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

Sterols. XXXVI. Ketones from Mares' Pregnancy Urine

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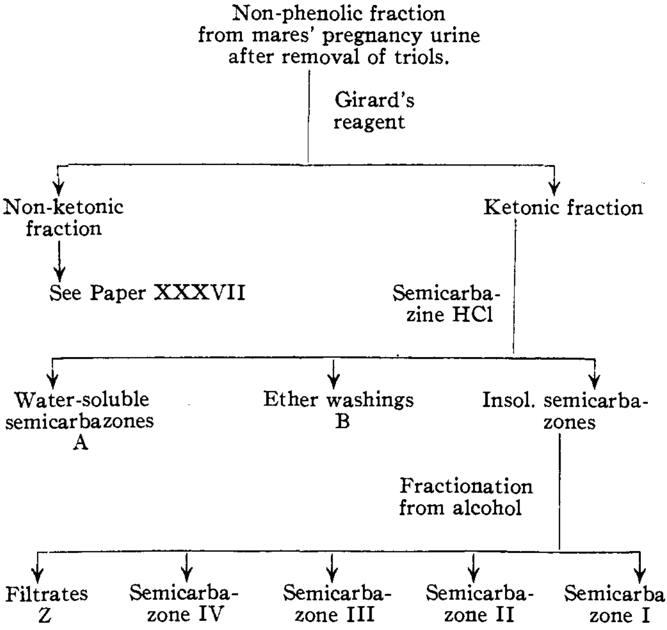
As part of an extensive investigation of urines from various animal sources, we have been studying during the past two years the steroid content of mares' pregnancy urine. We already have reported the presence of pregnanediol,[1] *allo*-pregnanediol,[1] uranetriol,[2] pregnanetriol,[2] and uranediol[3] in the carbinol fraction from mares' pregnancy urine. The presence of a pregnanetriol which is probably identical with our pregnanetriol, was reported earlier by Haslewood, Marrian and Smith.[4] More recently these authors also have reported[5] the presence of pregnanetriol-3,17,20 in the pregnancy urine of women afflicted with cortical tumors. In the description of our investigation of the carbinol fraction of mares' pregnancy urine we reported the separation of a ketonic fraction from this source. Since Heard[6] has announced the isolation of a number of ketones from mares' pregnancy urine, we feel obliged to report the progress we have made in the course of a similar study. We are able to confirm the presence in mares' urine of the ketone, m. p. 252°, reported by Heard and in addition we have found a number of other steroids present. While a direct comparison of our compound with Heard's ketone has not been made, we believe that the two are identical in view of their apparently similar properties. However, molecular weight determinations of our substance (mol. wt. 672) gave a value twice that of a steroid, indicating that it may be a combination of two sterol molecules through their oxygen atoms. This would account for the inactivity of these atoms. We are not planning further work on this compound since we feel that Dr. Heard is entitled to priority in its discovery.

The accompanying diagram shows the scheme of isolation of our various ketones. The crude semicarbazone mixture from 30,000 gallons (114,000 liters) of mares' pregnancy urine yielded a water solution (A) containing Heard's water-soluble semicarbazone, an ethereal solution (B) containing chiefly non-steroidal semicarbazones, and a solid crude semicarbazone mixture. The latter on systematic fractional crystallization from alcohol yielded solid semicarbazones I, II, III and IV and a mother liquor Z. Each fraction was hydrolyzed with alcoholic sulfuric acid, and further purifications were effected by high-vacuum distillation, half-succinate separation, and fractional crystallization.

FLOW SHEET



We were surprised to find that all of the semicarbazones yielded both hydroxylated and hydroxyl-free ketones. This occurrence of hydroxyl-free ketones in mares' pregnancy urine is in contrast to the almost complete absence of such ketones in human pregnancy urine.[7] Possibly the course of reduction of progesterone and other hormones in the mare follows a somewhat different path, or else reduction may be more complete in man.

From the most insoluble semicarbazone frac-

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[1] Marker, Kamm, Crooks, Oakwood, Lawson and Wittle, THIS JOURNAL, **59**, 2297 (1937).

[2] Marker, Kamm, Crooks, Oakwood, Wittle and Lawson, *ibid.*, **60**, 210 (1938).

[3] Marker, Rohrmann and Wittle, *ibid.*, **60**, 1561 (1938).

[4] Haslewood, Marrian and Smith, *Biochem. J.*, **28**, 1316 (1934).

[5] Marrian and Butler, *J. Biol. Chem.*, **119**, LXVI (1937).

[6] Heard, THIS JOURNAL, **60**, 493 (1938).

[7] Marker, Kamm and McGrew, *ibid.*, **59**, 616 (1937)

tion (I) we have isolated two substances: a ketone which corresponds in properties to Heard's ketone, $C_{19}H_{26}O_3$, m. p. 252°, but has a molecular weight of 672, and *allo*-pregnanedione. We have also some indication of the presence of at least one other ketone in this hydrolyzed semicarbazone mixture (I). The presence of *allo*-pregnanedione and pregnanedione in mares' pregnancy urine lends further support to the hypothesis that progesterone is the source of most of the steroidal carbinols and ketones, such as pregnanediol, *allo*-pregnanediol, pregnanetriol-3(α),4(β),20(α),[8] *allo*-pregnanol-3(α)-one-20, pregnanol-3-(α)-one-20, pregnanediol-3(β),20(α),[9] pregnenediol-3(β),-20(α),[9] pregnanedione, and *allo*-pregnanol-3(β)-one-20.

