ther. Considerable residue remained in the distillation vessel. The fraction subliming at 110–140° was crystallized repeatedly from dilute acetone and yielded *allo*-pregnanedione, m. p. 196–200°, which gave no depression in melting point when mixed with authentic *allo*-pregnanedione.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.3; H, 10.3.

The non-distillable residue in the sublimation flask was crystallized from benzene–pentane, and yielded a crude ketone (ketone I) which melted at 220°. This ketone approximated the properties of Heard's ketone (m. p. 252°) upon further crystallization.

Anal. Found: C, 76.06; H, 9.85; mol. wt. (several determinations), 672.

The semicarbazone prepared from this ketone melted at 300°.

allo-Pregnanol-3(β)-one-20.—A preliminary investigation of the hydroxyketone from semicarbazones II and III showed that one substance was present in both fractions, which were therefore combined. This hydroxy ketone was sublimed in a high vacuum at a bath temperature of 185°, and the sublimate crystallized from carbon tetrachloride to give a ketone melting at 193°. This ketone depressed the melting point of *allo*-pregnanol-20(β)-one-3 (m. p. 195°), showed no unsaturation to bromine in acetic acid and gave a heavy precipitation with an alcoholic solution of digitonin.

A portion oxidized with chromic acid in the usual manner gave *allo*-pregnanedione, m. p. 197–199°, and showed no depression with an authentic sample of *allo*-pregnanedione, m. p. 200°.

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

Uranol-11-one-3, Pregnanedione, and Ketone II from Semicarbazones II and III.—The ketones from semicarbazones II and III were distilled separately and fractions collected at bath temperatures of 135–160°, 160–200°, leaving considerable amounts of glassy residue. The fraction 135–160° was crystallized from diluted acetone to yield a solid melting over the range 100–135°. The material in the mother liquors was then crystallized repeatedly from dilute acetone, sublimed in a high vacuum, and then crystallized from ether–pentane to give pregnanedione, m. p. 118°, which did not depress the melting point of an authentic sample.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.5; H, 10.7.

The fraction subliming at 160–200° was a mixture which could be separated only with difficulty. Repeated crystallization from dilute alcohol and dilute acetone yielded a ketone which melted at 165°, and depressed the melting points of uranedione (m. p. 180°) and *allo*-pregnanedione (m. p. 200°).

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

It gave a gelatinous semicarbazone melting at 250° with decomposition.

Oxidation of a sample with chromic acid in the customary fashion yielded uranedione, m. p. 175–176°, which showed no depression with an authentic sample of uranedione.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.7; H, 10.5.

The glassy residues from sublimation of the neutral ketones up to 200° were combined and crystallized from benzene–pentane to yield about 100 mg. of a ketone melting at 115–120°. It did not precipitate with digitonin.

Anal. Found: C, 74.3; H, 9.65; mol. wt., 462.

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

The ketonic fraction of mares' pregnancy urine yielded pregnanedione, *allo*-pregnanedione, *allo*-pregnanol-3(β)-one-20, and uranol-11-one-3 in addition to the ketone reported by Heard.

STATE COLLEGE, PENNA. RECEIVED MARCH 16, 1938

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

Sterols. XXXVII. Uranediol from Mares' Pregnancy Urine

BY RUSSELL E. MARKER, EWALD ROHRMANN AND EUGENE L. WITTLE

In paper XXIX[1] of this series the isolation of uranetriol from mares' pregnancy urine was reported. The urane compounds were shown to differ in structure from pregnane compounds in the configuration at C_9. In the pregnane series the hydrogen at C_9 is *trans* to the methyl group at C_{10}, while in the urane series the hydrogen atom is considered to be *cis* to the methyl group. Clemmensen reduction of uranetrione gave the parent hydrocarbon, urane, which is different from either pregnane or *allo*-pregnane. Evidence was presented to show that the hydroxyl groups in uranetriol are located at the 3-, 11- and 20-positions. The presence of uranediol in mares' pregnancy urine was indicated by the fact that oxidation of a mixture of crude diols gave a diketone which on Clemmensen reduction yielded the hydrocarbon urane.

