phenone. This was recrystallized from methyl alcohol; m. p. 248°; yield 10.5 g.

Anal. Calcd. for $C_{23}H_{37}O_2Cl$: C, 72.5; H, 9.8. Found: C, 72.8; H, 9.6.

The next five compounds in the synthetic series were prepared by procedures analogous to those of the above corresponding homologs.

Methyl Ester of 3-Chloro-*nor-allo*-cholanic Acid.—M. p. 178°. *Anal.* Calcd for $C_{24}H_{39}O_2Cl$: C, 73.0; H, 10.0. Found: C, 73.3; H, 10.2.

3-Chloro-*allo*-*bis*-*nor*-cholanyl-diphenylcarbinol.—M. p. 183°. *Anal.* Calcd. for $C_{36}H_{47}OCl$; C, 80.9; H, 9.1. Found: C, 80.8; H, 9.3.

3-Chloro-*bis-nor-allo*-cholanic Acid.—M. p. 231°. *Anal.* Calcd. for $C_{22}H_{35}O_2Cl$: C, 71.9; H, 9.6. Found: C, 71.8; H, 9.3.

Methyl Ester of 3-Chloro-*bis-nor-allo*-cholanic Acid.—M. p. 151°. *Anal.* Calcd. for $C_{23}H_{37}O_2Cl$: C, 72.5; H, 9.8. Found: C, 72.5; H, 9.8.

3-Chloro-*allo-ter-nor*-cholanyldiphenylcarbinol.—M. p. 146°. *Anal.* Calcd. for $C_{34}H_{45}OCl$: C, 80.0; H, 9.0. Found: C, 80.9; H, 9.2.

***epi-allo*-Pregnanol-3-one-20.**—A solution of 11 g. of 3-chloro-*allo-ter-nor*-cholanyl-diphenylcarbinol was boiled for six hours with a mixture of 100 cc. of acetic acid and 100 cc. of acetic anhydride. The solvent was evaporated; the residue was dissolved in 400 cc. of chloroform and ozonized at 0°. The chloroform was evaporated *in vacuo;* 225 cc. of acetic acid was added and the solution distilled to 50 cc. Acetic acid (400 cc.) was added and the resulting solution heated with 15 g. of granulated zinc until a sample no longer gave a color with starch iodide paper. It was then filtered and evaporated to dryness *in vacuo*. The residue was boiled for ten hours with a solution of 30 g. of potassium acetate in 60 cc. of valeric acid; a large excess of alcoholic potassium hydroxide was added and the mixture boiled for an hour, diluted with water and shaken with ether. The residue from the ethereal solution was purified through the acid succinate and isolated as the semicarbazone. This after recrystallization from ethyl alcohol melted at 242° with decomposition. It was hydrolyzed by boiling for one and one-half hours with a mixture of 150 cc. of 95% alcohol, 15 cc. of concentrated sulfuric acid and 30 cc. of water. After dilution with water and extraction with ether the product was sublimed in a high vacuum at 130° and crystallized several times from 70% acetone. It melted at 170° and showed no depression of melting point with *epi-allo*-pregnanolone isolated from human pregnancy urine.

Summary

epi-allo-Pregnanolone was prepared by the Wieland degradation of 3-chloro-*allo*-cholanic acid and subsequent hydrolysis of the chlorine to a —OH group. This substance is identical with *epi-allo*-pregnanolone, which was isolated from human pregnancy urine and which possesses androgenic activity.

STATE COLLEGE, PENNA. RECEIVED APRIL 8, 1937
DETROIT, MICH.

