Dihydro-pseudotigogenin (VI).—To a solution of 500 mg. of pseudotigogenin in 50 cc. of glacial acetic acid was added 250 mg. of platinum oxide catalyst. The mixture was shaken with hydrogen at 45 pounds pressure for twenty hours. The catalyst was filtered, and the product was crystallized from acetic acid, m. p. 202–205°. When mixed with tetrahydro-pseudodiosgenin, m. p. 202–205°, it melted at 202–205°. It is very insoluble in acetone and ether.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.4, H, 11.2. Found: C, 77.5; H, 11.2.

The last two compounds are, of course, identical.

Δ^{16}-*allo*-Pregnenedione (IX).—To a solution of 250 mg. of tetrahydropseudodiosgenin in 25 cc. of acetic acid was added 250 mg. of chromic anhydride in 5 cc. of 95% acetic acid. It was allowed to stand at room temperature for ninety minutes; water was added and the product was then extracted with ether. The ethereal layer was washed with sodium carbonate solution. The product was crystallized from ether–pentane, m. p. 208–211°; yield, 65 mg. When mixed with an authentic sample of Δ^{16}-*allo*-pregnenedione, it gave no depression in melting point.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.2; H, 9.7.

Summary

Reactions of diosgenin have been studied.

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Sterols. CI. The Structure of Pseudosarsasapogenin

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It has been shown that steroidal sapogenins upon treatment with acetic anhydride at 200° are converted almost quantitatively into isomeric compounds called pseudosapogenins.[1] These compounds are important intermediates in the conversion of the sapogenins into pregnane compounds and the steroidal hormones. Upon oxidation they give Δ^{16}-pregnenone-20 derivatives, while upon treatment with acids they are converted back to the original sapogenins in almost quantitative yields. This reaction has been extended to include desoxysarsasapogenin.

Because of the various reactions involved, structure I was tentatively assigned to pseudosarsasapogenin.[1] Since the publication of our original suggestion of a tentative structure of the pseudosapogenins we have received several suggestions proposing formula II as the structure of these compounds.[2] In our original work we eliminated formula II because dihydropseudosarsasapogenin upon mild oxidation gives a diketo acid, which upon further oxidation gives Δ^{16}-pregnenedione-3,20 (VI). Because of the significance of this acid in the proof of the structure of pseudosarsasapogenin, we have rechecked the analysis of its semicarbazone and found that it contains 15.35% nitrogen. The theoretical value for a disemicarbazone of the diketo-acid is 15.44% nitrogen. The formation of a diketo acid by mild oxidation could not be explained on the basis of formula II.

Furthermore, if formula II were correct for the pseudosapogenins, the dihydropseudosarsasapogenin should be identical or possibly isomeric at C-20 or C-22 with dihydrosarsasapogenin (VIII) which is formed by the direct hydrogenation of sarsasapogenin[3] (VII). We have rechecked the mixed melting point of dihydropseudosarsasapogenin with dihydrosarsasapogenin, and as previously reported obtained a large melting point depression. This would not eliminate the possibility of isomerization in the reduction of II at C-20 or C-22, but even so the two compounds should show similarity in their reactions. We have repeated the oxidation of dihydrosarsasapogenin (VIII) under mild conditions and have obtained dehydrosarsasapogentic acid (IX),[3] a mono keto acid, as the major oxidation product, whereas when dihydropseudosarsasapogenin is oxidized under the same conditions it gives the diketo-acid and Δ^{16}-pregnenedione. Oxidation of dihydrosarsasapogenin under the identical conditions which we used to obtain a good yield of Δ^{16}-pregnenedione from dihydropseudosarsasapogenin gave only a negligible amount of non-acidic material from which we were unable to obtain even a trace of Δ^{16}-pregnenedione. It has previously been shown that oxidation of dihydrosarsasapogenin under more drastic conditions caused a cleavage between C-22 and C-23 as the major reaction[4] with no evidence of cleavage between

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 3592 (1939); **62**, 518 (1940); **62**, 521 (1940).

