liter of 95% ethanol, 100 cc. of concentrated hydrochloric acid was added and the mixture was refluxed for ninety minutes. It was cooled and the aglucone crystallized on standing a few hours. This was filtered and washed with 50% ethanol. It was light green in color. After crystallization from ethanol 90 g. of material melting at 192–200° was obtained. Material (30 g.) of the same melting point crystallized on concentrating the mother liquors to one-third volume.

This product was dissolved in an equal volume of acetic anhydride and refluxed thirty minutes. The colorless acetate was recrystallized from ethyl acetate and finally from acetone and melted at 199–200° with softening at 196°. The mixed m. p. with diosgenin acetate was 199–200°. The mixed m. p. with tigogenin acetate, m. p. 204°, was 183–195°.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.44; H, 9.79.

By refluxing fifteen minutes with ethanolic potash the free genin was obtained, and crystallized from ethanol and then from acetone. It shrank slightly at 204° and melted at 206–208°. The mixture with diosgenin melting at 206–208° had m. p. 206–208° with shrinking at 204°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.19. Found: C, 78.27; H, 10.29.

Reduction to Tigogenin.—A mixture of 1.5 g. of trillium genin acetate, 0.5 g. of Adams catalyst and 200 cc. of ether was shaken with hydrogen at 3 atm. at room temperature for ninety minutes. After filtering the catalyst, the product crystallized on concentrating the ether. It was washed with pentane; it crystallized from methyl alcohol as flat plates, m. p. 205–208°. The mixture with tigogenin acetate melted at 205–208°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.91; H, 10.20.

Hydrolysis of the acetate by refluxing with ethanolic potash gave a product which crystallized from acetone as long needles, m. p. 207–208°. The mixed m. p. with tigogenin (m. p. 204–205°) was 205–208°. The mixed melting point with diosgenin was 192–200°.

Isolation of Diosgenin from Dioscorea villosa.—The procedure was identical with that described for *Trillium erectum;* yield of crude acetate, 73 g., m. p. 162–167° from 25 lb. of roots. By repeated recrystallization from ethyl acetate and then from acetone a product was obtained with m. p. 196–198°. It gave no depression in melting point when mixed with an authentic sample of diosgenin acetate.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.09; H, 9.92.

The free genin was obtained by hydrolysis of the acetate. It crystallized from acetone as needles, m. p. 206–209°, and gave no depression in melting point when mixed with an authentic sample of diosgenin.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.19. Found: C, 77.95; H, 10.22.

The catalytic reduction to tigogenin was carried out as before using the genin acetate. The product was recrystallized from acetone, m. p. 196–199°. When mixed with tigogenin acetate there was no depression in m. p. Mixed with diosgenin acetate it melted at 173–195°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.20; H, 9.89.

Hydrolysis gave a product which crystallized from acetone as needles, m. p. 206–208°. Mixed with tigogenin it showed no depression. When mixed with diosgenin the m. p. was 194–206°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.64. Found: C, 78.10; H, 10.31.

Summary

Diosgenin has been isolated from *Dioscorea villosa* (L.) and from *Trillium erectum* (L.).

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

Sterols. CV. The Preparation of Testosterone and Related Compounds from Sarsasapogenin and Diosgenin

