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

Sterols. XV. The Synthetic Preparation of *epi-allo*-Pregnanolone, the Androgenic Principle of Human Pregnancy Urine

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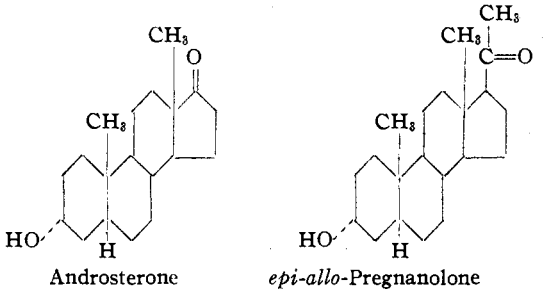
Recently, Marker, Kamm and McGrew[1] reported the isolation of a new sterol from human pregnancy urine and the identification of it as *epi-allo*-pregnanolone. Later, in a preliminary communication[2] we reported the synthesis of this compound and noted its androgenic properties. Because of the relatively large amount of this substance present in human pregnancy urine (1–2 mg. per gallon (4 liters)), it is possible that the entire male hormone activity of human pregnancy urine may be due to it.

It is interesting to observe the marked similarity between *epi-allo*-pregnanolone and androsterone.



Androsterone *epi-allo*-Pregnanolone

The only difference is that androsterone has a keto group in the 17-position, whereas *epi-allo*-pregnanolone has an acetyl group in that position. In view of this close similarity, this compound would be expected to have an activity similar to that of androsterone.

The starting material for the synthesis of *epi-allo*-pregnanolone was 3-chloro-*allo*-cholanic acid, a by-product from the preparation of chloroandrosterone.[3] This was degraded stepwise to a ketone in the 20-position by the method of Wieland.[4]

We find that the methyl esters of the various cholanic acids may be produced in good yields by boiling the acids with methyl alcohol and sulfuric acid. The resulting esters give better yields of the corresponding diphenyl carbinols when condensed with phenylmagnesium bromide at 40°, rather than at 100° as described by Heilbron, Samant and Simpson.[5] A preliminary experiment with cholestyl chloride indicated that no reaction occurs between the chlorine atom and the Grignard reagent at room temperature, the cholestyl chloride being recoverable unchanged in crystalline form; at 100°, however, no crystalline material was obtainable.

(1) Marker, Kamm and McGrew, THIS JOURNAL, **59**, 616 (1937).
(2) Marker, Kamm, Jones, Wittle, Oakwood and Crooks, *ibid.*, **59**, 768 (1937).
(3) Marker, Whitmore and Kamm, *ibid.*, **57**, 2358 (1935).
(4) Wieland, Schlichtling and R. Jacobi, *Z. physiol. Chem.*, **116**, 80 (1926).

Experimental

Methyl Ester of 3-Chloro-*allo*-cholanic Acid.—To a solution of 100 g. of 3-chloro-*allo*-cholanic acid m. 180° in 3 liters of methyl alcohol was added 15 cc. of concd. sulfuric acid. The solution was refluxed for two hours, distilling the alcohol in the meantime to 500 cc. The solution was cooled and the product recrystallized from methyl alcohol, m. p. 133°.

Anal. Calcd. for $C_{25}H_{41}O_2Cl$: C, 73.2; H, 10.1. Found: C, 72.8; H, 10.3.

3-Chloro-*allo*-nor-cholanyl-diphenylcarbinol.—A solution of 27 g. of methyl 3-chloro-*allo*-cholanate in 800 cc. of ether was added over a period of one hour to a stirred and gently refluxing solution of 0.27 mole of phenylmagnesium bromide in 600 cc. of ether. The solution was stirred and refluxed for four hours after which the ether was distilled until the temperature of the remaining solution reached 40°. Most of the solids dissolve at this point to form a clear solution. The solution was maintained at 40° for one hour, decomposed with one liter of dilute sulfuric acid, and extracted with ether. The ether solution was washed with water and concentrated to 200 cc. Two-hundred cc. of methyl alcohol was added and the solution was further concentrated to 250 cc. On cooling and shaking the carbinol crystallized as a white solid. This was recrystallized from methyl alcohol; m. p. 171°; yield 27 g.

Anal. Calcd. for $C_{36}H_{49}OCl$: C, 81.1; H, 9.2. Found: C, 81.1; H, 9.3.

3-Chloro-nor-*allo*-cholanic Acid.—To a solution of 19 g. of 3-chloro-*nor-allo*-cholanyl-diphenylcarbinol in 375 cc. of glacial acetic acid at 90° was added a solution of 14.3 g. of chromic oxide in 140 cc. of 90% acetic acid over a period of twenty-five minutes. The solution was stirred an additional three hours at 90°, cooled to room temperature and 1800 cc. of 10% hydrochloric acid was added slowly with shaking. The mixture was allowed to stand overnight and filtered. The solid was stirred with 100 cc. of boiling methyl alcohol, cooled and filtered. It was washed with cold methyl alcohol, leaving a crystalline product free of benzo-

(5) Heilbron, Samant and Simpson, *J. Chem. Soc.*, 1410 (1935).

phenone. This was recrystallized from methyl alcohol; m. p. 248°; yield 10.5 g.

