solution and then with water. The ether was evaporated and the residual alcohol removed by heating *in vacuo*. The sirupy residue was dissolved in acetone and allowed to crystallize at 0°. The white crystals which separated were recrystallized from acetone to give a product melting at 204°. This gave no depression in melting point with an authentic sample of estranediol obtained by the hydrogenation of estrone.

The filtrate remaining after removal of the high melting diol was evaporated to a volume of about 4 cc. and allowed to crystallize at 0°. The white crystals were recrystallized from acetone to give needles melting at 175°. This gave a depression of 18° when mixed with a sample of estranediol-3,17$\alpha$.

*Anal.* Calcd. for $C_{18}H_{30}O_2 \cdot C_{18}H_{30}O$: C, 79.9; H, 11.2. Found: C, 79.8; H, 11.0.

To a solution of 50 mg. of the molecular compound, m. p. 175°, in 15 cc. of glacial acetic acid was added a solution of 200 mg. of chromic oxide in 10 cc. of glacial acetic acid and 2 cc. of water. The resulting solution was allowed to stand at room temperature for forty minutes. The mixture was then poured into 200 cc. of water and the resulting mixture extracted with ether. The ether extract was washed with sodium carbonate solution and finally with water. The ether was evaporated and the crystalline residue distilled in high vacuum, the fraction distilling at 130–160° being collected. This fraction was crystallized from aqueous methanol to give crystals melting at 170° which gave no depression in melting point when mixed with the diketone (m. p. 170°) obtained by the oxidation of estranediol-3,17$\alpha$.

*Anal.* Calcd. for $C_{18}H_{26}O_2$: C, 78.8; H, 9.5. Found: C, 78.9; H, 9.9.

## Summary

Estrone has been reduced with aluminum isopropylate to give $\alpha$- and $\beta$-estradiols. $\alpha$-Estradiol upon catalytic hydrogenation yielded estranediol-3,17$\alpha$, and a molecular compound $C_{18}H_{30}O_2 \cdot C_{18}H_{30}O$. Oxidation of both compounds yielded estranedione.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

# Sterols. XLVIII.* Isolation of Androsterone and Pregnanol-3-$\alpha$ from Human Pregnancy Urine

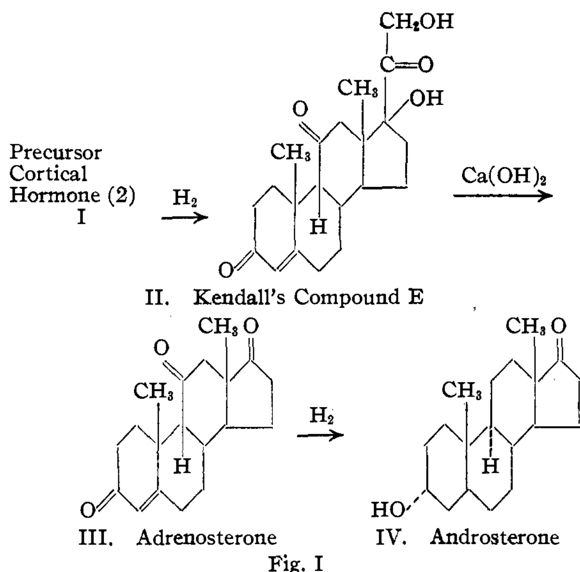
BY RUSSELL E. MARKER AND ELMER J. LAWSON