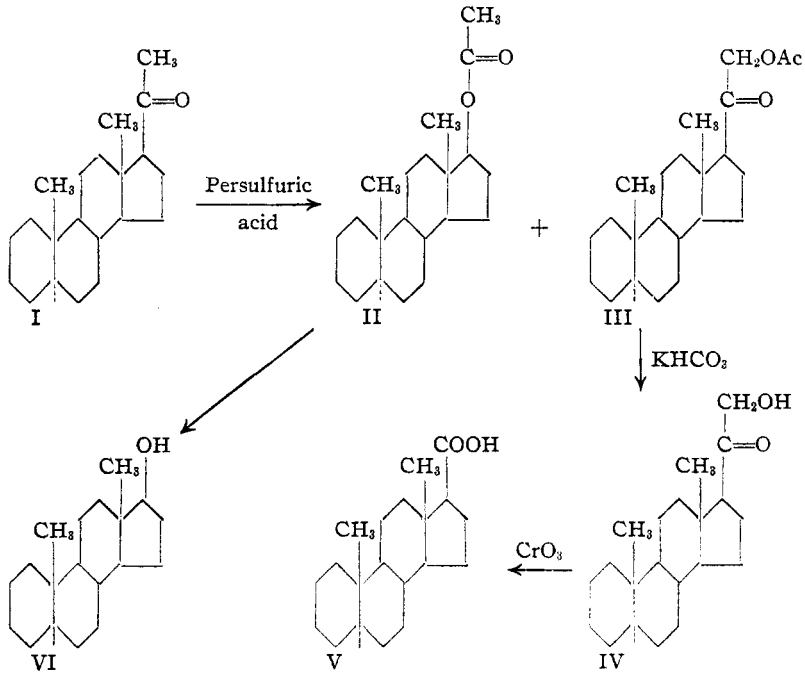
BY RUSSELL E. MARKER

It has been shown that the steroidal sapogenins upon treatment with acetic anhydride at 200° are converted into pseudosapogenins,[1,2,3] which are readily oxidized to give Δ^{16}-20-keto-pregnane compounds. Because of the ready availability of the sapogenins and the high yields obtained, they make a very desirable starting material for the preparation of the steroidal hormones. The preparation of progesterone from sarsasapogenin and diosgenin already has been described.[1,2,3]

We now report one of the methods for the conversion of the steroidal sapogenins into testosterone and related compounds. Heretofore, these compounds have been generally prepared from cholesterol. It was shown that the action of persulfuric acid on *allo*-pregnanone-20[4] caused an oxidation between C-17 and C-20 to produce an acetoxy group at C-17, which apparently was a mixture of isomers under the conditions then used. We have now modified our original procedure, carrying out the reaction at room tempera-

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 3592 (1939).
(2) Marker and Rohrmann, *ibid.*, **62**, 518 (1940).
(3) Marker, Tsukamoto and Turner, *ibid.*, **62**, 2525 (1940).
(4) Marker, Rohrmann, Wittle, Crooks and Jones, *ibid.*, **62**, 650 (1940).

ture in acetic acid over a period of seven to ten days, and using Von Baeyer's dry persulfuric acid mixture. When the reaction was carried out on *allo*-pregnanone-20 (I) under these conditions two products, II and III, were formed in the oxidation in good yield. The more insoluble product which could be readily crystallized from the reaction mixture in about 30–35% yield contains an acetoxy group at C-21. This was hydrolyzed readily to the hydroxy ketone (IV) with potassium bicarbonate in methanol, and upon treatment with acetic anhydride the original acetate was obtained. This formed a semicarbazone. Upon mild oxidation with chromic anhydride the hydroxy ketone (IV) gave a good yield of *etio-allo*-cholanic acid (V).



The mother liquors after the separation of the 21-acetoxy-*allo*-pregnanone-20 were hydrolyzed and the carbinol fraction separated by means of its half succinic ester. The non-carbinol fraction was almost pure unreacted *allo*-pregnanone-20. The carbinol fraction upon hydrolysis of the succinic ester yielded about 30–35% androstanol-17 (α), which is identical with the same product obtained by the reduction of 3-chloroandrostanone-17 by sodium in alcohol. Only one isomer was obtained by this mild persulfuric acid reaction.

The same reaction was carried out on pregnanol-3(α)-one-20 acetate, giving *etio*-cholanediol-3(α),-17(α) after alkaline hydrolysis. In this case no attempt was made to separate the 21-acetoxy compound formed in the oxidation, but from the alkaline hydrolysis layer there was obtained a small amount of *etio*-lithocholic acid. This latter product may have been formed either in the reaction, or by the alkaline cleavage of the hydroxy ketone.

Upon treatment with persulfuric acid of Δ^5-pregnenol-3(β)-one-20 acetate (prepared from diosgenin), first protecting the double bond by bromine, the non-ketonic fraction yielded Δ^5-androstenediol-3(β),17(α). This, on oxidation, gave androstenedione-3,17.

Preliminary experiments carried out on the oxidation of cholestanone-3 with persulfuric acid under the same conditions gave no unchanged cholestanone. The products obtained were not investigated. However, it was found that when 2-bromocholestanone-3 or 4-bromo-coprostanone-3 was treated with persulfuric acid under these conditions the initial products were recovered unchanged. In this case a bromine atom adjacent to a ketone group stabilizes it toward oxidation with persulfuric acid. Likewise, cholestanol acetate is unaffected by persulfuric acid, but cholestanol upon treatment with persulfuric acid in acetic acid gave cholestanol acetate almost quantitatively. Thus, compounds containing acetoxy groups, free hydroxy groups and ketonic groups with a bromine atom in the alpha position can be used for these oxidations without affecting the groups. This fact in connection with the preceding experiments suggested a means of preparing testosterone by the oxidation of 4-bromopregnanedione with persulfuric acid, followed by a splitting out of hydrogen bromide.

