl group on carbon atom number 17 from the allo to the iso-allo form produced a decrease in dextrotatory power. The introduction of an ethylenic linkage in the 14, 15-position caused an increase in dextrorotation while the introduction of an ethylenic linkage in the 1,2-, 4,5-, 5,6-, and 8,14-positions caused a

decrease in dextrorotation. The effect of the introduction of an ethylenic linkage in the 7,8-position was irregular. Reduction of the keto group at carbon atom number 17 to a carbinol group caused a decrease in dextrotatory power.

h. Absorption spectra. Heilbron, Morton, and Sexton (24) studied the absorption spectra of cholesterilene and several other cholesterol derivatives. Cholesterilene (prepared by the copper sulfate method) (38) was repeatedly recrystallized from ether-alcohol until it melted at 78°. It exhibited maxima at 249, 304, and 312 mu. In comparison with ergosterol, the maximum absorption for each of the three bands of cholesterilene was about 250 Å. units toward the visible. The molecular extinction coefficient of cholesterilene was 2400. On irradiation, the maxima disappeared. In the study of a group of cholesterol derivatives, selective absorption was observed only when at least two double bonds were present in a single molecule. On the basis of a comparison of their absorption spectra, Heilbron and Sexton (25) suggested that one double bond in cholesterilene must occupy the same position as the double bond of pseudo-cholestene (4,5-position).

Rosenheim and King (54) studied the absorption spectrum of cholesterilene. They came to the conclusion that the three maxima observed in the long wave region were due to impurities since their intensities decreased upon purification.

The absorption curves of cholesterilene (prepared by the pyrolytic decomposition of dicholesterylphosphoric acid) were observed (46). In the short wave region, they were found to correspond with the curve for cho-

lesterilene (prepared by the copper sulfate method) published by Heilbron, Morton, and Sexton (24). The high maximum at 240 mu. was always found but, in place of the three maxima in the long wave region, no selective absorption was observed.

Schoenheimer and Evans (60) studied the absorption spectrum of the hydrocarbon which they obtained by the treatment of allo- and epiallocholesterol with alcoholic hydrochloric acid. The absorption spectrum of this compound showed maxima at 229, 235, and 244 mu. Stavely and Bergmann (61) studied the absorption spectrum of cholesterilene (prepared by the copper sulfate method) and found that it had the same typical absorption maxima at 229, 235, and 244 mu. as the hydrocarbon obtained by Schoenheimer and Evans (60), which indicated the identity of these two hydrocarbons.

1. Crystal structure. Mauthner and Suida (38) studied the crystal structure of cholesterilene (prepared by the copper sulfate method). The angles $110:1\bar{1}0$ and $110:\bar{1}10$ measured $100^{\circ}13'$ and $79^{\circ}47'$. When the crystals were assumed to belong to the monoclinic system and the end plane was called 001, the geometrical constants were $a:b:c=1.1341:1:?$, $\beta=131^{\circ}59'40''$. The geometrical and optical properties, however, favored the rhombic crystal structure and the constants according to this system were $a:b:c=0.83597:1:0.75256$. On the basis of the data which were obtained, the authors were not able to definitely classify the crystal structure of cholesterilene.

4. Cholestadienes.

a. 2,4-Cholestadiene. The reactions of 2,4-cholestadiene (62) are very interesting when they are compared with those of cholesterilene.

On catalytic hydrogenation with platinum oxide, it yielded only pseudo-cholestane (m.p. 58-60°, $(\alpha)_D^{29}$ + 27.2°) and no cholestane was isolated. Titration with perbenzoic acid gave a value corresponding to 1.94 double bonds per molecule. When refluxed for eight hours with maleic anhydride in benzene, it yielded a crystalline substance, (m.p. 70-2°, $(\alpha)^{21}$ - 77.8°) (c=0.474) in chloroform (1 dm. tube) and 15 per cent of a crystalline acid, m.p. 268-70 . A much better yield (43 per cent) of this acid was obtained when the reaction was carried out in xylene in a sealed tube at 135°. Attempts to recover the diene from the addition product by distillation under reduced pressures were unsuccessful, due to the fact that the boiling point of the addition product was below its decomposition temperature. The 2,4-cholestadiene was treated with sodium and amyl alcohol. The reduction product was pseudo-cholestene (m.p. 77-8°, $(\alpha)_D^{20}$ + 66.9°). It was further identified by the preparation of its dibromide, m.p. 116-7°. The 2,4-cholestadiene, when refluxed with dilute hydrochloric acid for 26 hours, rearranged to give cholesterilene (m.p. 78°, $(\alpha)_D^{23}$ - 103.8°) (c=0.974) in chloroform (1 dm. tube).

b. <u>7-Dehydrocholestene isomer</u>. Dimroth and Trautmann (12) were not able to produce a maleic anhydride addition compound with their hydrocarbon, m.p. 91°, obtained by the dehydration of 7-hydroxycholestene with acetic anhydride. They concluded, therefore, that the two double bonds were present in different rings.

III. EXPERIMENTAL.

A. Preparation of Cholesterilene.

1. Cholesterol - copper sulfate.

Cholesterilene (m.p. 79.5-80°; $(\alpha)_D^{25}$ - 104.91° and n_D^{25} 1.45974 (c, 3.00 in CCl_4)) was prepared in a 68 per cent yield (13 gm.) by heating an intimate mixture of 20 grams of dry cholesterol and an equal weight of copper sulfate (preheated at 250-60°) at a temperature of 195-200° with constant stirring for 20 minutes, according to the method of Mauthner and Suida (38); a 20 per cent yield of dicholesteryl ether was obtained as a by product. The reaction product was extracted with hot benzene and the residue obtained by concentration of the benzene solution in vacuo was extracted with hot alcohol to separate the cholesterilene from the insoluble dicholesteryl ether. The cholesterilene was purified as presented in Table IV until the melting point, specific optical rotation, and index of refraction were unaffected by further purification. The compound produced no depression in mixed melting point with 3,5-cholestadiene, obtained by the action of quinoline on pseudo-cholestene dibromide.

