Summary

1. A study has been made of the behavior of certain aliphatic organic acids, chiefly typical hydroxy, unsaturated, or keto, toward reduction catalyzed by Raney nickel and in the presence of sodium hydroxide and under high hydrogen pressure.

2. At temperatures below 250° and hydrogen pressures not exceeding 330 atmospheres, α- and γ-hydroxy acids are not affected, whereas β-hydroxy acids are converted into the corresponding unsubstituted acids.

3. The unsaturated acids studied were hydrogenated rapidly at temperatures of 100°, or less.

4. α- and γ-keto acids were hydrogenated with ease to the corresponding hydroxy acids at quite moderate temperatures.

5. Of particular importance was the conversion, in alkaline solution at 250° and under a hydrogen pressure of 330 atmospheres, of formic acid into methane and carbon dioxide.

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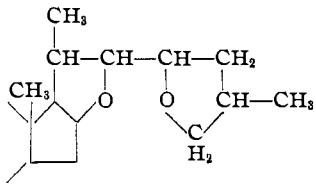
[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

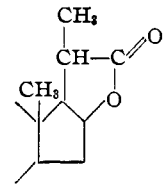
Sterols. LIII. The Structure of the Side Chain of Sarsasapogenin*

BY RUSSELL E. MARKER AND EWALD ROHRMANN

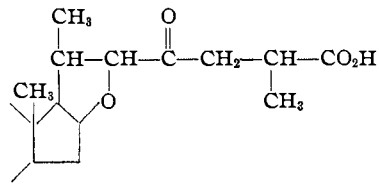
In the course of extensive experiments undertaken to obtain hormone intermediates from relatively inexpensive starting materials, we have made many observations on the sapogenins which can hardly be reconciled with the present conception of the side chain of these substances. Up to the present it has been assumed that this side chain contains two rather non-reactive oxygen atoms. In accordance with this assumption Tschesche and Hagedorn[1] suggested a double tetrahydrofuran structure, I.



I. Sapogenin side chain



II. C_{22} Lactone



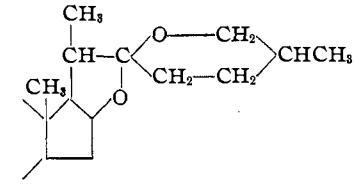
III. C_{27} Acid

Formulations of Tschesche and Hagedorn[1]

The existence of an oxide linkage at C-16 is well established from the degradation of the C_{22} lactone from tigogenin to *etio-allo*-bilianic acid by Tschesche and Hagedorn[1] and of the C_{22} lactone from sarsasapogenin to *etio*-bilianic acid by Farmer and Kon.[2] The linkage between C-16 and C-22 is based upon the formation of lactone II. The definite proof of the structure of this lactone will require a stepwise degradation to a known compound such as an *allo*-pregnane, a pregnane derivative or a known bile acid.

The corresponding C_{27} acid from sarsasapogenin was prepared by Fieser and Jacobsen[3] and was designated as sarsasapogenoic acid. In later work[4] they presented evidence interpreted as offering support to the formulation suggested by Tschesche and Hagedorn for the sapogenin and the C_{27} acid.

We have found that the two oxygen atoms in the sapogenin side chain are inert only in neutral or alkaline media. *In acid media they are unusually reactive.* While the evidence presented here is not yet complete, all of the results are consistent with the presence of a protected carbonyl group existing as a ketone spiro acetal. The following formulation is suggested on the basis of the accepted structure of the lactone (II) and on the assumption that the sapogenins possess the cholesterol skeletal side chain.



IV. Sapogenin side chain (suggested)

* Paper LII, THIS JOURNAL, **61**, 588 (1939).

(1) Tschesche and Hagedorn, *Ber.*, **68**, 1412 (1935).

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

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

(4) Fieser and Jacobsen, *ibid.*, **60**, 2753 (1938).

The present paper gives the relationship of our experimental results to this formulation. Our results include the following types of reactions: (1) catalytic hydrogenation, (2) Clemmensen reduction, (3) bromination, (4) selenium dioxide oxidation, and (5) isomerization by acid.