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

Sterols. XVI. Lanosterol and Agnosterol

BY RUSSELL E. MARKER, EUGENE L. WITTLE AND LAWSON W. MIXON

Windaus[1] has shown that "isocholesterol," which is obtained from the neutral fraction of sheep wool grease, can be separated into two compounds, lanosterol and agnosterol, by crystallization of their acetates. Lanosterol, $C_{30}H_{50}O$, has one reactive double bond which can be reduced by platinum catalyst to dihydrolanosterol, containing one inactive double bond. Agnosterol, $C_{30}H_{48}O$, differs from lanosterol in that it has one reactive double bond and *two* inactive bonds. Both have the same number of carbon atoms and give characteristic sterol tests, yet from some of their reactions they appear similar to the amyrins.[2]

Lanosteryl acetate upon reduction gave α-dihydrolanosteryl acetate. This contains one inert double bond which is isomerized by dry hydrogen chloride in chloroform to β-dihydrolanosteryl acetate. This isomerization is not complete, giving a mixture of the beta with a small amount of the alpha compound, which may be separated easily by treatment of the crude isomerization product with a small amount of chromic oxide in acetic acid. The alpha-compound is readily oxidized, whereas the beta-compound resists mild oxidation. This isomerization is similar to that of α-ergostenol into β-ergostenol.[3] α-Ergostenol contains an inert double bond, but upon isomerization to β-ergostenol the double bond becomes active and can be reduced by platinum and hydrogen. The same is true of apocholic acid.[4] However, this

(1) Windaus and Tschesche, *Z. physiol. Chem.*, **190**, 51 (1930).

(2) Doree and Garratt, *J. Soc. Chem. Ind.*, **52**, 355 (1933), and Doree and Petrow, *J. Chem. Soc.*, 1562 (1936).

(3) Reindel, Walter and Rauch, *Ann.*, **452**, 34 (1927); **460**, 212 (1928); Hart, Speer and Heyl, THIS JOURNAL, **50**, 2016 (1930).

(4) Yamasaki, *Z. physiol. Chem.*, **233**, 10 (1935).

is not the case with the dihydrolanosteryl acetates. Both of these isomers contain double bonds which are inert to hydrogenation. When α-ergostenol is treated with mercuric acetate, a new conjugated double bond is introduced into the molecule.[5] Both α- and β-dihydrolanosteryl acetates were recovered unchanged after this treatment. That these two compounds do not contain epimeric -OH groups is shown by the fact that each gives a different dihydrolanostenone upon oxidation. Both of these dihydrolanostenones gave the original dihydrolanosterols unchanged upon reduction by sodium in alcohol. This is unlike the reduction of lanostenone by sodium as reported by Doree,[2] who found an isomer of lanosterol upon reduction which was designated as lanosterol A.

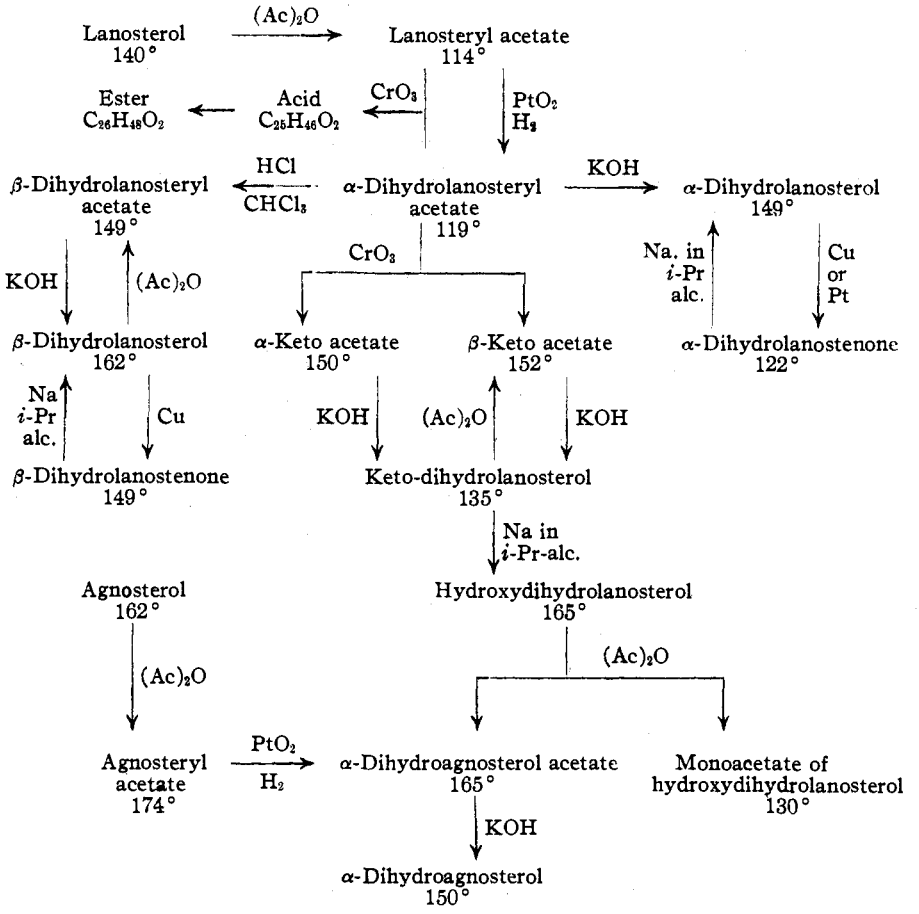
To determine if lanosterol contained an angular methyl group between rings A and B dehydrogenation with platinum black was tried according to the method of Honigmann[6] for the dehydrogenation of neoergosterol to dehydroneoergosterol. If no angular methyl group were present we would probably obtain a naphthol. We found no naphthol or phenol, but obtained a good yield of lanostenone. The same results were obtained when dihydrolanosterol was used, dihydrolanostenone being the sole product. We checked our catalyst before use by dehydrogenating neoergosterol to dehydroneoergosterol.

