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

Sterols. CXXXVII. Sapogenins. LVIII. Oxidation Products of Sarsasapogenin: Keto-sarsasapogenin

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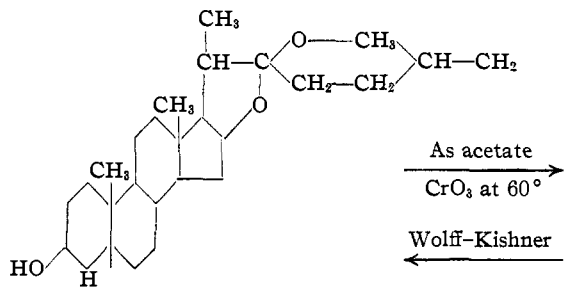
Fieser and Jacobsen[1] in their investigation of the chromic anhydride oxidation products of sarsasapogenin acetate obtained a neutral compound which they reported to be unreacted sarsasapogenin acetate, but later[2] recognized as the acetate of a neutral oxidation product. They suggested that the substance was a hydroxy sarsasapogenin. As the substance was obtained by the chromic anhydride oxidation, this seemed unlikely to us. Marker and Rohrmann[3] established the presence of a free carbonyl group in this compound, rather than the hydroxyl group as suggested by Fieser and Jacobsen.

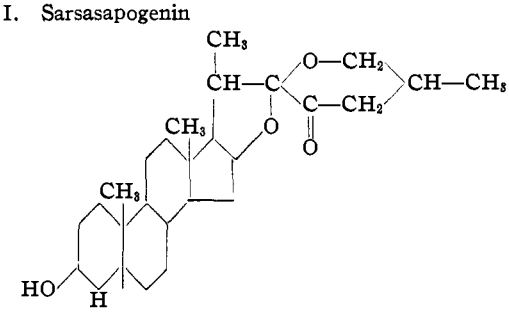
The present work was undertaken to clarify the structure of this compound and establish it as an intermediate in the oxidation of the sapogenin to the various oxidation products. This fact was suggested by the failure of Fieser and Jacobsen[1] to isolate the C_{22} lactone and C_{22} keto acid from the oxidation mixture of sarsasapogenoic acid under similar conditions used in the oxidation of sarsasapogenin acetate. However, Marker and Rohrmann[3] obtained the C_{22} keto acid as an oxidation product of sarsasapogenoic acid, but confirmed the results of Fieser and Jacobsen by showing the absence of sarsasapogenin lactone.

Although the previous workers[1] were able to isolate a constant melting compound, their analyses indicated a probably molecular mixture of sarsasapogenin acetate and the neutral acetate. By many recrystallizations it has been possible to separate this mixture into a compound melting at 173° with the empirical formula $C_{29}H_{44}O_5$. Purification was simplified by treating the mixture with acetic anhydride in a bomb tube at 200° for ten hours. The neutral compound, which is not readily attacked by the acetic anhydride, can be separated from the non-crystalline pseudosarsasapogenin acetate very easily.

We have obtained tetrahydrosarsasapogenin by the Clemmensen reduction of the neutral compound and sarsasapogenin by the Wolff-Kishner reduction. This suggests that the neutral compound contains the ketal structure as found in sarsasapogenin. Similar to the bromine atom in bromosarsasapogenin,[4] it is most probable that the oxygen atom is adjacent to the potential carbonyl group at C-22 (II). This is suggested from the fact that the chromic anhydride oxidation of bromosarsasapogenin failed to give a bromo-neutral compound which would be expected if the position of the oxygen atom was not C-23.