We have previously reported the preparation of pregnanedione[2] in good yields from sarsasapogenin. This was brominated according to the method of Butenandt and Schmidt,[5] obtaining 4-bromopregnanedione. After standing for ten days with persulfuric acid in acetic acid, the prod-

(5) Butenandt and Schmidt, *Ber.*, **67**, 1901 (1934).

uct was treated with pyridine to eliminate hydrogen bromide. The resulting mixture was sublimed in a high vacuum. As we were not interested in isolating the desoxycorticosterone formed in this reaction, it was eliminated by giving the mixture a mild hydrolysis with potassium bicarbonate in methanol. It was found in preliminary experiments that a 17-acetoxy group is but little affected by such treatment, whereas a 21-acetoxy group[6] is hydrolyzed readily. There were no steroidal acids in the carbonate solution from the hydrolysis. Mild oxidation of the neutral fraction gave Δ^5-3-keto-*etio*-cholenic acid, proving the presence of desoxycorticosterone in the original mixture. The neutral product from the oxidation was hydrolyzed by refluxing with a 1% methanolic potassium hydroxide solution. Again there were no acids recovered from the alkaline solution. The carbinol fraction was separated from the hydrolysis product by means of its half succinate. After hydrolysis this gave testosterone, which was purified by passing it through a short column of aluminum oxide.

The non-carbinol fraction after passage through a short column of aluminum oxide gave progesterone.

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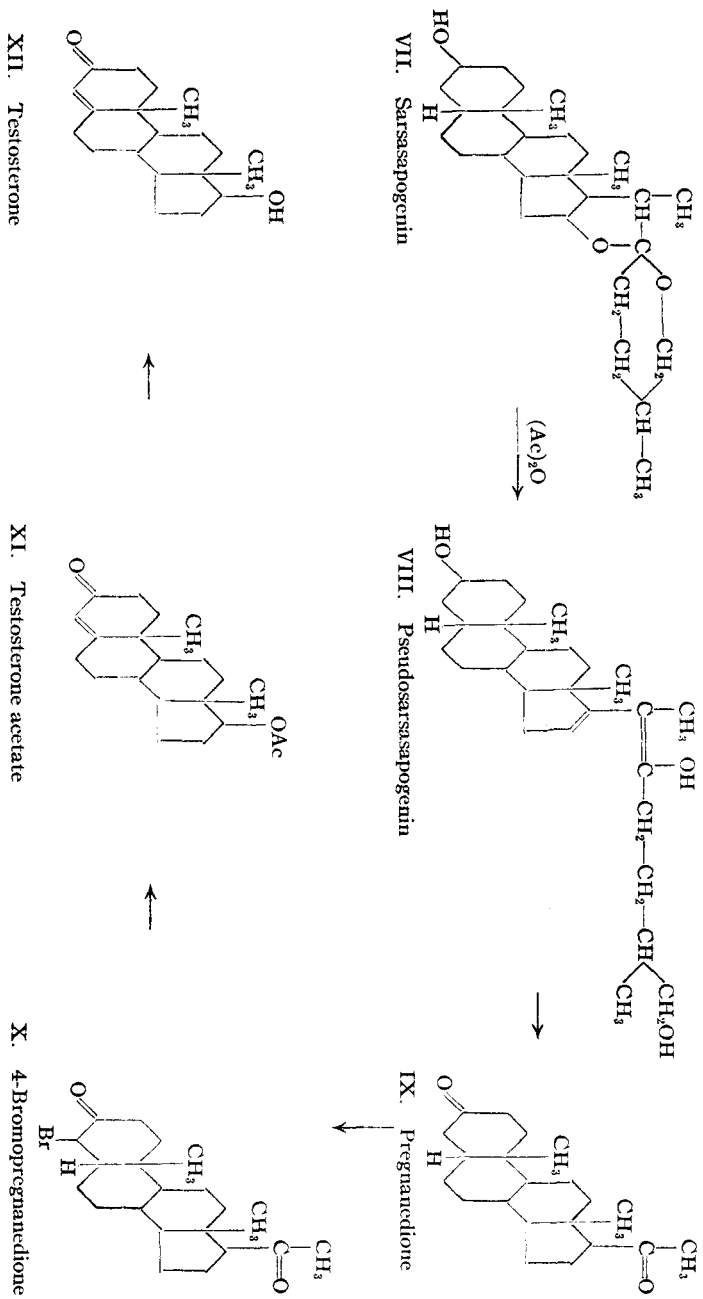

