needles of *epi*-sarsasapogenin, m. p. 205°, which gave no depression with samples of *epi*-sarsasapogenin obtained in the previous reactions.

epi-**Dihydrosarsasapogenin.**—A mixture of 360 mg. of *epi*-sarsasapogenin, 500 mg. of Adams catalyst and 80 cc. of glacial acetic acid was shaken with hydrogen at 3 atmospheres and 70° for ten hours. The mixture was filtered and the filtrate poured into water. The resulting mixture was extracted with ether and the ether evaporated on the steam-bath. The residue, which did not crystallize, was heated for ten minutes with an excess of alcoholic potassium hydroxide. The resulting solution was poured into water and the mixture extracted with ether. The ethereal extract was washed with water and the ether was evaporated on the steam-bath. The residue was crystallized from ether–pentane to give white needles, m. p. 136°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1. Found: C, 77.5; H, 11.0.

Bromo-*epi*-sarsasapogenin Acetate.—To a solution of 100 mg. of *epi*-sarsasapogenin acetate in 10 cc. of glacial acetic acid was added 1 drop of 48% hydrobromic acid and 0.25 cc. of 1.05 *M* bromine in acetic acid. Hydrogen bromide was liberated in the reaction. The solution was poured into water and the precipitate collected, washed with water and dried. The white solid was crystallized from acetone to give small white plates, m. p. 180°.

Anal. Calcd. for $C_{29}H_{45}O_4Br$: C, 64.76; H, 8.45. Found: C, 64.5; H, 8.6.

Summary

Sarsasapogenin has been epimerized with sodium and amyl alcohol to yield *epi*-sarsasapogenin, suggesting that the substance is a 3-β-hydroxy compound of the coprostane type. Catalytic hydrogenation of sarsasapogenone yielded largely *epi*-sarsasapogenin, while the aluminum isopropylate reduction of sarsapogenone gives the theoretical yields of a mixture of *epi*-sarsasapogenin and sarsasapogenin.

STATE COLLEGE, PENNA. RECEIVED NOVEMBER 25, 1938

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

Sterols. LVII. Ketonic Steroids from Cows' Pregnancy Urine and Bulls' Urine

BY RUSSELL E. MARKER

In recent papers in this series we have shown that the neutral non-ketonic fractions from cows' pregnancy urine[1] and bulls' urine[2] contain the three naturally occurring pregnanediols in amounts corresponding, respectively, to one-half, and to twice those present in human pregnancy urine. The occurrence of such large amounts of the pregnanediols in bulls' urine is remarkable, and suggests that in this case they may be of cortical origin or else the reduction products of some C_{21} male hormone.

We have now isolated androsterone and dehydroisoandrosterone from bulls' urine. Cows' pregnancy urine also yielded androsterone and gave indications of the presence of dehydroisoandrosterone. These ketones were isolated from the neutral ketonic fractions by a method similar to that of Callow and Callow.[3] The ketonic sirup was distilled in a high vacuum and the fraction collected at 100–200° precipitated with digitonin. The regenerated ketone mixture from the insoluble digitonide was benzoylated and the rather insoluble dehydroisoandrosterone benzoate isolated by fractional crystallization. Saponification of this benzoate yielded dehydroisoandrosterone. The filtrate from the digitonide was freed of digitonin and the ketonic sirup oximated. Fractional crystallization of the mixture of oximes yielded the sparingly soluble androsterone oxime, from which androsterone was obtained on hydrolysis. The amounts of these ketones isolated, 33 mg. of androsterone oxime and 5 mg. of dehydroisoandrosterone benzoate from 200 gal. (760 liters) of bulls' urine and about half these amounts from 200 gal. (760 liters) of cows' pregnancy urine, probably represent about one-third of the quantities actually present. It should, however, be observed that these amounts far exceed the values to be expected on the basis of a recent paper by Butz and Hall.[4] These workers subjected bulls' urine to acidic hydrolysis, and showed that the neutral ketonic sirup assayed 0.01 mg./g. computed as androsterone. This sirup was separated into approximately equal "cholestanone" and "cholestenone" fractions and the latter contained two-thirds of the original activity. According to their procedure the androgenic activity of bulls' urine appeared to be very slight. However, the amounts of androstenone and dehydroisoandrosterone which we find present far exceed the

(1) Marker, THIS JOURNAL, **60**, 2442 (1938).
(2) Marker, Wittle and Lawson, *ibid.*, **60**, 2931 (1938).
(3) Callow and Callow, *Biochem. J.*, **32**, 1759 (1938).
(4) Butz and Hall, *J. Biol. Chem.*, **126**, 265 (1938).

amounts based on this assay, and we are therefore inclined to believe that most of the androgenic material is liberated only after prolonged alkaline hydrolysis such as we have employed. On the other hand, it is apparent from the work of Butz and Hall that some material (α,β-unsaturated ketones) is lost in this treatment.

