(3 atm.) at 70° for eight hours. The mixture was filtered and the acetic acid was evaporated from the filtrate *in vacuo*. The residual sirup was refluxed for fifteen minutes with an excess of alcoholic potassium hydroxide and the solution poured into water. The resulting mixture was extracted with ether and the ethereal extract washed with water. The ether was evaporated on the steam-bath and the residue crystallized from aqueous acetone to give silky white needles, m. p. 109–110°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.5; H, 11.5. Found: C, 80.5; H, 11.5.

The substance gave no evidence of oxidation when heated at 90° with selenium dioxide in acetic acid–benzene solution.

Bromodesoxysarsasapogenin.—To a solution of 100 mg. of desoxysarsasapogenin in 60 cc. of glacial acetic acid acidified with 5 drops of 48% hydrobromic acid was added 0.25 cc. of 1.05 *M* bromine in glacial acetic acid. The bromine was taken up with difficulty and the solution was warmed to about 30°. The solution was poured into water and the precipitate collected and washed with water. The dried material was crystallized from acetone to give white crystals, m. p. 170°.

Anal. Calcd. for $C_{27}H_{43}O_2Br$: C, 67.6; H, 9.0. Found: C, 67.7; H, 9.1.

The substance showed no evidence of oxidation when heated for thirty minutes with selenium dioxide in benzene–acetic acid solution.

Clemmensen Reduction of Desoxysarsasapogenin to Tetrahydrodesoxysarsasapogenin.—A solution of 150 mg. of desoxysarsasapogenin in 50 cc. of 95% ethanol was mixed with 20 g. of amalgamated zinc. To this boiling mixture was added 10 cc. of concentrated hydrochloric acid over a period of nine hours. The solution was poured into water and the mixture extracted with ether. The ethereal extract was washed with water and the ether evaporated on the steam-bath. The sirupy residue was crystallized from ether–hexane to give silky white needles, m. p. 101°. The material gave a depression with dihydrosarsasapogenin.

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.1; H, 12.0. Found: C, 80.0; H, 11.5.

A similar reduction of 500 mg. of sarsasapogenone in 100 cc. of 95% ethanol gave white needles, m. p. 100°. This gave no depression with that obtained from desoxysarsasapogenin.

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.1; H, 12.0. Found: C, 79.8; H, 11.9.

The filtrate from this yielded 20 mg. of heavy white crystals, m. p. 118°. This gave no appreciable depression with the material, m. p. 100°.

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.1; H, 12.0. Found: C, 80.3; H, 11.8.

It is possible that this higher melting substance may be either a polymorphous form of the lower melting form or it may differ in configuration from the lower form.

Summary

The method of preparing desoxysarsasapogenin has been simplified and the reactions characteristic of sarsasapogenin have been extended to the desoxy compound.

STATE COLLEGE, PENNA. RECEIVED FEBRUARY 20, 1939

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

Sterols. LX. Oxidation Products of Sarsasapogenin. Structure of C_{22} Keto Acid

BY RUSSELL E. MARKER AND EWALD ROHRMANN

In studying the chromic anhydride oxidation products of sarsasapogenin we have obtained in addition to the C_{22} lactone of Farmer and Kon[1] and the C_{27} acid and C_{27} neutral product of Fieser and Jacobsen,[2] a keto acid of the composition $C_{22}H_{34}O_4$. This substance appears to be of some importance in further elucidating the structure of the sapogenin side chain.

Sarsasapogenin acetate was oxidized at 60° with chromic anhydride as described by Fieser and Jacobsen[2] and the C_{22} acid obtained from the hydrolyzed acidic fraction after removal of most of the sarsasapogenoic acid. The yield of acid obtained under these conditions is approximately 1–2% although slightly better yields were obtained when the oxidation was carried out at 80°. The acid forms a semicarbazone readily.

The acid upon catalytic hydrogenation in neutral medium with Adams catalyst yielded largely a neutral product, m. p. 197–198°, which was identical with the C_{22} lactone (II) first obtained by Farmer and Kon[1] by the direct oxidation of sarsasapogenin acetate with chromic anhydride. In the same hydrogenation a lactone, m. p. 186–188°, was obtained which appears to be a polymorphous form of the lactone, as it gives no depression with the higher melting form. When the hydrogenation was carried out in acidic ethanol, only the lower melting form was obtained in good yield. No appreciable acidic fraction was obtained in either case.

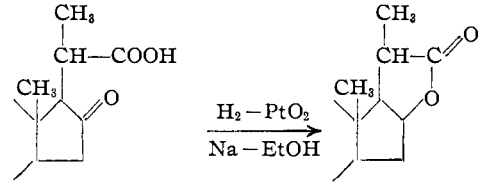
Upon reduction with sodium and ethanol the

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

(2) Fieser and Jacobsen, THIS JOURNAL, **60**, 28, 2753 (1938).

