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

Sterols. XXXVIII. Pregnenediol in Mares' Pregnancy Urine and its Conversion into Progesterone

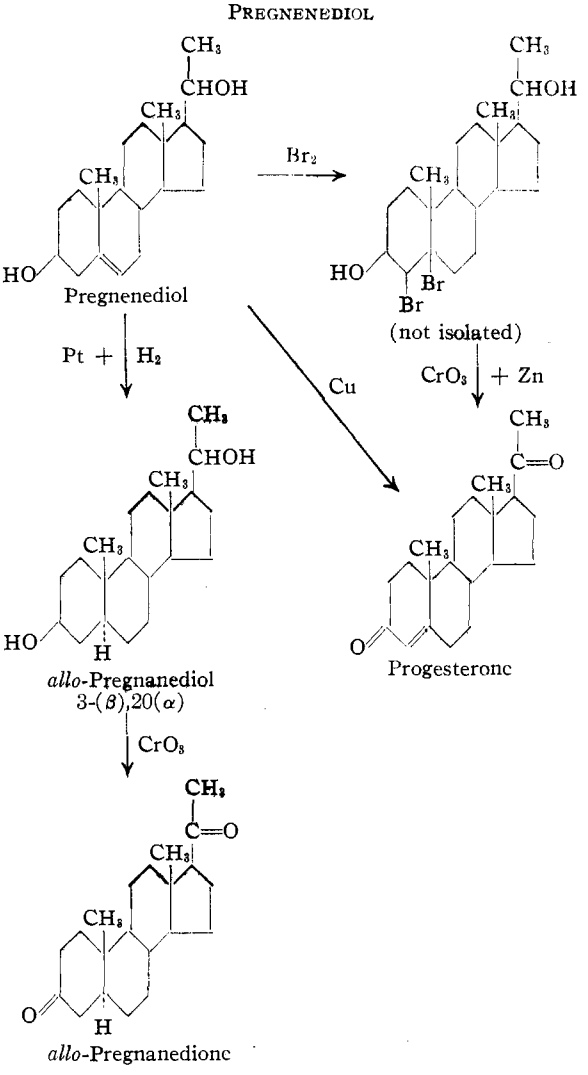
BY RUSSELL E. MARKER AND EWALD ROHRMANN

It is now fairly well established that pregnanediol and *allo*-pregnanediol are the final *in vivo* reduction products of progesterone. A number of intermediate reduction products, however, also have been isolated from both mares' and human pregnancy urines. *epi*-Pregnanolone[1] and *epi-allo*-pregnanolone[2] represent such partial reduction products which have been isolated from human urine. In addition to these, pregnanedione, *allo*-pregnanedione, and *allo*-pregnanol-3(β)-one-20 have been isolated from the ketonic fraction of mares' pregnancy urine.[3] The presence of these substances in pregnancy urine suggests that other possible reduction products might be present, specifically, compounds derived from $\Delta^{5,6}$-pregnene.

A preliminary study of the digitonin precipitated non-ketonic fraction of mares' pregnancy urine has shown the presence of two other reduction products of progesterone, namely, pregnenediol-3(β),20(α) and *allo*-pregnanediol-3(β),20(α). The fact that these products occur in the digitonin precipitated fraction indicates that the hydroxyl group at C_3 possesses the β-configuration. With the exception of *allo*-pregnanol-3(β)-one-20 all of the previously reported intermediate reduction products of progesterone have been of the *epi*- or (α)-configuration.

Pregnenediol was contaminated with another unsaturated compound, probably urenediol, which rendered complete purification difficult. Reduction of the pregnenediol with platinum catalyst yielded *allo*-pregnanediol-3(β),20(α) which upon oxidation with chromic anhydride gave *allo*-pregnanedione. Sublimation of the pregnenediol with copper yielded progesterone. Bromination followed by oxidation with chromic anhydride likewise yielded progesterone. The reactions involved are illustrated in the chart.