Sarsasapogenin on catalytic hydrogenation in acidic medium takes up one mol of hydrogen to give a compound having the composition $C_{27}H_{46}O_3$ and designated as dihydrosarsasapogenin (V). The hydrogenation takes place readily in acetic acid or in ethanol acidified with hydrochloric acid. Attempts to carry on the hydrogenation in a neutral medium have been completely unsuccessful. Tetrahydrofurfuryl acetate when treated with hydrogen in acidic ethanol or acetic acid under similar conditions gave no evidence of reduction. In view of this fact we are inclined to believe that the addition of hydrogen to sarsasapogenin is not due to the opening of a true tetrahydrofuran ring. We have applied this reaction to other sapogenins as well as to desoxysarsasapogenin and in every case one mole of hydrogen was taken up in acid solution to yield a dihydrosapogenin.[5]

Dihydrosarsasapogenin (V) forms a dibenzoate and a *bis*-3,5-dinitrobenzoate indicating that a new hydroxyl group was formed in the hydrogenation. The dihydro compound is readily precipitated by digitonin, indicating the presence of a 3-β-hydroxyl group. The hydrogenation of sarsasapogenin acetate in acidic ethanol and the oxidation of the resulting reaction product with chromic anhydride at room temperature readily yields after hydrolysis a monocarboxylic acid $C_{27}H_{44}O_4$ designated as sarsasapogentic acid (VI) melting at 189°. It forms a methyl ester but forms no semicarbazone. The oxidation of dihydrosarsasapogenin with chromic anhydride in acetic acid at room temperature readily gives a monocarboxylic acid, $C_{27}H_{42}O_4$. This acid which evidently contains a ketonic group at C-3 gives a positive Zimmermann test,[6] and forms a monosemicarbazone. This facile oxidation of dihydrosarsasapogenin to an acid with the same carbon content indicates that a primary hydroxyl group was formed during the hydrogenation.

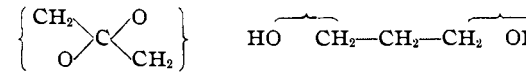
(5) To be published later.

(6) Zimmermann, *Z. physiol. Chem.*, **233**, 257 (1935).

All attempts to reduce sarsasapogenin by aluminum isopropylate or by sodium in amyl alcohol have been unsuccessful, the starting material being recovered unchanged.

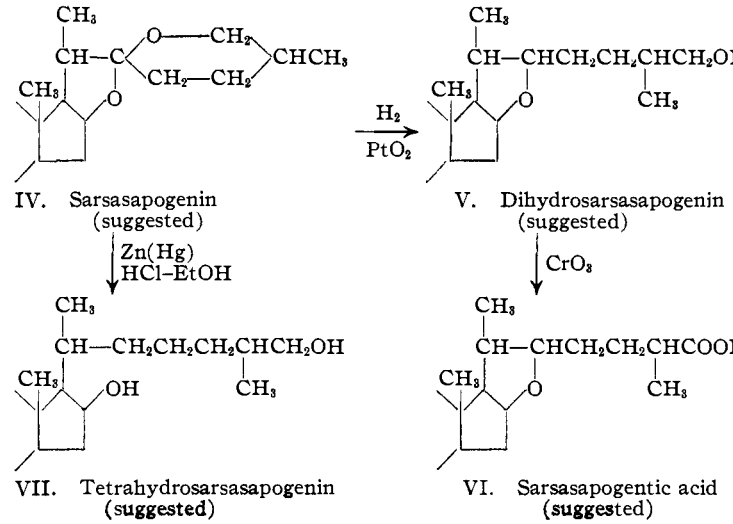
Clemmensen reduction of an alcoholic solution of sarsasapogenin with amalgamated zinc and hydrochloric acid gave a substance of the composition $C_{27}H_{48}O_3$, m. p. 193°, designated as tetrahydrosarsasapogenin (VII). This substance upon mild oxidation with chromic anhydride yielded largely an oily acid fraction which has not yet been crystallized. This acid fraction formed a disemicarbazone. Tetrahydrosarsasapogenin forms only a dibenzoate, suggesting that one of the hydroxyl groups may be hindered, which would be the case at the C-16 position.