We have now succeeded in isolating uranediol from the digitonin precipitated fraction of the ketone-free carbinol fraction of mares' pregnancy

---

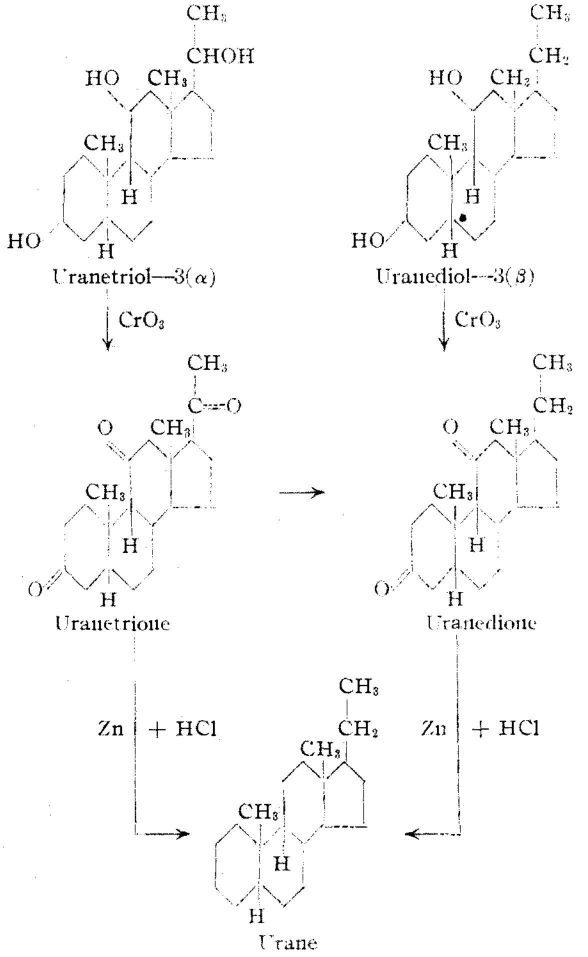
[1] Marker, *et al.*, THIS JOURNAL, **60**, 1061 (1938).

urine. Upon oxidation the diol yields a dione which is identical with that reported in paper XXIX.[1] The fact that the substance is precipitated by digitonin indicates the presence of a β-hydroxyl group in the C_3-position. The dione formed only a monosemicarbazone and a mono-2,4-dinitrophenylhydrazone which indicates that the second hydroxyl group is in the hindered C_{11}-position rather than in the C_{20}-position as in the pregnanediols.

Treatment of uranedione in acetic acid at room temperature with PtO_2 catalyst apparently reduces only the ketonic group at C_3, since epimerization of the resulting reduction products with sodium in xylene results in destruction of the material. Uranediol forms a diacetate.

Isomerization with sodium in xylene yielded material which gave very little precipitation with digitonin, indicating that the substance has the normal configuration at C_5.

The relationship of uranetriol to uranediol is given in the chart.



It is a striking fact that while uranetriol occurs in nature with the *epi* configuration with respect to the hydroxyl at C_3, uranediol possesses the normal or beta configuration at that position. The two compounds are closely related chemically as is shown by the fact that both can be reduced to the hydrocarbon, urane. It is probable, therefore, that they are closely related biologically. If not related to the active principles of the suprarenal cortex they may owe their origin to some as yet uncharacterized hormone.

The separation of uranediol from other digitonin-precipitated substances is greatly facilitated by the fact that it forms a digitonide which is relatively soluble in 95% ethanol. The soluble digitonide is separated by removing most of the alcohol and precipitating with ether. The digitonide so formed on decomposition with pyridine yields uranediol in relatively pure form. Mares' pregnancy urine contains approximately 5 mg. of the substance per gallon.

At present uranediol is the only compound which has been found in urine having the beta configuration at C_3 and the regular configuration at C_5. That other regular compounds with the beta configuration at C_3 are absent is indicated by the fact that when a mixture of digitonin-precipitated compounds was epimerized with sodium and xylene and the resulting $C_3(\alpha)$ compounds oxidized to ketones the only ketone obtained was uranedione.