Experimental Part

Action of Persulfuric Acid on *allo*-Pregnanone-20.—To a solution of 10 g. of *allo*-pregnanone-20 in 1 liter of acetic acid at 25° was added a mixture of von Baeyer's persulfuric reagent made from 30 g. of potassium persulfate, 33 g. of concentrated sulfuric acid and 90 g. of potassium sulfate. It was let stand with occasional shaking for seven days at 25°. At the end of this time a solution of 35 g. of potassium hydroxide in 35 cc. of water was added, and the inorganic salts were filtered and washed well with acetic acid. The acetic acid was removed *in vacuo*, and the residue was extracted with ether and washed well with water and sodium carbonate solution.

***allo*-Pregnanol-21-one-20.**—The ether was evaporated from the above product and the residue was crystallized from a mixture of acetone and methanol. It melted at 197–200°; yield, 3.4 g. This was the acetate of *allo*-pregnanol-21-one-20.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.6; H, 10.0. Found: C, 76.6; H, 10.1.

When treated with semicarbazide acetate in methanol for one hour, it gave a semicarbazone which was crystallized from dilute ethanol, m. p. 242–244° dec.

Anal. Calcd. for $C_{24}H_{39}O_3N_3$: C, 69.0; H, 9.4. Found: C, 69.1; H, 9.6.

To a solution of 300 mg. of the acetate of *allo*-pregnanol-21-one-20 in 30 cc. of boiling methanol was added 5 cc. of a 10% solution of potassium bicarbonate solution. It was

(6) Reichstein and Gätzi, *Helv. Chim. Acta*, **21**, 1185 (1938).

allowed to reflux for ninety minutes, after which the methanol was vacuum distilled to about 20 cc. The product was extracted with ether and washed well with water. The ether was evaporated and the residue was crystallized from methanol, in which it is rather soluble. It melted at 115–117°.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.8. Found: C, 79.6; H, 10.8.

A mixture of 50 mg. of *allo*-pregnanol-21-one-20, 1 cc. of acetic anhydride and 2 cc. of pyridine was let stand overnight at room temperature. The resulting product was crystallized from methanol–acetone, m. p. 197–199°. When mixed with the acetate of *allo*-pregnanol-21-one-20, m. p. 197–200°, it gave no depression in m. p.

***etio-allo*-Cholanic Acid.**—A solution of 100 mg. of *allo*-pregnanol-21-one-20 in 10 cc. of acetic acid was treated with a solution of 200 mg. of chromic anhydride in 10 cc. of 95% acetic acid at room temperature. It was let stand for forty-five minutes. Water was added and the acid was extracted with ether. The ethereal solution was freed of acetic acid as well as possible by washing with water. It was then shaken with dilute sodium hydroxide solution. The aqueous layer was acidified and extracted with ether. After removal of the ether, the residue was crystallized from ethyl acetate, m. p. 228–230°. When mixed with *etio-allo*-cholanic acid, m. p. 228–230°, it gave no depression in melting point.

Anal. Calcd. for $C_{20}H_{32}O_2$: C, 78.9; H, 10.6. Found: C, 79.1; H, 10.8.

When treated with diazomethane in ether it gave an ester which melted at 140–141° and gave no depression in melting point when mixed with the methyl ester of an authentic sample of *etio-allo*-cholanic acid.

Androstanol-17(α).—The mother liquors from the crystallization of the acetate of *allo*-pregnanol-21-one-20 were evaporated to dryness and dried by distilling 50 cc. of benzene from it. To the residue was added 5 g. of succinic anhydride and 10 cc. of pyridine. This was heated for two hours on a steam-bath. Ether was added and the pyridine was removed by shaking with dilute hydrochloric acid. The succinic esters were removed by shaking with potassium carbonate solution. This alkaline solution was acidified and the esters were extracted with ether and hydrolyzed by refluxing for thirty minutes with alcoholic potassium hydroxide. The carbinol fraction was extracted with ether and the solvent was removed. The residue was crystallized from methanol and from ether–pentane and finally from ether, yield, 2.1 g., m. p. 164–166°. When mixed with androstanol-17(α) prepared by the sodium reduction of an alcoholic solution of 3-chloro-androstanone-17, m. p. 164–166°, it gave no depression in melting point.

