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

Androsterone and Related Sterols

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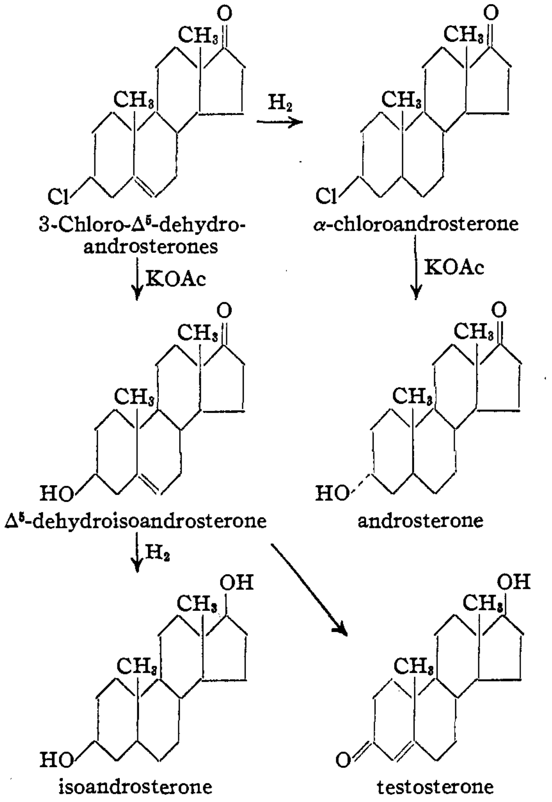
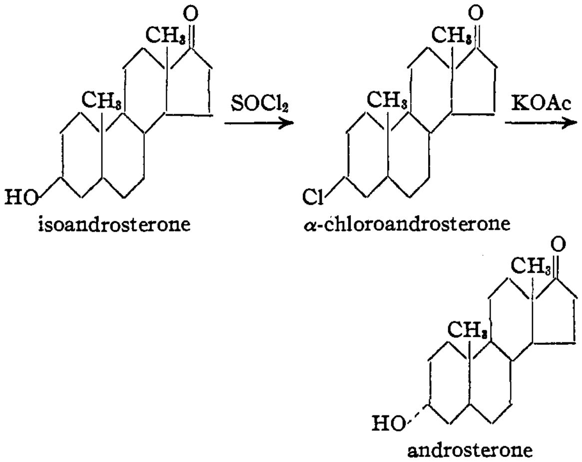
In the isolation of androsterone from male urine Butenandt and Dannenbaum[1] recovered an unsaturated chloro ketone which on reduction of the double bond and subsequent hydrolysis of the chloride by means of potassium acetate gave androsterone. Hydrolysis of the unsaturated chloride gave Δ^5-*trans*-dehydroandrosterone. The saturated chloroandrosterone has been synthesized in this Laboratory from α-cholestyl chloride and this has been hydrolyzed to androsterone.[2]

We have now synthesized from cholesterol the unsaturated chloro ketone isolated from male urine.[1] Cholesterol was converted into cholesteryl chloride, which was brominated and then oxidized by chromic oxide. The resulting products were then debrominated by means of zinc and the α-chloroandrostenone isolated as its semicarbazone. When the unsaturated chloro ketone was reduced by hydrogen it gave α-3-chloroandrosterone which was identical with the compound prepared by us by the oxidation of α-cholestyl chloride.[2]



This on hydrolysis gave androsterone, whereas when the unsaturated chloro ketone is first hydrolyzed to Δ^5-dehydroisoandrosterone and this is reduced, the product is isoandrosterone. Recently Butenandt and Hanisch[3] have synthesized testosterone from Δ^5-dehydroisoandrosterone. This same synthesis was independently confirmed by Ruzicka and Wettstein.[4] Thus it can be seen that the 3-chloro-Δ^5-dehydroandrosterone can be converted into all of the three known naturally occurring male sex hormones, androsterone, Δ^5-dehydroisoandrosterone, and testosterone.