Recently a new theory of the biogenesis of the steroidal hormones was proposed.[1] This theory assumes that the cortical hormones ($C_{21}$ and $C_{19}$) and the sex hormones ($C_{21}$, $C_{19}$ and $C_{18}$) arise, not from cholesterol, but from a precursor (I) which may be cortin itself, constructed possibly from sugar units, by reductive processes possibly involving ascorbic acid in the suprarenal glands. While dehydrative and hydrolytic processes are involved, there is no necessity in this theory to assume that any of the steroidal hormones are formed by oxidations. Examples of transformations of the type we postulated in the organism have been found recently. Steiger and Reichstein[2] have shown that their substance J, and a new stereoisomer, O, are not, as was thought at the time of the appearance of Paper XL,[1] 3,11,20-triols, but are instead 3,17,20-triols. When triol-J is heated with alcoholic sulfuric acid, dehydration occurs with the formation of *allo*-pregnanol-3$\beta$-one-20. This type of dehydration coupled with the reduction of the carbonyl grouping at $C_{20}$ is supposed to account for the degradation of the dihydroxyacetone residue to give ultimately a $CH_3$—$\overset{|}{C}H$—OH ($\alpha$) residue at $C_{17}$. It should be noted that while dehydration may remove the hydroxyl group either at $C_{17}$ or $C_{21}$ to give after reduction $C_{17,20}$ or $C_{20,21}$ glycols, the formation of $C_{17,21}$ glycols is not possible, and these should not be found in urines or glandular extracts.

Again, recent work by Mason[3] has shown that Kendall's compound E (Reichstein's Fa, Wintersteiner's F) is converted by the action of calcium hydroxide into adrenosterone. This reaction is simply a hydrolytic process, constituting in fact the reversal of an ordinary aldol condensation. Thus it is not necessary to employ an oxidative mechanism to account for the formation of the $C_{19}$ steroids from the $C_{21}$ steroids. The possibility of the oxidation of steroids in the suprarenals is also very remote in view of the presence of the highly reducing ascorbic acid. Certainly, no oxidation vigorous enough to rupture ordinary —C—C— bonds or oxidize methylene groups is likely to occur, or need be postulated.

An essential feature of the theory is the as-

(*) Paper XLVII, THIS JOURNAL, **60**, 2927 (1938).
(1) Marker, *ibid.*, **60**, 1725 (1938).
(2) Steiger and Reichstein, *Helv. Chim. Acta*, **21**, 546 (1938).
(3) Mason, *Proc. Staff Meet. Mayo Clinic*, **13**, 235 (1938).




II. Kendall's Compound E

III. Adrenosterone IV. Androsterone

Fig. I

sumption that carbonyl groups adjacent to tertiary carbon atoms can be reduced to methylene groups *in vivo* as well as *in vitro*. This type of reduction has been observed in the laboratory in the case of the reduction of 7-keto-cholesteryl chloride to $\alpha$-cholestyl chloride[4] and in the case of the reduction of uranetrione[5] to pregnanediol and uranediol. It is further assumed that while this reaction involves no inversion of the tertiary hydrogen at $C_{17}$, the tertiary hydrogen atom at $C_9$ is inverted from the $\beta$- to the $\alpha$-type. Evidence for the occurrence of this type of reduction can be adduced from the isolation from urines of uranediol[6] and uranolone[7] which contain no oxygen atoms at $C_{20}$, and from cortical extracts by Steiger and Reichstein[2] of the compounds J, O and K, which contain no oxygen atoms at $C_{11}$. There is no reasonable doubt that the parent structure of J, O and K is that of *allo*-pregnane since all these have been converted to androstanedione-3,17. The question of the configuration at $C_9$ of the parent ring system of the other cortical derivatives having oxygen atoms at $C_{11}$ is, as both Steiger and Reichstein[8] and one of us[1] have indicated, not definitely settled. The attempted correlation of corticosterone and digoxigenin[9,9a] has not shed any further light on this problem; indeed the non-identity of the $\Delta^4$,3,11-diketocholenic acids obtained might be used as an argument to indicate that cortical derivatives are of the *allo*-urane type.

(4) Marker, Kamm, Fleming, Popkin and Wittle, THIS JOURNAL, **59**, 619 (1937).

(5) Marker, Kamm, Oakwood, Wittle and Lawson, *ibid.*, **60**, 1061 (1938); Marker, Wittle and Oakwood, *ibid.*, **60**, 1567 (1938).

(6) Marker, Rohrmann and Wittle, *ibid.*, **60**, 1561 (1938).

(7) Marker, Lawson, Wittle and Crooks, *ibid.*, **60**, 1559 (1938).