The isolation of androsterone and dehydroisoandrosterone from cows' pregnancy urine as well as bulls' urine seems to indicate that in at least the former case these substances arise, not from testosterone, but from cortical substances by a mechanism such as we have discussed in an earlier paper.[5] Further instances of this apparently anomalous occurrence of the male hormones are the isolation of androsterone and dehydroisoandrosterone from the urine of normal women,[3] the isolation of androsterone from the urine of pregnant women,[6] and the isolation of dehydroisoandrosterone from the urine of a six-year old girl[7] with an abdominal tumor of adrenal origin.

We have also obtained estrone from bulls' urine on high vacuum distillation and subsequent fractional crystallization of the phenolic ketone fraction. The identity of the estrone was confirmed by conversion into its characteristic benzoate. This is another example of the apparently anomalous occurrence of estrone in male urines. The presence of large amounts of estrone in stallions' urine has been known for some time,[8,9] and recently[10] its isolation from human male urine has been reported. Furthermore, cortical extracts have been found to have estrogenic activity.[11,12] We believe that these facts can best be explained by the assumption that the estrone isolated in these cases is derived from cortical substances by a mechanism such as that presented in an earlier paper.[5]

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Experimental Part

The bulls' urine used in this investigation was collected from 16 mature bulls, and amounted to 200 gal. (760 liters). The hydrolysis of this urine and its separation into acidic, phenolic, ketonic, and non-ketonic fractions already has been described.

Similarly, the hydrolysis and preliminary fractionation of 200 gal. (700 liters) of cows' pregnancy urine has also been reported.

Androsterone Oxime and Dehydroisoandrosterone Benzoate from Bulls' Urine.—The ketonic fraction from 200 gal. of bulls' urine was isolated by means of Girard's reagent from the neutral steroidal fraction and weighed 2.5 g. This ketonic sirup was sublimed in a high vacuum and a fraction (1.9 g.) collected at 100–200° (bath temp.). This sublimate was dissolved in a small amount of alcohol and a hot alcoholic solution of 1 g. of digitonin added. The next day the digitonide was collected and washed well with cold alcohol. The filtrate was concentrated to 5 cc., and 100 cc. of ether added. The precipitated digitonin was removed by filtration, and the ethereal filtrate washed and evaporated. The residual sirup was heated for four hours with 500 mg. of sodium acetate and 500 mg. of hydroxylamine hydrochloride in 45 cc. of alcohol. Then water was added and the gum which collected on the walls of the flask was dissolved in 10 cc. of alcohol and carefully precipitated by the addition of 3 cc. of water. After standing for some time to cool slowly, a semicrystalline deposit appeared and this was collected and recrystallized thrice from 80% acetone to give 33 mg. of androsterone oxime, m. p. 213°, which did not depress with an authentic sample.

Anal. Calcd. for $C_{19}H_{31}O_2N$: C, 74.7; H, 10.2. Found: C, 74.4; H, 10.2.

The dried digitonide (860 mg.) obtained above was heated with 10 cc. of pyridine for hour on a steam-bath. The cooled solution was diluted with ether, the precipitated digitonin removed by filtration, and the ethereal filtrate freed of pyridine by washing with dilute hydrochloric acid and water. After removing the ether the semicrystalline residue (180 mg.) was dissolved in 2 cc. of dry pyridine and 5 drops of benzoyl chloride was added. The next day the mixture was heated for fifteen minutes on a steam-bath, cooled, and diluted with water and much ether. The ethereal extract, after washing with dilute hydrochloric acid and sodium carbonate solutions, was concentrated to a volume of 2 cc., a few drops of petroleum ether added, and set aside overnight in the refrigerator. The semicrystalline product was crystallized from methanol and then melted at 220–243°. A second recrystallization gave pure dehydroisoandrosterone benzoate (5 mg.), m. p. 246–247°, which did not depress with an authentic sample. The mother liquor gave 15 mg. of a less pure specimen, m. p. 228–244°.

The two samples of dehydroisoandrosterone benzoate (m. p. 246–247° and m. p. 228–243°) were combined and hydrolyzed with alcoholic potassium hydroxide. The product, isolated in the usual manner, was sublimed in a high vacuum at 140–150° and then crystallized from petroleum ether to give dehydroisoandrosterone, m. p. 140–142°, which gave no depression with an authentic sample.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.8. Found: C, 79.0; H, 10.0.