In addition to the reduction products of progesterone referred to above, there was isolated from the carbinol fraction of pregnant mares' urine the sterol β-equistanol, obtained previously from stallion urine.[4] We have found no evidence of the occurrence of cholesterol in mares' pregnancy urine.



Experimental Part

The insoluble digitonides A and B isolated in the work described in the preceding paper were used in this study. They were obtained from the neutral non-ketonic fraction from 1000 gallons (3700 liters) of mares' pregnancy urine. The Flow Sheet presented in Paper XXXVI should be consulted.

Decomposition of Digitonides from Carbinol Fraction I.—The 38 g. of digitonide A from carbinol fraction I was dissolved in 125 cc. of pyridine, the resulting solution was heated on the steam-bath for twenty-five minutes and poured into 1 liter of ether. The precipitate was filtered

(1) Marker and Kamm, THIS JOURNAL, **59**, 1373 (1937).
(2) Marker, Kamm and McGrew, *ibid.*, **59**, 616 (1937).
(3) Marker, Lawson, Wittle and Crooks, *ibid.*, **60**, 1559 (1938).
(4) Marker, Lawson, Rohrmann and Wittle, *ibid.*, **60**, 1555 (1938).

and washed with ether. The filtrate was then washed first with an excess of dilute hydrochloric acid and then with water. The ether was evaporated on the steam-bath and the residue sublimed in high vacuum at a temperature of 130 to 200°. The sublimed material appeared to be a complex mixture of substances which would not readily crystallize.

The residue from this distillation was reserved for another study.

Decomposition of Insoluble Digitonides from Carbinol Fraction II.—The 26 g. of insoluble digitonide obtained was dissolved in 150 cc. of hot pyridine and the solution heated on the steam-bath for thirty minutes and poured into 1.5 liters of ether. The precipitate was filtered and washed with ether. The filtrate was washed as usual, the ether was distilled off, and the orange colored residue dissolved in methanol and treated with Norite.

This material was combined with the sublimed fraction obtained from digitonide A.

Epimerization of Carbinols from Insoluble Digitonides A and B.—The residues obtained from the decomposition of digitonides A and B were combined and dissolved in 150 cc. of xylene, 7 g. of sodium was added and the mixture refluxed for seven hours. The excess sodium was destroyed with ethanol and the mixture washed with dilute hydrochloric acid and water. The xylene was evaporated *in vacuo* at 40°. The residue was dissolved in 250 cc. of boiling alcohol and to this was added a hot solution of 15 g. of digitonin in 250 cc. of alcohol. The mixture was cooled at 20° for one hour, filtered, and washed with alcohol to give insoluble digitonide C.

The alcohol was evaporated from the filtrate to a volume of about 50 cc., 500 cc. of ether was added and the small amount of precipitate was filtered and washed with ether to give a soluble digitonide D.

Equistanol from Digitonide D.—The 500 mg. of soluble digitonide D was dissolved in 5 cc. of pyridine, heated on the steam-bath for thirty minutes and the solution poured into 100 cc. of ether. The white precipitate was filtered and washed with ether. After washing the filtrate as before, the ether was evaporated from the steam-bath and the residue crystallized from methanol, giving white needles melting at 130°. This material gave no depression in melting point when mixed with a sample of equistanol from stallion urine.

Anal. Calcd. for $C_{30}H_{54}O$: C, 83.6; H, 12.6. Found: C, 84.0; H, 12.3.

Decomposition of Digitonide C.—Digitonide C (18 g.) was decomposed as before, the carbinols sublimed up to a temperature of 200°, and the sublimed material combined. The residue which did not distil at 200° was reserved for further study.

allo-Pregnanediol-3(β),20(α) from Mares' Pregnancy Urine.—The distilled solid from the decomposition of digitonide C containing only *allo* and unsaturated compounds was leached with a small amount of boiling acetone. The mixture was filtered and the insoluble residue was recrystallized from methanol to give small white plates, m. p. 216°, which gave no depression with an authentic sample of 3(β),20(α)-*allo*-pregnanediol.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.8; H, 11.3. Found: C, 78.4; H, 11.3.

