tionation. Rehydrogenation and refractionation of the tetradecahydrophenanthrene gave a product, b. p. 155–157° (27 mm.), having $n^{25}D$ of 1.5003 which agrees with the value given by Pinkney and Marvel.[5]

In the fractionation of the reaction mixtures there were intermediate fractions which are not reported in the table. Their amounts in most cases represented only a few per cent. of the weight of phenanthrene originally used. However, with copper–chromium oxide at temperatures of 200–300° these unidentified products amounted to about 30% of the weight of phenanthrene submitted to hydrogenation.

Summary

Methods have been given for the preparation of 9,10-dihydrophenanthrene, 1,2,3,4,5,6,7,8-octahydrophenanthrene, and tetradecahydrophenanthrene from "70% phenanthrene."

MADISON, WIS. RECEIVED NOVEMBER 23, 1936

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

Molecular Rearrangements in the Sterols. I. The Action of Anhydrous Potassium Acetate on Cholesteryl *p*-Toluenesulfonate in Acetic Anhydride Solution

BY EVERETT S. WALLIS, E. FERNHOLZ AND F. T. GEPHART

The epimerization of the hydroxyl group of cholesterol and other unsaturated sterols has become of great interest since Ruzicka[1] showed that the male hormone, androsterone, is a derivative of *epi*-dihydrocholesterol, and that its physiological activity is much greater than the corresponding epimer.

This difference in the activity of the two isomers stimulated our interest in the preparation of the epimer of dehydroandrosterone. In order to find a satisfactory method for the preparation of this latter compound we first thought it advisable to study possible methods for the epimerization of cholesterol, since the previous attempts of Stoll[2] to prepare *epi*-cholesterol had failed.

During the course of this investigation three papers have appeared which have an important bearing on this problem. Evans and Schoenheimer[3] have reported the preparation of *epi*-allocholesterol, which differs from *epi*-cholesterol only in the position of the double bond. Marker, Oakwood and Crooks[4] have described the preparation of *epi*-cholesterol by the action of oxygen on the Grignard reagent obtained from cholesteryl chloride. Beynon, Heilbron and Spring[5] have published experimental results obtained in an investigation of some reactions of the isomeric ethers of cholesterol.

These publications make it advisable to report certain experiments we have carried out on the problem of obtaining *epi*-cholesterol in good yields, and which also have interest regarding the constitution of the two series of isomeric ethers of cholesterol, discovered by Stoll.[2]

Stoll[2] found that when cholesteryl *p*-toluene sulfonate is boiled with an alcohol it reacts easily to form a normal levorotatory ether, but that an isomeric dextrorotatory ether is formed when the reaction is carried out in the presence of potassium acetate. Stoll[2] expressed the opinion that this new ether was a derivative of *epi*-cholesterol or of *epi*-allocholesterol.

In the same year Wagner-Jauregg and Werner[6] reported that cholesteryl chloride and bromide behaved in a similar manner, that is, when heated alone with alcohols the normal levorotatory ether is formed, but in the presence of potassium acetate the isomeric dextrorotatory ether is produced. They further observed that the isomeric methyl ether is converted into the normal ether when heated with hydrogen chloride in methyl alcohol at 130°. From this fact they concluded that in the formation of ethers from either cholesteryl halides or the *p*-toluene sulfonate the isomeric ethers are first formed, but that the acid produced in the reaction converts them into the so-called "normal" form. These investigators also attempted to hydrogenate the isomeric ether. Although they found that this reaction did not proceed smoothly, it is important to note in view of certain of our experiments about to be described that a small amount of the normal dihydrocholesteryl methyl ether was isolated.

In this connection the observations of Beynon, Heilbron and Spring[5] are also of special interest.

