Summary

1. Dry *n*-pentane, either in the form of vapor or liquid, undergoes little, if any, decomposition in the presence of freshly sublimed aluminum chloride. If, however, anhydrous hydrogen bromide or chloride, water, hydrated aluminum chloride, or alkyl chlorides are added, reaction immediately commences. In the liquid phase reaction, the butanes and isopentane have been identified as reaction products. The latter is the chief product. Undefined saturated higher boiling products and an insoluble unsaturated polymer are also produced. In the vapor phase reaction the amount of isobutane formed is greatly increased and with extensive reaction becomes the chief product. For the vapor phase reaction aluminum chloride and anhydrous hydrobromic acid provide the most active catalyst.

2. In moderate concentration, aluminum bromide is soluble in *n*-pentane. It is a much more active substance than aluminum chloride and does not require the addition of other substances to cause the decomposition of *n*-pentane. The extent of reaction has been found to depend on the aluminum bromide concentration as well as the reaction time. The reaction products were the same, qualitatively, as those obtained with the chloride catalyst. As the extent of reaction was increased, the amount of butanes formed increased linearly, whereas the amount of isopentane formed reached a maximum value and then slowly declined. As much as 55.9% isopentane was obtained from *n*-pentane. When water is added to the reaction mixture, or anhydrous hydrogen bromide bubbled through it, the reaction velocity is increased.

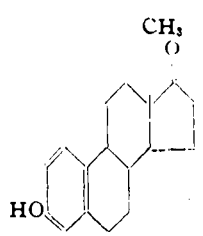
DETROIT, MICHIGAN RECEIVED JULY 21, 1936

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

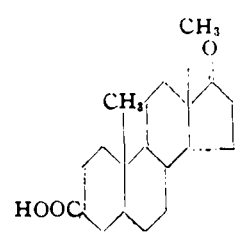
Sterols. VII. *Cis* and *Trans* 3-Carboxyandrostanone, An Oestrus-Producing Male Hormone Derivative, and *epi*-Cholesterol

BY RUSSELL E. MARKER, OLIVER KAMM, THOMAS S. OAKWOOD AND JOSEPH F. LAUCIUS

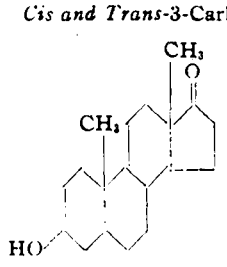
Because of the acidic nature of theelin, we prepared an androsterone derivative having a carboxyl group in the 3-position to see whether this compound would have female hormone properties in addition to male activity. The product was the mixture of *cis*- and *trans*-3-carboxyandrostanone. We made no attempt to separate the two stereoisomers. We found that the ethyl ester of this compound gave an oestrus response in rats when given in quantities of from 5–10 γ. The ethyl ester is much more active than the free acid. These compounds, however, were inactive when tested for male hormone properties by the cock's comb test in doses of 2 mg. Thus it is not necessary to have unsaturation in the molecule to have an oestrus-producing compound.



Theelin



Cis and Trans-3-Carboxyandrostanone



Androsterone

Because of the importance of obtaining *epi*-cholesterol in large quantities for research purposes avoiding the expensive digitonin process for the separation of the *cis* and *trans* isomers, we have looked for other means of separating these isomers, and found that by crystallizing the acetates from ethyl alcohol, a partial separation can be accomplished giving a product containing 80–90% of *epi*-cholesterol. The remaining cholesterol can then be removed completely by recrystallizing the benzoates from ethyl alcohol. We previously separated the two isomers by digitonin.[1]

We found that *epi*-cholesterol behaves very similarly in instability to *epi*-allocholesterol pre-

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

pared by Evans and Schoenheimer.[2] In an attempt to convert *epi*-cholesterol into *epi*-allocholesterol through its hydrochloride, we obtained cholesterylene almost quantitatively by dehydration and no *epi*-cholesterol hydrochloride. The same is true when *epi*-cholesterol is warmed with a small amount of hydrogen chloride in alcohol, dehydrating to cholesterylene. Evans and Schoenheimer found the same thing to happen when *epi*-allocholesterol was warmed with alcoholic hydrogen chloride. On attempting to prepare dibromo-*epi*-cholesteryl acetate by the addition of bromine in acetic acid to *epi*-cholesteryl acetate, there was a similar splitting giving a tetrabromocholestane. This product is a different isomer from that obtained by the addition of bromine to cholesterylene.

epi-Cholesterol does not irradiate with ultraviolet light to give products of antirachitic value.