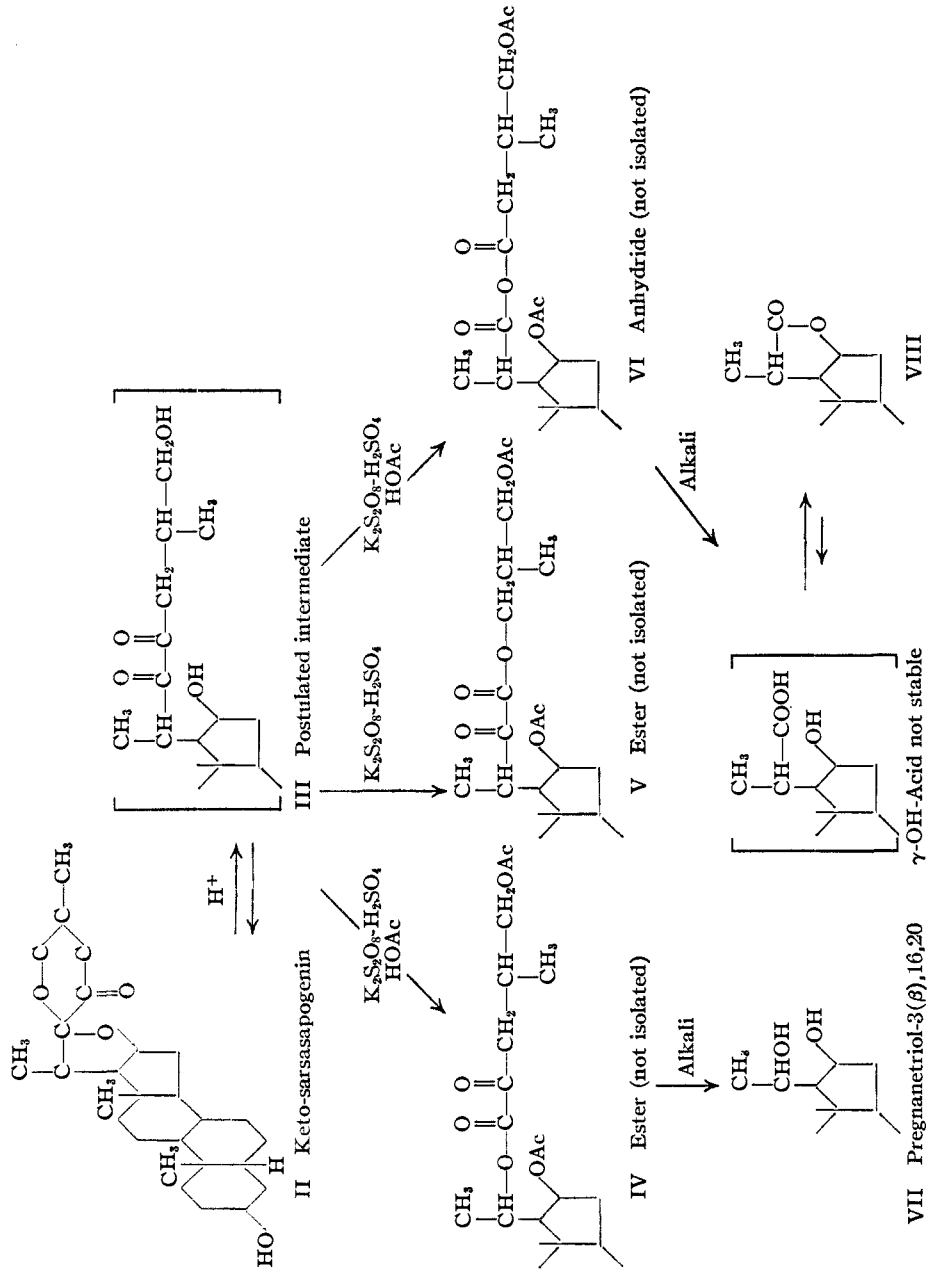
I. Sarsasapogenin



II. Keto-sarsasapogenin

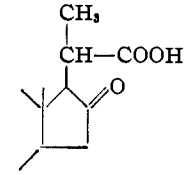
In order to determine the position of the oxygen atom, we have oxidized the neutral product with von Baeyer's dry persulfate mixture. Sarsasapogenin lactone (VIII) was obtained in excellent yield with a lesser amount of pregnanetriol-3,16,20 (VII). The results obtained would be expected if structure II is correct, for it is well known that persulfates in the presence of acid readily react with polycyclic ketones to yield lactones. A typical example of this type of oxidation is the conversion of camphor to α-campholide.[5] The ease with which the conversion of the neutral to the lactone takes place in greater yields than the pregnanetriol is in complete accordance with the ketal structure of the sapogenin side chain, as is indicated in the suggested mechanism given in the accompanying chart.

(1) Fieser and Jacobsen, THIS JOURNAL, **60**, 28 (1938).
(2) Fieser and Jacobsen, *ibid.*, **60**, 2753 (1938).
(3) Marker and Rohrmann, *ibid.*, **61**, 2072 (1939).
(4) Marker, *et al.*, *ibid.*, **63**, 1032 (1941).
(5) Baeyer and Villiger, *Ber.*, **32**, 3630 (1899).



Since the C_{22}–C_{23} bond in structure III is more highly activated than the C_{20}–C_{22} and C_{23}–C_{24} bonds because of the adjacent carbonyl groups, it should be expected that the persulfate would cause an oxidation of the former in preference to the latter two to produce the anhydride grouping. This proved to be the case, for on hydrolysis of the solid esters IV and V and the anhydride VI mixture, the lactone was obtained in the acid fraction in greater yields. The lactone is formed through the unstable γ-hydroxy acid intermediate obtained on hydrolysis of VI, and by oxidation.