It has been shown in a previous paper that α-cholestanol on treatment with phosphorus pentachloride gives β-cholestyl chloride, whereas when treated with thionyl chloride α-cholestyl chloride is formed. By taking advantage of this means of obtaining a Walden inversion we were able to convert isoandrosterone into androsterone.



Isoandrosterone on treatment with thionyl chloride gives α-chloroandrosterone which on hydrolysis gives androsterone.

Since the action of thionyl chloride and phosphorus pentachloride on the saturated sterols give

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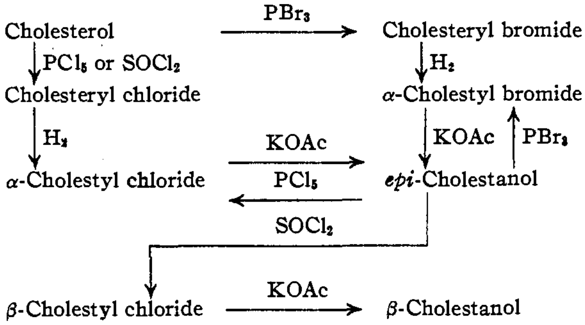
(1) Butenandt and Dannenbaum, *Z. physiol. Chem.*, **229**, 167 (1934).

(2) Marker, Whitmore and Kamm, THIS JOURNAL, **57**, 2358 (1935).

(3) Butenandt and Hanisch, *Ber.*, **68**, 1859 (1935).

(4) Ruzicka and Wettstein, *Helv. Chim. Acta.*, **18**, 1264 (1935).

chlorides of opposite configuration, the action of phosphorus tribromide on the sterols was investigated.



It was found that phosphorus tribromide with cholesterol gives cholesteryl bromide which on reduction gives a bromide which is identical with the bromide obtained by the action of phosphorus tribromide on *epi*-cholestanol. This on hydrolysis gives *epi*-cholestanol. This suggests that this bromide has the same configuration as the chloride produced from *epi*-cholestanol and phosphorus pentachloride and the opposite to that produced from *epi*-cholestanol and thionyl chloride.

Experimental

3-Chloro-5,6-dibromochlolestane.—To a solution of 200 g. of cholesteryl chloride in 500 cc. of dry ether was added dropwise with shaking a solution of 80 g. of bromine in 200 cc. of acetic acid. Then 2 liters of acetone was added and the mixture was cooled with ice. The product was filtered and washed with cold acetone; yield 240 g.; m. p. 130°.

3-Chloro-Δ^5-dehydroandrosterone.—To a mixture of 240 g. of 3-chloro-5,6-dibromocholestane and six liters of glacial acetic acid was added over a period of five hours with stirring at 70°, 400 g. of chromic oxide in 400 cc. of water and 800 cc. of acetic acid. Then 200 cc. of alcohol was added and the solution was cooled in ice and filtered. Unoxidized 3-chloro-5,6-dibromocholestane was recovered to the extent of 80 g. The acetic acid was evaporated under reduced pressure and the residue dissolved in a mixture of one liter of ether and two liters of water. The ether solution was washed with sodium bicarbonate. The ether was evaporated and the residue taken up in one liter of acetic acid and heated with 200 g. of zinc dust on a steam-bath for one-half hour. The acetic acid was distilled *in vacuo*, the residue dissolved in one liter of ether and washed with 200 cc. of 10% sodium hydroxide solution. The sodium salt of 3-chloro-Δ^5-dehydrocholanic acid precipitated. This was extracted with ether, the ether evaporated and the residue taken up in 200 cc. of alcohol. To this was added 13.6 g. of sodium acetate and 11.1 g. of semicarbazide hydrochloride. This was heated for one-half an hour on a steam-bath, and then the alcohol was evaporated under reduced pressure to a small volume. Ether was added and the solid material filtered and washed with ether. This was then recrystallized from alcohol-ether; m. p. 275 (dec.); yield 2 g.