The cholesterilene was purified by treatment with sodium and amyl alcohol as follows. To a boiling solution of 0.5 gram of cholesterilene dissolved in 70 cc. of n-amyl alcohol was added, over a period of four hours, 7.5 grams of sodium cut into small pieces. The sodium amoxide was

Table IV

Physical Constants of Cholesterilene ($CuSO_4$)

Treatment	M.P. in °C.	c	α obs.	$(\alpha)_D^{25}$	n_D^{25}
Recrystallization from Alcohol five times Treatment with Decolorizing Carbon twice	79-79.5	3.06	-3.02	- 98.62	1.45914
Recrystallization from Alcohol	79-80	3.03	-3.15	-103.88	1.46123
Treatment with Sodium and Amyl Alcohol	79-80	2.95	-3.06	-103.63	1.46123
Treatment with Decolorizing Carbon	79.5-80	2.98	-3.11	-104.27	1.46014
Recrystallization from Alcohol	79.5-80	3.00	-3.11	-103.58	1.45974
Recrystallization from Alcohol	79.5-80	3.01	- 3.14	-104.23	1.45894
Adsorption by Alumina	79.5-80	3.00	-3.15	-104.91	1.45974
Recrystallization from Alcohol	79.5-80	3.00	-3.15	-104.91	1.45974

decomposed by the addition of water and the alcohol layer was separated and dissolved in 50 cc. of ether. The ether solution was washed with water until free from alkali and dried over anhydrous sodium sulfate. The residue obtained by concentration of this solution in vacuo was recrystallized from ethyl alcohol.

The cholesterilene was purified by adsorption of impurities on activated alumina as follows. A solution of 0.5 gram of cholesterilene in 15 cc. of benzene-petroleum ether (1:1 by volume) was passed through a column (7 mm. in diameter x 70 mm. long) of activated alumina (ground to 50 mesh and preheated at 200° for 2 hours). The solvent was removed in vacuo and the residue was recrystallized from ethyl alcohol.

2. Cholesterol - potassium acid sulfate.

Cholesterilene and dicholesteryl ether were prepared by the action of freshly fused potassium acid sulfate on cholesterol (15) under the various conditions presented in Table V. The reaction product was extracted with ether and the ether extract was filtered and concentrated in vacuo. The residue was extracted with hot alcohol to separate the cholesterilene from the insoluble dicholesteryl ether.

Table V

Effect of Potassium Acid Sulfate on Cholesterol under Various Conditions

Cholesterol gm.	Potassium acid sulfate	Temp. in °C.	Time in min.	Cholesterilene gm.	Dicholesteryl ether
10	10	210-15	10	1.0*	4.0
10	10	210-15	15	4.2	1.4
10	10	210-15	20	4.0	1.5
10	10	205-10	15	5.0	1.6
10	15	205-10	15	trace	0
10	7.5	205-10	15	1.0*	4.5

*semi-crystalline.

The cholesterilene (m.p., 79.5-80°; $(\alpha)_D^{25}$-103.91 and n_D^{25} 1.45974 (c, 3.00 in CCl_4)) prepared by this method was purified as described for cholesterilene ($CuSO_4$) until the melting point, specific optical rotation, and index of refraction were unaffected by further purification.

Cholesterilene was prepared by the action of potassium acid sulfate on dicholesteryl ether. Five grams of dicholesteryl ether was heated with 5 gm. of freshly fused potassium acid sulfate at 205-10° for ten minutes and 0.7 gm. of cholesterilene and 2.5 gm. of dicholesteryl ether were recovered from the reaction product. When 5 gm. of dicholesteryl ether was heated with an equal weight of potassium acid sulfate at 210-15° for ten minutes, a trace of cholesterilene and 1.9 gm. of dicholesteryl ether were obtained. When 5 gm. of dicholesteryl ether and 5 gm. of potassium acid

sulfate were heated at 210-15° for fifteen minutes, the reaction product yielded a trace of cholesterilene and no dicholesteryl ether. In each case, some uncrystallizable oils were obtained.

3. Allo- and epiallocholesterol - hydrochloric acid.

Cholesterol was converted into cholesterol dibromide by the action of 4 cc. of bromine dissolved in 125 cc. of glacial acetic acid on 25 gm. of cholesterol dissolved in 250 cc. of anhydrous ether, according to the directions of Windaus (75). Without recrystallization, the cholesterol dibromide was oxidized, by treatment with chromium trioxide for 12 hours according to the method of Schoenheimer (59), to cholestenone (m.p., 79-80°, $(\alpha)_D^{20}$ + 79.2° (c, 3.00 in CCl_4)) in an over-all yield from cholesterol of 67 per cent. Ten grams of cholestenone were converted in 54 per cent yield into a mixture of allo- and epiallocholesterol (m.p., 140-1°) by the action of alumimum isopropoxide (60), prepared from 1.5 gm. of aluminum (1).