(8) Steiger and Reichstein, *Helv. Chim. Acta*, **21**, 161 (1938).

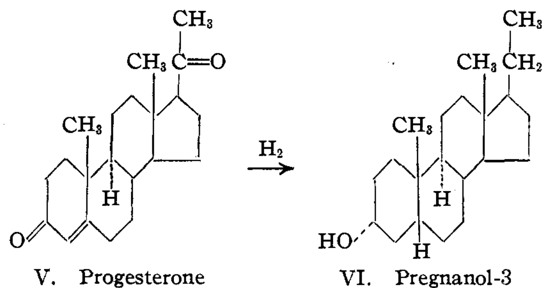
(9) Steiger and Reichstein, *ibid.*, **21**, 828 (1938).

(9a) Since this paper was submitted for publication, Mason and Hoehn [THIS JOURNAL, **60**, 2824 (1938)] have shown that the diketocholenic acid derived from digoxigenin is $\Delta^4$,3,12-diketocholenic acid.

Further support for our theory of the biogenesis of the steroidal hormones has now been found in the isolation of pregnanol-3-$\alpha$ and androsterone from human pregnancy urine.

We have reported previously the fractionation of semicarbazones of ketones from human pregnancy urine. At the time that work was carried out a semicarbazone fraction of intermediate solubility was obtained which was assumed to be impure *allo*-pregnanol-3$\alpha$-one-20 semicarbazone. An investigation of this fraction, however, has shown it to be androsterone, as proved by mixed melting points and its conversion to androstanedione. We believe that the occurrence of androsterone in human pregnancy urine and of estrone in stallions' urine indicates that these hormones are derived ultimately from sources other than the gonads, namely, from the suprarenal steroids by degradations of the type we have discussed previously. Thus, androsterone (IV) could arise from adrenosterone (III) as indicated in Fig. 1. To conceive of the androsterone as being derived from progesterone would involve a side-chain oxidation of a type more vigorous than is likely to occur in the glands.

The isolation of pregnanol-3-$\alpha$, which we have prepared from pregnanol-3$\alpha$-one-20,[10] was accomplished by precipitating the 3-$\beta$-OH sterols with digitonin and fractionating the 3-$\alpha$-OH sterols by means of chromatographic adsorption and high vacuum distillation. The pregnanol-3-$\alpha$, after isolation as its acetate which is not very soluble in cold methanol, showed no depression in melting point with an authentic sample. With the exception of cholesterol and equistanol, this is the first mono-oxygenated steroid isolated from glandular extracts or urines.




V. Progesterone VI. Pregnanol-3

Fig. II.

(10) Marker and Lawson, *ibid.*, **60**, 2438 (1938).

It undoubtedly arises from progesterone by reductive processes such as we have discussed, and its presence in urines must be considered valuable evidence in support of the proposed theory.

## Experimental Part

In the crystallization of the semicarbazones of the ketonic fraction of human pregnancy urine sterols, the more insoluble semicarbazone was that of *epi-allo*-pregnanolone. From the mother liquors of this was obtained the more soluble semicarbazone of *epi*-pregnanolone. A fraction of intermediate solubility between these two semicarbazones was crystallized from ethanol six times to a decomposition point of 255°. This semicarbazone became very insoluble as it became purer, thus resembling the semicarbazone of *epi-allo*-pregnanolone, and for that reason it was not further investigated until the present time. The yield was 1.5 g. from 150,000 liters of human pregnancy urine.

**Androsterone from Human Pregnancy Urine.**—To a solution of 500 mg. of the above semicarbazone in 25 cc. of ethanol was added a solution of 2.5 cc. of concd. sulfuric acid in 5 cc. of water, and the mixture was refluxed for one hour. Water was added and the mixture extracted with ether. The residue after evaporation of the ether was sublimed in a high vacuum at 135–150° and then crystallized from dilute methanol to give a product melting at 176°. It gave no depression in melting point when mixed with an authentic sample of androsterone, but depressed the melting point of *epi-allo*-pregnanolone to 140–145°.

