

Mares' Non-Pregnancy Urine.—The butanol extract of 300 gallons of mares' non-pregnancy urine (previously hydrolyzed with hydrochloric acid) was hydrolyzed with an excess of aqueous potassium hydroxide solution. The neutral fraction was extracted with ether, which on evaporation yielded 209 g. of tarry residue. This residue was dissolved in a minimum amount of ethyl alcohol and treated with a 2% digitonin ethyl alcohol solution. On decomposition of the digitonide and sublimation of the residue under high vacuum at 130–140°, 0.34 g. of cholesterol was obtained, m. p. 144–146°. There was no depression in melting point when mixed with an authentic sample of cholesterol.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.85; H, 12.00. Found: C, 83.62; H, 11.69.

When refluxed with acetic anhydride this sample gave an acetate melting at 119° which gave no depression when mixed with an authentic sample.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.2; H, 11.3. Found: C, 81.27; H, 11.1.

The neutral fraction (42.5 g.) from the succinic anhydride

treatment gave no crystalline products when sublimed under high vacuum at 100–170°.

The carbinol fraction (30 g.) from the succinic anhydride treatment gave 25.19 g. of a very viscous oil on sublimation under high vacuum. This oil was oxidized by dissolving in 100 cc. of acetic acid and adding 20 g. of chromic anhydride in 20 cc. of water and 200 cc. of acetic acid. The mixture was kept at room temperature for thirty minutes. The ether extract was washed well with water and then with potassium carbonate to remove any acids present. The neutral fraction (8.8 g.) in 100 cc. of ethanol was added to 7.2 g. of semicarbazide hydrochloride and 10.8 g. of sodium acetate in 140 cc. of ethanol and refluxed for three hours. No crystalline semicarbazones could be isolated, indicating the absence of *allo*-pregnenedione.

Summary

The androstane and pregnane content of *Cantharides*, Mexican flies, ant eggs, sheep feces, chicken feces and mares' non-pregnancy urine was investigated.

STATE COLLEGE, PENNA.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE AND FROM THE PHARMAZEUT. INSTITUT UND D. KLINIKAPOTHEKE D. MEDIZIN. FAKULTÄT ZU KANAZAWA, JAPAN]

Sterols. C. Diosgenin¹

BY RUSSELL E. MARKER, TAKEO TSUKAMOTO AND D. L. TURNER

Diosgenin (I) has been isolated from an extract of *Dioscorea tokoro* (Makino), and its nuclear structure proven by Tsukamoto, *et al.*^{2,3,4} It has been shown to have a 5,6 double bond and one nuclear hydroxyl group in the 3-position having the beta-configuration as in cholesterol. By catalytic reduction, Tsukamoto obtained tigenin, showing that it has the same side chain characteristic of the saturated steroidal sapogenins.

The ready availability of diosgenin and the fact that it contains a double bond in its nucleus suggested that it would be an ideal compound to apply the reactions^{5,6} which have been applied to sarsasapogenin and other steroidal sapogenins for the removal of the side chain leading to derivatives of the pregnane and androstane series.

(1) The isolation of the diosgenin was carried out in the Japanese laboratories and the experimental work reported in this paper was carried out at The Pennsylvania State College.

(2) Tsukamoto, Ueno and Ohta, *J. Pharm. Soc., Japan*, **56**, 135 (1936).

(3) Tsukamoto, Ueno and Ohta, *ibid.*, **57**, 9 (1937).

(4) Tsukamoto, Ueno, Ohta and Tschesche, *ibid.*, **57**, 283 (1937).