Without separation of the two isomers, the mixture of allo- and epiallocholesterol was dehydrated in 84 per cent yield to cholesterilene by means of alcoholic hydrochloric acid, according to the method of Schoenheimer and Evans (60). The reaction mixture, obtained by heating 1 gm. of allo- and epiallocholesterol with 8 drops of concentrated hydrochloric acid dissolved in 60 cc. of 95 per cent alcohol for 2 hours, was filtered and allowed to cool slowly to crystallize the cholesterilene.

The cholesterilene was purified further by repeated recrystallization from ethyl alcohol, by treatment with sodium and amyl alcohol, and by adsorption of impurities by activated alumina as described for cholesterilene

($CuSO_4$) until the physical constants (m.p., 79.5-80°; $(\alpha)_D^{25}$ - 123.23° and n_D^{25} 1.45974 (c, 3.00 in CCl_4)) were unaffected by further purification. The compound showed no depression in mixed melting point with 3,5-cholestadiene.

4. Cholesteryl chloride - quinoline.

Cholesteryl chloride (m.p., 95°, $(\alpha)_D^{20}$ - 28.7°(c, 300 in CCl_4)) was prepared in 87 per cent yield by heating a solution of 50 grams of dry cholesterol dissolved in 100 cc. of thionyl chloride (freshly distilled from linseed oil) for one hour in the absence of pyridine, according to the directions of Daughenbaugh and Allison (10), except that the product was removed from its aqueous suspension by filtration instead of by extraction with ether. It was observed that when cholesterol was treated with a commercial unpurified thionyl chloride, trichlorocholestane (m.p., 107-8°, $(\alpha)_D^{25}$ - 35.58°(c, 3.00 in CCl_4)) was obtained which showed no depression in mixed melting point with trichlorocholestane (m.p., 107-8°, $(\alpha)_D^{25}$ - 35.82 (c, 2.98 in CCl_4) prepared by the treatment of cholesteryl chloride with dry chlorine gas in chloroform solutions, according to the method of Mauthner and Suida (37). Mauthner (34) reported a melting point of 106° and a specific rotation of -34.7° for trichlorocholestane.

Anal. Calcd. for $C_{27}H_{43}Cl_3$: Cl, 22.375. Found: Cl, 22.29 and 22.31.

Cholesteryl chloride (m.p., 95-6°, $(\alpha)_D^{20}$ - 28.7°(c, 3.00 in CCl_4)) was also prepared in 83 per cent yield by the action of an equimolar quantity of phosphorous pentachloride on cholesterol at room temperature for 45 minutes, according to the directions of Mauthner and Suida (37).

Cholesterilene was prepared by the action of quinoline on cholesteryl chloride, according to the procedure described by Mauthner and Suida (40). An ether solution of the red-colored reaction product, obtained by slowly refluxing a solution of 12 gm. of cholesteryl chloride dissolved in 80 cc. of quinoline (dried over sodium hydroxide and distilled) for two hours, was washed with 3 N hydrochloric acid and with water, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was treated with decolorizing carbon in alcohol solution and crystallized repeatedly from alcohol.

The cholesterilene was purified further by repeated recrystallization from ethyl alcohol, by treatment with sodium and amyl alcohol, by adsorption of impurities by activated alumina, and by recrystallization from acetone-methanol as described for cholesterilene ($CuSO_4$) until its physical constants (m.p., 79.5-80°; $(\alpha)_D^{25}$ - 100.24° and n_D^{25} 1.45974 (c, 2.99 in CCl_4)) were unaffected by further purification. The compound showed no depression in mixed melting point with 3,5-cholestadiene.

5. Cholesteryl methyl xanthogenate - heated.

Cholesteryl methyl xanthogenate was prepared in 66 per cent yield by a method similar to that employed by Tschugaeff and Gasteff (68). Into a one-liter, round-bottomed flask, fitted with a reflux condenser, were placed 200 cc. of dry benzene, 84 cc. of tert-amyl alcohol, and 4.32 gm. (0.188 mole) of sodium. The mixture was refluxed on a water bath until the sodium had completely reacted (about 8 hours). A solution of 57.9 gm. (0.15 mole) of anhydrous cholesterol in 150 cc. of dry benzene was then

added and the solution was heated for four more hours. The mixture was cooled, 11.28 cc. (0.1865 mole) of carbon disulfide (dried over anhydrous sodium sulfate) was added, and the resulting mixture was heated for two hours. It was then cooled again, 15.39 cc. (0.164 mole) of freshly distilled dimethyl sulfate added, and heated for two more hours. At the end of this time, the reaction mixture was cooled and 100 cc. of ether was added. The ether solution was washed with water, dried over calcium chloride and concentrated. Alcohol was added until the solution was slightly turbid while hot and, when it was slowly cooled, long needles separated which were crystallized from ether-alcohol. A yield of 46.9 gm. of cholesteryl methyl xanthogenate (m.p., 126-7°) was obtained.

Cholesteryl methyl xanthogenate was also prepared in 75.5 per cent yield by a method similar to that originated by Bose and Doran (5). A mixture of 1.95 gm. (0.05 mole) of potassium and 35 cc. of dry benzene were heated in a 500 cc. round-bottomed flask until the potassium was completely melted. The flask was stoppered with a cork and shaken vigorously until the potassium was in a very finely divided state. A solution of 19.3 gm. (0.05 mole) of cholesterol (dried over phosphorous pentoxide) in 35 cc. of dry benzene was added and the resulting mixture refluxed on a water bath for 30 minutes. After it had been cooled to 40°, 12 cc. (0.20 mole) of carbon disulfide (dried over sodium sulfate) and 18.45 cc. (1.98 mole) of freshly-distilled dimethyl sulfate were added and the mixture was refluxed for three hours longer. An ether solution of the cooled product was dried over sodium sulfate and concentrated <u>in vacuo</u> to half its volume. Alcohol was added to the hot solution until it became slightly turbid and, when

this mixture was cooled, the ester separated in long needles, which were recrystallized from ether-alcohol. This method yielded 18 grams of cholesteryl methyl xanthogenate, m.p., 126°.