In the Clemmensen reduction process the ketone spiro acetal has apparently been reduced to a glycol



Dihydrosarsasapogenin was unaffected by a similar Clemmensen reduction.

Sarsasapogenin acetate, sarsasapogenin, and sarsasapogenone all react with one mol of bromine in acetic acid solution to give crystalline monobromo derivatives with evolution of hydrogen bromide. Sarsasapogenin acetate did not react with more than one mol of bromine. The great ease of bromination is hard to reconcile with structure I. Bromosarsasapogenin upon Clemmensen reduction yields tetrahydrosarsasapogenin, indicating that the bromo compound still contains the ketone spiro acetal grouping. Tetra-



IV. Sarsasapogenin (suggested)

V. Dihydrosarsasapogenin (suggested)

VII. Tetrahydrosarsasapogenin (suggested)

VI. Sarsasapogentic acid (suggested)

hydrofuryl acetate did not take up bromine under similar conditions.

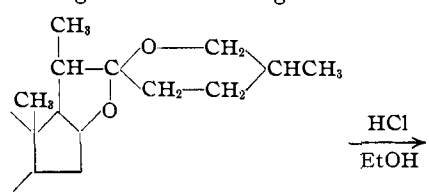
Sarsasapogenin is oxidized readily by selenium dioxide. The reaction is complex and no oxidation products have been isolated. The great ease of oxidation with selenium dioxide gives additional evidence of the presence of a $-CH_2\overset{|}{C}=O$ group or its equivalent. The fact that bromosarsasapogenin does not react with selenium dioxide strongly suggests that the bromine is substituted on a methylene carbon adjacent to a potential carbonyl group. Neither dihydrosarsasapogenin nor tetrahydrosarsasapogenin is oxidized by selenium dioxide. Tetrahydrofuryl acetate gives no evidence of oxidation under similar conditions.

The fact that the catalytic hydrogenation of sarsasapogenin takes place only in acidic media prompted a study of the action of hydrochloric acid upon the substance. Sarsasapogenin when refluxed with an alcoholic solution of hydrochloric acid is readily isomerized to a substance of the same composition, m. p. 185°. The product is much more soluble in the usual solvents than is sarsasapogenin. It forms an acetate, m. p. 152°, which gives a large depression with sarsasapogenin acetate, m. p. 145°. It forms a benzoate, m. p. 175°, which likewise gives a depression with sarsasapogenin benzoate, m. p. 170°. The isomerization product is readily oxidized at room temperature with chromic anhydride to yield a ketone, m. p. 189°. The same ketone was obtained by refluxing sarsasapogenone with an alcoholic solution of hydrochloric acid. This ketone is very probably identical with the isosarsasapogenone obtained by Fieser and Jacobsen[3] by heating sarsasapogenone with hydrobromic acid in chloroform solution in a sealed tube. The isomerization product from sarsasapogenin has been designated tentatively as isosarsasapogenin (VIII).

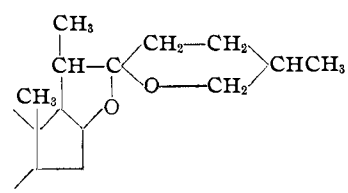
Isosarsasapogenone on mild Clemmensen reduction yields a desoxy compound. This was found to be identical with the product obtained by refluxing desoxysarsasapogenin with an alcoholic solution of hydrochloric acid and is designated as desoxyisosarsasapogenin. Isosarsasapogenin on mild Clemmensen reduction using amalgamated zinc yields a tetrahydro compound identical with the tetrahydrosarsasapogenin obtained by the Clemmensen reduction of sarsasapogenin.