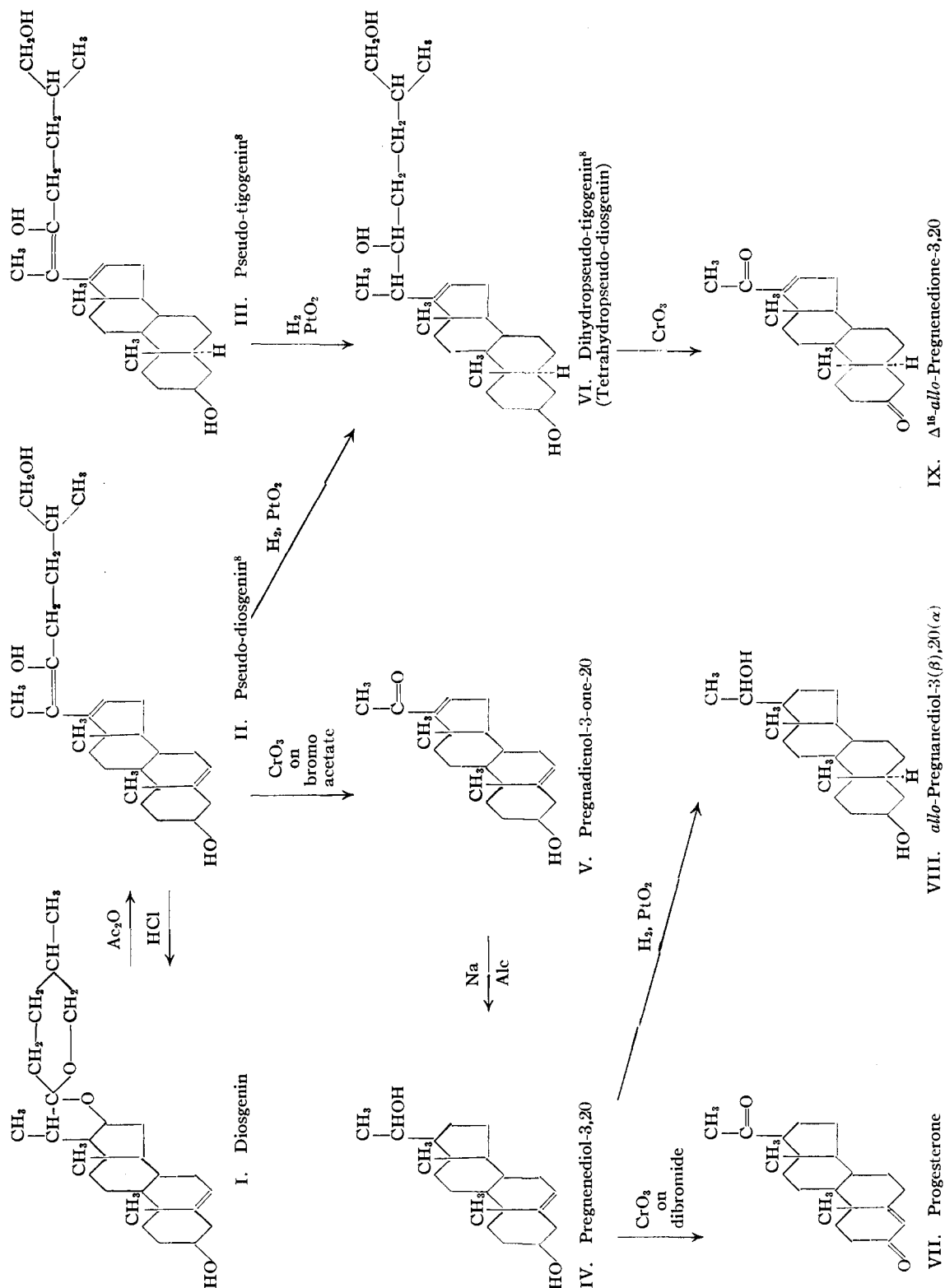
(5) Marker and Rohrmann, *THIS JOURNAL*, **61**, 3592 (1939). **62**, 518, 521, 896, 898 (1940).

(6) Marker, Rohrmann and Jones, *ibid.*, **62**, 648 (1940).

It is also a compound which can be correlated readily with the other sapogenins.