Cholesterilene was prepared by heating 23.8 grams of cholesteryl methyl xanthogenate, under atmospheric pressure, at a temperature of 200°, according to the method of Tschugaeff and Gasteff (68). The heating was continued at this temperature until all bubbling ceased; the resulting residue was repeatedly recrystallized from ether-alcohol and yielded 9.63 grams of cholesterilene. Cholesterilene was also prepared by heating 10 grams of cholesteryl methyl xanthogenate at a temperature of 200-5° for 45 minutes, under the vacuum of a water pump. Considerable frothing was observed in both this and the previous run. The amount of frothing seemed to be unaffected by the pressure at which the xanthogenate was heated. An alcohol extract of the cooled residue was treated with decolorizing carbon and cooled. A yield of 6 grams of cholesterilene, after repeated recrystallization from alcohol, was obtained.

The two samples of cholesterilene obtained by heating cholesteryl methyl xanthogenate at atmospheric pressure and at reduced pressure were combined and purified further by repeated recrystallization from ethyl alcohol, by treatment with sodium and amyl alcohol, and by adsorption of impurities by activated alumina as described for cholesterilene ($CuSO_4$) until the physical constants (m.p., 79.5-80°; $(\alpha)_D^{25}$ - 123.23° and n_D^{25} 1.45974 (c, 3.00 in CCl_4)) were unaffected by further purification. The compound showed no depression in mixed melting point with 3,5-cholestadiene.

Table VI

Physical Properties of Cholesterilene and 3,5-Cholestadiene

No.	Method of Preparation	M.P. in °C.	c	α observed	$(\alpha)_D^{25}$	n_D^{25}
1	Cholesterol + Copper Sulfate	79.5-80	3.00	-3.15	-104.91	1.45974
2	Cholesterol + Potassium Acid Sulfate	79.5-80	3.00	-3.12	-103.91	1.45974
3	Allo- and Epiallocholesterol + Hydrochloric Acid	79.5-80	3.00	-3.70	-123.23	1.45974
4	Cholesteryl Chloride + Quinoline	79.5-80	2.99	-3.00	-100.24	1.45974
5	Cholesteryl Methyl Xanthogenate - Heated	79.5-80	3.00	-3.70	-123.23	1.45974
6	Pseudo-Cholestene Dibromide + Quinoline	79.5-80	3.00	-3.10	-103.24	1.45974

Note: - All specific optical rotations and indices of refraction in this series of experiments were determined according to the following procedure. A solution was prepared by dissolving approximately 30 mg. of the compound in enough carbon tetrachloride to make 1.0058 cc. of solution. The optical rotations were measured with a Hilger Polarimeter using a 1 dm. micropolarimeter tube. The indices of refraction were measured with a Pulfrich Refractometer which was manufactured by Adam Hilger, Ltd. Both measurements were made at 25° C. using the same solution $n_D^{25} = 1.45625$ for the CCl_4 used. Compound numbers 1 to 5 refer to cholesterilene and number 6 to 3,5-cholestadiene.

B. Preparation of 3,5-Cholestadiene.

Cholestene hydrochloride was prepared in 89 per cent yield by the treatment of a chloroform solution of cholestene with hydrogen chloride gas, according to the directions given by Mauthner (33). A solution of 6 grams of cholestene dissolved in 100 cc. of chloroform (dried over anhydrous sodium sulfate) was saturated with dry hydrogen chloride gas at 0° during a period of three hours and then the solution was allowed to stand stoppered for two days at room temperature. The chloroform was removed by

distillation in a vacuum and 50 cc. of alcohol was added to the oily residue dissolved in 50 cc. of ether. On standing for a short time, plates separated which, after repeated recrystallization from ether-alcohol, did not yield a compound of definite melting point but melted at 80-90°.

Cholestene hydrochloride was likewise prepared in 60.5 per cent yield by treatment of a solution of 5 grams of cholestene dissolved in 80 cc. of anhydrous ether and 80 cc. of absolute alcohol with dry hydrogen chloride gas, according to the directions given by Mauthner (35). The reaction mixture was cooled in an ice-salt mixture and the needles, which were removed by filtration and recrystallized from ether-alcohol, melted at 92-3°.

Pseudo-cholestene was prepared by refluxing 6.25 grams of cholestene hydrochloride (prepared by saturating a solution of cholestene in chloroform with gaseous hydrogen chloride) with 6.25 grams of freshly fused potassium acetate in 150 cc. of absolute alcohol for 15 hours, according to the method of Mauthner (35). During this time, considerable solid potassium chloride separated. The solution was filtered hot and, when it was cooled, yielded pseudo-cholestene in the form of very fine needles. The pseudo-cholestene was recrystallized by the addition of alcohol to a hot ether solution, which had been filtered to remove inorganic salts, until the solution became turbid. When the solution was cooled, it yielded fine needles, which were repeatedly recrystallized from alcohol. A yield of 5.08 grams (89 per cent) of pseudo-cholestene (m.p., 78-9°, $(\alpha)_D^{20}$ + 61.7° (c, 3.00 in CCl_4)) was obtained.