Pregnenediol-3(β),20(α) from Mares' Pregnancy Urine.—The filtrate obtained after separation of the *allo*-pregnanediol-3(β),20(α) was evaporated to dryness and the residue fractionally crystallized from acetone. Pure material was difficult to isolate due to contaminating substances of an unsaturated character, possibly urenetriol or urenediol. The material melted poorly at 160–170°. Recrystallized from benzene and then from acetone it melted at 172–176°. It readily took up bromine in acetic acid solution.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.8. Found: C, 78.9; H, 11.1.

Reduction of Pregnenediol-3(β),20(α).—Pregnenediol (200 mg.) was dissolved in 50 cc. of ether, 0.5 g. of Adams platinum catalyst was added and the material reduced for one hour at room temperature and 45 pounds (3 atm.) pressure. The catalyst was filtered and the ether evaporated from the filtrate. The resulting residue was crystallized from 90% ethanol to give material melting at 217°. This gave no depression in melting point when mixed with an authentic sample of *allo*-pregnanediol-3(β),20(α).

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.8; H, 11.3. Found: C, 78.7; H, 11.2.

allo-Pregnanedione from allo-Pregnanediol-3(β),20(α).—100 mg. of the *allo*-pregnanediol-3(β),20(α) obtained by reduction of pregnenediol-3(β),20(α) was dissolved in 10 cc. of glacial acetic acid; 200 mg. of chromic anhydride in 10 cc. of 90% acetic acid was added and the mixture allowed to stand at room temperature for thirty minutes. The mixture was then poured into water and extracted with ether. The ether extract was washed with aqueous sodium carbonate and water. The ether was evaporated and the residue crystallized from acetone to give white crystals melting at 199°. This gave no depression in melting point when mixed with an authentic sample of *allo*-pregnanedione.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.6; H, 10.2.

Progesterone from Pregnenediol by Copper Oxidation.—Pregnenediol (100 mg.) was mixed thoroughly with 0.5 g. of Baker precipitated copper. The resulting mixture was heated at 230° at 20 mm. for thirty minutes. The mixture was then sublimed in high vacuum at 125°. The sublimed material was dissolved in 4.5 cc. of alcohol and 1.8 cc. of water was added to the resulting solution. After clarification with Norite the filtrate was cooled in salt–ice and the walls of the container were scratched vigorously. White crystals formed, m. p. 118–119°. This gave no depression in melting point when mixed with a sample of natural progesterone.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.1; H, 9.6. Found: C, 80.0; H, 9.5.

Progesterone from Pregnenediol by Chromium Oxidation.—Pregnenediol (200 mg.) was dissolved in 25 cc. of glacial acetic acid and the theoretical amount of bromine in acetic acid was added. To this solution was added 150 mg. of chromic anhydride dissolved in 20 cc. of 90% acetic acid. The mixture was allowed to stand for thirty minutes at room temperature, 5 cc. of alcohol and 1 g. of zinc dust were added and the mixture heated on the steam-bath for thirty minutes. The mixture was filtered and the filtrate diluted with water and extracted with 250 cc. of ether. The ether extract was washed with sodium carbonate solu-

tion and water, the ether evaporated, and the residue sublimed in high vacuum at 125°. After crystallization from 70% ethanol it melted at 118°.

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

Summary

Pregnenediol-3(β),20(α) and *allo*-pregnanediol-3(β),20(α) have been isolated from mares' pregnancy urine. Pregnenediol-3(β),20(α) upon oxidation gave progesterone and upon catalytic reduction gave *allo*-pregnanediol-3(β),20(α). β-Equistanol, previously obtained from stallion urine, has now been detected also in mares' pregnancy urine.