(2) Private communications.

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

(4) Marker and Rohrmann, *ibid.*, **61**, 3477 (1939).

C-20 and C-22 which would be necessary to obtain pregnenone-20 derivatives.

Pseudosarsasapogenin, on treatment with ozone for a short time, absorbed the theoretical amount of ozone for one double bond, giving pregnenolone. The same product was obtained when the diacetate of pseudosarsasapogenin was ozonized. However, when dihydropseudosarsasapogenin was treated with ozone, it was observed that the ozone was liberated from the solution as soon as ozonolysis was started, and a good yield of unchanged product was obtained after passing ozone through the solution for thirty-five minutes. Only a 5% yield of crude semicarbazones could be obtained on treatment of the mother liquors of crystallization with semicarbazide acetate. These were very inhomogeneous and could not be crystallized into pure products.

Hydrogen peroxide in acetic acid reacts upon pseudosarsasapogenin to give a compound of the formula $C_{27}H_{44}O_5$, in good yield. However, when dihydropseudosarsasapogenin was treated with hydrogen peroxide under the same conditions the same compound was obtained in about the same yield. Treatment of dihydrosarsasapogenin (VIII) with hydrogen peroxide under the same conditions gave only unchanged material. Because of the ease of isomerization of pseudosarsasapogenin to sarsasapogenin under the influence of acids, we then treated sarsasapogenin acetate with hydrogen peroxide. The major product obtained was pregnanetriol-3,16,20, a compound which was obtained previously by the action of persulfuric acid upon sarsasapogenin acetate.[5] Dihydropseudosarsasapogenin absorbs bromine rather rapidly, but not so fast as a double bond in the 5 position. Dihydrosarsasapogenin does not exhibit this tendency.

Should formula II be correct for pseudosarsasapogenin, mild oxidation to obtain Δ^{16}-pregnenedione would involve going through the intermediate oxidation product X, followed by hydrolysis of the ester at C-16 and subsequent dehydration of the resulting carbinol. The treatment used to obtain Δ^{16}-pregnenedione is probably not drastic enough to involve such a hydrolysis and dehydration. In order to study the reactivity of an ester at C-16, we prepared tetrahydrosarsasapogenin as previously described.[3] This compound, although having hydroxyl groups at C-3, C-16 and C-27, under the usual methods of esterification forms esters only with the hydroxyl groups at C-3 and C-27, leaving a free hydroxyl group at C-16 which can be oxidized to a ketonic group. By heating tetrahydrosarsasapogenin with acetic anhydride at 200° we were able to obtain a product which upon prolonged refluxing with potassium hydroxide in alcohol retains its acetyl group at C-16 without hydrolysis, whereas the diesters of tetrahydrosarsasapogenin are readily saponified. The unreactivity of a substituent at C-16 evidently depends to a great extent upon the nature of the side chain on the sterol molecule, for the acetoxy group at C-16 in the triacetate of *allo*-pregnanetriol-3,16,20 can be hydrolyzed by the ordinary procedures.[6]

As double bonds in a molecule are readily reacted upon by potassium permanganate in acetic acid, this reaction was applied to pseudosarsasapogenin. The non-acid fraction consisted almost entirely of Δ^{16}-pregnenedione-3,20.

The reactions presented here, in conjunction with those previously published,[1] indicate that formula I is to be preferred to formula II for pseudosarsasapogenin.

We wish to thank Parke, Davis and Company for their generous assistance. We also wish to thank Dr. H. M. Crooks and Dr. E. L. Wittle of the Parke, Davis and Company Laboratories for their valuable suggestions in this investigation.

