chromic oxide. This was kept at 15–20° for thirteen hours and then at 25–30° for eight hours. Methyl alcohol was added and the solvents evaporated under reduced pressure. Water was added and the precipitate crystallized from acetone. It melted at 132°.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.8. Found: C, 79.3; H, 9.7.

A mixture of the two samples of androstandione gave a melting point of 128–129°.

Summary

allo-Pregnandiol, a reduction product of progesterone, was converted into androstanedione, an oxidation product of androsterone, thus establishing a complete structural relationship between the female sex hormone, progesterone, and the male sex hormone, androsterone.

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

Sterols. IX. Isolation of *epi*-Pregnanol-3-one-20 from Human Pregnancy Urine

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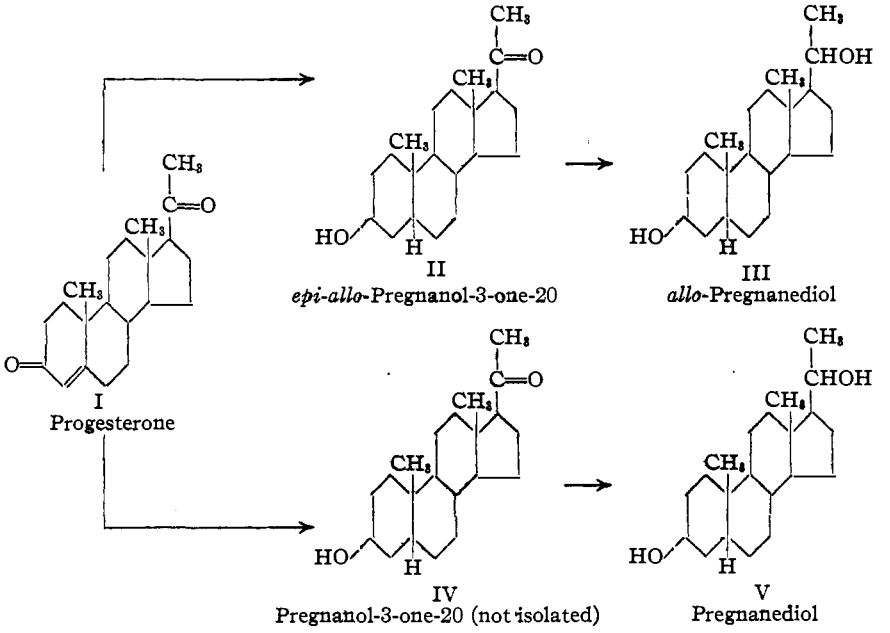
A reinvestigation of the sterol derivatives contained in human pregnancy urine fails to show the presence of progesterone. Since our experiments were conducted on a large scale using the sterol fraction from 10,000 gallons of human pregnancy urine, it seems reasonable to conclude that the corpus luteum hormone is not excreted as such by the human subject during pregnancy. Whether or not the *normal* female excretes the hormone is the subject of an investigation now in progress.

It is known that the neutral carbinol fraction from human pregnancy urine consists mainly of a mixture of pregnanediol and *allo*-pregnanediol, which differ only in the configuration of rings A and B of the sterol nucleus. We have now found, however, that sterols which may be considered as reduction products intermediate between progesterone and the pregnanediols *are* eliminated. In this article we describe the isolation of a compound of that type, namely, a pregnanolone.



Butenandt[1] in the isolation of progesterone from hog corpora lutea obtained *allo*-pregnanol-3-one-20 melting at *195°* which he prepared later by the degradation of stigmasterol. Since stigmasterol possesses the normal arrangement (of cholesterol) in respect to the hydroxyl group and since his method of synthesis did not produce an inversion of this group, the naturally occurring allopregnanolone also has the hydroxyl group in the normal arrangement.

The epimeric form of *allo*-pregnanol-3-one-20 has been obtained from the residues of human pregnancy urine after removal of the theelin, theelol and other known sterol derivatives. It is present in quantities of 1-2 mg. per gallon of urine. This product does not absorb bromine

(1) Butenandt and co-workers, *Ber.*, **67**, 1441, 1897 (1934).

and is not precipitated by digitonin. Upon oxidation it yields *allo*-pregnanedione and upon reduction, *allo*-pregnanediol-3,20. The formula of the new compound and its relationship to other sterol derivatives are represented herewith.