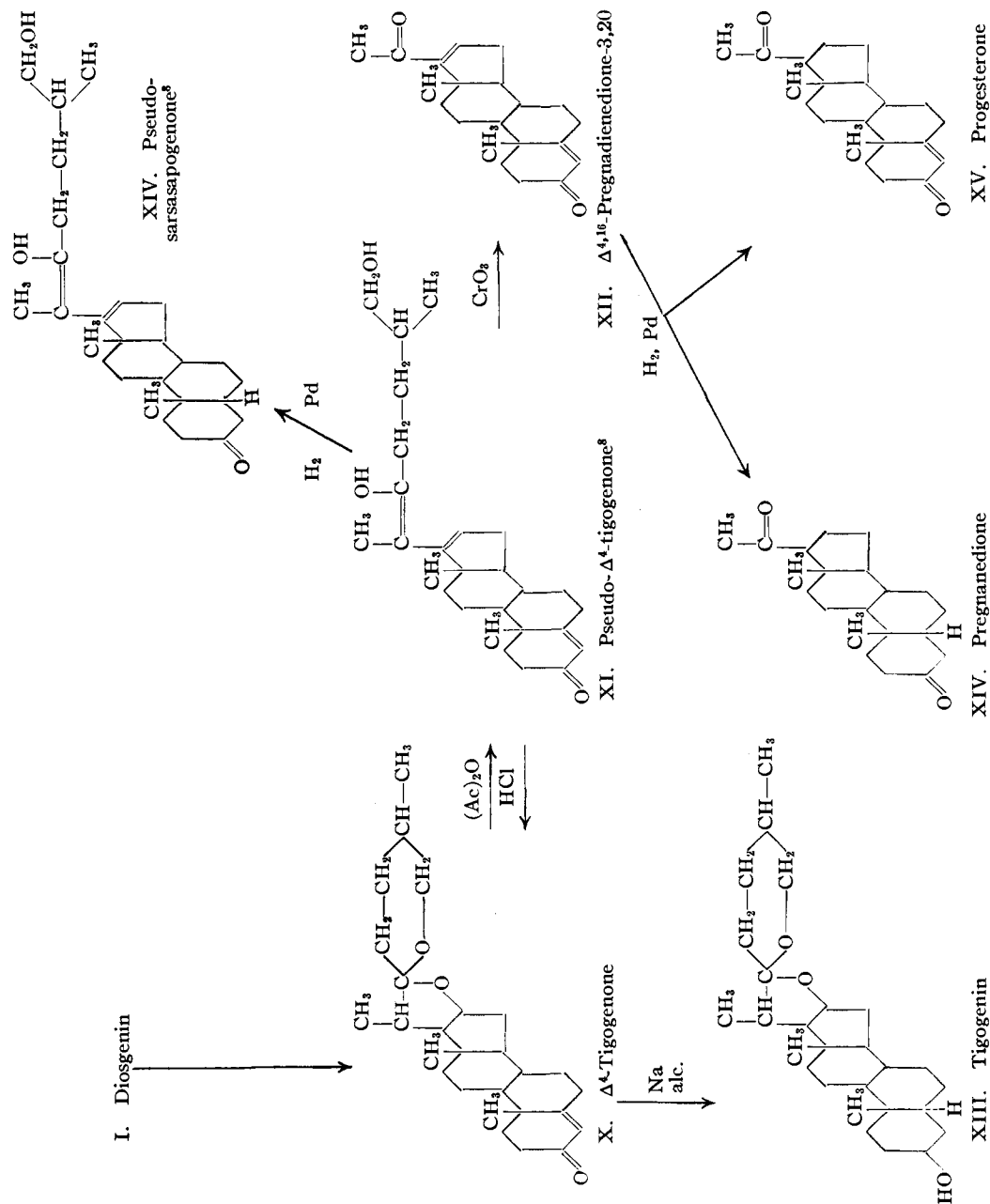
When diosgenin (I) is heated at 200° with acetic anhydride it is converted into pseudodiosgenin (II). As is characteristic of the other pseudosapogenins, pseudodiosgenin is easily reconverted to diosgenin by alcoholic hydrochloric acid. In preliminary experiments on pseudosarsasapogenin⁷ it was found that bromine in acetic acid converted much of the pseudosapogenin back to the sapogenin. This probably was due to the catalytic effect of hydrobromic acid formed in the reaction. To overcome this difficulty, we acetylated pseudodiosgenin and brominated it at a low temperature in acetic acid containing sodium acetate. We then added chromic acid to this solution. After debromination and hydrolysis we obtained $\Delta^{5,16}$ -pregnadienol-3-one-20 (V). However, the yield in this case was much lower than that obtained in the oxidation of the saturated pseudosapogenins.^{5,6} This is probably due to the difficulty of bromination of the pseudo compound without converting it back to the original sapogenin. When

(7) Unpublished results from this Laboratory.



(8) The formulas of the pseudo and dihydro compounds are tentative, and need further structural proof.