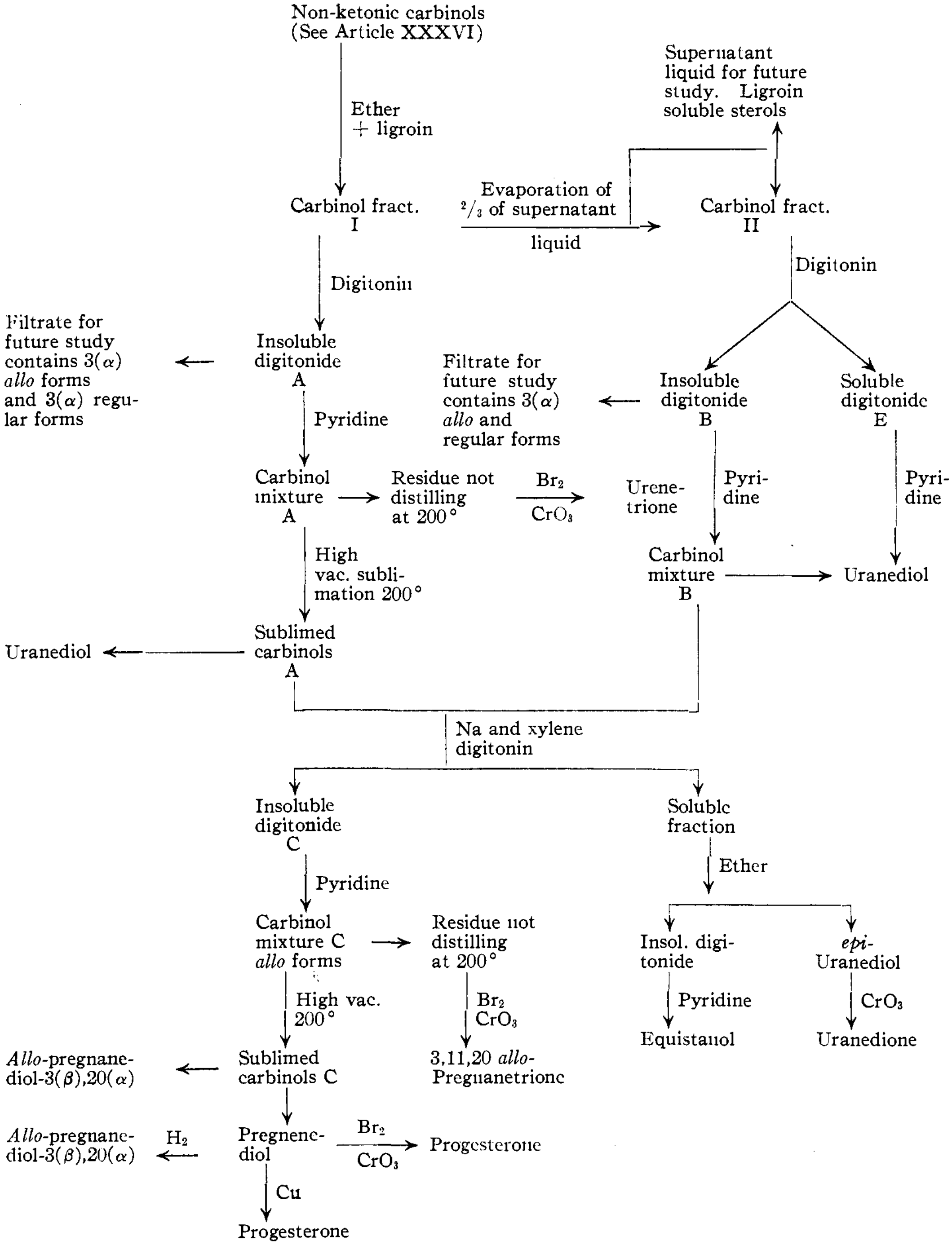
The steps involved in the experimental work reported in this paper and in the two following articles are outlined in the attached Flow Sheet.

Experimental Part

Precipitation of Mares' Pregnancy Urine Carbinols with Digitonin.—A concentrate of mares' urine carbinols corresponding to 1000 gallons (3700 liters) of urine from which all ketones had been removed previously with Girard's reagent was dissolved in 400 cc. of ether. To this solution was added 2 liters of ligroin and the mixture cooled in a salt–ice bath for one hour, and the upper liquid decanted from the precipitated tar. This gave carbinol fraction I. The supernatant liquid was distilled until approximately two-thirds of the solvent was removed. The resulting mixture was cooled in a salt–ice bath and the supernatant liquid poured from the precipitated tar. This gave carbinol fraction II.

Fraction I was made up to a volume of 2 liters with 95% ethanol. The solution was heated to boiling on the steam-bath and a boiling solution of 30 g. of digitonin in 1 liter of 95% ethanol was added. A precipitate formed at once. The mixture was cooled to 20°, and after three hours was filtered. The solid was washed with 500 cc. of 95% ethanol, resuspended in 500 cc. 95% ethanol and refiltered. This

FLOW SHEET



fraction, referred to as digitonide-A, was obtained in a yield of 38 g.

Carbinol fraction II was made up to a volume of 750 cc. with 95% ethanol. It was heated to boiling and a hot solution of 25 g. of digitonin in 1.5 liters of 95% ethanol was added. A precipitate formed after a few minutes. The mixture was cooled to 18°, filtered after two hours and washed with 95% ethanol. This fraction, referred to as digitonide-B, was obtained in a yield of 26 g. The filtrate was evaporated to a volume of about 50 cc. and 1 liter of ether was added. The precipitate was filtered and washed with ether. The residue of soluble digitonide-E weighed 8 g.

Digitonides A and B were reserved for other work. The filtrates likewise were reserved for investigation of the non-digitonin-precipitated substances.

Uranediol from Mares' Urine.—The soluble digitonide-E from carbinol fraction II was dissolved in 50 cc. of hot pyridine and the mixture heated thirty minutes on the steam-bath. The resulting solution was then poured into 1000 cc. of ether and the precipitate filtered and washed with ether. The filtrate was washed first with dilute hydrochloric acid and then with water. The ether was distilled, the crystals which separated were recrystallized from 15 cc. of acetone and washed with cold ether. After two additional recrystallizations from acetone the product was obtained in the form of white needles, m. p. 210°.