Pseudo-cholestene (m.p., 78-9°) was likewise prepared in 88 per cent yield by refluxing cholestene hydrochloride (prepared from cholestene and gaseous hydrogen chloride in ether-alcohol) with an equal weight of potassium acetate in absolute alcohol for fifteen hours. Salkowski test was negative; after 2 hours, the chloroform layer was slightly fluorescent and the sulfuric acid layer was colorless; and after 15 hours, the chloroform layer had developed a red color and the sulfuric acid layer displayed a green fluorescence.

The dibromide of pseudo-cholestene was prepared by the treatment of an ether solution of pseudo-cholestene with a solution of bromine in glacial acetic acid, according to the directions given by Mauthner (35). To a solution of 10 grams (0.027 mole) of pseudo-cholestene in 80 cc. of anhydrous ether was added 4.32 gm. (0.027 mole) of bromine in glacial acetic acid (solution of 5 gm. of bromine in enough acetic acid to make 50 cc.). When the mixture was allowed to stand for a few minutes, the dibromide separated in the form of needles. The mixture was cooled in ice and yielded more of the dibromide which was recrystallized from ether-alcohol. When allowed to stand longer, the mother liquors turned dark, yellow to green. A yield of 11.8 gm. (82.5 per cent of pseudo-cholestene dibromide (m.p., 116-7°)) was obtained.

3,5-Cholestadiene (m.p., 76-7°, $(\alpha)_D^{20}$ - 70.8° (c, 3.00 in CCl_4)) was prepared by refluxing a mixture of pseudo-cholestene dibromide and quinoline slowly for two hours. A mixture of 11.7 grams of pseudo-cholestene dibromide and 100 cc. of quinoline was refluxed slowly for two hours, during which time the mixture acquired a deep red color. A solution of the

reaction product in 200 cc. of ether was extracted with 3 N hydrochloric acid to remove the excess quinoline, washed with water until free from acid, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. An alcohol solution of the thick, viscous oil which remained was treated with decolorizing carbon and the fine needles, which separated when the solution was cooled, were repeatedly recrystallized from alcohol. A yield of 4.76 gm. (58.8 per cent) of 3,5-cholestadiene was obtained.

The compound was purified further by repeated recrystallization from ethyl alcohol and from acetone-methanol, by treatment with sodium and amyl alcohol, and by adsorption of impurities by activated alumina until the physical constants (m.p., 79.5-80°; $(\alpha)_D^{25}$ - 103.24° and n_D^{25} 1.45974 (c, 3.00 in CCl_4)) were unaffected by further purification. It showed no depression in mixed melting point with cholesterilene prepared by the following methods; (1) cholesterol and copper sulfate, (2) allo- and epiallocholesterol and hydrochloric acid, (3) cholesteryl chloride and quinoline, and (4) cholesteryl methyl xanthogenate heated.

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

An attempt was made to prepare 3,5-cholestadiene by a method similar to that employed for the preparation of pseudo-cholestene. A saturated solution of equal weights of pseudo-cholestene dibromide and freshly fused potassium acetate in absolute alcohol was refluxed for fifteen hours. The product was obtained as an oil which contained organic bromine.

C. Preparation of 4,6-Cholestadiene.

Cholestene was prepared by the reduction of cholesteryl chloride with sodium in tert-amyl alcohol, by a method similar to that employed by Mauthner and Suida (37). Twenty grams (0.0494 mole) of cholesteryl chloride and 400 cc. of tert-amyl alcohol were placed in a 1-liter round-bottomed flask, fitted with a reflux condenser, and the mixture was refluxed very slowly while 20 gm. (0.87 mole) of sodium, cut into small pieces, were added, a little at a time, to the hot mixture until all of the sodium had reacted. The excess tert-amyl alcohol was removed by distillation under the vacuum of a water pump. The residue was cooled and treated with water, while the flask was kept in running water, until all of the sodium tert-amoxide had been decomposed. The resulting mixture was extracted with ether and the ether extract was washed with water until free from alkali. The ether solution was dried over anhydrous sodium sulfate, filtered, and concentrated to a small volume. Alcohol was then added to the hot ether solution which yielded long needles when cooled slowly. A yield of 15.76 gm. (86.1 per cent) of product, melting at 85-90° was obtained by recrystallization from ether-alcohol.

Since the product contained organic chlorine, the above treatment was repeated. Twenty grams of this crude material were treated with 300 cc. of tert-amyl alcohol and 15 gm. of sodium in the same manner as described above. When the product was recrystallized by the slow addition of alcohol to a concentrated solution of it in ether, cholestene (m.p., 91-2°, $(\alpha)_D^{20}$ - 48.3° (c = 3.00 in CCl_4)) separated in the form of long needles. The yield

obtained from this second reduction was 18.43 gm., which represents a recovery of 92.15 per cent. The over-all yield, based on the original weight of cholesteryl chloride, was 79.3 per cent.

When iso-amyl alcohol was substituted for tert-amyl alcohol in the preparation of cholestene, it was found that the decomposition of the sodium iso-amoxide, the complete removal of the excess iso-amyl alcohol, and the subsequent crystallization of the cholestene, were rendered more difficult.

Cholestene was converted into α- and β-dibromides according to the directions of Mauthner and Suida (37). To a solution of 10 gm. (0.027 mole) of cholestene in 75 cc. of chloroform was added slowly from a burette 4.32 gm. (0.027 mole) of bromine dissolved in chloroform. This bromine solution contained 5 grams of bromine dissolved in enough chloroform to make 50 cc. The chloroform was removed and the resulting crystalline residue was recrystallized by the addition of an equal volume of alcohol to an ether solution of the residue. A yield of 11.9 gm. (83.2 per cent) of the β-dibromide, which melted at 105-6°, was obtained. A small amount of the isomeric α-dibromide (m.p., 140°) was obtained when the mother liquors were allowed to stand at room temperature for two days.

