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

Sterols. XLVII.* The Reduction Products of Estrone

BY RUSSELL E. MARKER AND EWALD ROHRMANN

In paper XLII[1] of this series the isolation and properties of two isomeric estranediols from human non-pregnancy urine were described. These diols were designated tentatively as diol-A, melting at 242° and diol-B, melting at 204°. Oxidation of these diols yielded different diketones, indicating that the two diols must differ at least in regard to the configuration at C_5 or C_{10}. It is not surprising that one of these diols isolated from the non-pregnancy urine was identical with an estranediol first obtained by Dirscherl[2] by the catalytic hydrogenation of estrone.

By the catalytic hydrogenation of estrone in an acid medium Dirscherl obtained two estranediols, one melting at 206° and the other at 155°. In addition to these he obtained two monohydroxy estrane compounds which apparently were formed by the elimination of the hydroxyl group at C_3. In his work no attempt to establish the configuration of the hydroxyl groups was made.

In an attempt to obtain more information concerning the configuration of the estranediols we have carried out the hydrogenation of α-estradiol in acid medium. This reaction yielded a diol melting at 204° which was found to be identical with the diol obtained by Dirscherl from the catalytic hydrogenation of estrone. This diol must therefore possess the α-configuration at C_{17}. In addition to this diol a compound of the composition $C_{18}H_{30}O_2 \cdot C_{18}H_{30}O$ melting at 175° was obtained. This substance appears to be a molecular compound. Upon oxidation with chromic acid at room temperature, it yielded a diketone melting at 170° which was identical with the diketone obtained by the oxidation of α-estranediol.

The α-estradiol used in the hydrogenation experiment was prepared in good yield by the aluminum isopropylate reduction of estrone. The compound was separated from the β-estradiol by means of its insoluble digitonide.[3]

In the catalytic hydrogenation of estrone and α-estradiol we have obtained no positive evidence of the formation of the estranediol melting at 242° obtained from human non-pregnancy urine.[1] This fact suggests that whereas catalytic hydrogenation of estrone produces compounds having the same configuration at C_5 and C_{10}, the reductive processes taking place in the body during the utilization of estrone may result in the formation of estranediols differing in their configurations at these asymmetric centers.

(*) Paper XLVI, THIS JOURNAL, **60**, 2442 (1938).

(1) Marker, Rohrmann, Lawson and Wittle, THIS JOURNAL, **60**, 1901 (1938).

(2) Dirscherl, *Z. physiol. Chem.*, **239**, 53 (1936).

(3) Wintersteiner, THIS JOURNAL, **59**, 765 (1937).

Experimental Part

Reduction of Estrone with Aluminum Isopropylate.—A mixture of 1 g. of estrone, 70 cc. of dry isopropyl alcohol and 1 g. of aluminum isopropylate was refluxed on a steam-bath for five hours. Approximately 50 cc. of the mixture was then slowly distilled off over a period of five hours. One gram of potassium hydroxide in 30 cc. of hot methanol was then added to the hot residue and the mixture allowed to stand for thirty minutes. The mixture was then poured into water and acidified with hydrochloric acid. The acidic mixture was extracted with ether and the ether extract was washed with water. The ether was removed, the crystalline residue dissolved in 15 cc. of hot 80% alcohol, and a boiling solution of 2 g. of digitonin in 80 cc. of 95% alcohol and 20 cc. of water was added. The resulting solution was cooled and allowed to stand for six hours. The white crystals of the digitonide which separated were filtered and washed with a little 95% alcohol. The dried crystalline digitonide weighed 1.5 g.

The digitonide was dissolved in 15 cc. of hot pyridine and the resulting solution heated on the steam-bath for twenty-five minutes. The hot solution was then poured into 200 cc. of ether and 15 cc. of methanol and the white precipitate was filtered and washed with ether. The filtrate was washed first with dilute hydrochloric acid and then with water. The ethereal solution was evaporated on the steam-bath and the crystalline residue after crystallization from acetone melted at 174°. This product gave no depression in melting point when mixed with an authentic sample of α-estradiol.

The filtrate from the digitonide separation was evaporated to a volume of 25 cc., 200 cc. of ether added, and the mixture thoroughly shaken. The mixture was filtered and the residue washed with ether containing 5% methanol. The filtrate was evaporated to dryness and the residue was crystallized from acetone to give β-estradiol melting at 215° (uncorr.).

Reduction of α-Estradiol.—To a solution of 1 g. of α-estradiol, m. p. 175°, dissolved in 100 cc. of absolute alcohol containing 1 cc. of concentrated hydrochloric acid, was added 500 mg. of platinum oxide catalyst. This was shaken with hydrogen at about five pounds (0.3 atm.) pressure and room temperature for five hours. The mixture was filtered and the filtrate poured into 300 cc. of water. The resulting mixture was extracted with ether and the ether extract washed first with sodium hydroxide

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-3,17α.

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-3,17α.

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 α- and β-estradiols. α-Estradiol upon catalytic hydrogenation yielded estranediol-3,17α, and a molecular compound $C_{18}H_{30}O_2 \cdot C_{18}H_{30}O$. Oxidation of both compounds yielded estranedione.

STATE COLLEGE, PENNA. RECEIVED OCTOBER 7, 1938

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

Sterols. XLVIII.* Isolation of Androsterone and Pregnanol-3-α 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-3β-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 $CH_3—\underset{|}{C}H—OH$ (α) 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).