Anal. Calcd. for $C_{19}H_{32}O$: C, 82.5; H, 11.7. Found: C, 82.2; H, 11.6.

The non-carbinol fraction from the above was crystallized from methanol. It melted at 128–131° and gave no depression in melting point when mixed with *allo*-pregnan-one-20.

Action of Persulfuric Acid on Pregnanol-3(α)-one-20 Acetate.—To a solution of 2 g. of pregnanol-3(α)-one-20 acetate in 300 cc. of acetic acid was added 5 g. of von Baeyer's dry persulfate mixture with shaking. It was kept at 25° and daily 5 g. additional persulfate mixture was added with shaking. At the end of eight days an excess of 50% potassium hydroxide solution was added to neutralize the inorganic acids. The salts were filtered and the filtrate concentrated *in vacuo*. The residue was extracted with ether and water. The ether was evaporated and the residue was hydrolyzed by boiling with a 5% methanolic potassium hydroxide solution for one hour. The product was extracted well with ether and washed with water. The ether was evaporated and the residue was dissolved in 25 cc. of absolute ethanol and refluxed with 3 g. of Girard reagent for thirty minutes. From the ketonic fraction was obtained only a small amount of product which crystallized from dilute methanol. It melted at 142–144° and gave no depression in melting point when mixed with *epi*-pregnanolone.

The non-ketonic material was crystallized from ethyl acetate and from ether. It melted at 233–235°. When mixed with an authentic sample of *etio*-cholanediol-3(α), 17(α), m. p. 233–235°, prepared by the sodium reduction of *etio*-cholanolone, it gave no depression in melting point, yield, 514 mg.

Anal. Calcd. for $C_{19}H_{32}O_2$: C, 78.1; H, 11.0. Found: C, 78.0; H, 10.9.

When refluxed with acetic anhydride it gave an acetate, m. p. 124–125°, which gave no depression in melting point when mixed with an authentic sample of the diacetate of *etio*-cholanediol-3(α),17(α).

The alkaline solution from the above hydrolysis of the total reaction product was acidified and extracted with ether. A small amount of acid was obtained which was crystallized from ether. It melted at 275–276° and gave no depression in melting point when mixed with *etio*-lithocholic acid, m. p. 274–276°.

Anal. Calcd. for $C_{20}H_{32}O_3$: C, 74.9; H, 10.0. Found: C, 74.7; H, 10.0.

The Action of Persulfuric Acid on Δ^5-Pregnenol-3-one-20 Acetate.—To a solution of 1.2 g. of Δ^5-pregnenolone acetate (prepared from diosgenin) in 200 cc. of glacial acetic acid was added 3.4 cc. of a molar solution of bromine in acetic acid. To this was added 10 g. of von Baeyer dry persulfate mixture. It was allowed to stand for five days and an additional 10 g. of the mixture was added, after which it was allowed to stand for two days at 25°. To this was added 10 cc. of a 50% solution of potassium hydroxide. The inorganic salts were filtered and washed with acetic acid. The filtrate was warmed on a steam-bath for one hour with 10 g. of zinc dust with stirring. The acetic acid solution was decanted and the excess solvent removed *in vacuo*. The residue was extracted with ether and the ether evaporated. The product was refluxed for thirty minutes with a 1% solution of alcoholic potassium hydroxide solution. The neutral product was extracted with ether, and the solvent was evaporated. The residue was treated with Girard reagent to remove ketones. The non-ketonic fraction (210 mg.) was dissolved in absolute benzene and run through a three-inch (8-cm.) tube of aluminum oxide. It was washed off with ether, and the filtrate was crystallized from slightly diluted methanol, m. p. 176–178°.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.6; H, 10.4. Found: C, 78.4; H, 10.6.

As we had no sample of Δ^5-androstenediol-3,17 for comparison of mixed melting points, the product was dissolved in acetic acid, brominated and oxidized with chromic acid. After debromination with zinc dust, the product was crystallized from dilute methanol and dilute acetone, m. p. 167–170°. Mixed with an authentic sample of androstenedione, m. p. 170–171°, it melted at 167–170°.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.6; H, 9.2. Found: C, 79.4; H, 9.1.