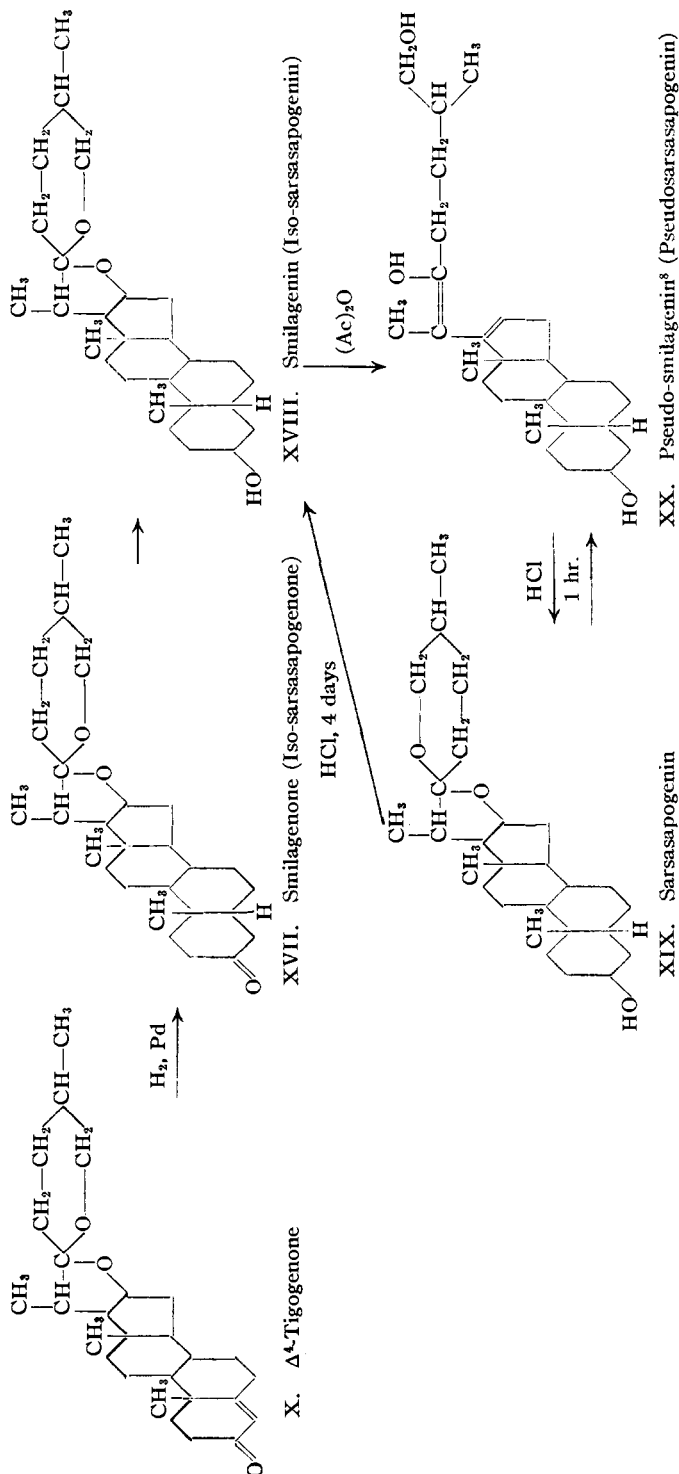
$\Delta^{5,16}$ -pregnadienol-3-one-20 (V) was reduced with sodium in absolute alcohol, the conjugated car-



bonyl group at C-20 was reduced to a carbinol and the 16-17 double bond was saturated. This is similar to the reduction of Δ^{16} -pregnenedione-3,20,^{5,6} in which the major product is pregnanediol-3(α),-20(α). There was probably a small amount of isomeric pregnanediols formed in our reduction as indicated by the melting point of the product, but upon hydrogenation of IV by platinum oxide catalyst and hydrogen, a good yield of *allo*-pregnanediol-3(β),20(α) (VIII) was obtained. Protection of the double bond in Δ^5 -pregnenediol-3,20 (IV)

by addition of bromine, oxidation of the product and debromination gave progesterone (VII).

Reduction of pseudodiosgenin with Adams catalyst and hydrogen formed tetrahydropseudodiosgenin (VI). The same product was formed when pseudotigogenin (III) was similarly reduced. Oxidation of tetrahydropseudodiosgenin (VI) by chromic anhydride gave Δ^{16} -*allo*-pregnenedione-3,20 (IX), which is analogous to the oxidation of dihydropseudosarsasapogenin^{5,6} to Δ^{16} -pregnenedione-3,20.



Diosgenin (I) on bromination, oxidation and debromination gave Δ^4 -tigogenone (X). Reduction of (X) with sodium in alcohol gave tigogenin (XIII), showing that it had the conjugated ketone system in Ring A. Δ^4 -Tigogenone (X) was

formed in better yield by the oxidation of diosgenin (I) with aluminum isopropylate in cyclohexanone. Upon heating (X) with acetic anhydride at 200° , followed by alkaline hydrolysis, a product which could not be crystallized from the ordinary laboratory solvents was obtained; it always precipitated as a slightly colored oil. This was not unchanged (X), as the latter crystallizes very readily even from ether. When the oily pseudo- Δ^4 -tigogenone (XI) was heated with alcoholic hydrochloric acid it gave a good yield of Δ^4 -tigogenone (X). To prove the identity of the pseudo- Δ^4 -tigogenone, it was reduced by palladium and hydrogen, giving crystalline pseudo-sarsasapogenone (XIV), a product previously prepared from sarsasapogenin.⁵ As the conjugated ketone system in Ring A of sterols is resistant to attack by mild treatment with chromic anhydride, pseudo- Δ^4 -tigogenone proved to be a very desirable derivative of diosgenin for conversion to pregnane compounds. This eliminated the troublesome bromination of the pseudo compounds before oxidation. Oxidation of the oily pseudo- Δ^4 -tigogenone (XI) at room temperature with chromic acid, gave $\Delta^{4,16}$ -pregnadienedione (XII) in good yield. The latter compound upon reduction with sodium in alcohol gave *allo*-pregnenediol-3(β),20(α).