In the study of the oxidation of lanosterol by chromic oxide, Doree obtained a keto acid and two lanostenones, but on vigorous oxidation using a large excess of chromic oxide he obtained acetone and a trace of a higher ketone which was detected by the odor, but was not isolated. To try to isolate this ketone and thus identify the side chain of lanosterol, we carried out the oxidation of dihydrolanosterol acetate on a large scale using the same conditions under which androsterone and methylheptanone are formed from *epi*-cholestanol acetate. We were able to obtain only a trace of a ketone having a fruity odor. The material which did not steam distil formed no semicarbazone, but consisted of about equal portions of acids and neutral non-ketonic material. From the acidic portion was isolated a considerable amount of a monocarboxylic acid of 25 carbon atoms, determined by combustion and molecular weight to have the formula of $C_{25}H_{46}O_2$. This acid is completely saturated and is obtained in large amounts also from lanosteryl acetate. During the process of oxidation these compounds have lost five carbon atoms of the ring containing the acetate group and the unsaturated bonds. If the acetate group is in the first ring on these compounds, then the active double bond must also be in that ring to lose 5 carbon atoms and give a saturated monocarboxylic acid identical to that formed from dihydrolanosteryl acetate. Getting a monocarboxylic acid from this ring system could be explained by a mechanism similar to that of the opening of ring B of ergosterol by ultraviolet light to give calciferol.

To throw some light on the oxidation of α-dihydrolanosterol acetate we studied controlled milder chromic oxide oxidations, using less chromic oxide, a shorter time for the oxidation and a lower temperature. This compound was oxidized very readily to give a mixture of two keto-acetates which are separated easily by crystallization. The least soluble compound, α-ketodihydrolanosteryl acetate, melts at 150° and the more soluble β-keto-dihydrolanosteryl acetate melts at 152°, but a mixture gives a large depression in melting point. These compounds upon hydrolysis with potassium hydroxide gave an identical keto-dihydrolanosterol. Both of these hydrolysis products when treated with acetic anhydride give only β-keto-dihydrolanosteryl acetate. This converts the alpha compound into the beta compound. Reduction of the ketone group of keto-dihydrolanosterol by sodium in isopropyl alcohol gives a hydroxydihydrolanosterol. This dihydroxy compound on treatment with acetic anhydride is partially dehydrated giving a mixture of the monoacetate of hydroxydihydrolanosterol and a monoacetate in which one of the -OH groups had been removed under the influence of the acetic anhydride. The latter compound melted at 165° and gave no depression in melting point when mixed with α-dihydroagnosterol acetate prepared by catalytic reduction of natural agnosterol acetate according to the method of Windaus.[1] Hydrolysis of the acetate with potassium hydroxide gave α-dihydroagnosterol which did not give a depression in melting point with Windaus' dihydroagnosterol.