In our study of the sterol residues from human pregnancy urine it was found that the major portion consisted of carbinols together with some ketosterols. The carbinols were separated from the non-carbinol material by means of the sodium salts of their half esters with phthalic acid. The ketonic carbinols were then separated from the plain carbinols through the water soluble betaine hydrazones. The ketonic carbinols were then further separated by crystallization of their semicarbazones. The non-carbinol fraction gave practically no ketones when treated with the betaine hydrazine reagent, thus demonstrating the absence of progesterone in these residues.

Experimental

Separation of Carbinols from Hydrocarbons.—The residue from 10,000 gallons of human pregnancy urine after the separation of theelin and theelol by the Doisy method[2] was hydrolyzed by refluxing with an excess of alkali. This was then steam distilled until no more volatile oils came over. The residue was cooled, filtered, the solid precipitate washed well with water, dried and finally shaken with ice-cold ether. The ether-insoluble portion consists of a mixture of pregnanediol and *allo*-pregnanediol.

The ether-soluble portion was concentrated, leaving 777 g. of tarry residue. This was dissolved in benzene and the carbinols present converted into their acid phthalates according to the following procedure. To 50 g. of the tar was added 25 g. of phthalic anhydride and 25 cc. of dry pyridine. The solution was heated on a steam-bath for two hours, ether was added and the pyridine removed by washing with dilute sulfuric acid. The ether solution was shaken with a solution of 50 g. of sodium carbonate in 200 cc. of water. The ether layer contained the non-carbinol fraction. The carbonate solution, which was extracted thoroughly with ether, was acidified with sulfuric acid and then again extracted with ether. The ether was evaporated and the phthalates saponified by refluxing for two hours with 200 cc. of alcohol containing 50 g. of potassium hydroxide in 40 cc. of water. Water was added and the alcohol distilled. The alkaline solution with its suspended solids was extracted with ether and thus yielded 31.4 g. of a carbinol fraction.

Separation of Ketonic Carbinols from Carbinol Fraction.—To 31.4 g. of total carbinols was added 30 cc. of ethyl alcohol and 5 g. of betaine hydrazine chloride. The reaction mixture was boiled for fifteen minutes on a steam-bath, poured into 200 cc. of water containing ice and the solution thoroughly extracted with ether. The ether layer contained the non-ketonic carbinols, whereas the aqueous layer contained the ketonic carbinols. An excess of hydrochloric acid was added to the aqueous layer and the solution warmed on a steam-bath. The oil which separated was extracted with ether. This gave 2.0 g. of ketonic carbinols. The total urine residue gave 31 g. of ketonic carbinols.

Semicarbazone of *epi-allo*-Pregnanol-3-one-20.—Thirty-one grams of the ketonic carbinol fraction was sublimed in 3-g. quantities in high vacuum, collecting the portion from 140–200° which weighed 23 g. To a solution of 8 g. of the sublimate in 100 cc. of alcohol was added 13.6 g. of sodium acetate and 11.1 g. of semicarbazide hydrochloride. The alcohol was distilled to dryness and the semicarbazone residue washed with hot water and finally with ether. The white solid (7.3 g.) was crystallized from alcohol to a constant melting point of 248–250°, dec.

Anal. Calcd. for $C_{22}H_{37}N_3O_2$: C, 70.3; H, 9.9. Found: C, 69.9; H, 9.9.

***epi-allo*-Pregnanol-3-one-20.**—To a solution of 3 g. of semicarbazone in 150 cc. of alcohol was added 15 cc. of sulfuric acid in 30 cc. of water. The product was refluxed for one-half hour, poured into water and thoroughly extracted with ether. The ether was distilled and the residue sublimed in high vacuum at 130°. The sublimate was crystallized from 70% alcohol and then from 70% acetone, m. p. 162–164°. It does not absorb bromine in the cold, and does not precipitate with digitonin; $[\alpha]^{30}D$ +91.0°, c = 1% in alcohol.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.8. Found: C, 79.3; H, 10.8.

Oxidation of *epi-allo*-Pregnanol-3-one-20 to *allo*-Pregnanedione.—To a solution of 180 mg. of *epi-allo*-pregnanol-3-one-20 in 16 cc. of glacial acetic acid was added 58 mg. of chromic anhydride in 30 cc. of 90% acetic acid. After standing for sixteen hours at 15–20° the solution was diluted with water and the precipitate was collected, dried and sublimed in high vacuum at 115°. The sublimate after crystallization from acetone melted at 198–200° and did not depress the melting point of *allo*-pregnanedione (m. p. 200°).

