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

Sterols. LXXIX. Oxidation Products of Dihydrosarsasapogenin

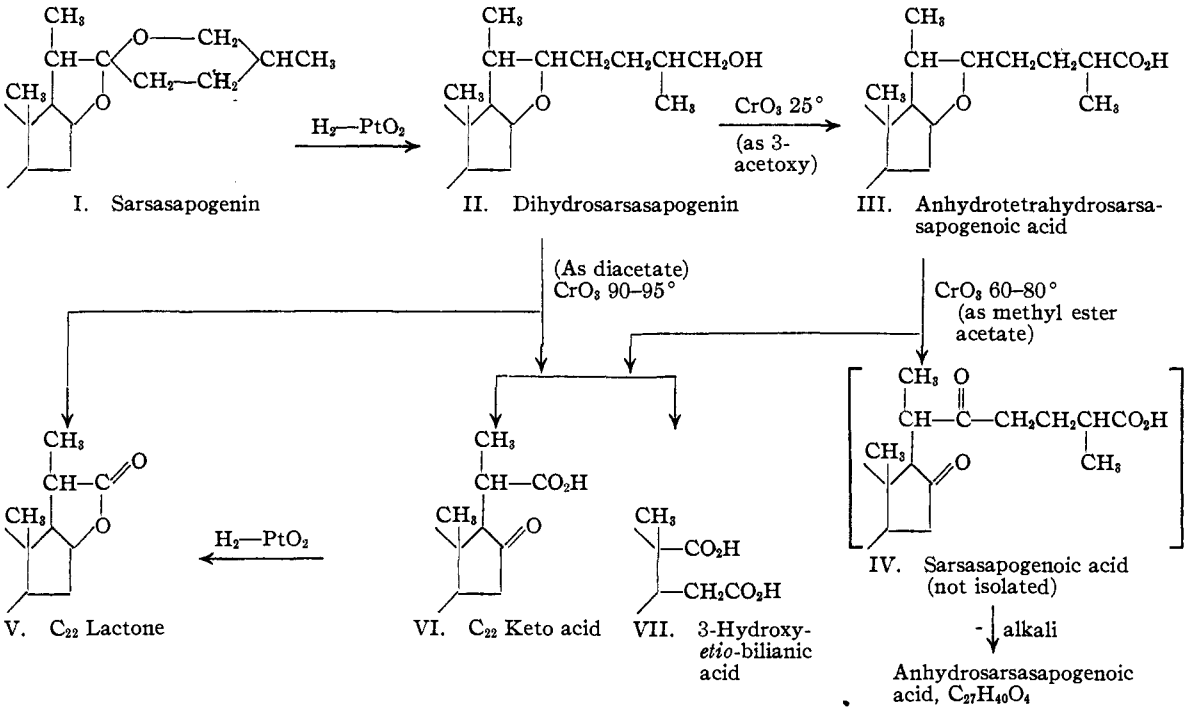
BY RUSSELL E. MARKER AND EWALD ROHRMANN

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).



hydrosarsasapogenin[1] (II) has been obtained from a study of its oxidation products. The oxidation of the diacetate with chromic anhydride at 90–95° yielded a neutral fraction from which the C_{22} lactone[3] was isolated. The acidic fraction (extractable with 3% sodium hydroxide) yielded the C_{22} keto acid[4] (IV) and the C_{19} dibasic acid[5] (VII).

We wish to thank Parke, Davis and Company for the generous help and assistance rendered during the course of this work.

Experimental Part[6]

Oxidation of the Diacetate of Dihydrosarsasapogenin.—A mixture of 20 g. of dihydrosarsasapogenin, m. p. 165–166°, and 200 cc. of acetic anhydride was refluxed for thirty minutes. The acetic anhydride was evaporated *in vacuo* and the residual sirup dissolved in 400 cc. of acetic acid. The well stirred solution was heated at 90–95° on a steam-bath while 44 g. of chromic anhydride in 250 cc. of 80% acetic acid was added over a period of two hours, after which the mixture was heated for an additional two hours. The mixture was concentrated *in vacuo* to a volume of about 100 cc. The residual material was diluted with water and the precipitated solids taken up in ether. The ethereal solution, after thorough washing with water, was washed twice with 3% sodium hydroxide solution to remove the acidic fraction.

The ether solution containing the neutral material was evaporated to a sirup which was hydrolyzed by refluxing with an excess of ethanolic potassium hydroxide. The resulting solution was diluted with water and the precipitated solid taken up in ether. The alkaline water layer was washed several times with ether. Evaporation of the combined ether extracts gave approximately 2 g. of a neutral sirup which was not investigated further. The alkaline water layer was acidified with hydrochloric acid and the mixture extracted with ether. Evaporation of the ether gave almost 600 mg. of crude lactonic material (noncrystalline). This was sublimed in high vacuum and the material distilling at 140–160° was crystallized from ether–pentane to give fine white needles, m. p. 200–201.5°. This gave no depression with the hydroxy lactone of sarsasapogenin, m. p. 200–201.5°.

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.25; H, 9.9. Found: C, 76.1; H, 9.9.

The sodium hydroxide washings containing the acidic material from the oxidation was heated on the steam-bath for twenty minutes to complete the hydrolysis. The cooled mixture was acidified with hydrochloric acid and the precipitated acids taken up in ether. Upon standing the ethereal solution deposited 600 mg. of small compact white crystals which were recrystallized once from methanol to give a product, m. p. 285–288° dec. This gave no depression with a sample of the C_{22} keto acid previously reported,[4] m. p. 285–287° dec.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.9; H, 9.5. Found: C, 72.5; H, 9.3.