In order to determine the importance of the neutral as an intermediate in the oxidation of the sapogenin, we have oxidized the acetate at 60° with chromic anhydride. A good yield of the C_{22} lactone (VIII) was obtained in addition to a small amount of the C_{22} keto acid (IX) and some un-

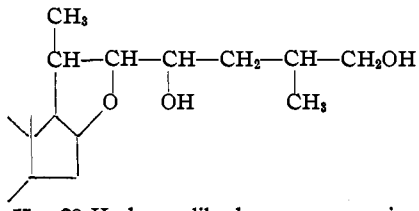


IX C_{22} Keto acid

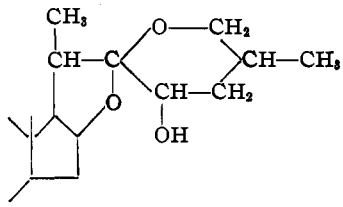
changed starting material. *No sarsasapogenoic acid was obtained.*

Since the lactone and keto acid have not been obtained from the oxidation mixture of sarsasapogenoic acid under similar conditions, and only the keto acid under more vigorous conditions,[6] it is evident that their source in the oxidation of sarsasapogenin acetate is the intermediate neutral acetate.

Reduction of the neutral acetate with Adams catalyst in acidic medium yielded on hydrolysis a compound, m. p. 219–221°, with the empirical formula $C_{27}H_{46}O_4$ which we have designated as 23-hydroxy-dihydrosarsasapogenin (X). Sodium and ethanol reduction yielded a substance, m. p.



X. 23-Hydroxy-dihydrosarsasapogenin



XI. 23-Hydroxy-sarsasapogenin

234–238°, which is apparently 23-hydroxysarsasapogenin (XI).

We wish to thank Parke, Davis and Company for their assistance.

Experimental

Isolation of 23-Keto-sarsasapogenin from the Oxidation of Sarsasapogenin Acetate.—Sarsasapogenin acetate, 50 g., was oxidized with chromic acid at 60° as described by Fieser and Jacobsen.[1] The neutral fraction, 28.5 g., was hydrolyzed with a 3% ethanolic potassium hydroxide solution by refluxing for thirty minutes. The solution was decanted into water and extracted with ether. The ethereal extract was washed well with water and evaporated. The residue was crystallized several times from acetone to yield a product of m. p. 218–221°. Repeated crystallization raised the melting point to 223–225°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.85. Found: C, 75.6; H, 10.0.

A mixture of 10 g. of the crude neutral material and 30 cc. of acetic anhydride was heated in a bomb tube at 200° for ten hours. The excess acetic anhydride was evaporated *in vacuo*, and the residue was crystallized from acetone as needles, m. p. 172–173°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.6; H, 9.4. Found: C, 73.2; H, 9.7.

Hydrolysis of the above acetate yielded a product which crystallized from acetone in white plates, m. p. 225–226°. This gave no depression in melting point when mixed with the material obtained from the crystallization of the original neutral product, and gave a 30° depression with sarsasapogenin.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.5; H, 9.8.

The keto compound when treated with semicarbazide hydrochloride under the usual conditions gave a semicarbazone which was very insoluble in methanol, ether and ethyl acetate, m. p. 291–293° dec.

Anal. Calcd. for $C_{28}H_{45}O_4N_3$: C, 68.9; H, 9.3. Found: C, 68.9; H, 9.3.

Reduction of 23-Keto-sarsasapogenin. (a) Wolff-Kishner.—To a solution of 1 g. of sodium in 25 cc. of absolute alcohol was added 600 mg. of semicarbazone of 23-keto-sarsasapogenin and the mixture was heated in a bomb tube for eight hours at 180°. The contents were washed out of the tube with water and ether, the layers separated, and the ethereal extract was washed with water. The residue from evaporation was crystallized from acetone as white needles, m. p. 198–200°. This gave no depression in melting point when mixed with sarsasapogenin. A yield of 200 mg. of pure product was obtained.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.6; H, 10.8.

When refluxed with acetic anhydride, sarsasapogenin acetate was obtained which crystallized from ethyl acetate in white plates, m. p. 141–143°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.1; H, 10.2.

(b) **Catalytic Hydrogenation.**—A mixture of 1.0 g. of the pure neutral acetate, 1 g. of Adams catalyst, 100 cc. of glacial acetic acid and 30 cc. of absolute ethanol was shaken with hydrogen at 2 atm. and room temperature for fifteen hours. The mixture was filtered and the filtrate was diluted with water. The precipitated solid was extracted with ether and the ethereal extract was washed with dilute sodium carbonate solution. The ether was evaporated and the residue was refluxed for thirty minutes with a 3% ethanolic potassium hydroxide solution. The product was crystallized from ethyl acetate as white plates, m. p. 219–221°. This gave a 30° depression when mixed with 23-keto-sarsasapogenin.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.6; H, 10.6.