Isosarsasapogenin, on catalytic hydrogenation in hot acetic acid, yielded a product identical with dihydrosarsasapogenin obtained by the catalytic hydrogenation of sarsasapogenin. This suggests that isosarsasapogenin differs from sarsasapogenin in the configuration about the spiro carbon, the action of acids such as hydrochloric acid producing an inversion.

Such an isomerization of sarsasapogenin to isosarsasapogenin would involve the opening and closing of an oxide ring



IV. Sarsasapogenin (suggested)



VIII. Isosarsasapogenin (suggested)

A comparison of the melting points of sarsasapogenin, smilagenin,[2,7] and isosarsasapogenin and derivatives is of interest.

TABLE I

		Acetate	Benzoate	Ketone	Desoxy
Sarsasapogenin, °C.	200	145	171	224	216
Smilagenin, °C.	184	152	181	157	133
Isosarsasapogenin, °C.	185	152	175	189	140

Isosarsasapogenin acetate readily reacts with bromine in acetic acid solution to liberate hydrogen bromide and yield bromoisosarsasapogenin acetate. Isosarsasapogenin is oxidized readily by selenium dioxide.

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. We also wish to thank Drs. Elmer J. Lawson and Eugene L. Wittle for many helpful suggestions offered during the course of this work.

Experimental Part

Isolation of Sarsasapogenin.—To 100 lb. (45 kg.) of ground Mexican sarsaparilla root in a large drum was added 30 gallons (120 liters) of 95% ethanol. The mixture was stirred well several times and then allowed to stand for about eight hours, filtered and the residue washed well

(7) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).

with 95% ethanol. The combined filtrate and washings were evaporated *in vacuo* to a sirup. The sirup, after defatting with ligroin and ether as described by Simpson and Jacobs,[8] was dissolved in 12 liters of 20% ethanol and the solution heated for forty-five minutes at 80–90° with 2 liters of concentrated hydrochloric acid. The mixture was then cooled in salt–ice mixture and the tarry solid collected. The tar was dissolved in 10 liters of 95% ethanol and the resulting solution refluxed on the steam-bath for two hours with 600 cc. of concentrated hydrochloric acid. The mixture was then diluted with three liters of water and cooled in an ice-bath. The solid material was collected, washed with water and dried. The dried residue was refluxed for thirty minutes with a large excess of acetic anhydride. The resulting solution was cooled thoroughly and the crystalline acetates collected and washed well with cold methanol, in which sarsasapogenin acetate is very sparingly soluble. The tan colored crystals were treated with Norite in ethyl acetate and crystallized twice from this solvent to give white plates melting at 142°; yield 80 g.

A second crop of crystals was obtained from the mother liquors. Upon hydrolysis with an excess of alcoholic potassium hydroxide and crystallization from acetone this yielded an additional 10 g. of sarsasapogenin as white needles, m. p. 194–196°.

Somewhat better yields were obtained from an extract prepared by Parke, Davis and Company on a larger scale. In this case, 450 kg. of root gave 56 kg. of extract from which 1480 g. of sarsasapogenin acetate, m. p. 145°, was obtained.

Alkaline hydrolysis of the ligroin soluble fraction obtained above yielded a white crystalline compound melting at 135° which gave no depression with an authentic sample of β-sitosterol.

Catalytic Hydrogenation of Sarsasapogenin Acetate.—A mixture of 7 g. of sarsasapogenin acetate, 1 g. of Adams catalyst, and 150 cc. glacial acetic acid was shaken with hydrogen at 3 atmospheres and 70° for sixteen hours. After evaporation of the solvent *in vacuo*, all attempts to crystallize the residue were unsuccessful. The material was refluxed with an excess of alcoholic potassium hydroxide for thirty minutes and the resulting solution poured into water. The mixture was extracted with ether and the ethereal extract washed with water. The residue from the ether crystallized from acetone to give white needles of dihydrosarsasapogenin, m. p. 165°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1; mol. wt., 418. Found: C, 77.6; H, 11.0; mol. wt., 390.

Similar results were obtained when the hydrogenation was carried out at room temperature and 3 atmospheres pressure in ethanol acidified with hydrochloric acid. The hydrogenation also takes place readily in a 4:1 mixture of acetic acid and ethanol at room temperature.