We found that dihydroagnosterol can be prepared much more easily by not isolating the intermediates. As α- and β-keto-dihydrolanosterol acetates give the same keto-dihydrolano-

(5) Heilbron, Johnstone and Spring, *J. Chem. Soc.*, 2253 (1929).
(6) Honigmann, *Ann.*, **511**, 292 (1934).



sterol on hydrolysis, the crude mixture of acetates was reduced with sodium in isopropyl alcohol. This total reduction product was then boiled with acetic anhydride giving α-dihydroagnosterol acetate.

Experimental

Lanosterol.—To 10 liters of ether was added 2.5 pounds (1.1 kg.) of melted lanolin (Merck Anhydrous). To this was added with shaking a solution of 2.5 liters of ethyl alcohol containing 125 g. of dissolved sodium. After standing one day at 25° water was added and the upper portion of the ether layer separated. The aqueous portion was well extracted with ether, and the ether solution washed with water. The ether was evaporated and the residue was dissolved in 3 liters of acetone. It was let stand for three hours, and then filtered from a small amount of insoluble material. To the filtrate was added 3 liters of acetone and 3 liters of methyl alcohol. The solution was cooled to 0° for twenty-four hours and the agnosterol–lanosterol mixture was filtered. This was again crystallized from methyl alcohol–acetone giving 45 g. of crude "isocholesterol." To separate the agnosterol from the lanosterol, this product was heated for five hours on a steam-bath with 200 cc. of acetic anhydride. The acetic anhydride was distilled *in vacuo* and the residue was crystallized from ethyl acetate according to the method of Windaus,[1] to give agnosteryl acetate and lanosterol acetate. Lanosteryl acetate melted at 114°. The acetate was hydrolyzed by refluxing with an excess of dilute potassium hydroxide to give lanosterol, m. p. 140°.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.4; H, 11.8. Found: C, 84.3; H, 11.7.

α-Dihydrolanosteryl Acetate.—To a solution of 50 g. of lanosteryl acetate in 500 cc. of warm acetic acid was added 8.0 g. of platinum oxide. The solution was shaken four hours at 70° with hydrogen under a pressure of 45 pounds (3 atm.). The catalyst was filtered and the acetic acid was evaporated to 100 cc. After cooling to room temperature the product was filtered, washed with methyl alcohol and crystallized from ethyl acetate; m. p. 119°; yield 30 g.

Anal. Calcd. for $C_{32}H_{54}O_2$: C, 81.6; H, 11.5. Found: C, 81.5; H, 11.4.

Crude isocholesteryl acetate may be hydrogenated in the same manner. Dihydrolanosteryl acetate may be separated from dihydroagnosteryl acetate by crystallization from acetic acid or ethyl acetate. The lanosterol derivative is much more insoluble in these solvents.

α-Dihydrolanosterol.—This was prepared by hydrolysis of the acetate by alcoholic potassium hydroxide solution followed by crystallization from ethyl acetate, m. p. 148°.

Anal. Calcd. for $C_{30}H_{52}O$: C, 84.0; H, 12.2. Found: C, 84.1; H, 12.4.

α-Dihydrolanostenone.—A mixture of 2 g. of α-dihydrolanosterol and 4 g. of precipitated copper was heated for one-half hour at 250° and 2 mm. pressure. The product was heated further to 300° and distilled. The distillate was crystallized from ethyl acetate–methyl alcohol to give α-dihydrolanostenone; yield 1.6 g.; m. p. 122°.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.4; H, 11.8. Found: C, 84.0; H, 11.6.

This gave a 2,4-dinitrophenylhydrazone, m. p. 213°.

Anal. Calcd. for $C_{36}H_{54}N_4O_4$: C, 71.2; H, 9.0. Found: C, 71.0; H, 9.0.

Reduction of α-Dihydrolanostenone to α-Dihydrolanosterol.—To a boiling solution of 1.0 g. of α-dihydrolanostenone in 150 cc. of dry isopropyl alcohol was added 5 g. of sodium over a period of one hour. The solution was poured into water and extracted with ether. The ether was evaporated and the residue was crystallized from acetone–methyl alcohol mixture, yield 0.8 g., m. p. 143°. No depression in melting point with α-dihydrolanosterol m. p. 148°, was observed. The acetate melted at 116°, and gave no depression in melting point with α-dihydrolanosteryl acetate, m. p. 118°. The low melting points of these products are probably due to the presence of a small amount of the *epi*-isomer formed by the sodium reduction.