(1) Ruzicka and co-workers, *Helv. Chim. Acta*, **17**, 1395 (1934).
(2) Stoll, *Z. physiol. Chem.*, **207**, 147 (1932).
(3) Evans and Schoenheimer, THIS JOURNAL, **58**, 182 (1936).
(4) Marker, Oakwood and Crooks, *ibid.*, **58**, 481 (1936); see also, Marker, Kamm, Oakwood and Laucius, *ibid.*, **58**, 1948 (1936).
(5) Beynon, Heilbron and Spring, *J. Chem. Soc.*, 907 (1936).
(6) Wagner-Jauregg and Werner, *J. physiol. Chem.*, **213**, 119 (1932).

These authors made a study of the hydrolysis of the two series of cholesteryl ethers with the idea in mind that the "abnormal" ethers might be derivatives of *epi*-cholesterol. They found that whereas the normal ethers are not hydrolyzed by halogen acids in acetic acid solution the isomeric ethers react quite readily with halogen acids to produce the normal cholesteryl halides. They also observed that by the action of bromine the alkoxyl group in the isomeric ethers is replaced by bromine. In each case studied 3,5,6-tribromocholestane was produced. The normal ethers reacted normally to give a stable dibromide.

In this paper we wish to report certain experimental results obtained by us in a search for a satisfactory method for the preparation in good yields of the epimeric modifications of certain unsaturated sterols. Our method of attack was also based upon certain experiments of Phillips[7] carried out on alcohols containing only one asymmetric carbon atom, and it involved an investigation of the action of potassium acetate on cholesteryl *p*-toluene sulfonate. But in order to avoid the complication of the formation of ethers, and since we had observed that in acetic acid solution the normal cholesteryl acetate is formed, the reaction was carried out with anhydrous potassium acetate in acetic anhydride solution. This indeed yielded a new acetate, isomeric with cholesteryl acetate, which at first we were inclined to believe was *epi*-cholesteryl acetate. Experiments soon showed, however, that this was not the case.

This new acetate which we shall now call *i*-cholesteryl acetate is *strongly dextrorotatory*, $[\alpha]^{20}D$ +47.8°, and melts at 73°. Hydrolysis yields *i*-cholesterol. This compound crystallizes from alcohol in long needles which melt at room temperature. The substance resolidifies and passes into another crystalline form which melts at 74–75°. *i*-Cholesterol is strongly *dextrorotatory*, $[\alpha]^{20}D$ +23.9°. It is not precipitated by digitonin. Reacetylation yields the above *i*-cholesteryl acetate.

Experimental results obtained in a study of the catalytic hydrogenation of this new acetate are of special interest. Palladium black was found to be entirely ineffective in bringing about the hydrogenation. Platinum black brought about a very slow reaction. On working up the products there was isolated, besides the starting material, some cholestane. The Adams platinum oxide catalyst produced an entirely different result. When the platinum oxide was reduced in presence of the *i*-cholesteryl acetate a rapid hydrogenation took place and dihydrocholesteryl acetate was formed in good yield. This fact strongly indicates that an inversion of the hydroxyl group does not occur during the formation of *i*-cholesterol. It also suggests a close relationship to the "abnormal" ethers discussed above since, as has already been pointed out, Wagner-Jauregg and Werner[6] isolated dihydrocholesteryl methyl ether from the hydrogenation products of the "abnormal" ether.

There are other important facts which should be recorded. *i*-Cholesteryl acetate does not react with perbenzoic acid. Neither does it decolorize readily a solution of bromine in carbon tetrachloride. The Liebermann reaction, however, is quite strong. This inertness toward bromine and perbenzoic acid, and the relatively great stability toward catalytic hydrogenation, suggest that a double bond as such is not present in *i*-cholesteryl acetate and *i*-cholesterol.

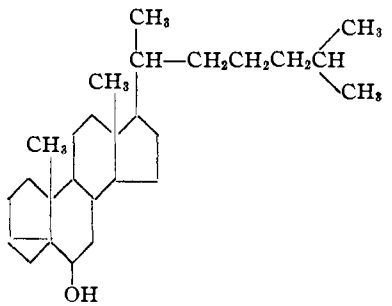
Experiments were carried out to determine the nature of the hydroxyl group in *i*-cholesterol. It was found that *i*-cholesterol could be oxidized easily with chromic acid in acetic acid solution. From the products formed we were able to isolate an oxime in a 20% yield. This leads to the conclusion that *i*-cholesterol is a secondary alcohol.