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

The compound did not discolor bromine and gave a 20° depression in melting point when mixed with 3(β),20(α)-*allo*-pregnanediol.

Preparation of Uranediol Diacetate.—Approximately 75 mg. of uranediol was refluxed with 4 cc. of acetic anhydride for thirty-five minutes. The acetic anhydride was evaporated in vacuum and the crystalline residue recrystallized from methanol to a constant melting point. It crystallized in the form of small white plates, m. p. 160°.

Anal. Calcd. for $C_{25}H_{40}O_4$: C, 74.4; H, 10.0. Found: C, 74.6; H, 10.0.

Oxidation of Uranediol to Uranedione.—Uranediol (100 mg.) was dissolved in 30 cc. of glacial acetic acid. A solution of 150 mg. of chromic anhydride in 10 cc. of 90% acetic acid then was added at room temperature. A precipitate separated at first, but dissolved after the addition of chromic anhydride was completed. The mixture was allowed to stand at room temperature for forty-five minutes. Water was added and the mixture extracted twice with 75-cc. portions of ether. The combined ether extracts were washed with water and then with saturated sodium carbonate solution. The ether was evaporated and the crystalline residue dissolved in 5 cc. of methanol and cooled in a salt–ice bath. One-half cc. of water was added slowly, the precipitate collected and recrystallized from 90% methanol. Recrystallization from acetone gave a product which melted at 177.5° and which gave no depression in melting point when mixed with a sample of uranedione prepared from uranetriol.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.78; H, 10.3.

Uranedione Semicarbazone.—A solution of 20 mg. of uranedione, 30 mg. of semicarbazide hydrochloride and 30 mg. of sodium acetate in 10 cc. of alcohol was refluxed on the steam-bath for one hour, diluted with water and filtered. The product was washed with ether and crystallized from alcohol to yield uranedione monosemicarbazone, m. p. 245°, dec.

Anal. Calcd. for $C_{22}H_{35}O_2N_3$: C, 70.7; H, 9.4. Found: C, 70.4; H, 9.1.

Uranedione Mono-2,4-dinitrophenylhydrazone.—A solution of 30 mg. of uranedione and 40 mg. of 2,4-dinitrophenylhydrazine in 10 cc. of refluxing alcohol was acidified with two drops of concd. sulfuric acid and refluxed for five minutes. The solution was allowed to cool, the crystalline product separated by filtration and crystallized from ethyl acetate to give the mono-2,4-dinitrophenylhydrazone of uranedione, m. p. 200° dec.

Anal. Calcd. for $C_{27}H_{36}O_5N_4$: C, 65.4; H, 7.2. Found: C, 65.0; H, 7.3.

Epimerization of Steroids in the Mother Liquors.—The mother liquors from the crystallization of uranediol were evaporated and the residue dissolved in 200 cc. of xylene. Sodium (5 g.) was added and the mixture refluxed for eight hours. The excess sodium was destroyed with 95% ethanol and the resulting solution washed with dilute hydrochloric acid and water. The xylene was evaporated in vacuo at 40°. The sticky residue obtained was dissolved in 100 cc. of 95% ethanol and a boiling solution of 10 g. of digitonin in 250 cc. of 95% ethanol was added. A flocculent precipitate separated almost at once. The mixture was cooled at 20° for two hours, filtered and washed with 95% ethanol; 10.5 g. of white digitonide was obtained. The ethanol was distilled from the filtrate until a volume of about 50 cc. remained and 250 cc. of ether was added. The precipitate was collected and washed with ether. The ether was evaporated from the filtrate, leaving a sticky residue.

The residue was dissolved in 100 cc. of glacial acetic acid, 2.5 g. of chromic anhydride in 60 cc. of 60% acetic acid was added, and the mixture allowed to stand at room temperature for forty-five minutes. The mixture was then poured into 800 cc. of water and extracted with 800 cc. of ether. The ether extract was washed with water and aqueous sodium carbonate. The residue obtained by evaporation of the ether was sublimed in high vacuum at 130–150°. The sublimed material consisted almost entirely of uranedione, indicating the absence in pregnancy urine of other substances having a beta configuration at C_3 and the coprostane configuration at C_5.

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

Uranediol, the presence of which in mares' pregnancy urine previously has been demonstrated, now has been isolated. It is shown to have the structure of uranediol-3(β),11. This is the first sterol derivative to be obtained from urine possessing the β-configuration on C_3 and the coprostane configuration on C_5. On oxidation it yields uranedione which, however, forms only a mono-semicarbazone and a mono-2,4-dinitrophenylhydrazone.

State College, Penna. Received March 16, 1938