4,6-Cholestadiene (m.p., 78-9°, $(\alpha)_D^{20}$ + 10.5° (c, 3.00 in CCl_4)) was prepared by slowly refluxing for two hours a mixture of β-cholestene dibromide and quinoline. Five grams of β-cholestene dibromide and 45 cc. of quinoline were placed in a 200 cc. round-bottomed flask fitted with a reflux condenser, and refluxed slowly for two hours. The solution, at the end of that time, had acquired a deep red color. The mixture was cooled and 100 cc. of ether was added. The ether solution was treated with 3 N

hydrochloric acid to remove the quinoline and washed with water until it was free from acid. It was then dried over anhydrous sodium sulfate, filtered, and the ether was removed *in vacuo*. The resulting oil, after it had been repeatedly recrystallized from methyl alcohol and from ether-methanol, yielded 2.26 gm. (65.1 per cent) of 4,6-cholestadiene in the form of fine needles.

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

An attempt was made to prepare 4,6-cholestadiene by the treatment of cholestene dibromide with potassium acetate. A saturated, absolute alcohol solution of equal weights of cholestene dibromide and freshly fused potassium acetate was slowly refluxed for fifteen hours, during which time considerable solid separated. The solution was filtered and cooled but the product could not be obtained in a crystalline form.

D. Preparation of 7-Dehydrocholestene Isomer.

An average yield of 15 per cent of 7-ketocholestene was obtained by oxidizing cholestene with chromic acid, according to the directions of Windaus (77) except that, after the oxidation, a little alcohol was added and the acetic acid was removed by vacuum distillation before the mixture was extracted with ether. Ten grams (0.027 mole) of cholestene and 500 cc. of glacial acetic acid were heated to a temperature of 80° in a 1-liter, 3-necked, round-bottomed flask, fitted with a mechanical stirrer, thermometer, and a dropping funnel. While the mixture was continuously stirred and

maintained at 80°, a solution of 14 gm. (0.14 mole) of chromium trioxide was added slowly over a period of two hours. The mixture was cooled to 75° and a little alcohol added to destroy the excess chromium trioxide. After the removal of the acetic acid, by distillation under the vacuum of a water pump, the resulting green residue was extracted thoroughly with 200 cc. of ether. The ether extract was washed twice with water and then washed repeatedly with 2 N sodium hydroxide until the green color of the solution had disappeared and a brown layer was no longer formed. It was then washed free from alkali with water and dried over anhydrous sodium sulfate. The ether was removed in vacuo and the resulting yellow oil was dried in a vacuum dessicator over calcium chloride, which caused it to crystallize. This solid was recrystallized from a very small amount of alcohol and yielded 7-ketocholestene, m.p., 125-6°, in the form of needles.

7-Ketocholestene was also obtained in 15.2 per cent yield by the direct ether extraction of the cooled reaction mixture and in 8.9 per cent yield by ether extraction of the oxidation mixture, which was concentrated without the addition of alcohol.

7-Hydroxycholestene was not isolated but 7-dehydrocholestene isomer (12) was obtained in 59.4 per cent yield by the treatment of the aluminum iso-propoxide reduction product of 7-ketocholestene with dilute alcoholic hydrochloric acid. Ten grams of 7-ketocholestene were reduced by the action of aluminum iso-propoxide (50), prepared from 1.5 grams of aluminum (1). Several attempts were made to crystallize the product from ether-methanol but only oils could be obtained.

The crude reduction product was dissolved in 150 cc. of boiling 95

per cent alcohol and 20 drops of concentrated hydrochloric acid were added. The resulting mixture was refluxed for two hours, filtered, and cooled. The product, which separated from the solution, was repeatedly recrystallized from alcohol and obtained in the form of long needles (m.p., 90.5-91.5°, $(\alpha)_D^{20}$ + 3.67° (c, 3.00 in CCl_4)). It gave no depression in mixed melting point with 7-dehydrocholestene isomer obtained by the method of Dimroth and Trautmann (12).

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

E. Oxidation of Cholesterilene with Chromic Acid

Cholesterilene (prepared by the copper sulfate method) was oxidized with chromic acid according to the method of Fantl (19) who isolated the oxidation product, oxycholestenone, in the form of the monophenylhydrazone, from cholesterilene (prepared by the zinc dust distillation of cholesterol). To a cooled solution of 0.25 gram of cholesterilene in 3 cc. of benzene and 1 cc. of glacial acetic acid was added, with cooling and stirring, a solution of 0.35 gram of chromium trioxide dissolved in 2 cc. of dilute sulfuric acid (1 volume of concentrated sulfuric acid diluted with 3 volumes of water). The reaction mixture was shaken at room temperature for 6 hours. Ether was added to the reaction mixture and the ether solution was washed repeatedly with 5 per cent sodium hydroxide and with water until free from alkali, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was dissolved in ethyl alcohol, cooled to room temperature, and

then 1 drop of glacial acetic acid and 1 drop of fresh phenylhydrazine were added. The monophenylhydrazone of oxycholestenone which separated was recrystallized from chloroform-ethyl alcohol until it melted at 270-1°.

3,5-Cholestadiene was oxidized with chromic acid by the same procedure as was followed for cholesterilene and the phenylhydrazone, which was obtained from the neutral fraction of the oxidation product, was found to melt at 270-1° and to give no depression in melting point with the monophenylhydrazone of oxycholestenone, obtained by the oxidation of cholesterilene.