A solution of 1.0 g. of the semicarbazone in 30 cc. of alcohol was treated with 15 cc. of 5 *N* sulfuric acid. This was refluxed for three hours, poured into water, extracted with ether and crystallized from 90% alcohol; m. p. 156–157°. The product absorbs bromine, thus showing unsaturation. The melting point compares with 157° reported by Butenandt[1] for his product obtained as a by-product from the extraction of male urine.

Anal. Calcd. for $C_{19}H_{27}OCl$: C, 74.3; H, 9.0. Found: C, 73.6; H, 9.1.

α-3-Chloroandrosterone.—A solution of 100 mg. of 3-chloro-Δ^5-dehydroandrosterone in 25 cc. of ether was treated with 100 mg. of platinum oxide catalyst. This was shaken with hydrogen at 3 atm. pressure for thirty minutes. The product was crystallized from methyl alcohol; m. p. 165–168°. Mixed melting point with 3-chloroandrosterone prepared by oxidation of α-chloestyl chloride showed no depression.

Anal. Calcd. for $C_{19}H_{29}OCl$: C, 73.8; H, 9.5. Found: C, 73.4; H, 9.7.

Androsterone.—A mixture of 6 g. of 3-chloroandrosterone,[2] 8 g. of potassium acetate and 30 cc. of *n*-valeric acid was refluxed with a bath at 200° for fourteen hours. It was cooled and 15 g. of sodium hydroxide in 30 cc. of water slowly added. To this was added 200 cc. of 95% alcohol. The product was refluxed two hours, then most of the alcohol was distilled under reduced pressure. Water was added and the product extracted with ether. The ether was evaporated and the residue dried thoroughly. To this was added 8 g. of succinic anhydride and 12 cc. of pyridine. This was heated at 100° for one hour. It was taken up in ether, shaken with dilute hydrochloric acid and then with water. The ethereal solution was then washed three times with sodium carbonate solution. This solution was then extracted with ether, and 10 g. of sodium hydroxide in 25 cc. of water added to the aqueous layer. It was heated two hours on a steam-bath. The androsterone was extracted with ether, treated with norit in methyl alcohol, then crystallized from 65% methyl alcohol; m. p. 178°. Mixed melting point with androsterone prepared from *epi*-cholestanol showed no depression. A mixed melting point with isoandrosterone gave a depression of 36°.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.6; H, 10.4. Found: C, 78.2; H, 10.4.

α-3-Chloroandrosterone from Isoandrosterone.—Isoandrosterone was prepared from beta-cholestanol by the method of Ruzicka[5] and co-workers.

A mixture of 100 mg. of isoandrosterone and 1 cc. of thionyl chloride stood overnight. Water was added and the product extracted with ether. The ether solution was shaken with sodium carbonate solution, treated with norit, and the ether evaporated. The residue was crystallized from methyl alcohol; m. p. 167–169°. A mixture with α-chloroandrosterone[1] showed no depression.

Anal. Calcd. for $C_{19}H_{29}OCl$: C, 73.9; H, 9.5. Found: C, 74.4; H, 9.7.

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(5) Ruzicka, *Helv. Chim. Acta*, **17**, 1395 (1934).

Cholesteryl Bromide.—To 20 g. of cholesterol was added 50 cc. of benzene and 6 cc. of phosphorus tribromide. The product was heated for two hours on a steam-bath, most of the benzene was distilled off and ether was added. It was shaken with water, separated and the ether was evaporated and the residue crystallized from absolute alcohol; m. p. 96°.

Cholestyl Bromide from Cholesteryl Bromide.—A solution of 5 g. of cholesteryl bromide in 50 cc. of dry ether, 500 mg. of platinum oxide catalyst and 5 cc. of glacial acetic acid was shaken for one hour with hydrogen at 45 pounds pressure. The catalyst was filtered and the product crystallized from absolute alcohol; m. p. 115°.