In certain reactions a conversion of *i*-cholesterol to cholesterol was observed. For example, in the course of the preparation of the 3,5-dinitrobenzoate of *i*-cholesterol the interesting observation was made that when the reaction was carried out by heating the mixture of pyridine, dinitrobenzoyl chloride and *i*-cholesterol for one hour on the water-bath it was possible to isolate in a yield of 18% the normal cholesteryl dinitrobenzoate (m. p. 193° $[\alpha]^{18}D$ −14.6°). If, however, the reaction was carried out at room temperature only small amounts of the normal cholesteryl dinitrobenzoate were produced. In another experiment *i*-cholesteryl acetate was dissolved in acetic acid, and after the addition of two drops of sulfuric acid the mixture was heated on the water-bath for one hour. Partial conversion to cholesteryl acetate took place, and hydrolysis gave a product precipitable with digitonin.

All these facts lead to the conclusion that the reaction of anhydrous potassium acetate on

(7) Phillips, *J. Chem. Soc.*, **123**, 44 (1925). Other papers by Phillips and co-workers have appeared since this time.

cholesteryl *p*-toluene sulfonate in acetic anhydride solution is accompanied by a molecular rearrangement. The exact nature of this rearrangement is still obscure. At the present time, however, we believe that in *i*-cholesterol and its derivatives the carbon skeleton is different, and that the reaction may involve a type of rearrangement common in certain terpenes, but until now unknown in sterol chemistry. Such a rearrangement would produce a compound whose structure could be represented in the following manner.



This type of formulation would explain the apparent non-reactivity of the double bond, since it is not present as such in the molecule. The assumed structure represents the molecule as a secondary alcohol, and therefore explains the formation of a ketone on oxidation with chromic acid. It also accounts for the conversion of *i*-cholesterol and its acetate under suitable conditions into cholesterol and its derivatives.

In conclusion we wish to state that regardless of the true nature of the structure of *i*-cholesterol, the experimental facts above described strongly suggest that a close relationship exists between this new compound and the so-called "abnormal" ethers of Stoll. This relationship is being investigated further.

Experimental Part

Preparation of Cholesteryl *p*-Toluene Sulfonate.—This compound was prepared by the method of Freudenberg[8] with the following modifications. After dissolving by gentle warming 58.2 g. of dried cholesterol in 70 cc. of dried pyridine, 58.2 g. of *p*-toluenesulfonyl chloride was added. Within five minutes a white crystalline precipitate began to form. After the mixture had stood overnight, the product was taken up in ether, worked up in the usual manner, and finally recrystallized from dry ether; yield, 72.1 g. (89.3%); m. p. 131.5–132.5°.

Preparation of *i*-Cholesteryl Acetate.—One hundred grams of anhydrous potassium acetate was dissolved in 1500 cc. of boiling acetic anhydride (pure). The solution was cooled to 50° whereupon potassium acetate crystallized in a finely divided state. To this mixture was added 35 g. of cholesteryl *p*-toluenesulfonate. The flask was then placed on the steam-bath, and stirred for approximately thirty-six hours. During this time the temperature inside the flask varied from 70–80°. The dark brown mixture was poured into cold water; 800 cc. of ether was added and the ether layer was washed repeatedly with a cold aqueous solution of potassium carbonate. (In a later experiment the acetic anhydride was removed by distillation under diminished pressure.) After complete removal of the acetic anhydride and acetic acid the ether layer was dried with anhydrous sodium sulfate, decolorized with animal charcoal and the ether removed by distillation. An oily product was obtained. This material was dissolved in alcohol, and small amounts of ether were added. Crystallization was carried out in such a manner as to obtain four crops of crystals: 3.6 g., m. p. 71.5°; 0.5 g., m. p. 70–71°; 4.7 g., m. p. 64–66°; 7.2 g., m. p. 59–62°. Several recrystallizations of the third and fourth fractions gave 0.4 g. of material, m. p. 71°, and 7.5 g., m. p. 67–68°. The combined weight of fractions of m. p. 68–71.5° was 12.0 g. (43.2%). The other products of the reaction consisted mainly of an unsaturated hydrocarbon and cholesteryl acetate.