IV. DISCUSSION

Cholesteryl methyl xanthogenate was prepared by two different methods and a comparison was made of the yields which were obtained by these methods. One method involved the reaction of sodium tert-amoxide on cholesterol while the other involved the reaction of powdered potassium on cholesterol. The latter method required less time and also produced a larger yield of product.

Cholesterilene was prepared by heating cholesteryl methyl xanthogenate at atmospheric pressure and also under the vacuum of a water pump. No significant difference in the amount of frothing or in the quality of the products prepared by the two methods was observed.

Cholesterol was heated with potassium acid sulfate under various conditions of temperature, time of reaction, and ratio of the reacting substances. It was observed that, when cholesterol was heated at 210-15° with an equal weight of potassium acid sulfate for ten minutes, the yield of cholesterilene was small and the yield of dicholesteryl ether was comparatively large but, when the heating was continued five minutes longer, the yield of cholesterilene was increased and the yield of dicholesteryl ether was correspondingly decreased. An increase of the time of heating to 20 minutes did not appreciably affect the yields of cholesterilene and dicholesteryl ether. When cholesterol was heated with one and one-half times its weight of potassium acid sulfate at 205-10° for 15 minutes, no dicholesteryl ether and only a trace of cholesterilene were obtained. When

cholesterol was heated with three-fourths its weight of potassium acid sulfate, a considerable quantity of dicholesteryl ether and only a small amount of cholesterilene were obtained. Cholesterilene was obtained by heating dicholesteryl ether with an equal weight of potassium acid sulfate at 205-10° for ten minutes.

4,6-Cholestadiene was prepared by the removal of two molecules of hydrogen bromide from one molecule of cholestene dibromide (5,6-dibromocholestane) by the action of quinoline. It was found to give a positive specific optical rotation, which would indicate that one of the double bonds is in the 4,5-position. Because of the method of preparation, the compound would logically have one double bond in each of rings A and B and the second double bond would be in the 6,7-position, in conjugation with the first. A crystalline product was not obtained when potassium acetate in alcohol solution was used in place of quinoline.

3,5-Cholestadiene was prepared by the removal of two molecules of hydrogen bromide from one molecule of pseudo-cholestene dibromide (4,5-dibromocholestane) by the action of quinoline. The specific optical rotation of this compound was negative, which would indicate that one of the double bonds is in the 5,6-position. Because of the method of preparation, it would logically have one double bond in each of rings A and B and the second double bond would be in the 3,4-position, in conjugation with the first. No depression in mixed melting point was observed when this compound was mixed with cholesterilene prepared by each of the following methods: (1) cholesterol and copper sulfate, (2) allo- and epiallocholesterol and alcoholic hydrochloric acid, (3) cholesteryl chloride and quinoline, and (4) cholesteryl

methyl xanthogenate-heated. When pseudo-cholestene dibromide was treated with potassium acetate in alcohol solution, the removal of hydrogen bromide was incomplete and the product could not be obtained in a crystalline form. 3,5-Cholestadiene was oxidized with chromic acid and the neutral fraction of the oxidation product yielded oxycholestenone, which was isolated as the monophenylhydrazone. Cholesterilene (prepared by the copper sulfate method) was oxidized by the same procedure and the monophenylhydrazone of oxycholestenone which was obtained by treatment of the neutral fraction of the oxidation product with phenylhydrazine was shown by melting point and mixed melting point to be identical with that obtained from the oxidation of 3,5-cholestadiene.

7-Dehydrocholestene isomer was prepared by the action of alcoholic hydrochloric acid on the reduction product obtained by the action of aluminum isopropoxide on 7-ketocholestene. The physical constants of this compound differed from those of 4,6-cholestadiene since the latter melted lower but possessed a more positive specific rotation.

Cholesteryl chloride was prepared from cholesterol by the action of phosphorous pentachloride and of thionyl chloride. The results indicated that the thionyl chloride method produced a larger yield of product. The physical constants confirmed that the products formed by these two methods were identical, thus eliminating the possibility of a Walden Inversion in either case. This is in agreement with the observations of Marker, Whitmore, and Kamm (32). It was observed that cholesteryl chloride could not be prepared when cholesterol was treated with thionyl chloride, as purchased and without purification. Instead, a compound was formed which was shown by

melting point, mixed melting point, optical rotation, and analysis to be trichlorocholestane. When, however, the thionyl chloride was freshly distilled from linseed oil, a satisfactory yield of cholesteryl chloride was obtained. A certain grade of thionyl chloride was unsatisfactory for the conversion of cholesterol into cholesteryl chloride, even after the thionyl chloride had been distilled from linseed oil.

The preparation of cholestene hydrochloride (5-chlorocholestane) by the addition of hydrogen chloride to cholestene in chloroform and in ether-alcohol solution was investigated. The former method produced a larger yield of product which, however, did not possess a definite melting point as did the compound obtained by the addition of hydrogen chloride to cholestene in ether-alcohol solution. These two products were treated with potassium acetate in absolute alcohol and pseudo-cholestene was obtained from both compounds. The yield, however, was much larger in the case of the hydrochloride, which was prepared from cholestene and hydrogen chloride in chloroform. The pseudo-cholestene, prepared from this cholestene hydrochloride required fewer recrystallizations for its purification than the pseudocholestene prepared from the hydrochloride of constant melting point.