Attempts to hydrogenate sarsasapogenin acetate in absolute ethanol at room temperature were unsuccessful.

bis-3,5-Dinitrobenzoate of Dihydrosarsasapogenin.—A solution of 500 mg. of dihydrosarsasapogenin and 600 mg. of 3,5-dinitrobenzoyl chloride in 10 cc. of pyridine was heated on the steam-bath for six hours and then allowed to stand at room temperature for six hours. The solution was diluted with water and extracted with ether and the pyridine removed with dilute hydrochloric acid. Evaporation of the ether gave a residue which crystallized from ethyl acetate to give pale tan plates, m. p. 220°.

Anal. Calcd. for $C_{41}H_{50}O_{13}N_4$: C, 61.2; H, 6.2. Calcd. for $C_{34}H_{48}O_8N_2$: C, 69.8; H, 8.3. Found: C, 61.1; H, 5.9.

Hydrolysis of the product with alcoholic potassium hydroxide gave dihydrosarsasapogenin.

Oxidation of Dihydrosarsasapogenin.—To a solution of 1.5 g. of dihydrosarsasapogenin in 40 cc. of glacial acetic acid, was added a solution of 1 g. of chromic anhydride in 20 cc. of 80% acetic acid and the resulting mixture was allowed to stand at room temperature for ninety minutes. The mixture was then poured into water and extracted with ether. The ether extract was washed first with water, then with dilute sodium carbonate solution. The sodium carbonate washings were acidified with hydrochloric acid and the resulting mixture extracted with ether. The ether extract was washed well with water and the ether evaporated on the steam-bath. The residue was crystallized from acetone–methanol to give white crystals of 3-ketosarsasapogentic acid, m. p. 198°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.6. Found: C, 75.6; H, 9.9.

Semicarbazone of 3-Keto-sarsasapogentic Acid.—To 100 mg. of the acid obtained from the oxidation of dihydrosarsasapogenin dissolved in 7 cc. of 80% ethanol was added 300 mg. of semicarbazide hydrochloride and 400 mg. of sodium acetate. The resulting solution was boiled on the steam-bath for one hour. It was then cooled with salt–ice and the white crystals collected and washed with water. The material was recrystallized from 95% ethanol to give a product of m. p. 180°, dec.

Anal. Calcd. for $C_{28}H_{45}O_4N_3$: C, 68.9; H, 9.3. Found: C, 68.8; H, 9.3.

Oxidation of 3-Acetoxydihydrosarsasapogenin to Sarsasapogentic Acid.—Five grams of sarsasapogenin acetate was hydrogenated in ethanol acidified with hydrochloric acid and the unhydrolyzed sirup obtained was dissolved in 150 cc. of glacial acetic acid. To this solution was added a solution of 2 g. of chromic anhydride in 60 cc. of 80% acetic acid. The mixture was allowed to stand at room temperature for one hour when it was diluted with water. The precipitated solid was extracted with ether and the ethereal extract washed well with water and then with 5% sodium carbonate solution.

The sodium carbonate washings were acidified with hydrochloric acid and extracted with ether. The ethereal extract was washed with water and the ether evaporated on the steam-bath. The residue was saponified by heating on the steam-bath for twenty minutes with an excess of 3% aqueous sodium hydroxide solution. The solution was then acidified with hydrochloric acid and the precipitated acid extracted with ether. The ether was evaporated on the steam-bath and the residual oil crystallized from aqueous methanol to give white crystals, m. p. 187°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2; neut. equiv., 432.5. Found: C, 74.6; H, 10.1; neut. equiv., 443.

The acid gives a precipitate with an alcoholic solution of digitonin.

(8) Jacobs and Simpson, *J. Biol. Chem.*, **108**, 501 (1934).