It was found to be impossible to free the *i*-cholesteryl acetate from cholesteryl acetate by the process of recrystallization. Therefore, 7.5 g. of the above acetate of m. p. 67–68° was dissolved in 100 cc. of alcohol, and treated with 8 g. of potassium hydroxide. The solution was refluxed for one-half hour on the water-bath, poured into water and the product extracted with ether. The dried ether solution was evaporated to dryness and taken up in a small amount of 90% alcohol. A solution of 6 g. of digitonin in 500 cc. of 90% alcohol was then added. The mixture was cooled to room temperature and the digitonide was filtered and dried; weight 4.8 g. (corresponding to 1.2 g. of cholesterol).

The filtrate was evaporated to dryness, the residue was digested with ether, and, after removing the solvent, it was crystallized from alcohol. After thorough cooling in an ice–salt mixture the *i*-cholesterol which separated was collected rapidly, and placed in a vacuum desiccator where it melted and resolidified. The product weighed 3.5 g. and melted constantly at 74–75°, $[\alpha]^{20}D$ +23.9° (22.6 mg. dissolved in 2 cc. of chloroform solution gave an αD +0.27°, 100-mm. tube).

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.85; H, 11.92. Found. C, 83.75; H, 11.87.

The acetate was prepared by heating 1.5 g. of *i*-cholesterol with 10 cc. of acetic anhydride for one hour on the water-bath. The crystalline product which separated on cooling was recrystallized from alcohol; yield 1.4 g., m. p. 73°, $[\alpha]^{20}D$ +47.8° (25.1 mg. in 2 cc. chloroform solution gave αD +0.60).

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.23; H, 11.29. Found: C, 81.32; H, 11.39.

Catalytic Hydrogenation of *i*-Cholesteryl Acetate.—(a) After reducing 1.07 g. of platinum oxide suspended in 50 cc. of acetic acid with hydrogen, 1 g. of *i*-cholesteryl acetate was added. Hydrogen was absorbed slowly and after five hours the reaction was stopped. After removal of the solvent the material was crystallized from alcohol. This

(8) Freudenberg and Hess, *Ann.*, **448**, 128 (1926).

gave 0.29 g. of starting material, m. p. 70°. The material remaining in the mother liquor was hydrolyzed with alcoholic potassium hydroxide and on crystallization from acetone there was obtained a substance, m. p. 78°, which did not depress the melting point of authentic cholestane; $[\alpha]^{20}D$ +22.5° (22.2 mg. in 2 cc. chloroform solution gave $[\alpha]^{20}D$ +0.25 1 dm. tube; lit. +24.7°).

(b) In a second experiment 1.332 g. of *i*-cholesteryl acetate was shaken with 1.30 g. of PtO_2 and 50 cc. of glacial acetic acid in an atmosphere of hydrogen, at room temperature, and at ordinary pressure. In fifteen minutes 310 cc. of hydrogen was absorbed. After hydrolysis with alcoholic potassium hydroxide, there was obtained a crystalline product which after thorough drying melted at 140°, and was identified by mixed melting point determination as dihydrocholesterol (cholestanol); yield, 1.1 g., or 85%.