Anal. Calcd. for $C_{23}H_{37}O_2Cl$: C, 72.5; H, 9.8. Found: C, 72.8; H, 9.6.

The next five compounds in the synthetic series were prepared by procedures analogous to those of the above corresponding homologs.

Methyl Ester of 3-Chloro-*nor-allo*-cholanic Acid.—M. p. 178°. *Anal.* Calcd for $C_{24}H_{39}O_2Cl$: C, 73.0; H, 10.0. Found: C, 73.3; H, 10.2.

3-Chloro-*allo*-*bis*-*nor*-cholanyl-diphenylcarbinol.—M. p. 183°. *Anal.* Calcd. for $C_{36}H_{47}OCl$: C, 80.9; H, 9.1. Found: C, 80.8; H, 9.3.

3-Chloro-*bis-nor-allo*-cholanic Acid.—M. p. 231°. *Anal.* Calcd. for $C_{22}H_{35}O_2Cl$: C, 71.9; H, 9.6. Found: C, 71.8; H, 9.3.

Methyl Ester of 3-Chloro-*bis-nor-allo*-cholanic Acid.—M. p. 151°. *Anal.* Calcd. for $C_{23}H_{37}O_2Cl$: C, 72.5; H, 9.8. Found: C, 72.5; H, 9.8.

3-Chloro-*allo-ter-nor*-cholanyldiphenylcarbinol.—M. p. 146°. *Anal.* Calcd. for $C_{34}H_{45}OCl$: C, 80.0; H, 9.0. Found: C, 80.9; H, 9.2.

***epi-allo*-Pregnanol-3-one-20.**—A solution of 11 g. of 3-chloro-*allo-ter-nor*-cholanyl-diphenylcarbinol was boiled for six hours with a mixture of 100 cc. of acetic acid and 100 cc. of acetic anhydride. The solvent was evaporated; the residue was dissolved in 400 cc. of chloroform and ozonized at 0°. The chloroform was evaporated *in vacuo;* 225 cc. of acetic acid was added and the solution distilled to 50 cc. Acetic acid (400 cc.) was added and the resulting solution heated with 15 g. of granulated zinc until a sample no longer gave a color with starch iodide paper. It was then filtered and evaporated to dryness *in vacuo*. The residue was boiled for ten hours with a solution of 30 g. of potassium acetate in 60 cc. of valeric acid; a large excess of alcoholic potassium hydroxide was added and the mixture boiled for an hour, diluted with water and shaken with ether. The residue from the ethereal solution was purified through the acid succinate and isolated as the semicarbazone. This after recrystallization from ethyl alcohol melted at 242° with decomposition. It was hydrolyzed by boiling for one and one-half hours with a mixture of 150 cc. of 95% alcohol, 15 cc. of concentrated sulfuric acid and 30 cc. of water. After dilution with water and extraction with ether the product was sublimed in a high vacuum at 130° and crystallized several times from 70% acetone. It melted at 170° and showed no depression of melting point with *epi-allo*-pregnanolone isolated from human pregnancy urine.

Summary

epi-allo-Pregnanolone was prepared by the Wieland degradation of 3-chloro-*allo*-cholanic acid and subsequent hydrolysis of the chlorine to a —OH group. This substance is identical with *epi-allo*-pregnanolone, which was isolated from human pregnancy urine and which possesses androgenic activity.

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Sterols. XVI. Lanosterol and Agnosterol

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Windaus[1] has shown that "isocholesterol," which is obtained from the neutral fraction of sheep wool grease, can be separated into two compounds, lanosterol and agnosterol, by crystallization of their acetates. Lanosterol, $C_{30}H_{50}O$, has one reactive double bond which can be reduced by platinum catalyst to dihydrolanosterol, containing one inactive double bond. Agnosterol, $C_{30}H_{48}O$, differs from lanosterol in that it has one reactive double bond and *two* inactive bonds. Both have the same number of carbon atoms and give characteristic sterol tests, yet from some of their reactions they appear similar to the amyrins.[2]

Lanosteryl acetate upon reduction gave α-dihydrolanosteryl acetate. This contains one inert double bond which is isomerized by dry hydrogen chloride in chloroform to β-dihydrolanosteryl acetate. This isomerization is not complete, giving a mixture of the beta with a small amount of the alpha compound, which may be separated easily by treatment of the crude isomerization product with a small amount of chromic oxide in acetic acid. The alpha-compound is readily oxidized, whereas the beta-compound resists mild oxidation. This isomerization is similar to that of α-ergostenol into β-ergostenol.[3] α-Ergostenol contains an inert double bond, but upon isomerization to β-ergostenol the double bond becomes active and can be reduced by platinum and hydrogen. The same is true of apocholic acid.[4] However, this

(1) Windaus and Tschesche, *Z. physiol. Chem.*, **190**, 51 (1930).

(2) Doree and Garratt, *J. Soc. Chem. Ind.*, **52**, 355 (1933), and Doree and Petrow, *J. Chem. Soc.*, 1562 (1936).

(3) Reindel, Walter and Rauch, *Ann.*, **452**, 34 (1927); **460**, 212 (1928); Hart, Speer and Heyl, THIS JOURNAL, **50**, 2016 (1930).

(4) Yamasaki, *Z. physiol. Chem.*, **233**, 10 (1935).