Methyl Ester of Sarsasapogentic Acid.—A solution of 100 mg. of the hydroxy acid obtained as described above in a mixture of 2 cc. of methanol and 8 cc. of ether, after cooling in ice, was treated with an excess of an ethereal solution of diazomethane. The resulting mixture was allowed to stand at room temperature for fifteen minutes, after which the solvent was boiled off on the steam-bath. The residue was crystallized from ether–hexane to give white plates melting at 124°.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.3; H, 10.4. Found: C, 75.2; H, 10.4.

Bromosarsasapogenin Acetate.—A solution of 1 g. of sarsasapogenin acetate in 35 cc. of glacial acetic acid after cooling to 20° and acidification with two drops of 48% hydrobromic acid was treated with 2.2 cc. of 1.05 molar bromine in acetic acid solution added dropwise over a period of ten minutes. The bromine was taken up readily with the liberation of hydrogen bromide. The mixture after standing for ten minutes was poured into water and the precipitated material filtered and washed with water. The residue was crystallized from acetone–ethyl acetate mixture to give fine white needles melting at 208°, dec.

Anal. Calcd. for $C_{29}H_{45}O_4Br$: C, 64.8; H, 8.5. Found: C, 64.8; H, 8.5.

Attempts to make the sarsasapogenin acetate react with more than one mole of bromine were unsuccessful.

On larger runs the acetate usually was obtained as large white prisms melting at 195°, dec. This appears to be identical with the material, m. p. 208°, dec., as it has the same composition and behaves the same in the various reactions carried out with it.

Clemmensen Reduction of Sarsasapogenin Acetate to Tetrahydrosarsasapogenin.—A solution of 500 mg. of sarsasapogenin acetate in 100 cc. of 95% ethanol was treated with 20 g. of amalgamated zinc and the mixture was heated to boiling. Then 15 cc. of concentrated hydrochloric acid was added slowly over a period of four hours to the boiling mixture. The mixture was refluxed for an additional hour. The solution was poured into water and the resulting mixture extracted with ether. The ether extract was washed with sodium carbonate solution and then with water and the ether evaporated on the steam-bath. The residue was crystallized from ethyl acetate to give compact white crystals, m. p. 193°. This gave a 20° depression with sarsasapogenin.

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.1; H, 11.5; mol. wt., 420. Found: C, 76.8; H, 11.5; mol. wt., 390.

Similar results were obtained with sarsasapogenin except that the yields were somewhat better, 500 mg. of sarsasapogenin yielding 400 mg. of product. Bromosarsasapogenin acetate was reduced in a similar manner to give a good yield of the tetrahydro product, m. p. 193°.

Unamalgamated zinc gives mainly unchanged sarsasapogenin and a poor yield of the tetrahydro compound.

When the reaction was carried out over a longer time, crystalline products were very difficult to isolate. This suggests that the tetrahydro compound is somewhat sensitive to acids.

Attempts to dehydrate the tetrahydro compound with potassium acid sulfate, zinc chloride, acetic anhydride–acetyl chloride, or sublimation to yield a crystalline anhydro compound were unsuccessful. The substance sublimes unchanged at 180° under high vacuum.

Attempts to prepare a crystalline acetate by the usual methods were unsuccessful. The substance did not give any evidence of oxidation with selenium dioxide.

Benzoate of Tetrahydrosarsasapogenin.—A solution of 100 mg. of tetrahydrosarsasapogenin in 5 cc. of dry pyridine was treated with 7 drops of benzoyl chloride. The mixture was allowed to stand at room temperature for eight hours and then heated on the steam-bath for one hour. The solution was poured into water and the resulting mixture allowed to stand overnight. The mixture was then extracted with ether and the ether extract washed with dilute hydrochloric acid, dilute sodium carbonate solution and finally with water. The ether was evaporated on the steam-bath and the residue crystallized from aqueous acetone to give small white plates, m. p. 149°.

Anal. Calcd. for $C_{41}H_{56}O_5$: C, 78.4; H, 9.0. Found: C, 78.2, 78.4; H, 9.3, 9.1.

