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

# Sterols. LXXVIII. Sterols from Sow Pregnancy Urine

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The fact that mares pregnancy urine has been shown recently to exhibit some interesting differences[1] from the other pregnancy urines which have been studied indicates the desirability of further investigations of pregnancy urines of other animals.

The present preliminary investigation of sow pregnancy urine indicates that this urine is strikingly different from the other urines studied. Sow pregnancy urine differs from other urines mainly in the absence of the three common pregnanediols which have been found in all of the other pregnancy urines heretofore investigated. However, both pregnanol-3($\alpha$)-one-20 and *allo*-pregnanol-3($\beta$)-one-20 were isolated from the ketonic sterol fraction. The presence of these two products and the absence of pregnanediols suggests that the sow in the course of the *in vivo* reduction of progesterone[2] is unable to effect the reduction of a C-20 carbonyl group to a C-20 carbinol. This also receives support from the fact that progesterone has been isolated from sow ovaries.[3]

The *epi*-ketone fraction remaining after the removal of pregnanol-3($\alpha$)-one-20 when treated with hydroxylamine acetate yielded an oxime which appears to be identical with androsterone oxime, thus indicating the presence of androsterone. This lends further support to the theory that these products are derived from the adrenal compounds. Callow[4] has recently suggested that *trans*-dehydroandrosterone is probably derived from the adrenal cortical secretions and not from testosterone. It should be pointed out that from the generalizations previously made in regard to the origin of these substances,[2,5] both androsterone and *trans*-dehydroandrosterone may be derived from the adrenal cortical secretions and it is not necessary to assume that they are reduction products of $\Delta^{4,5}$-androstenedione-3,17.

Both cholesterol and the characteristic urinary hydrocarbon[1,6,7] of the composition $C_{28}H_{58}$ were isolated from the sow urine. $\beta$-Equistanol[8,9,10] was not encountered.

There appear to be other C-3 beta sterols present in the sow pregnancy urine but these could not be isolated from the quantity of urine studied. No apparent differences were observed in urine collected during the second month of pregnancy and during the last month of pregnancy.

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## Experimental Part

To 30 gallons (110 liters) of sow pregnancy urine, collected from Chester sows during the last month of pregnancy was added enough concentrated hydrochloric acid to bring it to a *p*H of 1. The urine was then heated at 100° for three hours, thoroughly extracted with butanol, and the solvent removed *in vacuo*. The residue, consisting of a black tar, was steam distilled for two hours from aqueous sodium hydroxide. The neutral products were extracted with ether and the solvent removed.

The total neutral fraction (14 g.) was dissolved in pyridine (20 cc.) and 10 g. of succinic anhydride was added. The mixture was heated for ninety minutes on the steam-bath, after which ether and water were added and the pyridine removed by dilute hydrochloric acid. The succinates were removed from the ether by potassium carbonate solution, and hydrolyzed by boiling with alcoholic potassium hydroxide.

**Hydrocarbon Fraction.**—The non-carbinol fraction was treated with Girard's reagent in alcohol, but only a negligible amount of ketones was obtained. The non-ketonic fraction was then sublimed in high vacuum at 80–100° and the sublimate crystallized from acetone to give white plates, m. p. 60–63°. It gave no depression in melting point when mixed with the urinary hydrocarbon obtained from mares pregnancy and human pregnancy urine, m. p. 61–63°.

*Anal.* Calcd. for $C_{28}H_{58}$: C, 85.3; H, 14.8. Found: C, 85.1; H, 14.8.

The carbinol fraction was dissolved in a small amount of alcohol and treated with 10 g. of digitonin dissolved in 500 cc. of hot 90% ethanol. The digitonide was allowed to stand overnight, filtered, washed and dried; yield, 8.6 g. The digitonide was heated on the steam-bath for one hour with 25 cc. of dry pyridine and the solution poured into ether and filtered. The pyridine was removed from the filtrate by dilute hydrochloric acid and the ether was evaporated to 1.9 g. of oily residue.

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 2537 (1939).
(2) Marker, THIS JOURNAL, **60**, 1725 (1938).
(3) Fieser, "Chemistry of Natural Products Related to Phenanthrene," 2d ed., Reinhold Publishing Corp., New York, N. Y., 1937, pp. 241 ff.
(4) Callow, *Biochem. J.*, **33**, 559 (1939).
(5) Marker and Lawson, THIS JOURNAL, **60**, 2928 (1938).
(6) Marker, *ibid.*, **60**, 2442 (1938).
(7) Marker, *ibid.*, **61**, 1287 (1939).
(8) Marker, Lawson, Rohrmann and Wittle, *ibid.*, **60**, 1555 (1938).
(9) Marker, Wittle and Lawson, *ibid.*, **60**, 2931 (1938).
(10) Marker and Rohrmann, *ibid.*, **60**, 1565 (1938).