Experimental Part

Pseudodesoxysarsasapogenin.—A mixture of 9 g. of desoxysarsasapogenin and 40 cc. of acetic anhydride was heated in a sealed tube for ten hours at 200°. The acetic anhydride was evaporated *in vacuo* and the residue was hydrolyzed by refluxing with alcoholic potassium hydroxide for thirty minutes. Water was added and the product was extracted with ether. The solvent was removed and the residue was crystallized from aqueous acetone, m. p. 130°, yield 5.3 g.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.9; H, 11.1. Found: C, 80.8; H, 11.0.

Dihydropseudodesoxysarsasapogenin.—A mixture of 600 mg. of pseudodesoxysarsasapogenin, 200 mg. of platinum oxide catalyst and 80 cc. of glacial acetic acid was shaken with hydrogen at room temperature and a pressure of 3 atm. for sixteen hours. The mixture was filtered and the solvent was evaporated *in vacuo*. The residue was hydrolyzed with hot methanolic potassium hydroxide and poured into water. The product was extracted with ether and washed well with water. The solvent was evaporated and the residue was crystallized from acetone, m. p. 128–129°. A mixture with the starting material melted at 105–112°.

(5) Marker, Rohrmann, Crooks, Wittle, Jones and Turner, THIS JOURNAL, **62**, 525 (1940).

(6) Marker and Wittle, *ibid.*, **61**, 855 (1939).

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.5; H, 11.5. Found: C, 80.7; H, 11.4.

Reaction of Hydrogen Peroxide with Pseudosarsasapogenin.—A mixture of 1 g. of pseudosarsasapogenin, 10 cc. of 30% hydrogen peroxide and 230 cc. of acetic acid was heated for five hours at 70°. The solution was concentrated *in vacuo* and diluted with water. It was extracted with ether, washed well with water and the solvent was evaporated. The residue was hydrolyzed with hot methanolic potassium hydroxide. The resulting solution was poured into water and extracted with ether. The ether was evaporated and the residue was crystallized from aqueous methanol as tiny white needles, melting at 253–254°, yield 250 mg.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.26; H, 9.9. Found: C, 72.39, 72.56; H, 10.01, 9.99.

The same product was obtained when dihydropseudosarsasapogenin was treated with hydrogen peroxide in the same manner.

The alkaline solution from the above hydrolysis was acidified and extracted with ether. The extract was washed with water and the ether was evaporated. The residue was crystallized from aqueous methanol to give a small amount of lactonic material melting at 282–285°.

Anal. Found: C, 72.57; H, 9.44.

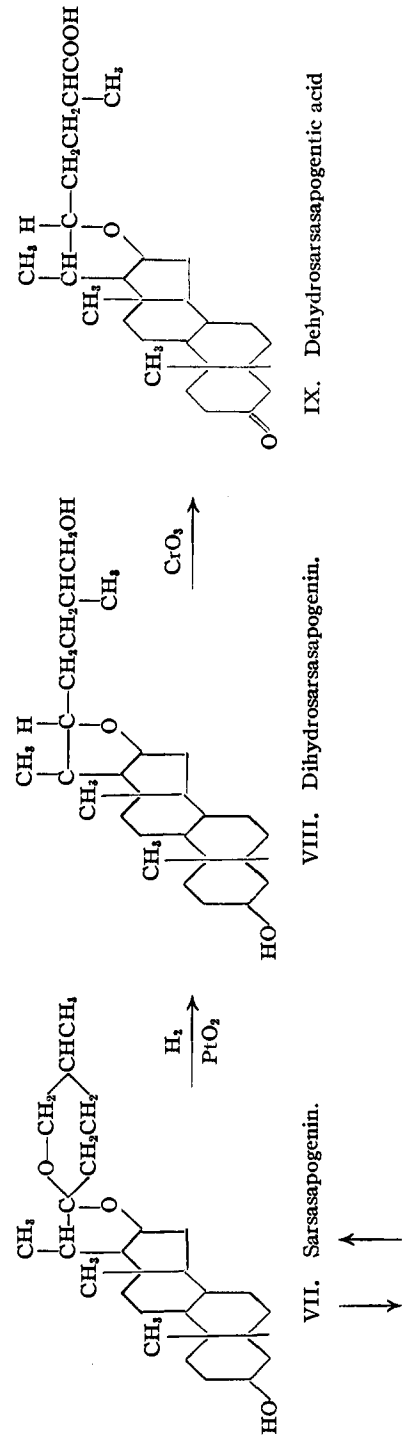
When refluxed with acetic anhydride the neutral product gave non-crystalline material. Oxidation gave both neutral and acidic products, neither of which was obtained crystalline. It is stable to short heating at its melting point. No change was observed when it was allowed to stand for twenty-four hours with alcoholic hydrogen chloride. It was unaffected by catalytic hydrogenation in acetic acid with platinum oxide catalyst.