Marker and Rohrmann⁵ have shown that in the reduction of the conjugated ketone system at C-16,C-20 by palladium-barium sulfate catalyst the primary product is a saturated ketone of the normal configuration at C-17. We have now tried the partial reduction of $\Delta^{4,16}$ -pregnadienedione-3,20 (XII) with palladium-barium sulfate catalyst, stopping the reduction after absorption of approximately half of the theoretical amount of hydrogen required to hydrogenate both double bonds. By letting the partially reduced product stand in 60% acetone at 0° overnight, $\Delta^{4,16}$ -pregnadienedione-3,20 (XII) and Δ^{16} -pregnenedione-3,20 separated because of their slight solubility in this mixture as compared to progesterone. By repeated crystallization of the

filtrate from dilute acetone and pentane, progesterone was obtained although in low yield. This was due both to the difficulty of separating progesterone from the mixture, and the accompanying formation of pregnanedione-3,20 and Δ^{16} -pregnenedione during the partial reduction. However, when the total mother liquors from the progesterone were reduced completely with palladium-barium sulfate catalyst and hydrogen, a good yield of pregnanedione (XIV) was obtained.

Sarsasapogenin,⁹ treated with hydrochloric acid in alcohol, is converted to smilagenin (iso-sarsasapogenin), which is isomeric with sarsasapogenin at the C-22 carbon atom, the spiro-ketal carbon. It has been shown¹⁰ that sarsasapogenin has the same side chain as neo-tigogenin (iso-tigogenin) (isomeric at C-22 to tigogenin), by the conversion of the former into the latter. In the same manner smilagenin was converted into tigogenin. These experiments showed that tigogenin and, therefore, gitogenin, digitogenin and diosgenin, have the same side chain as isosarsasapogenin (smilagenin), differing in configuration at C-22 from neo-tigogenin and sarsasapogenin. We now have additional evidence of this correlation through reactions not involving the side chain. When Δ^4 -tigogenone (X) was reduced with palladium-barium sulfate catalyst, it gave smilagenone (XVII) and not sarsasapogenone, confirming the earlier work⁵ on the correlation of the structure of the side chains of the steroidal sapogenins. When smilagenone was reduced it gave smilagenin (XVIII). It had previously been shown⁵ that smilagenin and sarsasapogenin gave the identical pseudo-sarsasapogenin (XX) which upon treatment with hydrochloric acid for a short time gave sarsasapogenin. It is interesting that sarsasapogenin and neo-tigogenin can be converted into their isomeric compounds, smilagenin and tigogenin, by refluxing for four days with alcoholic hydrochloric acid, whereas tigogenin, smilagenin, gitogenin, digitogenin, chlorogenin and diosgenin, are little affected by such treatment.

Evidently these last six compounds have the stable configuration around C-22. Treatment with ethanolic hydrogen chloride for a short time converts the pseudo forms of chlorogenin, tigogenin and diosgenin into the original genins, which are stable to longer treatment with hydrogen chloride. On the other hand, pseudosarsa-

sapogenin (pseudosmilagenin) on mild treatment with hydrogen chloride gives sarsasapogenin which is changed to smilagenin on longer treatment. It thus appears that the *allo*-configuration at C-5 influences the ring closure of the pseudo-genin in such a way as to form the more stable genin directly, whereas pseudosarsasapogenin, the only genin of the coprostane or normal configuration at C-5 allows ring closure to give first sarsasapogenin the less stable form, which is converted by longer treatment with acid to smilagenin.

We wish to thank Parke, Davis and Company.

Experimental Part

The crude diosgenin was isolated from *Dioscorea tokoro* (Makino). It was purified by refluxing with alcoholic potassium hydroxide for thirty minutes, cooling and extracting with ether. The solvent was removed and the residue was crystallized from acetone and from acetic acid, m. p. 200–202°. It was unaffected by refluxing for four days with ethanolic hydrochloric acid.

Δ^4 -Tigogenone (X).—(a) To a cooled solution of 2 g. of diosgenin in 50 cc. of glacial acetic acid was added 4.8 cc. of a *N* bromine solution in acetic acid. It was allowed to stand for fifteen minutes, and then 1 g. of chromic anhydride in 10 cc. of 90% acetic acid was added, keeping the temperature at 25°. After standing for thirty minutes, water was added, the product was extracted with ether, and the ethereal solution was washed well with water. To this was added 100 cc. of acetic acid and 2 g. of zinc dust and the mixture was heated on a steam-bath, distilling off the ether. It was then heated for one hour with an additional 2 g. of zinc dust. The product was filtered and concentrated *in vacuo*. It was extracted with ether, and the acids were removed by washing with sodium carbonate solution. It was crystallized first from ether, and finally from pentane, giving a product melting at 186–188°. When mixed with diosgenin, m. p. 202–205°, it melted at 140–148°.