(c) **Sodium and Ethanol.**—To a boiling solution of 500 mg. of the neutral acetate in 100 cc. of absolute ethanol was added 6 g. of sodium in small pieces over a period of one hour. The solution was cooled, diluted with water and extracted with ether. The ethereal extract was washed with water, and the solvent was removed. The residue crystallized from aqueous methanol in white prisms, m. p. 234–236°. Further crystallization did not raise the melting point. This gave a 30° depression in melting point with the starting material, and also with the material obtained from the catalytic hydrogenation. Unfortunately the sample was lost when shipped for analysis.

(6) Marker and Rohrmann, THIS JOURNAL, 61, 1285 (1939).

(d) **Clemmensen Reduction.**—To a boiling solution of 1.0 g. of the neutral compound in 300 cc. of 95% ethanol and 40 g. of amalgamated 20-mesh zinc was added 48 cc. of concentrated hydrochloric acid over a period of nine hours. The solution was decanted into water and the precipitated solid was extracted with ether. The ethereal solution was washed with water, evaporated and the residue was crystallized from acetone–ethyl acetate as white prisms, m. p. 192–194°. This gave no depression in melting point when mixed with a sample of tetrahydrosarsasapogenin, yield 900 mg.

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.1; H, 11.5. Found: C, 77.6; H, 11.5.

The Action of Persulfuric Acid on 23-Keto-sarsasapogenin.—To a solution of 2.0 g. of pure 23-keto-sarsasapogenin in 100 cc. of acetic acid at 25° was added a mixture of von Baeyer persulfuric reagent made from 10 g. of potassium persulfate, 11 g. of concentrated sulfuric acid and 30 g. of potassium sulfate. It was allowed to stand with occasional shaking for sixteen days at 25°. At the end of this time the solution was filtered, poured into water and the white solid was extracted with ether. The ethereal extract was washed well with water and with one liter of a 3% potassium hydroxide solution. No acid fraction was obtained on acidification of the sodium hydroxide washings.

The ether extract was again washed with water and evaporated. The solid residue was hydrolyzed by refluxing with ethanolic potassium hydroxide. Water was added to the alkaline solution and the precipitated solid was taken up in ether and crystallized from acetone in small white needles, m. p. 221–222°. This gave no depression in melting point when mixed with pregnane-3(β),16,20-triol. A yield of 460 mg. was obtained.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.9; H, 10.8. Found: C, 75.0; H, 10.7.

The alkaline washings from the above hydrolysis was acidified and the white solid was extracted with ether. The ether extract was washed well with water, evaporated and the residue was crystallized from ether–pentane in long white needles, m. p. 200–202°. This gave no depression in melting point when mixed with a sample of the hydroxy lactone obtained from the chromic acid oxidation of sarsasapogenin, yield 980 mg.

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.25; H, 9.9. Found: C, 76.2; H, 9.9.

When refluxed with acetic anhydride an acetate was obtained which crystallized from ether–pentane, m. p. 182–183.5°. This gave no depression in melting point when mixed with an authentic sample of the acetate of the hydroxy lactone.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.2; H, 9.3. Found: C, 73.9; H, 9.0.

Chromic Acid Oxidation of 23-Keto-sarsasapogenin Acetate.—To 2.7 g. of pure 23-keto-sarsasapogenin acetate dissolved in 61 cc. of glacial acetic acid at 60° was added a solution of 1.75 g. of chromic anhydride in 40 cc. of 80% acetic acid over a period of four hours. The solution was stirred for an additional hour at 60° and then was poured into water. The solid was extracted with ether and the ethereal extract was washed thoroughly with water. The acid fraction was removed from the ether layer by washing with one liter of 3% sodium hydroxide. The alkaline washings were heated on the steam-bath to complete hydrolysis, cooled and acidified with dilute hydrochloric acid. The solid was extracted with ether. The ethereal extract was washed with water, the solvent removed and the residue was slurried with acetone. The precipitate was filtered and washed well with acetone giving a fine white powder, m. p. 284° dec. This gave no depression in melting point when mixed with the C_{22}-keto acid obtained from the oxidation of sarsasapogenin acetate. A yield of 250 mg. was obtained.

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

The ethereal extract remaining after the removal of the acids was washed with water and evaporated. The residue was treated in the same manner as for the neutral material from the persulfuric acid oxidation. The sodium hydroxide washings yielded a compound which crystallized from ether–pentane in long white needles, m. p. 200–202°. This gave no depression in melting point when mixed with sarsasapogenin lactone, obtained from the chromic acid oxidation of sarsasapogenin acetate, yield 1.05 g.

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.3; H, 9.9. Found: C, 76.6; H, 10.1.

The neutral material, 100 mg., was found to be unoxidized 23-keto-sarsasapogenin acetate.

Summary

The structure of 23-keto-sarsasapogenin and its transformation products is discussed in terms of the spiro-ketal structure for the sapogenin side chain.

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