The ketones were spearated from the beta-sterols by means of Girard's reagent in the usual manner. These were sublimed in high vacuum and the fraction distilling from 100–200° was collected. The sublimate was crystallized from ether–pentane to give white crystals, m. p. 191–193°. This gave no depression in melting point when mixed with *allo*-pregnanol-3(β)-20-one, m. p. 193–194°.

*Anal.* Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 9.9. Found: C, 79.0; H, 9.6.

No other crystalline products could be obtained from the mother liquors.

**beta-Non-ketonic Sterols.**—The fraction of non-ketonic, digitonin precipitable sterols was sublimed in high vacuum, the fraction subliming at 100–200° being collected. This was crystallized from ether–pentane and then from ethanol, to give white crystals, m. p. 146–147°. This gave no depression in melting point when mixed with an authentic sample of cholesterol, m. p. 147–148.5°.

*Anal.* Calcd. for $C_{27}H_{46}O$: C, 83.9; H, 12.0. Found: C, 83.8; H, 11.9.

No additional crystalline products were obtained from the mother liquors. The solvent was evaporated and the residual tar oxidized by chromic anhydride in acetic acid at 25°. The small ketone fraction obtained was treated with semicarbazide acetate. None of the extremely insoluble disemicarbazone of *allo*-pregnanedione could be isolated, indicating the absence of *allo*-pregnanediol-3(β), 20(α).

**Epimeric Ketones.**—The fraction of sterols not precipitated by digitonin was treated with Girard's reagent in alcohol in the usual manner. The hydroxy ketones were sublimed *in vacuo*, the fraction subliming at 100–200° being collected. The sublimate was crystallized repeatedly from ether–pentane to give white crystals, m. p. 139–142°. This gave no depression in melting point when mixed with epipregnanolone, m. p. 144°.

*Anal.* Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 9.9. Found: C, 79.1; H, 9.7.

The filtrate was evaporated and the residue dissolved in 5 cc. of ethanol. To this was added 600 mg. of sodium acetate and 500 mg. of hydroxylamine hydrochloride and the solution heated four hours at 90°. Water was added and the gum which deposited was crystallized from 60% ethanol and then from 80% acetone to give white crystals, m. p. 210–212°. This gave no depression in melting point when mixed with the oxime of androsterone, m. p. 212–214°.

*Anal.* Calcd. for $C_{19}H_{31}O_2N$: C, 74.7; H, 10.2. Found: C, 74.2; H, 10.1.

The epimeric non-ketonic sterol fraction was sublimed in high vacuum. The sublimate could not be obtained crystalline. The total sublimate was oxidized by chromic anhydride in acetic acid at 25° and the ketonic fraction isolated from the mixture by means of Girard's reagent. Only a small amount of ketones was obtained which resisted crystallization after sublimation. These were treated with semicarbazide hydrochloride and sodium acetate in alcohol. The semicarbazones which were formed were extremely soluble in cold ethanol. Since *allo*-pregnanedione forms a very insoluble semicarbazone, even in boiling alcohol, the absence of this product is indicated.

Fifteen gallons (55 liters) of urine collected during the second month of pregnancy of the hogs gave the same results as those reported above.

## Summary

Sow pregnancy urine differs from other pregnancy urines studied in that the pregnanediols appear to be absent.

Pregnanol-3(α)-one-20, *allo*-pregnanol-3(β)-one-20, cholesterol and the urinary hydrocarbon were isolated.

Evidence of the presence of androsterone was obtained.

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# Sterols. LXXIX. Oxidation Products of Dihydrosarsasapogenin

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In order to obtain further evidence concerning the structure of the side chain of sarsasapogenin (I),[1] we have oxidized with chromic anhydride the methyl ester acetate of anhydrotetrahydrosarsasapogenoic acid (III) (obtained both from sarsasapogenoic acid by catalytic hydrogenation and from 3-acetoxydihydrosarsasapogenin by mild oxidation) and by treatment of the neutral fraction from the oxidation with aqueous ethanolic alkali have obtained a good yield of an acid identical with anhydrosarsasapogenoic acid. This indicates that the methyl ester acetate of sarsasapogenoic acid was formed in the oxidation and was in turn converted to the anhydro acid by the alkali treatment. In this reaction sarsasapogenoic acid could only have arisen from the oxidative opening of an oxide ring to yield a diketo compound, thus supporting IV which we recently proposed[2] for sarsasapogenoic acid.

Further support of the structure assigned to di-

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 846 (1939).

(2) Marker and Rohrmann, THIS JOURNAL, **61**, 2072 (1939).