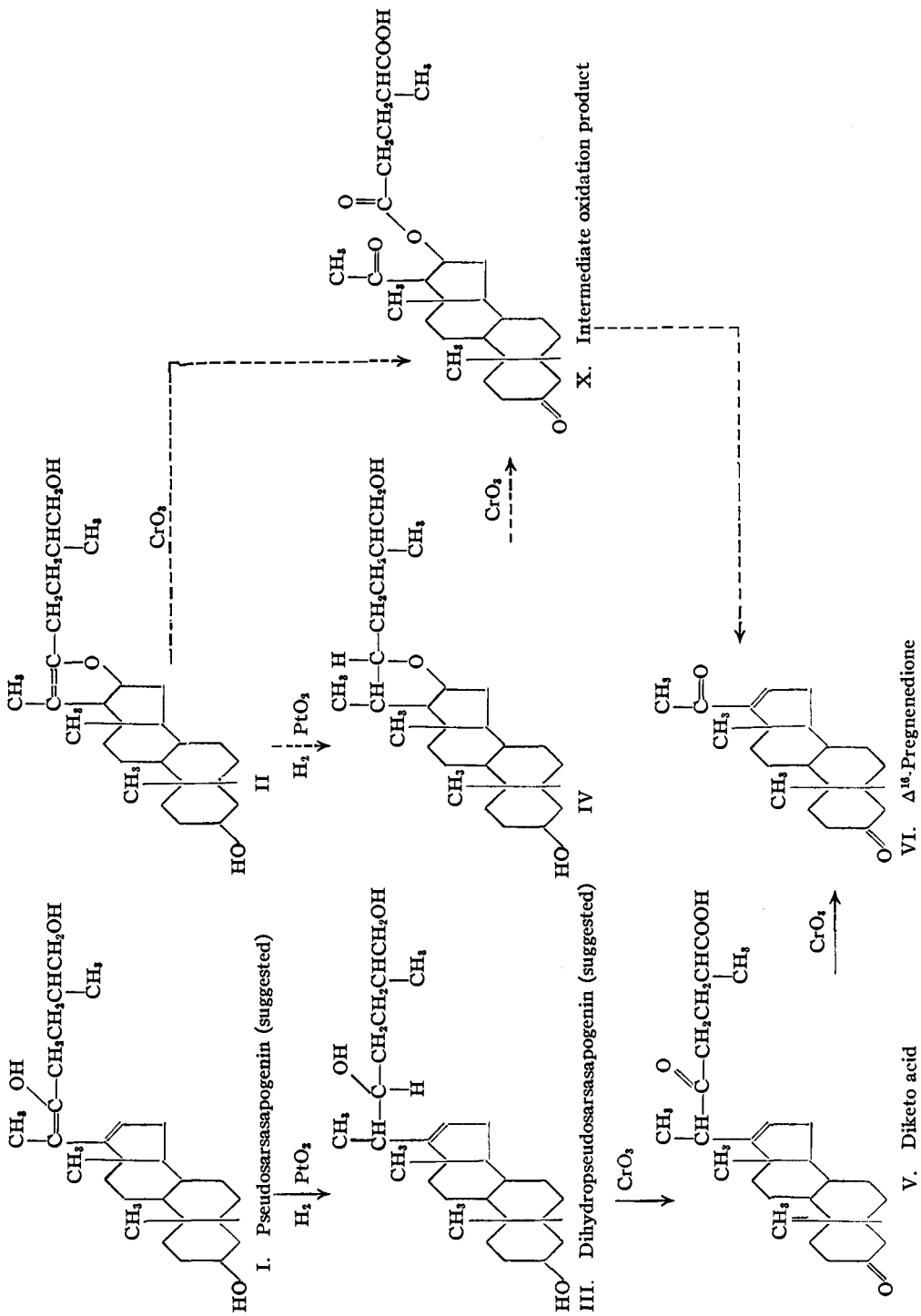
Reaction of Hydrogen Peroxide with Sarsasapogenin Acetate.—A mixture of 6 g. of sarsasapogenin acetate, 50 cc. of 30% hydrogen peroxide and 300 cc. of acetic acid was heated for seven hours at 70°. The solution was concentrated *in vacuo*, diluted with water and extracted with ether. The extract was washed with water and the solvent was evaporated. The residue was hydrolyzed with hot methanolic potassium hydroxide and then poured into water. The mixture was extracted with ether and the extract washed well with water. The solvent was evaporated and the residue was crystallized from acetone to give 1.55 g. of pregnanetriol, melting at 221–223°. This gave no depression in melting point when mixed with an authentic sample of pregnanetriol-3,16,20 prepared by the action of persulfuric acid on sarsasapogenin acetate.