(b) To a solution of 10 g. of diosgenin in a mixture of 500 cc. of dry toluene and 75 cc. of cyclohexanone was added 25 g. of aluminum isopropylate. After refluxing for ten hours, water and ether were added and the ethereal solution was washed well with dilute hydrochloric acid, and then with dilute sodium hydroxide. The ether was evaporated and the residue was steam distilled for two hours. The product was crystallized first from pentane and this was followed by crystallization from ether-pentane, m. p. 186–188°. Mixed with the product obtained by the chromic anhydride oxidation it gave no depression in melting point; yield, 7.4 g.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 78.6; H, 9.8. Found: C, 78.6; H, 9.9.

Isosarsasapogenone (Smilagenone) (XVII).—A mixture of 200 mg. of Δ^4 -tigogenone in 100 cc. of ether and 2 g. of palladium-barium sulfate catalyst was shaken under a pressure of 10 pounds of hydrogen at room temperature for ten hours. The catalyst was filtered and the ether was evaporated. The residue was crystallized from aqueous

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

(10) Marker and Rohrmann, *ibid.*, **62**, 647 (1940).

acetone to give a product melting at 172–174°. After several crystallizations from pentane it melted at 184–187°. When mixed with smilagenone, m. p. 187–188.5°, it melted at 185–188°; yield, 120 mg.

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 78.2; H, 10.2. Found: C, 78.3; H, 10.3.

Isosarsasapogenin (XVIII).—A mixture of 200 mg. of smilagenone, 25 cc. of dry isopropyl alcohol and 2 g. of aluminum isopropylate was refluxed for five hours. The solvent was then slowly distilled over a period of five hours. The product was extracted with ether and the ethereal solution washed well with dilute hydrochloric acid, and then with dilute sodium hydroxide and water. The ether was evaporated and the residue treated with 50 cc. of a 2% digitonin solution in 85% alcohol. After standing overnight the precipitated digitonide was filtered and dried. It was decomposed with pyridine in the usual manner and the product was crystallized from alcohol, m. p. 183–185°. When mixed with smilagenin, m. p. 184–185°, it melted at 183–185°. When mixed with sarsasapogenin, m. p. 199–200°, it melted at 174–178°.

Anal. Calcd. for $C_{27}H_{44}O_8$: C, 77.8; H, 10.7. Found: C, 77.6; H, 10.6.

Tigogenin (XIII) and Tigogenone.—To a solution of 250 mg. of Δ^4 -tigogenone (X) in 20 cc. of absolute ethanol was added 2 g. of sodium in small pieces. After the sodium had dissolved, the product was cooled and extracted with ether and water. The ether was removed and the residue was treated with a 2% solution of digitonin in 80% ethanol. After standing overnight the digitonide was filtered, dried and decomposed by heating with pyridine. The product thus obtained was crystallized from ethanol, m. p. 200–202°. Mixed with tigogenin, m. p. 204–206°, it melted at 202–205°.

This product was dissolved in 50 cc. of acetic acid and 100 mg. of chromic anhydride in a small amount of 90% acetic acid was added at room temperature. The reaction mixture was allowed to stand for thirty minutes, then water was added and the product was extracted with ether. The ethereal solution was washed well with sodium carbonate solution, and the ether was removed by distillation. The residue was crystallized from ether and from acetone to give a product of m. p. 201–204°. Mixed with tigogenone, m. p. 202–205°, it melted at 202–205°.

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 78.2; H, 10.2. Found: C, 77.9; H, 10.0.

Pseudo- Δ^4 -tigogenone (XI).—A mixture of 6 g. of Δ^4 -tigogenone (X) and 25 cc. of acetic anhydride was heated in a bomb tube at 200° for ten hours. The excess acetic anhydride was removed *in vacuo* and the residue was heated for thirty minutes on a steam-bath with 500 cc. of a 1% alcoholic potassium hydroxide solution. Water was added and the product was extracted with ether. The ether was treated with Norit and evaporated, leaving a yellow oil. This would not crystallize from the usual solvents nor from aqueous solvents, or from ether–pentane. When seeded with Δ^4 -tigogenone it did not crystallize.