Experimental

***Cis* and *Trans*-3-Carboxyandrostanone.**—The methyl ester of *cis*- and *trans*-3-carboxycholestane was prepared according to the method of Marker, Oakwood and Crooks.[1]

A solution of 100 g. of the methyl ester in 3700 cc. of glacial acetic acid was placed in a 5-liter flask equipped with a stirrer and dropping funnel. The temperature of the flask was kept at 45–50° during the addition of a solution of 115 g. of chromic anhydride in 350 cc. of 90% acetic acid. After addition was complete (three hours) stirring and warming was continued for seven hours. At the end of this time 200 cc. of methyl alcohol was added to destroy any excess chromic acid. The acetic acid was distilled from the reaction mixture under reduced pressure, keeping the temperature of the bath below 50°. After most of the acetic acid was distilled, 500 cc. of water was added and the distillation continued for an additional two hours. The residue was extracted with five 500-cc. portions of ether and the ether extract was successively washed once with 200 cc. of 10% hydrochloric acid, once with 100 cc. of a saturated solution of sodium bicarbonate, and then twice with 100-cc. portions of a 5% sodium hydroxide solution. The extract was dried with sodium sulfate and the ether distilled. The oil was dissolved in 1500 cc. of 95% alcohol to which was added 5 g. of semicarbazide hydrochloride and 5 g. of sodium acetate. The alcohol was distilled and the residue digested with 1500 cc. of dry ether for three hours. The insoluble crystalline residue was filtered, washed with dry ether, then boiled two hours with 50 cc. of distilled water. The semicarbazone was filtered, washed with ether, then extracted with acetone in a Soxhlet extractor; yield 2.5 g.; m. p. 250–260° (dec.).

A mixture of 1.0 g. of the semicarbazone, 115 cc. of alcohol, 15 cc. of concd. sulfuric acid and 24 cc. of water was boiled for one hour. The solution was poured into 1500 cc. of distilled water and extracted with three 300-cc. portions of dry ether. The ether extract was washed twice with water and then the ether was distilled. The residue was treated overnight with a solution of 5 g. of potassium hydroxide in 50 cc. of methyl alcohol. The alcoholic solution was poured into a liter of distilled water. This was concentrated *in vacuo* until 300 cc. was distilled to remove the alcohol. The solution was extracted with alcohol-free ether to remove neutral organic material. The aqueous portion was acidified with 5 *N* sulfuric acid, and then was extracted three times with 300-cc. portions of ether. On evaporation of the ether a solid remained. This was crystallized from acetone; yield 300 mg.; m. p. 253°.

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.5; H, 9.5. Found: C, 75.7; H, 9.5.

The ether solution which was obtained from the filtration of the semicarbazone was evaporated to dryness. On recrystallization from methyl alcohol–ether, 35 g. of unoxidized methyl ester of *cis*- and *trans*-3-carboxycholestane, m. p. 69°, was obtained.

Ethyl Ester of *cis*- and *trans*-3-Carboxyandrostanone.—These acids esterify very readily in a small amount of alcohol. When ordinary ether containing ethyl alcohol was used to extract the *cis*- and *trans*-3-carboxyandrostanone from the hydrolysis in the above experiment, the resulting product was the ethyl ester of *cis*- and *trans*-3-carboxyandrostanone. It was crystallized from dilute alcohol; m. p. 108–110°.

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.2; H, 9.9. Found: C, 75.8; H, 10.2.

Separation of *epi*-Cholesterol from the mixture of *epi*-Cholesterol and Cholesterol.—A mixture of *epi*-cholesterol and cholesterol was obtained by passing oxygen into cholesterylmagnesium chloride. 10.2 g. of magnesium turnings was covered with 50 cc. of dry ether. To this was added 1 cc. of ethyl bromide. After the Grignard had started a solution of 170 g. of well purified cholesteryl chloride dissolved in 1 liter of dry ether was added dropwise with vigorous stirring over a period of six hours. The ether solution was kept at a reflux temperature during the addition. It was then stirred vigorously for twelve hours. At the end of this time practically all of the magnesium was in solution. The product was cooled to 0° and oxygen passed into the reaction mixture for four hours. The oxygen was under a slight pressure during addition. The mixture was poured into dilute sulfuric acid and the mixture of cholesterols was extracted with ether. After washing with water, the ether layer was filtered from a small amount of insoluble white solid. The ether was then distilled leaving a white product. From 1360 g. of cholesteryl chloride was obtained 950 g. of the crude mixture of cholesterols.

A mixture of 50 g. of the above cholesterols, 20 g. of succinic anhydride and 40 cc. of dry pyridine was heated over steam for two hours. After cooling to room temperature, the product was dissolved in 300 cc. of ether, and 200 cc. of water containing 60 cc. of concd. hydrochloric acid was added. After extraction of the pyridine 300 cc. more ether was added and the half-succinic ester extracted with a solution of 25 g. of sodium carbonate in one liter of water at 33°. The alkaline extract was extracted twice with ether, then acidified with hydrochloric acid. The succinate was extracted with ether. After distilling the ether, the residue was refluxed with 25 g. of sodium hydroxide in one liter of water with stirring. The purified

(2) Evans and Schoenheimer, THIS JOURNAL, **58**, 182 (1936).

