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

Sterols. LVI. Sarsasapogenin Derivatives. *epi*-Sarsasapogenin*

BY RUSSELL E. MARKER AND EWALD ROHRMANN

While the nuclear configuration of sarsasapogenin was proved definitely by Farmer and Kon[1] by their degradation of the substance to *etio*-bilianic acid, no very conclusive chemical evidence has been offered which would establish the position of the nuclear hydroxyl group. Askew, Farmer and Kon[2] on the basis of surface film measurements concluded that the nuclear hydroxyl must be in the first ring and that it probably occupied the favored C-3 position since it precipitated with digitonin. Simpson and Jacobs[3] previously had assigned the nuclear hydroxyl group to the hindered C-11 position.

Askew, Farmer and Kon[2] reduced sarsasapogenone with sodium and moist ether and obtained a substance different from sarsasapogenin, this difference being only in the configuration of the nuclear hydroxyl, since it yielded sarsasapogenone on mild oxidation. The substance gave no precipitate with digitonin.

We have made additional observations which show that sarsasapogenin behaves as a 3β-hydroxy compound having the regular configuration at C-5. By epimerization of sarsasapogenin with sodium and amyl alcohol, a product of the same composition, m. p. 206°, was obtained. This substance which is evidently identical with the *epi*-sarsasapogenin of Farmer and Kon[2] yielded an acetate, m. p. 194°, and gave sarsasapogenone on mild oxidation with chromic anhydride.

The catalytic hydrogenation of sarsasapogenone in neutral medium with Adams catalyst gave approximately a 75% yield of *epi*-sarsasapogenin. With aluminum isopropylate sarsasapogenone gave almost quantitative yields of equal amounts of *epi*-sarsasapogenin and sarsasapogenin, the substances being easily separated by crystallization, the *epi* compound being less soluble.

epi-Sarsasapogenin behaves similarly to sarsasapogenin on catalytic hydrogenation in acidic medium, reaction with bromine, and oxidation with selenium dioxide.[4] All attempts to prepare a crystalline tetrahydro compound by Clemmensen reduction in ethanol solution were unsuccessful, only non-crystalline material being obtained.

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

Experimental Part

Epimerization of Sarsasapogenin with Sodium and Amyl Alcohol to *epi*-Sarsasapogenin.—To a solution of 1 g. of sarsasapogenin acetate in 50 cc. of boiling *n*-amyl alcohol was added 4 g. of sodium in small pieces over a period of two hours. The resulting solution was refluxed for an additional six hours, cooled, and then washed with an excess of dilute hydrochloric acid, and after removing the amyl alcohol the crystalline residue was treated with Norite and crystallized from acetone to give white needles, m. p. 207°. This substance, *epi*-sarsasapogenin, depressed the melting point of sarsasapogenin. The yield was 300 mg. of pure product.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.7; H, 10.6.

Oxidation of *epi*-sarsasapogenin with chromic anhydride at room temperature yielded sarsasapogenone, m. p. 226°, which gave no depression with an authentic sample.

Treatment with acetic anhydride yielded an acetate which crystallized as white plates, m. p. 194°.

Reduction of Sarsasapogenone with Aluminum Isopropylate.—A mixture of 2 g. of sarsasapogenone, 2 g. of aluminum isopropylate and 70 cc. of dry isopropyl alcohol was refluxed on the steam-bath for eight hours. The solvent was then slowly distilled off over a period of four hours. The residue was heated for twenty minutes with a solution of 2 g. of potassium hydroxide in 60 cc. of methanol. The mixture was then poured into water and acidified with hydrochloric acid. The precipitated solid was extracted with ether and the extract washed well with water. The ether was evaporated on the steam-bath and the residue was crystallized from acetone–ethyl acetate to give white needles of *epi*-sarsasapogenin, m. p. 206°. This gave no depression with the *epi*-sarsasapogenin obtained by the sodium and amyl alcohol isomerization of sarsasapogenin; yield 950 mg.

Crystallization of the mother liquors from the above gave sarsasapogenin as white plates, m. p. 198°, which gave no depression with a sample of sarsasapogenin; yield 900 mg.

Hydrogenation of Sarsasapogenone.—A mixture of 400 mg. of sarsasapogenone, 500 mg. of Adams catalyst and 80 cc. of absolute ethanol was shaken with hydrogen at three atmospheres and room temperature for two hours. The mixture was filtered and the filtrate diluted with water. The resulting mixture was extracted with ether and the ether evaporated on the steam-bath. The residue was crystallized from acetone to give 300 mg. of white

(*) Paper LV, THIS JOURNAL, **61**, 855 (1939).

(1) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).

(2) Askew, Farmer and Kon, *ibid.*, 1399 (1936).

(3) Simpson and Jacobs, *J. Biol. Chem.*, **109**, 573 (1935); **110**, 565 (1935).

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

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

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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).