β-Dihydrolanosteryl Acetate.—A solution of 30 g. of α-dihydrolanosteryl acetate in 300 cc. of chloroform was treated with a stream of dry hydrogen chloride for two hours. The chloroform was evaporated *in vacuo* and the residue was crystallized from ethyl acetate and acetone–methyl alcohol. The melting point of the product was not sharp as it contained a mixture of the alpha and beta derivatives as in the case of the isomerization of α-ergostenol to β-ergostenol. It was found that β-dihydrolanosteryl acetate was resistant to mild chromic acid oxidation, whereas α-dihydrolanosteryl acetate was oxidized very readily by this reagent.

To a solution of 15.5 g. of the mixture in 470 cc. of acetic acid at 80° was added rapidly a solution of 4.7 g. of chromic anhydride in 50 cc. of 90% acetic acid. The solution was kept at 80° for ten minutes and then poured into two liters of water. The compound was extracted with ether, and the residue after distilling the ether was crystallized from equal portions of ethyl acetate and methyl alcohol, m. p. 149° (needles).

Anal. Calcd. for $C_{32}H_{54}O_2$: C, 81.6; H, 11.6. Found: C, 81.7; H, 11.4.

β-Dihydrolanosterol.—A solution of 0.8 g. of β-dihydrolanosteryl acetate in 50 cc. of alcohol containing 1.5 g. of potassium hydroxide was refluxed for thirty minutes. After extraction with ether and evaporation of the solvent the residue was crystallized from ethyl acetate and ethyl alcohol, m. p. 162°.

Anal. Calcd. for $C_{30}H_{52}O$: C, 84.0; H, 12.2. Found: C, 83.7; H, 12.1.

Acetylation of this product with acetic anhydride gave the original acetate, m. p. 147°. Mixed melting points gave no depression with the original.

β-Dihydrolanostenone.—A mixture of 450 mg. of β-dihydrolanosterol and 600 mg. of precipitated copper was heated at 250° at 2 mm. pressure for thirty minutes. The temperature was then raised to 300°, the product was distilled and crystallized from ethyl acetate and acetone–methyl alcohol; yield 350 mg.; m. p. 149°.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.4; H, 11.8. Found: C, 84.6; H, 11.5.

It gave a 2,4-dinitrophenylhydrazone, m. p. 230°.

Reduction of β-Dihydrolanostenone with Sodium.—A reduction with sodium by the same method used for the reduction of α-dihydrolanostenone gave β-dihydrolanosterol, m. p. 155°, which did not give a depression in melting point when mixed with pure β-dihydrolanosterol. In this case as before the sodium reduction product is lower in melting point, probably due to the presence of a small amount of the epimeric form of the compound which cannot be separated by crystallization.

Treatment of the Dihydrolanosteryl Acetates with Mercuric Acetate.—Refluxing α-dihydrolanosteryl acetate or β-dihydrolanosteryl acetate in ethyl alcohol with mercuric acetate for one hour produced no change. The solutions remained colorless and the original materials were recovered unchanged. This treatment introduces an extra double bond into ergostenol.

Dehydrogenation of α-Dihydrolanosterol with Platinum Black.—The platinum black was prepared according to the method used by Honigmann[6] for the dehydrogenation of neoergosterol to dehydroneoergosterol. Its activity was tested by carrying out this dehydrogenation.

A mixture of 2 g. of α-dihydrolanosterol and 400 mg. of platinum black was heated in an atmosphere of carbon dioxide. The temperature was slowly raised to 270° at which point hydrogen bubbles came off. It was then kept at 300° for one hour, when evolution of gas had ceased. The product was cooled and the organic material dissolved in ether and filtered. After evaporation of the ether the product was crystallized from acetone, m. p. 122°. Mixtures with α-dihydrolanostenone gave no depression in melting point. This was the major product and no naphthol could be obtained from the mother liquors with picric acid.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.4; H, 11.8. Found: C, 84.1; H, 11.6.