Anal. Calcd. for $C_{27}H_{47}Br$: C, 71.8; H, 10.4. Found: C, 71.7; H, 10.4.

Cholestyl Bromide from Epi-chloestanol.—To a solution of 3 g. of *epi*-cholestanol in 15 cc. of dry benzene was added 1 cc. of phosphorus tribromide. The product was heated on a steam-bath for two hours, most of the benzene distilled off, extracted with ether and the extract shaken with sodium carbonate solution. It was then crystallized from alcohol–ether and finally from acetone; m. p. 115°. A mixture with cholestyl bromide from the first preparation gave no melting point depression.

Anal. Calcd. for $C_{27}H_{47}Br$: C, 71.8; H, 10.4. Found: C, 71.6; H, 10.3.

Hydrolysis of Cholestyl Bromide.—This bromide was hydrolyzed by the procedure described for the hydrolysis of α-chloroandrosterone to androsterone. It was purified through its half succinic ester; m. p. 184°. Mixed with *epi*-cholestanol it gave no depression. Mixed with *beta*-cholestanol it gave a depression of 32°.

Anal. Calcd. for $C_{27}H_{48}O$: C, 83.4; H, 12.5. Found: C, 82.7; H, 12.4.

We wish to thank Mr. H. M. Crooks of this Laboratory for the micro analyses on the compounds reported in this paper.

Summary

3-Chloro-Δ^5-dehydroandrosterone has been prepared from cholesterol. This can be converted into any of the three known naturally occurring male hormones. Isoandrosterone has been converted into androsterone. The hydrolysis of cholestyl bromide gives *epi*-cholestanol.

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Studies on Lignin and Related Compounds. XXIII. The Preparation and Methylation of Spruce Lignin Sulfonic Acids[1]

BY GEORGE H. TOMLINSON, 2ND, AND HAROLD HIBBERT

Introduction

In the manufacture of sulfite pulp, the wood is heated under pressure with a solution of calcium bisulfite and free sulfurous acid. The essential feature of this process is the removal of the lignin from the wood as a result of the formation of water soluble lignin sulfonic acids, the latter existing as their calcium salts in the so-called waste sulfite liquor.

Since this liquor can be fractionated it has been generally conceded that it contains at least two distinct sulfonic acids. One of these, generally termed α-lignin sulfonic acid, is precipitated from the liquor by the addition of aromatic amines. Klason has stated[2] that this fraction is identical with that salted out by sodium or calcium chloride, and that it represents 65% of the total product. The β-lignin sulfonic acid remaining in the filtrate may be precipitated as the lead salt by the addition of basic lead acetate. Klason considered that the two fractions are essentially very similar, and that fractionation is rendered possible by the presence of an acrolein group in the α-lignin in contrast with an acrylic acid group in the β-lignin sulfonic acid.

Klason's experimental evidence for this is not convincing, and a critical examination by Hägglund[3] showed that the addition of an aromatic amine to waste sulfite liquor results in the precipitation of a considerably larger quantity of product than is obtained by the addition of sodium chloride. The yield of the α-fraction was also found to be dependent on the nature of the amine used. Thus β-naphthylamine gives a higher yield than quinoline, while fuchsin[4] precipitates a portion not precipitable with naphthylamine. Hägglund concludes[3] that there can be no sharp chemical nor physical distinction between two α- and β-fractions and that, on the average, the lignin sulfonic acids in the α-fraction exist in a higher de-

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[1] Abstracted from a thesis "The Formation of Vanillin from Lignin Sulfonic Acids and its relation to the Structure of Lignin" presented by George Tomlinson to McGill University in partial fulfilment of the requirements for the Ph.D. degree, April, 1935.

[2] Klason, *Ber.*, **53B**, 1864 (1920); **64B**, 2733 (1931).

[3] Hägglund, *Zellstoff u. Papier*, **13**, 261 (1933).

[4] Hennig, *Papier Fabr.*, **30**, Tech.-wiss. Teil, 179 (1932).