C_{22} acid yielded approximately equal amounts of the two polymorphic forms of the lactone and no appreciable acidic fraction was obtained.

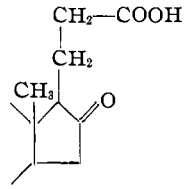
The reaction and properties of the acid are consistent with the behavior of γ-keto and δ-keto acids. The facile reduction of the acid to the C_{22} lactone strongly indicates that the substance is a γ-keto acid represented by structure I.



I. C_{22} γ-keto acid II. C_{22} lactone

It is of course entirely possible that the two supposed polymorphic forms of the lactone are actually stereoisomers differing in the configuration of the potential hydroxyl group at C-16. The fact that hydrogenation in acidic media gives only the low melting lactone is suggestive of such a possibility. We are planning to investigate this phase of the problem further, as soon as our supply of the acid is replenished.

The only other logical alternative structure would be that of a δ-keto acid (III).



III. C_{22} δ-keto acid

While there is as yet no conclusive evidence which would eliminate this possibility, there are some indications[2,3] that a methyl group is attached to C-20 as in structure I.

The presence of a carbonyl group in the C-16 position suggested the possibility of easily degrading the keto acid to 3β-hydroxy-bisnorcholanic acid. The Clemmensen reduction of the acid in ethanolic solution with amalgamated zinc yielded in addition to considerable amounts of unchanged acid a neutral substance, $C_{24}H_{38}O_4$, m. p. 164°. This substance is evidently an ethyl ester as it yielded the original keto acid on alkaline hydrolysis.

The possibility of sarsasapogenoic acid or the C_{27} neutral oxidation product described by Fieser and Jacobsen[2] being intermediates in the formation of the C_{22} keto acid is being investigated at the present time.

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

Isolation of Keto Acid from Chromic Anhydride Oxidation of Sarsasapogenin Acetate.—Sarsasapogenin acetate was oxidized with chromic anhydride at 60° as described by Fieser and Jacobsen.[2] The acidic fraction after removal of most of the sarsasapogenoic acid was taken up in ether and allowed to stand at room temperature for several days, when small compact white crystals were deposited. These were collected, washed with ether and recrystallized from methanol to yield a product with m. p. 285–287° dec. (gas evolution).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.9; H, 9.5; neut. equiv., 362. Found: C, 72.6, 72.5; H, 9.4, 9.2; neut. equiv., 380, 369.

The yield of acid under the above conditions appears to be approximately 1%. Better yields (about 3%) were obtained by carrying out the oxidation at 80–85°, using two parts of chromic anhydride to one part of sarsasapogenin acetate.

The acid is very sparingly soluble in ether, acetone, chloroform and ethyl acetate but is fairly soluble in methanol and ethanol.

Methyl Ester of Keto Acid.—A suspension of 200 mg. of the keto acid in 20 cc. of methanol–ether (1:1) was treated with an excess of an ethereal solution of diazomethane. The solvent was evaporated and the residue crystallized from ether–pentane to give clusters of small white plates, which melted at 124–126°, solidified at 127° and remelted at 159°.

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.4; H, 9.6. Found: C, 73.2; H, 9.6.

The methyl ester was recovered unchanged after shaking with hydrogen (3 atm.) and Adams catalyst in ether–ethanol (1:1) solution at room temperature for six hours.

Reduction of C_{22} Acid. (a) By Catalytic Hydrogenation in Neutral and Acidic Media.—A mixture of 190 mg. of the acid, 400 mg. of Adams catalyst, 50 cc. of absolute ethanol and 50 cc. of ether was shaken with hydrogen at 45 pounds pressure (3 atm.) for fifteen hours. The mixture was filtered, the filtrate diluted with water and extracted with ether. The ethereal extract was washed twice with dilute sodium carbonate solution. The crystalline residue remaining upon evaporation of the ether was recrystallized several times from ether–pentane to give white needles, m. p. 197–198°. This gave no depression with an authentic sample of the hydroxy lactone (m. p. 198–200°).

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.25; H, 9.9. Found: C, 76.3; H, 10.1.

The filtrates from the above crystallizations yielded thick white needles, m. p. 186–188°. A mixed melting point with the hydroxy lactone, m. p. 198–200°, started to shrink at 190° and melted at 197–199°. The substance is apparently a polymorphic form of the lactone.

Hydrogenation of 200 mg. of the acid in 100 cc. of ab-

(3) Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).

solute ethanol acidified with 2 cc. of concentrated hydrochloric acid with 300 mg. of Adams catalyst at 45 pounds pressure (3 atm.) at room temperature for six hours gave only the lower melting lactone (100 mg.), m. p. 186–188°.