A solution of 300 mg. of the oily pseudo- Δ^4 -tigogenone in 10 cc. of methanol and 1 cc. of concentrated hydrochloric acid was refluxed for thirty minutes. Water was added and the product was extracted with ether, treated with

Norit, and the solvent evaporated. The residue readily crystallized from ether–pentane to give a product, m. p. 186–188°. It gave no depression in melting point when mixed with Δ^4 -tigogenone, m. p. 186–188°; yield, 220 mg.

Anal. Calcd. for $C_{27}H_{40}O_8$: C, 78.6; H, 9.8. Found: C, 78.6; H, 9.9.

Pseudosarsasapogenone.—To a solution of 100 mg. of the oily pseudo- Δ^4 -tigogenone in 50 cc. of ether was added 200 mg. of palladium–barium sulfate catalyst. The mixture was shaken with hydrogen at 5 pounds pressure for two hours. The catalyst was filtered and the solvent removed. The residue was crystallized from dilute acetone, m. p. 166°. When mixed with pseudosarsasapogenone, m. p. 166°, it gave no depression in melting point.

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 78.2; H, 10.2. Found: C, 78.0; H, 10.2.

$\Delta^4,16$ -Pregnadienedione-3,20 (XII).—To a solution of 4 g. of the oily pseudo- Δ^4 -tigogenone in 200 cc. of glacial acetic acid was added 50 cc. of a 90% solution of glacial acetic acid containing 4 g. of chromic anhydride. The temperature was kept at 25–28° for ninety minutes. Water was added and the product was extracted with ether and washed free of acids with water and dilute sodium carbonate solution. After removal of the ether, the residue was sublimed in a high vacuum at 130–133°. The sublimate was crystallized from ether–pentane and melted at 171–179° after the first crystallization, giving 1.4 g. It was recrystallized from ether to a constant melting point of 182–185°. Mixed with Δ^4 -tigogenone, m. p. 186–188°, it melted at 142–150°.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 80.7; H, 9.0. Found: C, 80.6; H, 9.2.

***allo*-Pregnane-3,20-diol-3(β),20(α) (VIII).**—To a solution of 50 mg. of $\Delta^4,16$ -pregnadienedione-3,20 in 20 cc. of absolute ethanol was added 2 g. of sodium in small pieces. After the sodium dissolved, water and ether were added. The ethereal solution was washed well with water, and after removal of the solvent the residue was crystallized from dilute acetone, m. p. 212–216°. Mixed with *allo*-pregnane-3,20-diol-3(β),20(α), m. p. 215–217°, it melted at 215–217°.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.8; H, 11.3.

Reduction of $\Delta^4,16$ -Pregnadienedione-3,20 with Palladium.—A solution of 1 g. of $\Delta^4,16$ -pregnadienedione-3,20 in 100 cc. of ether was added to 500 mg. of palladium–barium sulfate catalyst, and the mixture was shaken with hydrogen at room temperature under a pressure of 5 pounds for twenty minutes. The solution was filtered and the ether was evaporated. The residue was dissolved in 20 cc. of 60% acetone and allowed to stand at 0° overnight. The material which precipitated out was filtered off and the filtrate was extracted with ether. The ether was removed and the residue repeatedly crystallized from pentane and dilute acetone to give a product melting at 119–121°. Mixed with pregnanedione, m. p. 120°, it melted at 100–105°. When mixed with progesterone, m. p. 119–121°, it melted at 119–121°; yield, 70 mg.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.3; H, 9.7.

The total residue from the progesterone was dissolved

in 100 cc. of ether and 2 g. of palladium-barium sulfate was added. The mixture was shaken with hydrogen at 10 pounds pressure for six hours. The solution was filtered and the ether was evaporated. The residue was crystallized from dilute acetone, m. p. 118–120°; yield, 380 mg. Mixed with pregnanedione, m. p. 119–120°, it melted at 118–120°.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.6; H, 10.1.

Pseudo-diosgenin (II).—A mixture of 5 g. of diosgenin and 25 cc. of acetic anhydride was heated in a bomb tube at 195–200° for ten hours. 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, treated with Norit, and then crystallized from acetone. This was followed by crystallizations from methanol and ether, m. p. 190–192°. A polymorphous form melts at 172–174°. Mixed with diosgenin, m. p. 202–205°, the melting point was 150–162°; yield, 3.8 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.4; H, 10.3.