Testosterone.—Treatment of cholestanone with persulfuric acid gave no unchanged cholestanone. When 2-bromocholestanone and 4-bromocoprostanone were treated in the same manner they were recovered unchanged. Treatment of cholestanol acetate with persulfuric acid gave only unchanged material. Treatment of cholestanol with persulfuric acid gave cholestanol acetate.

To a solution of 4 g. of 4-bromopregnanedione-3,20 in 400 cc. of glacial acetic acid was added 50 g. of von Baeyer powdered persulfate mixture. It was let stand at 25° for ten days with occasional stirring. The solids were filtered and washed well with ether. The filtrate was extracted with ether and the ethereal solution washed several times with water, followed by washing with sodium carbonate solution. The solvent was removed under vacuum and the residue was refluxed with 25 cc. of dry pyridine for ten hours. Water and ether were added and the ethereal layer was washed well with dilute sulfuric acid to remove the pyridine. The ether was removed and the residue was sublimed in a high vacuum, collecting a fraction distilling at 120–150° (bath temperature). This weighed 1.72 g.

Attempts to separate this mixture by chromatographic adsorption on a column of aluminum oxide were unsuccessful. In order to remove the desoxycorticosterone, the total product was dissolved in 100 cc. of methanol and a solution of 700 mg. of potassium bicarbonate in 5 cc. of water was added. It was refluxed for one-half hour, and the methanol was distilled *in vacuo* to about 20 cc. The product was extracted with ether and washed well with water. Upon acidification of the aqueous layer no acids were obtained. The solvent was removed from the neutral fraction and the residue was dissolved in 20 cc. of glacial acetic acid. To this was added a solution of 500 mg. of chromic anhydride in 10 cc. of 90% acetic acid. It was allowed to stand for forty-five minutes and then was extracted with ether and water. After removal of the excess acetic acid by shaking with water, the ethereal solution was shaken with a 1% solution of sodium hydroxide. Upon acidification of this an acid was obtained which upon crystallization from ether–pentane melted at 249–253°.

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.9; H, 8.9. Found: C, 75.4; H, 9.3.

Reduction of this acid by sodium in alcohol gave a product which upon crystallization from dilute methanol melted at 250°. It gave no depression in melting point when mixed with an authentic sample of 3(β)-hydroxy-*etio-allo*-cholanic acid, m. p. 250–252°.

The solvent was removed from the neutral fraction of the above oxidation and the residue was refluxed for thirty minutes with 50 cc. of a 1% solution of potassium hydroxide in methanol. The product was extracted with ether, and the solvent was removed giving 780 mg. of residue. The alkaline layer upon acidification gave no acids. The neutral product was dissolved in 5 cc. of pyridine and 500 mg. of succinic anhydride was added. It was heated on a steam-bath for one hour. At the end of this time it was dissolved in ether and the pyridine was removed by shaking with dilute hydrochloric acid. The ethereal solution was washed with potassium carbonate solution, and the aqueous layer was acidified and extracted with ether. The ether was evaporated and the residue was refluxed with 50 cc. of a 1% methanolic potassium hydroxide solution for forty-five minutes. Water was added and the product was well extracted with ether. The solvent was removed and the residue was dissolved in 20 cc. of benzene (dry) and passed through a 2-inch (5-cm.) column of aluminum oxide. Very little material passed through in the benzene. It was then washed with 50% ether–benzene and finally with 200 cc. of anhydrous ether. Both these fractions yielded crystalline products and were combined. It was crystallized from ether–pentane to give a product melting at 151–152°, yield 125 mg., although there was a considerable amount of material in the mother liquors. When mixed with an authentic sample of testosterone, m. p. 151–153°, it gave no depression in melting point.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.8. Found: C, 79.0; H, 9.9.

The ethereal non-carbinol fraction from the testosterone succinate treatment was evaporated to dryness, giving a residue which was oily. This was dissolved in 20 cc. of benzene and passed through a 2-inch column of aluminum oxide. It was washed with a 50% solution of ether–benzene. The product (280 mg.) was crystallized from pentane and dilute acetone. It melted at 124–126°, and gave no depression in melting point when mixed with an authentic sample of progesterone.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 79.9; H, 9.7.

Summary

Testosterone and related compounds have been prepared from sarsasapogenin and diosgenin.

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