Titration of *i*-Cholesteryl Acetate with Perbenzoic Acid.—Two samples of *i*-cholesteryl acetate were dissolved in chloroform, an excess of perbenzoic acid dissolved in the same solvent was added and the solutions were kept at 0°. Blanks were also run for comparison. Sample 1: 39.6 mg. consumed 0.079 mg. oxygen in forty-eight hours. Theoretical for 1 atom of oxygen 1.64 mg. oxygen. Sample 2: 56.0 mg. consumed 0.198 mg. oxygen in six days. Theoretical for 1 atom of oxygen 2.32 mg. of oxygen. It readily can be seen from these results that practically no reaction took place.

It was also observed that both *i*-cholesterol and *i*-cholesteryl acetate would not decolorize a dilute solution of bromine in carbon tetrachloride.

Oxidation with Chromic Acid.—To a solution of 0.93 g. of *i*-cholesterol in 30 cc. of specially purified acetic acid (potassium permanganate method), a solution of 0.64 g. of chromic acid in 20 cc. acetic acid was added drop by drop. The solution became temporarily cloudy and a slight rise in temperature was observed. After standing overnight at room temperature the mixture was extracted with ether. The ether was shaken with an aqueous solution of 2 *N* sodium hydroxide to remove the acidic material. Attempts to obtain a crystalline material from this acidic portion were unsuccessful. The neutral part crystallized but had an unsharp melting point; yield 0.33 g.

This crude ketone was boiled for two hours with 0.3 g. of hydroxylamine hydrochloride and 0.5 g. of sodium acetate in 30 cc. of ethyl alcohol. The product was precipitated with water. Recrystallizations from dilute alcohol gave small leaflets which when thoroughly dried melted at 143–144°; yield 0.2 g.

Anal. Calcd. for $C_{27}H_{45}NO$: C, 81.12; H, 11.35; N, 3.51. Found: C, 80.95; H, 11.58; N, 3.88.

Conversion of *i*-Cholesterol into Normal Cholesteryl *m*-Dinitrobenzoate.—A mixture of 0.574 g. of *i*-cholesterol (m. p. 74–75°), freed from cholesterol by means of digitonin, 0.6 g. of *m*-dinitrobenzoyl chloride, and 5 cc. of pyridine was heated for one hour in the water-bath. The material was taken up in ether and recrystallized from a mixture of acetone and alcohol; yield 0.16 g., m. p. 193°; $[\alpha]^{18}D$ −14.5°. The product was identified by a mixed melting point determination as cholesteryl *m*-dinitrobenzoate.

Anal. Calcd. for $C_{34}H_{48}O_6N_2$: C, 70.30; H, 8.34. Found: C, 70.28; H, 8.37.

Conversion of *i*-Cholesteryl Acetate into Normal Cholesteryl Acetate.—After dissolving 0.1 g. of pure *i*-cholesteryl acetate (m. p. 73°) in 10 cc. of acetic acid, two drops of sulfuric acid were added, and the solution was heated on the water-bath for one hour. The solution was then worked up in the usual manner and a crystalline material was obtained which melted unsharply at 95–103°. Two recrystallizations from alcohol gave 0.03 g. of material which melted at 112–114° and showed no depression of the melting point when mixed with cholesteryl acetate (m. p. 114°).

Hydrolysis with alcoholic potassium hydroxide gave a product precipitable with digitonin.

We wish to take this opportunity to express our thanks to Merck & Company, Inc., Rahway, N. J., for all analyses published in this article, and for a grant-in-aid for this work.

Summary

The action of anhydrous potassium acetate on cholesteryl *p*-toluenesulfonate in acetic anhydride solution has been studied.

A new acetate and a new alcohol isomeric with cholesterol have been isolated. Certain properties of these two compounds, designated as *i*-cholesterol and *i*-cholesteryl acetate, have been described.

Evidence is submitted which leads to the conclusion that the reaction is accompanied by a molecular rearrangement.

It is further pointed out that the properties of this new alcohol suggest that a close relationship exists between it and the isomeric ethers of cholesterol discovered by Stoll.

PRINCETON, N. J. RECEIVED NOVEMBER 17, 1936