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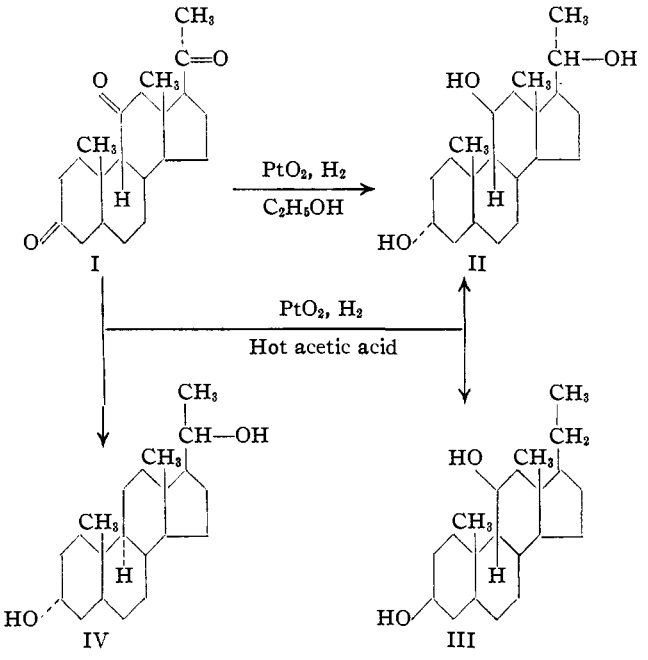
Sterols. XXXIX. The Reduction of Uranetrione

BY RUSSELL E. MARKER, EUGENE L. WITTLE AND THOMAS S. OAKWOOD

Uranetrione (I) is a 3,11,20-triketo-urane which has been found in previous work[1] to give uranedione (3,11-diketo-urane)[2] and pregnanedione when subjected to a series of reactions, the first step being catalytic reduction. In this paper a study of the reduction of uranetrione is presented.

Catalytic reduction of uranetrione (I) with platinum oxide in ethyl alcohol gave a product which on treatment with digitonin yielded a digitonide corresponding to approximately 10% of the product. The major unprecipitated fraction was found to contain a trihydroxy compound (II), as shown by the formation of a triacetate which is unaffected by further vigorous reduction or by chromic acid oxidation. This fraction, which did not precipitate with digitonin, was practically pure triol, showing that reduction of the carbonyl group at C-11 proceeded with the formation of only one of the two possible epimers. This behavior on reduction is therefore similar to that of the carbonyl group at C-20 which also gives only one epimer. The formation from uranetrione of a triol which forms a triacetate is in contrast to the reduction of a cortical steroid studied by Kendall.[3] His acid 1B (3-β-hydroxy-11-keto-aetio-*allo*-cholanic acid) was hydrogenated catalytically, and the resulting dihydroxy-aetio-*allo*-cholanic acid partially oxidized to give acid 1D (3-keto-11-hydroxy-aetio-*allo*-cholanic acid), which was stereoisomeric at C-11 with his dihydro acid 2. Acid 1D does not react with thionyl chloride or with acetylating reagents, and has a configuration at C-11 opposite to that of the natural cortical steroids.



According to our early prediction, uranetriol[1] now can be assigned definite stereochemical configurations. According to the arguments presented in an earlier paper,[1] a C-11α-hydroxyl group in a urane derivative is much more blocked than the epimeric C-11β-hydroxyl group, and, unlike the latter, will not form an acetate. Since triol (II), like uranetriol, forms a triacetate and does not precipitate with digitonin the configurations of the 3- and 11-hydroxyls in both these triols are established to be of the α- and β-configuration, respectively. The two triols, which

(1) Marker, Kamm, Oakwood, Wittle and Lawson, THIS JOURNAL, **60**, 1061 (1938).

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

(3) Mason, Hoehn, McKenzie and Kendall, *J. Biol. Chem.*, **120**, 719 (1937).