Oxidation of the Dibenzoate of Tetrahydrosarsasapogenin.—To a solution of 500 mg. of tetrahydrosarsasapogenin dibenzoate in 30 cc. of glacial acetic acid was added a solution of 300 mg. of chromic anhydride in 10 cc. of 80% acetic acid. After standing at room temperature for forty minutes the mixture was diluted with water and the precipitated solid extracted with ether and the ethereal extract washed with sodium carbonate and the ether evaporated. The residue was hydrolyzed with alcoholic potassium hydroxide and after treatment with Norite was crystallized from aqueous acetone to give white plates, m. p. 143°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1. Found: C, 77.4; H, 10.9.

The oxidation of the free tetrahydrosarsasapogenin with chromic anhydride at room temperature gave largely an acidic fraction which would not crystallize. Treatment of the crude acid mixture with semicarbazide acetate gave a product which appears to be a disemicarbazone acid.

Anal. Calcd. for $C_{29}H_{48}O_4N_6$: C, 63.9; H, 8.9. Found: C, 64.3; H, 8.8.

Attempts to prepare a crystalline methyl ester from the acid fraction were unsuccessful.

Isosarsasapogenin.—To a boiling solution of 1.5 g. of sarsasapogenin in 150 cc. of 95% ethanol was added a mixture of 100 cc. of 95% ethanol and 45 cc. of concentrated hydrochloric acid. After refluxing for four days on the steam-bath 20 cc. of more concentrated hydrochloric acid was added and the refluxing continued for an additional day. The solution was then poured into water and the resulting mixture extracted with ether. The ether extract was washed with water and the ether evaporated on the steam-bath. The residue was crystallized from acetone to give silky white needles with m. p. 185°. The substance depressed the melting point of sarsasapogenin.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.8; H, 10.7.

Isosarsasapogenin Acetate.—A mixture of 100 mg. of isosarsasapogenin and 2 cc. of acetic anhydride was refluxed for twenty minutes. Dilution with water gave a solid which crystallized from methanol as white needles,

m. p. 152°. These gave a depression of about 30° with sarsasapogenin acetate.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.0; H, 10.1.

Isosarsasapogenin Benzoate.—To a solution of 100 mg. of isosarsasapogenin in 7 cc. of dry pyridine was added an excess of benzoyl chloride and the resulting mixture was warmed on the steam-bath for two hours. The solution was then poured into water and the mixture extracted with ether. The ethereal solution was washed successively with dilute hydrochloric acid, dilute sodium carbonate solution, and water. The ether was evaporated on the steam-bath and the residue crystallized from aqueous acetone to give white needles, m. p. 146°. Recrystallization from 95% ethanol gave white needles, m. p. 175°. This gave a 15° depression with sarsasapogenin benzoate.

Anal. Calcd. for $C_{34}H_{48}O_4$: C, 78.4; H, 9.3. Found: C, 78.3; H, 9.3.

Bromoisosarsasapogenin Acetate.—The bromo compound was prepared as described in previous experiments and crystallized twice from methanol–acetone to give flat white needles, m. p. 170°.

Anal. Calcd. for $C_{29}H_{45}O_4Br$: C, 64.8; H, 8.5. Found: C, 65.2; H, 8.7.

Isosarsasapogenone from Isosarsasapogenin.—To a solution of 400 mg. of isosarsasapogenin in 40 cc. of glacial acetic acid was added 300 mg. of chromic anhydride dissolved in 10 cc. of 80% acetic acid. The mixture was allowed to stand at room temperature for one hour. It was then diluted with water and extracted with ether. The ethereal extract was washed with dilute sodium carbonate solution and with water and the ether evaporated on the steam-bath. The residue was crystallized from aqueous acetone to give white needles, m. p. 188.5°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.3. Found: C, 78.2; H, 10.3.

Isosarsasapogenone from Sarsasapogenone.—A solution of 200 mg. of sarsasapogenone in 50 cc. of 95% ethanol and 20 cc. of concentrated hydrochloric acid was refluxed on the steam-bath for sixty hours. The solution was then poured into water and the mixture extracted with ether. The ethereal solution was washed well with water and the ether evaporated on the steam-bath. The residue was treated with a little Norite and crystallized from aqueous acetone to give white needles, m. p. 185°. This gave no depression with the isosarsasapogenone obtained above.