Androsterone Oxime and Dehydroisoandrosterone Benzoate from Cows' Pregnancy Urine.—The ketonic sirup

(5) Marker, THIS JOURNAL, **60**, 1725 (1938).
(6) Marker and Lawson, *ibid.*, **60**, 2927 (1938).
(7) Callow, *Chemistry and Industry*, **55**, 1030 (1936).
(8) Deulofeu and Ferrari, *Z. physiol. Chem.*, **226**, 192 (1934).
(9) Haussler, *Helv. Chim. Acta*, **17**, 531 (1934).
(10) Dingemanse, Lacquer and Muhlbock, *Nature*, **141**, 927 (1938).
(11) Engehart, *Klin. Wochr.*, **9**, 2114 (1930).
(12) Callow and Parkes, *J. Physiol.*, **87**, 28P (1936).

from 200 gal. of cows' pregnancy urine was sublimed in a high vacuum and a fraction (900 mg.) collected at 100–200°. This sublimate was treated in the usual manner with digitonin to give 230 mg. of digitonide. The digitonide was decomposed with pyridine but attempts to isolate pure dehydroisoandrosterone benzoate were unsuccessful, for only a few milligrams of the benzoate, m. p. 218–240°, was obtained. This impure benzoate gave no depression with authentic dehydroisoandrosterone benzoate.

The filtrate from the digitonide was worked up as described for the isolation of androsterone oxime from bulls' urine and yielded androsterone oxime (17 mg.), m. p. and mixed m. p. 212–214°.

Anal. Calcd. for $C_{19}H_{31}O_2N$: C, 74.7; H, 10.2. Found: C, 74.5; H, 10.2.

Hydrolysis of Androsterone Oxime from Cows' Pregnancy Urine and Bulls' Urine.—Since the amounts of androsterone oxime from cows' pregnancy urine and bulls' urine were very small, the two specimens were combined.

The combined androsterone oxime in 10 cc. of alcohol and 5 cc. of 4 *N* sulfuric acid was refluxed for four hours. After sublimation in a high vacuum and crystallization from 80% methanol the product (10 mg.) melted at 177–180° and gave no depression with androsterone, m. p. 183°.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.6; H, 10.4. Found: C, 78.3; H, 10.4.

Estrone from Bulls' Urine.—The alkaline hydrolysate obtained in the course of the treatment of 100 gal. (380 liters) of bulls' urine was saturated with carbon dioxide and the precipitated tar removed with ether. After evaporation of the ether on a steam-bath the residual tar (350 g.) was dissolved in 1 liter of alcohol and heated with 30 g. of Girard's reagent for thirty minutes. The solution was diluted with water and ether, the water layer extracted with ether, and then hydrolyzed with hydrochloric acid. The tar (3.2 g.) thus obtained still contained much non-ketonic material; so it was treated again with Girard's reagent (3 g.) to give 820 mg. of ketones. This was freed of non-phenolic impurities by dissolving it in alkali, extracting the alkaline solution with ether, and removing the phenolic ketones by ether extraction of the acidified solution.

The phenolic ketone mixture was then distilled in a high vacuum and the fraction collecting at 140–180° crystallized from alcohol–water. Since the product thus obtained was somewhat oily even after treatment with Norite, it was sublimed in a high vacuum, and a fraction collected at 150–160°. After crystallization from 50% alcohol this yielded 9 mg. of estrone, m. p. 257–258°, which gave no depression with an authentic sample.

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.9; H, 8.3. Found: C, 79.8; H, 8.4.

The product (5 mg.) was benzoylated by the Schotten–Baumann method to give a benzoate, m. p. 203–205°, which gave no depression with estrone benzoate.

Summary

Androsterone, dehydroisoandrosterone, and estrone have been isolated from bulls' urine. Androsterone has been isolated from cows' pregnancy urine, and indications of the presence of dehydroisoandrosterone in the same urine also have been obtained. These results are discussed in the light of the author's theory of the biogenesis of the steroidal hormones.

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

Sterols. LVIII. The Position of the Nuclear Hydroxyl Groups in Chlorogenin

BY RUSSELL E. MARKER AND EWALD ROHRMANN

Chlorogenin, a steroid sapogenin having the composition $C_{27}H_{44}O_4$, was isolated and described by Liang and Noller,[1] the substance being obtained along with tigogenin from the acid hydrolysis products of extracts of *Chlorogalum pomeridianum*. The substance is isomeric with gitogenin, obtained from digitalis plants, but differs in the position of the two nuclear hydroxyl groups since the compound yields a diketone (II) of the composition $C_{27}H_{42}O_4$ on mild oxidation. Noller[2] assigned one of the hydroxyl groups to the favored C-3 position while the other was assigned tentatively to the sterically hindered C-12 position since the diketone formed only a mono-*o*-phenylenediamine derivative. Chlorogenin was reported to give no precipitate with digitonin and from this it was inferred that the hydroxyl group at C-3 had the α-configuration. In still more recent work Noller[3] gave surface film measurements which indicated that the two hydroxyl groups were in different rings and on the basis of previous evidence preference was given to structure (I).

Noller[2] first suspected that chlorogenin was related structurally to the digitalis sapogenins since the substance was found together with tigogenin. That such a relationship is probable

(1) Liang and Noller, THIS JOURNAL, **57**, 525 (1935).

(2) Noller, *ibid.*, **59**, 1092 (1937).

(3) Noller, *ibid.*, **60**, 1629 (1938).