cholesterols were extracted with ether; 820 g. was obtained from 1150 g. of crude material. This was converted into the acetate by boiling for one hour with three times excess of acetic anhydride. The excess acetic anhydride was removed by vacuum distillation. The acetates were boiled with 6 liters of 95% ethyl alcohol and the solution cooled to 5°. The precipitate was filtered and recrystallized, m. p. 101–104°. One more crystallization of the product gave pure cholesteryl acetate, m. p. 114°, which gave no depression in melting point when mixed with cholesteryl acetate prepared from pure cholesterol. The alcoholic mother liquors from the above crystallization were evaporated to dryness and hydrolyzed by means of alcoholic sodium hydroxide, giving a product consisting of approximately 90% of *epi*-cholesterol and 10% cholesterol. This mixture was then converted into the benzoate, and freed of cholesterol benzoate by crystallization from alcohol. The benzoate was then hydrolyzed and the *epi*-cholesterol crystallized from alcohol, m. p. 141.5°. Mixed with pure *epi*-cholesterol, m. p. 141.5°, it gave no depression in melting point, whereas when mixed with cholesterol, m. p. 147°, a depression of 19° was observed; $[\alpha]^{30}D$ −35.0° in 1% alcohol.

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

Action of Bromine on *epi*-Cholesteryl Acetate.—To a solution of 100 mg. of *epi*-cholesteryl acetate in 3 cc. of ether was added a solution of 40 mg. of bromine in 5 cc. of acetic acid. A solid crystallized out. This was filtered and recrystallized from acetic acid, m. p. 110°. It gave a tetrabromocholestane.

Anal. Calcd. for $C_{27}H_{46}Br_4$: C, 47.2; H, 6.3. Found: C, 47.4; H, 6.7.

This bromide is different from the tetrabromide formed from cholesterylene and bromine, giving a depression in melting point when mixed.

Action of Hydrochloric Acid on *epi*-Cholesterol.—A solution of 1 g. of *epi*-cholesterol in 30 cc. of alcohol containing 1 g. of hydrochloric acid was refluxed for sixteen hours. The insoluble oil was sublimed under high vacuum, then crystallized from alcohol, m. p. 76–77°, uncorr. Mixed with cholesterylene, m. p. 74–76°, prepared by the action of quinoline on cholesteryl chloride it gave no depression in melting point; $[\alpha]^{30}D$ −78.3° compared to $[\alpha]^{30}D$ −80.0° for authentic cholesterylene, concentration 1% in benzene.

When an attempt was made to prepare *epi*-allocholesterol by the addition of hydrochloric acid in the cold to an alcoholic solution of *epi*-cholesterol the same dehydration occurred, giving cholesterylene.

Summary

A mixture of *cis*- and *trans*-3-carboxyandrostanone and its ethyl ester was prepared and found to produce oestrogenic activity in rats, but lacked male activity in the cock's comb test.

epi-Cholesterol and cholesterol can be separated into their components by crystallization of their acetates followed by crystallization of their benzoates. *epi*-Cholesterol on treatment with alcoholic hydrogen chloride forms cholesterylene. *epi*-Cholesterol acetate on treatment with bromine gives a tetrabromocholestane which is a different isomer from the product obtained by the action of bromine on cholesterylene.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Mechanism of Carbohydrate Oxidation. XXII.[1] The Preparation and Reactions of Glyceraldehyde Diethyl Mercaptal

BY HAROLD W. ARNOLD AND WILLIAM LLOYD EVANS

During the course of our work on the synthesis of β-*d*-glucosidoglyceraldehyde derivatives[2] it seemed desirable to have available a monomeric glyceraldehyde derivative containing an unblocked hydroxyl group only on the third carbon atom. The following scheme was used in attempting to prepare such a derivative:

Glyceraldehyde diethyl mercaptal was prepared as a distillable oil by the usual procedure employed in preparing sugar mercaptals. It readily reacted with triphenylmethyl (trityl) chloride to form a crystalline trityl ether. From the work of Helferich and his students[3] it appears probable that the compound prepared was 3-trityl glyceraldehyde diethyl mercaptal. A few exceptions to Helferich's rule concerning the preferential reaction of trityl chloride with primary alcohol groups have been found[4] but they do not invalidate the rule because of the vigorous reaction conditions employed in discovering them.

The 3-trityl glyceraldehyde diethyl mercaptal could be easily acetylated or benzoylated to give

(1) No. XXI of this series, THIS JOURNAL, **58**, 1890 (1936).

(2) H. W. Arnold and W. L. Evans, *ibid.*, **58**, 1890 (1936).

(3) B. Helferich, P. S. Speidel and W. Toeldte, *Ber.*, **56**, 766 (1923); B. Helferich, L. Moog and A. Jünger, *ibid.*, **58**, 872 (1925); B. Helferich, W. Klein and W. Schäfer, *Ann.*, **447**, 19 (1926).

(4) R. C. Hockett and C. S. Hudson, THIS JOURNAL, **53**, 4456 (1931); *ibid.*, **56**, 945 (1934).