Bromosarsasapogenin acetate and dihydrosarsasapogenin were recovered unchanged when treated in the above manner. Dihydropseudosarsasapogenin rapidly decolorizes a bromine solution in acetic acid.

Oxidation of Pseudosarsasapogenin with Potassium Permanganate.—To a solution of 3 g. of pseudosarsasapogenin in 200 cc. of acetic acid was added a solution of 6 g. of potassium permanganate in 500 cc. of 60% acetic acid over a period of fifteen minutes. The temperature was maintained at 15° during the addition. It was then allowed to stand at room temperature for five hours, poured into water and extracted with ether. The extract was washed with water and then thoroughly shaken with a 5% solution of sodium hydroxide. The ether layer was washed with water and the solvent evaporated. The residue was treated with Norit in methanol and was then crystallized from aqueous methyl alcohol. It melted at 194–196°. When mixed with an authentic sample of pregnenedione it gave no depression in melting point.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.1; H, 9.6.





It gave a semicarbazone which after crystallization from aqueous ethanol did not melt below 295°.

Ozonolysis of the Diacetate of Pseudosarsasapogenin.—A solution of 2 g. of pseudosarsasapogenin in 30 cc. of acetic anhydride was refluxed for fifteen minutes. The solvent was evaporated *in vacuo* and the oily residue was dissolved in 100 cc. of chloroform. The solution was ozonized at 0° for twenty minutes. Ozone was completely absorbed for sixteen minutes as indicated by a negative starch–potassium iodide test given by the vapors escaping from the solution. The solution, after addition of 10 cc. of acetic acid, was steam distilled and the oily residue was dissolved in ether. The ether was washed with dilute potassium hydroxide solution and water and the solvent was evapo-

rated. The residue was sublimed in a high vacuum, and the fraction subliming between 150 and 185° was converted to the semicarbazone by refluxing it for two hours with 1 g. of semicarbazide hydrochloride and 1.2 g. of sodium acetate in 20 cc. of ethanol. The crude semicarbazone melted at 207°, but after several crystallizations from methanol it melted at 237°, yield 400 mg. The mixed melting point with the semicarbazone of Δ^{16}-pregnenol-3(β)-one-20 acetate of m. p. 237° prepared by chromium trioxide oxidation of pseudosarsasapogenin acetate was 236°.