The filtrate remaining after removal of the C_{22} keto acid was evaporated and the residual sirup crystallized from chloroform to give 1.9 g. of white crystals, m. p. 219–222°. This gave no depression with a sample of the C_{19} dibasic acid,[5] m. p. 220–222°.

Anal. Calcd. for $C_{19}H_{30}O_5$: C, 67.4; H, 8.9. Found: C, 67.2; H, 9.0.

When refluxed for thirty minutes with acetic anhydride

(3) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).
(4) Marker and Rohrmann, THIS JOURNAL, **61**, 1285 (1939).
(5) Marker and Rohrmann, *ibid.*, **61**, 2722 (1939).
(6) Micro-analyses by Dr. John R. Adams, Jr., of this Laboratory.

the acid formed the **acetate anhydride,** m. p. 202–203.5°. This gave no depression with a previously reported sample,[5] m. p. 203–204°.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.6; H, 8.3. Found: C, 69.7; H, 8.3.

Conversion of Anhydrotetrahydrosarsasapogenoic Acid to Anhydrosarsasapogenoic Acid.—The methyl ester of anhydrotetrahydrosarsasapogenoic acid was prepared by refluxing the acid (prepared from 3-acetoxydihydrosarsasapogenin) in methanol solution acidified with sulfuric acid. The ester, m. p. 124–126°, was identical with that obtained by the action of diazomethane on the acid.

A mixture of 7 g. of the methyl ester and 60 cc. of acetic anhydride was refluxed for thirty minutes. The acetic anhydride was evaporated *in vacuo* and the residual sirup dissolved in 200 cc. of acetic acid. To this well-stirred solution heated at 55–60° was added 10 g. of chromic anhydride in 80 cc. of 80% acetic acid over a period of two hours. The mixture was heated for an additional two hours at 55–60° and then for one hour at 80°. The excess chromic anhydride was destroyed with 5 cc. of ethanol and the mixture was evaporated *in vacuo* to a volume of approximately 75 cc. The mixture was diluted with water and the solid extracted with ether. The ethereal extract was washed with water and then twice with 3% sodium hydroxide solution.

The ethereal solution containing the neutral material was evaporated to give approximately 2 g. of sirup. This was dissolved in 30 cc. of 95% ethanol and to this solution was added 3 g. of potassium hydroxide and 15 cc. of water. The solution, after refluxing for two hours, was diluted with water and the resulting clear solution acidified with hydrochloric acid. The precipitated acid was taken up in ether. The ethereal solution was evaporated to a volume of about 30 cc. when 575 mg. of compact white crystals separated. These were recrystallized once from methanol–ether to give a product with m. p. 243–245° dec. This gave no depression with a sample of anhydrosarsasapogenoic acid prepared from sarsasapogenoic acid, m. p. 242–244°.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.6; H, 9.4.

The acidic fraction from the oxidation yielded the C_{22} keto acid, m. p. 283–285°.

A similar oxidation of the methyl ester acetate of anhydrotetrahydrosarsasapogenoic acid (1.45 g.) prepared from sarsasapogenoic acid by catalytic hydrogenation yielded the C_{22} keto acid, m. p. 284–286° dec.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.9; H, 9.5. Found: C, 72.6; H, 9.4.

The acidic filtrate from this yielded 3-hydroxy-*etio*-bilianic acid, m. p. 219–221°.

Anal. Calcd. for $C_{19}H_{30}O_5$: C, 67.4; H, 8.9. Found: C, 67.2; H, 9.0.

The neutral fraction from the oxidation upon treatment with aqueous ethanolic potassium hydroxide as described previously yielded anhydrosarsasapogenoic acid, m. p. 243–245° dec.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.9; H, 9.4.

Summary

The chromic anhydride oxidation products of dihydrosarsasapogenin have been studied.

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

Sterols. LXXX. Reactions of Chlorogenin

BY RUSSELL E. MARKER AND EWALD ROHRMANN

In a previous paper[1] we presented evidence indicating that the nuclear hydroxyl groups of the steroid sapogenin, chlorogenin,[2] were located at C-3 and C-6 and that the hydroxyl group at C-3 was of the β-configuration. Noller[3] had previously postulated that the hydroxyl groups were at C-3 and C-12 and that the group at C-3 was of the α-configuration. We also presented evidence indicating that chlorogenin belongs to the *allo* series in its configuration at C-5. Further evidence concerning the nuclear structure is afforded by the fact that tigogenone and chlorogenone upon Clemmensen reduction yield the same desoxy compound.[4] The catalytic hydrogenation of the desoxy compounds in an acidic medium likewise gives the same dihydrodesoxy compound. This indicates that chlorogenin differs from tigogenin only in the presence of an additional hydroxyl group.

In a previous paper[4] we alluded to the highly interesting studies of Tsukamoto, Ueno and Ota[5] on diosgenin (I), an unsaturated steroid sapogenin which yields tigogenin upon reduction. Of especial interest are the experiments of Tsukamoto, Ueno, Ota and Tschesche[6] on the oxidation of dios-

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 946 (1939).
(2) Liang and Noller, *ibid.*, **57**, 525 (1935).
(3) Noller, *ibid.*, **59**, 1092 (1937).
(4) Marker and Rohrmann, *ibid.*, **61**, 1516 (1939).
(5) Tsukamoto, Ueno and Ota, *C. A.*, **31**, 3493 (1937); *ibid.*, **32**, 2537 (1938); Tsukamoto and Ueno, *ibid.*, **32**, 7470 (1938).
(6) Tsukamoto, Ueno, Ota and Tschesche, *J. Pharm. Soc., Japan*, **57**, 283 (1937).