$\Delta^{5,16}$ -Pregnadienol-3-one-20 (V).—A solution of 4 g. of pseudodiosgenin in 15 cc. of acetic anhydride was refluxed for thirty minutes. After evaporation of the acetic anhydride, the residue failed to crystallize from acetic acid, acetone or methyl alcohol, which is characteristic of the acetates of the other pseudogenins. The total residues were added to 150 cc. of glacial acetic acid containing 5 g. of sodium acetate, and cooled to 15°. To this was added slowly with vigorous stirring 9.6 cc. of *N* bromine solution in acetic acid. To this product was added a solution of 4 g. of chromic anhydride in 50 cc. of 90% acetic acid, and the mixture was allowed to stand at 25–28° for ninety minutes. Water was added and the product was extracted with ether. To the ethereal solution was added 200 cc. of acetic acid and 5 g. of zinc dust. The ether was distilled off and two more 5-g. portions of zinc dust were added at one-hour intervals, keeping the temperature at 95°. The product was filtered, ether and water were added and the ethereal solution was washed well with water and sodium carbonate solution. The ether was evaporated and the residue was dissolved in alcohol and treated with Girard reagent to remove the ketones. The ketone fraction consisting of 1.2 g. was hydrolyzed by warming for fifteen minutes with 1 *N* alcoholic potassium hydroxide solution. The product obtained was sublimed in a high vacuum at 140° and the sublimate was recrystallized from acetone and ethyl acetate, m. p. 212–214°; yield, 280 mg. average from five runs.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.0; H, 9.8.

Δ^5 -Pregnenediol (IV).—To a solution of 275 mg. of $\Delta^{5,16}$ -pregnadienol-3-one-20 in 100 cc. of absolute ethanol was added 8 g. of sodium in small portions. After the sodium had dissolved, the product was extracted with water and ether, and sublimed in a high vacuum at 120–130°. The sublimate was crystallized from ether-pentane to give a product melting unsharply at 170–174°. This probably contained an isomeric compound formed on the reduction, and was not pure.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.8. Found: C, 79.2; H, 10.7.

Progesterone (VII).—To a solution of 63 mg. of pregnenediol in 10 cc. of glacial acetic acid was added 2 cc. of a 0.1 *M* bromine solution in acetic acid. To this was added a solution of 60 mg. of chromic anhydride in 5 cc. of 90% acetic acid. It was allowed to stand for thirty minutes at room temperature, water was added and the product was extracted with ether. To the ethereal solution was added 20 cc. of acetic acid and 1 g. of zinc dust. This was heated at 95° for one hour after the ether was removed. The zinc dust was filtered off and the filtrate was vacuum distilled. The residue was dissolved in ether and washed with water and sodium carbonate solution. After removal of the ether, the residue was sublimed in a high vacuum. The sublimate was crystallized from ether-pentane and from dilute acetone, m. p. 119–121°. Mixed with progesterone, m. p. 120–121°, 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, 9.6.

***allo*-Pregnenediol-3(β),20(α) (VIII).**—A solution of 50 mg. of pregnenediol in 50 cc. of ether mixed with 50 mg. of platinum oxide catalyst was shaken under 45 pounds (3 atm.) of hydrogen for three hours at room temperature. The catalyst was filtered and the ether was evaporated. The residue was crystallized from dilute acetone, m. p. 214–216°. When mixed with *allo*-pregnenediol-3(β),20(α), m. p. 216–217°, it melted at 215–217°.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.6; H, 11.4.

Diosgenin (I) from Pseudodiosgenin (II).—A solution of 1 g. of pseudodiosgenin in 100 cc. of absolute alcohol containing 5 cc. of concentrated hydrochloric acid was refluxed for two hours. The product was extracted with ether and washed well with water. After removal of the ether, the residue was crystallized from acetic acid, m. p. 203–206°. Mixed with diosgenin, m. p. 203–205°, it melted at 203–206°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.3; H, 10.4.

When refluxed with acetic anhydride it gave an acetate which when crystallized from acetic acid melted at 195–198°. It gave no depression in melting point when mixed with an authentic sample melting at the same temperature.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.1; H, 9.9.

Tetrahydro-pseudodiosgenin (VI).—To a solution of 1 g. of pseudodiosgenin in 100 cc. of glacial acetic acid was added 500 mg. of platinum oxide catalyst. The mixture was shaken with hydrogen at 45 pounds pressure for fifteen hours at room temperature. The solution was filtered, and the filtrate was evaporated to about 10 cc. Upon cooling, the product crystallized and it was recrystallized from acetic acid and from ether, m. p. 202–205°. Mixed with tigogenin, m. p. 204–206°, it melted at 180–188°. Mixed with pseudodiosgenin, it melted at 165–174°; yield, 730 mg. It was recovered unchanged after boiling with alcoholic hydrochloric acid for two hours.

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

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 $C_{27}H_{46}O_2$: C, 80.2; H, 9.6. Found: C, 80.2; H, 9.7.

Summary

Reactions of diosgenin have been studied.

STATE COLLEGE, PENNA.
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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.

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

(2) Private communications.

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 sarsasapogenin³ (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 reaction⁴ with no evidence of cleavage between

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

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