In another experiment under the same conditions the acetate of pregnenol-3(β)-one-20 was isolated directly from the material sublimed in the high vacuum. This product was crystallized from methanol as long flat blades, m. p. 139°, which gave no depression in melting point when mixed with the acetate of pregnenol-3(β)-one-20 prepared by the chromium trioxide oxidation of pseudosarsasapogenin diacetate.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.0; H, 9.6. Found: C, 77.0; H, 9.6.

Ozonolysis of Pseudosarsasapogenin.—A solution of 3 g. of pseudosarsasapogenin in 150 cc. of chloroform absorbed ozone readily for fifteen minutes. The solution was distilled with steam after the addition of 10 cc. of acetic acid. The oily residue was taken into solution with ether and washed well with sodium carbonate solution. The residue left on evaporation of the ether was refluxed for two hours with 100 cc. of alcohol containing 2 g. of semicarbazide hydrochloride and 2.5 g. of sodium acetate. The mixture was diluted with ether and the ethereal layer was repeatedly washed with water. The semicarbazone which finally precipitated from the ethereal solution was filtered off. It weighed 600 mg., and after recrystallization from dilute ethanol melted at 256°, giving no depression in melting point when mixed with the semicarbazone of Δ^{16}-pregnenol-3(β)-one-20 prepared by the chromium trioxide oxidation of pseudosarsasapogenin acetate.

For further characterization the semicarbazone was converted to Δ^{16}-pregnenedione-3,20 by refluxing 100 mg. for thirty minutes with 10 cc. of ethanol containing 2 cc. of water and 1 cc. of sulfuric acid. The solution was diluted with water and extracted with ether. The ethereal solution was washed well with water and the solvent was evaporated. The residue was dissolved in 10 cc. of acetic acid and 50 mg. of chromium trioxide in 5 cc. of 80% acetic acid was added to the solution. After standing at room temperature for twenty minutes, water was added and the mixture was extracted with ether. The ethereal solution was washed with potassium hydroxide solution and water and was evaporated to dryness. The residue crystallized from ether–acetone as white crystals, m. p. 195°, which gave no depression in melting point when mixed with an authentic sample of Δ^{16}-pregnenedione-3,20, m. p. 195°.

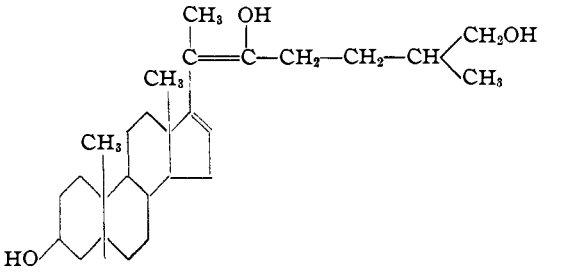
Upon treatment of 3 g. of dihydropseudosarsasapogenin in chloroform with ozone, it was found that the ozone immediately escaped from the solution. However, ozone was passed through the solution for thirty-five minutes. Upon working up the product, unchanged dihydropseudosarsasapogenin was obtained as the only crystalline product. The mother liquors from these gave 150 mg. of a mixture of semicarbazone upon treatment with semicarbazide acetate but these could not be separated.

Action of Acetic Anhydride on Tetrahydrosarsasapogenin.—A mixture of 2 g. of tetrahydrosarsasapogenin and 25 cc. of acetic anhydride was heated for ten hours in a sealed tube. The acetic anhydride was evaporated *in vacuo* and the residue was refluxed for forty-five minutes with 100 cc. of 5% alcoholic potassium hydroxide solution. This was poured into water and the mixture was extracted with ether. The extract was washed with water and the ether was evaporated. The residue from the evaporation of the ether was sublimed in high vacuum and crystallized from acetone to give 2 g. of 16-acetoxy-tetrahydrosarsasapogenin, melting at 155°.

Anal. Calcd. for $C_{29}H_{50}O_4$: C, 75.27; H, 10.89. Found: C, 75.28; H, 10.90.

Summary

The structure of pseudosarsasapogenin was further investigated, the evidence supporting the previously suggested structure;



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