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

The sodium carbonate washings in both of these hydrogenations yielded only traces of acidic material which was not investigated further.

(b) **By Sodium and Ethanol.**—To a boiling solution of 200 mg. of the keto acid in 75 cc. of absolute ethanol was added 5 g. of sodium in small pieces over a period of ninety minutes. The solution was cooled, diluted with water and acidified with hydrochloric acid. The separated solid was extracted with ether and the ethereal extract washed with 3% sodium hydroxide solution. Evaporation of the ether gave a crystalline residue which crystallized from ether–pentane as white needles, m. p. 197–199°. This gave no depression with an authentic sample of the hydroxy lactone, m. p. 198–200°.

The filtrate upon further crystallization from ether–pentane yielded the lower melting form of the lactone, m. p. 185–187°. This gave no depression with either the higher melting form or the lower melting form obtained above by catalytic hydrogenation.

The sodium hydroxide washings yielded only a trace of acids upon acidification with hydrochloric acid. This was not investigated further.

(c) **Attempted Clemmensen Reduction.**—A boiling mixture of 500 mg. of the acid, 75 cc. of 95% ethanol and 20 g. of amalgamated 20-mesh zinc was treated with 16 cc. of concentrated hydrochloric acid, added over a period of nine hours. The resulting solution was diluted with water and extracted with ether. The ethereal extract after washing with water was shaken with 3% sodium hydroxide solution. The remaining ethereal extract was washed with water and the ether evaporated. The crystalline residue was crystallized from ether–pentane to give white needles, m. p. 163–164°. This gave a 20° depression with the lactone, m. p. 200°.

Anal. Calcd. for $C_{24}H_{38}O_4$: C, 73.8; H, 9.8. Found: C, 73.6; H, 9.8.

A sample of the material when refluxed for fifteen minutes with an excess of alcoholic potassium hydroxide solution yielded, upon crystallization from ether, compact white crystals, m. p. 285° dec. This gave no depression with the original keto acid, indicating that the substance is the ethyl ester of the keto acid.

The sodium hydroxide washings containing the acidic fraction from the Clemmensen reduction was acidified with hydrochloric acid and extracted with ether. The ether was evaporated and the residue crystallized from methanol to give compact white crystals, m. p. 284° dec. This gave no depression with the original keto acid. No other acidic products appeared to be present.

Semicarbazone of Keto Acid.—A solution of 100 mg. of the keto acid, 100 mg. of semicarbazide hydrochloride and 150 mg. of sodium acetate in 10 cc. of 95% ethanol and 2 cc. of water was refluxed on the steam-bath for one hour. The solution was then diluted with water and the white solid collected and crystallized from ether to give a product of m. p. 204–207° dec.

Anal. Calcd. for $C_{23}H_{37}O_4N_3$: C, 65.8; H, 8.9. Found: C, 65.40; H, 8.9.

Summary

A monobasic keto acid of the composition $C_{22}H_{34}O_4$ has been obtained by the chromic anhydride oxidation of sarsasapogenin acetate. Catalytic reduction of the acid yields the C_{22} hydroxy lactone previously obtained by the chromic anhydride oxidation of sarsasapogenin acetate. Reduction of the acid with sodium and alcohol gives similar results.

STATE COLLEGE, PENNA. RECEIVED MARCH 6, 1939

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

Sterols. LXI. The Steroidal Content of Steers' Urine

BY RUSSELL E. MARKER

The occurrence of large amounts of the pregnanediols in bulls' urine[1] has prompted us to investigate the steroid content of animals with various sex organs removed in order to show the effect of their removal upon the type of sterols present. For this purpose our first investigation was carried out on the urine of steers that had their testes removed in infancy.

The steers' urine was hydrolyzed and separated into phenolic and neutral fractions and these examined in the usual manner. The phenolic fraction, after treatment with Girard's reagent, yielded a ketonic oil from which a small amount of estrone, characterized by its benzoate, was obtained. The neutral fraction was separated into hydroxylated and non-hydroxylated portions, and the former further separated into ketonic and non-ketonic parts. From the non-hydroxylated fraction the characteristic urinary hydrocarbon, $C_{28}H_{58}$, m. p. 62–63°, was obtained. The hydroxyketone fraction yielded androsterone and dehydroisoandrosterone in amounts comparable to those present in bulls' urine.[2] An examination of the carbinol fraction revealed the absence of the

(1) Marker, Wittle and Lawson, THIS JOURNAL, **60**, 2931 (1938).

(2) Marker, *ibid.*, **61**, 944 (1939).