*Anal.* Calcd. for $C_{19}H_{30}O_2$: C, 78.6; H, 10.4; mol. wt., 290. Found: C, 78.8; H, 10.5; mol. wt. (Rast), 283.

**Oxidation of Androsterone to Androstanedione.**—Twenty milligrams of androsterone isolated from human pregnancy urine was dissolved in 5 cc. of acetic acid and a solution of 7 mg. of chromic oxide in 2 cc. of 90% acetic acid was added. After standing for one hour at room temperature, water was added and the product extracted with ether. After removal of the solvent the residue was sublimed in high vacuum at 120° and then crystallized from dilute methanol to give androstanedione, m. p. 128°, which did not depress in melting point when mixed with an authentic sample.

*Anal.* Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.8. Found: C, 79.0; H, 9.9.

**Isolation of Pregnanol-3-$\alpha$ from Human Pregnancy Urine.**—The sterol fraction from 40,000 liters of human pregnancy urine after hydrolysis by alkali, removal of the pregnanediol and *allo*-pregnanediol by crystallization from acetone, and the ketones by Girard's reagent, was dissolved in 500 cc. of ether. To this was added 5 liters of ligroin and the ether removed by distillation. The mixture was cooled and the ligroin layer decanted from the tar which separated. The tarry residue was dissolved in 2 liters of ethyl alcohol and 200 g. of digitonin in 8 liters of hot alcohol was added. It was allowed to stand overnight and the digitonide was filtered and washed well with cold alcohol. The filtrate was concentrated to about 500 cc. and 5 liters of ether was added. It was again filtered from digitonin and the filtrate was evaporated to dryness *in vacuo*. This treatment removed all sterols having hydroxyl groups of the beta configuration.

The residue was dissolved in 250 cc. of pyridine and 200 g. of succinic anhydride was added. After heating for one hour on a steam-bath, ice was added and the succinate extracted with a large volume of ether. The pyridine was removed by washing with dilute hydrochloric acid and the ethereal layer was then extracted with sodium carbonate solution and a 2% solution of sodium hydroxide. The aqueous layers were combined, acidified and then extracted with ether. The solvent was removed and the residue saponified by refluxing with alcoholic potassium hydroxide solution. This carbinol fraction was extracted with ether and the solvent evaporated to dryness. On standing for several months some crystalline material appeared. It was treated with sufficient ether (cold) to filter. Upon washing with cold ether 23 g. of crystalline substance was obtained. The filtrate gave no precipitate when treated with a solution of alcoholic digitonin. The solvent was removed from the filtrate and the residue (115 g.) was dissolved in 2 liters of dry benzene. It was treated chromatographically with 800 cc. of aluminum oxide in 24-mm. diameter tubes. The absorbed material was washed with one liter of dry benzene, then with 5 liters of dry ether. Evaporation of the ether wash gave 12 g. of residue, which was sublimed *in vacuo* and the fraction distilling up to 120° collected. Upon washing with acetone this sublimate crystallized. It was converted into its acetate by boiling for half an hour with acetic anhydride. The acetate was crystallized several times from methanol, but could not be completely purified. After hydrolysis with alcoholic potassium hydroxide the sterol was dissolved in benzene, passed through a tube of aluminum oxide and washed out by ether and acetone. The eliminated material was crystallized from dilute methanol to a constant melting point of 146°. The product when mixed with *epi*-pregnanol-3 gave no depression in melting point. The amount of purified product was 26 mg., although much larger amounts are present.

*Anal.* Calcd. for $C_{21}H_{36}O$: C, 82.8; H, 11.8. Found: C, 82.5; H, 11.6.

Work on the identification of the *epi*-sterols from the other fractions is in progress at present and will be reported in THIS JOURNAL when the chemical structure of the products has been ascertained.

We wish to thank Dr. Oliver Kamm and Parke, Davis and Co. for their generous help and assistance in various phases of this work.

## Summary

The theory of the biogenesis of the steroidal hormones is discussed, and additional supporting evidence cited. The isolation of androsterone and pregnanol-3-$\alpha$ from human pregnancy urine is described.

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