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.5; H, 10.1.

Acetate of *epi-allo*-Pregnanol-3-one-20.—To 1 g. of *epi-allo*-pregnanol-3-one-20 was added 5 cc. of acetic anhydride. The mixture was refluxed for thirty minutes and the excess acetic anhydride distilled under reduced pressure. The residue was sublimed at 130° with a mercury vapor pump. It was crystallized from 70% alcohol and then from 70% acetone, m. p. 139–140°; $[\alpha]^{30}D$ +112°, c = 1 g. per 100 cc. in alcohol.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.8; H, 10.1. Found: C, 77.0; H, 10.0.

3 - *epi* - *allo* - Pregnanediol - 20 - trans.—*epi* - *allo* - Pregnanol-3-one-20 (100 mg.) was hydrogenated in acetic acid solution (100 cc.) using platinum oxide (100 mg.) at a pressure of 45 lb. (3 atm.). The catalyst was filtered off and the acetic acid distilled under reduced pressure. The residue was crystallized from acetone which yielded a product melting at 205–207°. Mixed with *allo*-pregnanediol (m. p. 242°) it gave a depression in melting point to 194°.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.8; H, 11.3. Found: C, 78.8; H, 11.2.

(2) Doisy, *et al.*, *J. Biol. Chem.*, **87**, 357 (1930).

Diacetate of 3-*epi-allo*-Pregnanediol-20-*trans*.—A solution of 100 mg. of 3-*epi-allo*-pregnanediol-20-*trans* in 5 cc. of acetic anhydride was refluxed for one hour. The acetic anhydride was evaporated under reduced pressure and the residue recrystallized from 60% acetone. Its melting point was found to be 124°.

Anal. Calcd. for $C_{25}H_{40}O_4$: C, 74.4; H, 10.0. Found: C, 74.5; H, 10.2.

Discussion

Although the human corpus luteum contains only traces of progestin,[3] the quantity being so small that it is difficult to imagine that it can be of great significance in the maintenance of pregnancy, it has been demonstrated recently[4] that during pregnancy the placenta may produce its own progestin.

The new sterol that we have isolated, *epi-allo*-pregnanol-3-one-20, probably represents the first stage of the reduction in the body of progesterone to *allo*-pregnanediol; at any rate, it seems logical to expect that the conjugated ketone system would be the easier to reduce, giving as the first reduction products pregnanolone and *allo*-pregnanolone, which on further reduction yield the pregnanediols. So far we have succeeded in isolating only one isomer of pregnanolone (II), but we have evidence that the one represented by formula IV is present also in urine residues.

From our present knowledge of the sterols it is apparent that the utilization of the sterol sex hormones by the body in performing their functions as sex hormones is accompanied by oxidation and reduction reactions. This is shown by the fact that testosterone, which occurs in the testes, after performing its duty as a sex hormone, is eliminated in male urine as its reduction products, of which dehydro-iso-androsterone and androsterone so far have been isolated. In the case of progesterone which functions as a hormone during pregnancy, the products which are eliminated from the body also are its reduction products, the pregnanolones and the pregnanediols. It may be significant that pregnancy urine contains no progesterone.

During pregnancy theelin and theelol are eliminated in the form of their conjugation products with glucuronic acid. The fact that very little theelin is found in the urine of the non-pregnant female who uses it as a hormone would also suggest that theelin may be thrown off from the normal animal in a more extensively altered form just as in the stallion the male sex hormone is eliminated as a theelin conjugate. There is a possibility, therefore, that unchanged progesterone may be obtained from non-pregnancy female urine. The physiological action of the sex hormones, therefore, appears to be chemical as well as physical.

(3) J. P. Pratt, *Arch. Path.*, **19**, 381 (1935).

(4) McGinty, McCullough and Wolter, *Proc. Soc. Exptl. Biol. Med.*, **34**, 176 (1936).

Summary

epi-allo-Pregnanol-3-one-20, a new ketonic sterol, was isolated from human pregnancy urine. This on oxidation gave *allo*-pregnanedione. Catalytic reduction gave *allo*-pregnanediol-3-(*cis*)-20(*trans*). The acetates of these compounds are described.

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