Desoxyisosarsasapogenin from Isosarsasapogenone.—To a boiling mixture of 100 mg. of isosarsasapogenone, 35 cc. of 95% ethanol and 10 g. of 20-mesh zinc was added 7 cc. of concentrated hydrochloric acid over a period of about four hours. The solution was poured into water and the mixture extracted with ether. The ethereal solution was washed well with water and the ether evaporated on the steam-bath. The residue was crystallized from methanol–acetone to give white plates, m. p. 140°.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.9; H, 11.1. Found: C, 80.5; H, 11.1.

Desoxyisosarsasapogenin from Desoxysarsasapogenin.—A mixture of 200 mg. of desoxysarsasapogenin, 100 cc. of 95% ethanol and 25 cc. of concentrated hydrochloric acid was refluxed on the steam-bath for sixty-five hours. The product was worked up as described above for the isomerization of sarsasapogenone to isosarsasapogenone. The product crystallized from methanol–acetone as white plates, m. p. 141°. This gave no depression with the desoxy compound obtained in the preceding experiment.

Hydrogenation of Isosarsasapogenin.—A mixture of 250 mg. of isosarsasapogenin, 500 mg. of Adams catalyst and 80 cc. of glacial acetic acid was shaken for twelve hours with hydrogen (3 atm.) at 70°. The mixture was worked up as described for dihydrosarsasapogenin. The material was crystallized from aqueous acetone to give white plates, m. p. 162°. This gave no depression with dihydrosarsasapogenin.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 77.4; H, 11.1. Found: C, 77.4; H, 10.9.

Clemmensen Reduction of Isosarsasapogenin.—A solution of 300 mg. of this substance in ethanol and hydrochloric acid was reduced with amalgamated zinc as described previously for the reduction of sarsasapogenin to tetrahydrosarsasapogenin. The product was crystallized from acetone to give compact white crystals, m. p. 193°. This gave no depression with a sample of tetrahydrosarsasapogenin, m. p. 193°.

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.0; H, 11.5. Found: C, 77.0; H, 11.4.

Oxidation of Sarsasapogenin Acetate with Selenium Dioxide.—One gram of sarsasapogenin acetate was dissolved in 15 cc. of glacial acetic acid and 10 cc. of benzene was added to the resulting solution. A solution of 700 mg. of selenium dioxide dissolved in 0.5 cc. of water and 5 cc. of glacial acetic acid was then added and the mixture refluxed for ninety minutes on the steam-bath. Red selenium was precipitated almost at once. Finally 2 g. of sodium acetate was added and the refluxing continued for ten minutes. The mixture contained a black deposit of selenium. No crystalline products were isolated.

Dihydrosarsasapogenin and tetrahydrosarsasapogenin upon similar treatment gave no sign of oxidation. This was likewise true for both crystalline forms of the bromosarsasapogenin acetate.

Experiments on Tetrahydrofurfuryl Acetate.—About 5 g. of tetrahydrofurfuryl acetate was added to a mixture of 1 g. of Adams catalyst, 75 cc. of absolute ethanol and 1 cc. of concentrated hydrochloric acid. The mixture was shaken with hydrogen at three atmospheres at room temperature for eight hours. The amount of hydrogen taken up corresponded only to that required to saturate the catalyst, so the product was not investigated further.

Five drops of tetrahydrofurfuryl acetate was dissolved in 2 cc. of glacial acetic acid and a drop of 48% hydrobromic acid was added. The resulting mixture did not absorb bromine in acetic acid solution.

About 5 drops of tetrahydrofurfuryl acetate was dissolved in 2 cc. of 90% acetic acid and treated with 100 mg. of selenium dioxide on the steam-bath for one hour. There was no evidence of the red color characteristic of selenium dioxide oxidations.

Summary

Evidence is given for the existence of a ketone spiro acetal grouping in the side chain of sarsasapogenin.

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