Semicarbazones II and III proved to contain the same components. From the hydrolyzed semicarbazones there was obtained a new hydroxyketone, uranol-11-one-3, the known pregnanedione, and *allo*-pregnanol-3(β)-one-20. The structure of uranol-11-one-3 is proved by its composition and oxidation to uranedione. It did not form a half-succinate ester, in agreement with the usual postulated inertness of a hydroxyl group at C_{11}. It gave a Zimmermann test showing the presence of a carbonyl group at C_3. The isolation of this substance lends support to the hypothesis that uranedione and the uranediols (α,α and β,α) may be related to some hormone of as yet unknown function,[10] although it is possible that these substances may be derived from the cortical hormone. The isolation of *allo*-pregnanol-3(β)-one-20 from mares' pregnancy urine is interesting in view of the fact that it was first isolated from corpus luteum extracts.[11–14] The structure of our substance was proved by the composition, melting point, precipitation with digitonin, and oxidation to *allo*-pregnanedione. This is the first 3(β)-hydroxyketone to be isolated from urine, although its occurrence had been foreseen in the light of a proposed course of the reduction of progesterone.

We have not as yet completed our investigation of the ketones from semicarbazones A, B, and Z, since the mixtures have proved to be rather intractable. Semicarbazones B and Z yield a mixture of neutral and hydroxylated ketones. The hydroxyketone mixture from semicarbazone Z does not precipitate digitonin, and therefore no *allo*-pregnenol-3(β)-one-20 is present. Distillation of the neutral and hydroxylated ketones from B gives chiefly ketones subliming below 125°, and possessing characteristic odors. Apparently almost no steroidal ketones are present in fraction B.

Because of the small amounts of ketones present in mares' pregnancy urine, a more extensive investigation of 150,000 gallons (570,000 liters) of urine is now in progress, and the results of this study will be reported shortly.

Experimental Part

Isolation of Ketonic Fractions.—The non-phenolic fraction from 30,000 gallons (114,000 liters) of mares' pregnancy urine from which the uranetriol and pregnanetriol had been removed was dissolved in about 2.5 liters of alcohol and heated for one half hour with 400 g. of Girard's reagent. Ice and water were added and the non-ketonic material extracted thoroughly with ether. The aqueous layer was acidified with an excess of hydrochloric acid and warmed for one hour on a steam-bath. The ketonic matter was extracted with ether and the ether extract washed and concentrated. The residual oil was treated again with Girard's reagent to remove a small amount of inert material which was carried through mechanically. About 30 g. of ketonic material was obtained.

The ketone mixture was dissolved in 300 cc. of alcohol and boiled for one hour with 50 g. of semicarbazide hydrochloride and 60 g. of sodium acetate. The mixture was distilled to remove almost all the alcohol and the resulting paste was stirred with water, filtered, and washed with water and with ether. The water washings were set aside as semicarbazone fraction A, and the ether washings as semicarbazone fraction B. The solid semicarbazone mixture, amounting to about 15–20 g., was fractionated from alcohol. This gave semicarbazone solution Z, and semicarbazones I, II, III, and IV. Semicarbazone solutions A and Z were evaporated to dryness to yield semicarbazones A and Z. These six semicarbazone fractions were hydrolyzed separately by boiling for one hour with fifty parts of alcohol and fifteen parts of 1:2 dilute sulfuric acid. Each hydrolysate was diluted with water, extracted with ether, the ether layer washed with water, and then evaporated to dryness. With the exception of the ketones from semicarbazone I, the residues were converted into the acid succinates in order to separate hydroxylated ketones from OH-free ketones.

allo-Pregnanedione and Ketone I from Semicarbazone I.—Hydrolysis of semicarbazone I gave 1.4 g. of solid ketones which could not be separated readily by crystallization. Accordingly, the total ketonic material was distilled in a high vacuum and fractions collected at bath temperature up to 110, 110–140 and 140–180°. The first and third fractions were too small to be investigated fur-

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(8) Marker, Kamm, Wittle, Oakwood and Lawson, This Journal, **60**, 1067 (1938).

(9) Marker and Rohrmann, *ibid.*, **60**, 1565 (1938).

(10) Marker, Lawson, Rohrman and Wittle, *ibid.*, **60**, 1555 (1938).

(11) Wintersteiner and Allen, *J. Biol. Chem.*, **107**, 321 (1934).

(12) Hartmann and Wettstein, *Helv. Chim. Acta*, **17**, 1365 (1934).

(13) Butenandt, Westphal and Hohlweg, *Z. physiol. Chem.*, **227**, 84 (1934).

(14) Slotta, Ruschig and Fels, *Ber.*, **67**, 1270 (1934).

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.

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Sterols. XXXVII. Uranediol from Mares' Pregnancy Urine

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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

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