Samples of cholesterilene were prepared by five different methods which were representative of the three general methods of preparing this compound which consist of (1) the direct dehydration of cholesterol, allocholesterol or their epimers, of (2) the removal of hydrogen halide from cholesterol halides, and of (3) the pyrolytic decomposition of cholesteryl esters. These samples were purified by repeated recrystallization from different solvents and mixed solvents such as ethyl alcohol and

acetone-methanol, by treatment with decolorizing carbon in alcohol solution, by treatment with sodium and amyl alcohol, and by adsorption of impurities by activated alumina until the physical constants were unaffected by further purification. The melting points, specific optical rotations, and refractive indices of the various purified samples of cholesterilene and of 3,5-cholestadiene were determined under identical conditions. A melting point of 79.5-80° and a refractive index of 1.45974 were obtained for 3,5-cholestadiene and for cholesterilene prepared by the action of copper sulfate on cholesterol, by the action of potassium acid sulfate on cholesterol, by the action of alcoholic hydrochloric acid on a mixture of allo- and epi-allocholesterol, by the action of quinoline on cholesteryl chloride, and by the pyrolytic decomposition of cholesteryl methyl xanthogenate. The observed specific optical rotations (see Table VI) of the different samples of cholesterilene and of 3,5-cholestadiene were found to give higher negative values than those previously reported in the literature (see Table II) which were, however, observed under various conditions. The observed specific optical rotation of cholesterilene, prepared by the action of quinoline on cholesteryl chloride was -100.24° as compared to -86.09° which was previously reported (40) and the value observed for 3,5-cholestadiene was -103.24° while the previously reported value (61) for this compound was -63.57°. The specific optical rotation of 3,5-cholestadiene and that of cholesterilene prepared by the various methods was found to be greater than -100°. It was observed for each sample of cholesterilene and for 3,5-cholestadiene that, during successive stages of purification by the various methods of treatment, as the melting point rose and reached constant value, the

negative value for the specific optical rotation rose and reached a constant value and the index of refraction also became constant. This would indicate that the variation in previously reported melting points and at least a part of the variation in the reported values for the specific optical rotations of cholesterilene when prepared by different methods was due to the presence of impurities which were removed by the various methods of purification.

Various facts reported in the literature which complicate an attempt to determine the structure of cholesterilene are: (a) cholesterilene cannot be reduced with sodium and alcohol, whereas a conjugated double bond system usually adds hydrogen by 1,4-addition; (b) cholesterilene does not yield a normal maleic anhydride addition product--this may be due to the molecular strain expected in the hypothetical addition product when the bonds are in the 3,4- and 5,6-positions which would not be expected with bonds located in the 5,6- and 7,8-positions or the 7,8- and 14,15-positions; (c) cholesterilene adds 1 mole of bromine but does not yield a crystalline bromine derivative; (d) cholesterilene adds 2 moles of hydrogen when catalytically reduced to yield cholestane and pseudo-cholestane which have the proper optical rotations--this observation shows the presence of 2 double bonds and prevents a possible explanation that the variation in optical rotation is due to racemization; and (3) the three maxima in the absorption spectra of cholesterilene have been reported to decrease in intensity upon purification.

V. SUMMARY

3,5- and 4,6-Cholestadienes were prepared by the removal of two molecules of hydrogen bromide from pseudo-cholestene dibromide (4,5-dibromocholestane) and β-cholestene dibromide (5,6-dibromocholestane) respectively. Alcoholic potassium acetate was found to be ineffective for the complete removal of hydrogen bromide but quinoline was found to act satisfactorily for the conversion of the dibromocholestanes to the cholestadienes. The cholestadiene (3,5-cholestadiene) obtained from 4,5-dibromocholestane was found to be laevorotatory, which indicates that one of the double bonds is in the 5,6-position; the other double bond would be in conjugation in the 3,4-position. The cholestadiene (4,6-cholestadiene) obtained from 5,6-dibromocholestane was found to be dextrorotatory, which indicates 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.

3,5-Cholestadiene was purified by various methods until its melting point, specific optical rotation and refractive index were unaffected by further purification. Cholesterilene was prepared by five different methods and the samples of cholesterilene obtained were purified by various methods. The melting points, specific optical rotations and refractive indices observed were compared with those of 3,5-cholestadiene and the melting points and refractive indices of these products were found to be of the same value. The numerical agreement of the two highest laevorotations

observed, namely those of the products obtained by the action of hydrochloric acid upon a mixture of allo- and epiallocholesterol and by the pyrolysis of cholesteryl methyl xanthogenate, leads to the indication that the products (including 3,5-cholestadiene) obtained by other methods still contain impurities which could not be removed by the procedure employed although the specific rotations observed are higher than previously reported. The treatment of cholesterilene and 3,5-cholestadiene with chromium trioxide yielded oxycholestenone (cholesten-4-dione-3,6) isolated as the monophenylhydrazone, which indicated their identity.

7-Dihydrocholestene isomer was prepared by the action of alcoholic hydrochloric acid on the reduction product (7-hydroxycholestene) of 7-ketocholestene. The difference in melting point and specific optical rotation values indicated that the two compounds were not identical.

The preparation of cholesteryl methyl xanthogenate by the method which involved the reaction of finely divided potassium on cholesterol was found to require less time and to produce a larger yield of product than the method which involved the reaction of sodium tert-amoxide on cholesterol. The preparation of cholestene hydrochloride (5-chlorocholestane) was found to be more convenient in chloroform solution than in ether-alcohol solution. The preparation of cholesterilene and dicholesteryl ether by the treatment of cholesterol with potassium acid sulfate under various conditions of temperature, time and proportion of the reagent was studied and cholesterilene was obtained by the treatment of dicholesteryl ether with potassium acid sulfate.

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