In the same manner lanostenone was obtained by the dehydrogenation of lanosterol with platinum black; m. p. 116°.

Oxidation of α-Dihydrolanosteryl Acetate with Chromic Oxide.—A solution of 50 g. of α-dihydrolanosteryl acetate in one liter of acetic acid was heated to 90° with stirring on a steam-bath. A solution of 100 g. of chromic oxide in 500 cc. of 90% acetic acid was dropped in over a period of three hours. The mixture was heated an additional three hours, and let stand overnight. Alcohol was added and the solvent was evaporated under reduced pressure. The residue was then steam distilled. The steam distillate upon extraction with ether, and distillation of the ether gave only a trace of ketone with a fruity odor. There was not enough to make a derivative. The residue from the steam distillation was extracted with ether and the ether solution was washed with sodium bicarbonate solution. The ether solution was shaken with a 10% sodium hydroxide solution, and the sodium hydroxide extract was washed well with ether, acidified, and the acid was crystallized from acetic acid and finally from ethyl acetate to a constant melting point of 81°.

Anal. Calcd. for $C_{28}H_{46}O_2$: C, 79.3; H, 12.3. Found: C, 79.2; H, 12.8.

The molecular weight as determined by the camphor method was 374; calcd. for $C_{28}H_{46}O_2$ it is 378.

The major portion of the acidic material consisted of this acid, which forms a barium salt, and is unaffected by boiling with acetic anhydride. The acid does not absorb bromine in acetic acid solution even at 60° overnight nor does it give ketone derivatives.

The ether extract of the sodium salts of this acid contained about the same amount of neutral products as the total acids. The neutral product did not form a semicarbazone.

Fifty grams of lanosteryl acetate was oxidized by the same method used for the oxidation of α-dihydrolanosteryl acetate. No volatile ketones were recovered. The acidic portion of the oxidation products was dissolved in ether and extracted with sodium bicarbonate solution. The ether solution was then extracted with 10% sodium hydroxide solution. The sodium hydroxide extract was washed well with ether, acidified, and the acid was crystallized from acetic acid and finally from ethyl acetate to a constant melting point of 81°. The major portion of the acids consisted of this one individual which was identical to the acid obtained on the oxidation of α-dihydrolanosterol acetate. No depression in melting point was observed upon mixtures.

Anal. Calcd. for $C_{28}H_{46}O$: C, 79.3; H, 12.3. Found: C, 79.0; H, 12.7.

This acid gave a methyl ester m. p. 67°.

α-Keto-dihydrolanosteryl Acetate.—To a solution of 15 g. of α-dihydrolanosteryl acetate in 450 cc. of acetic acid at 80°, was added a solution of 4.5 g. of chromic oxide in 50 cc. of 90% acetic acid. This mixture was heated at 80° for ten minutes, and was poured into 150 cc. of water. This was extracted with ether, and the ether extract washed with sodium carbonate solution. The ether was evaporated and the residue was dissolved in 250 cc. of acetic acid and cooled. A small amount of gelatinous precipitate was filtered and discarded. On further cooling, the acetic acid solution gave a first fraction of 4 g. of crystalline product which was crystallized from ethyl acetate, acetone, methyl alcohol and ethyl alcohol. The compound crystallized in large plates giving 1 g. of pure product of m. p. 150°, which was unaffected by boiling with acetic anhydride and was distilled unchanged at 200° under vacuum.

Anal. Calcd. for $C_{32}H_{52}O_3$: C, 79.3; H, 10.8. Found: C, 79.5; H, 10.6.

β-Keto-dihydrolanosteryl Acetate.—The acetic acid filtrate from α-keto-dihydrolanosteryl acetate was evaporated to dryness, and the residue was crystallized from ethyl acetate–methyl alcohol. The filtrate from this crystallization gave 4 g. of a yellow solid upon evaporation of the solvent. Further crystallization from ethyl acetate, methyl alcohol, acetone and ethyl alcohol gave a product crystallizing in angular plates, m. p. 152°. This compound gave a 10° depression in melting point when mixed with α-keto-dihydrolanosteryl acetate.

Anal. Calcd. for $C_{32}H_{52}O_3$: C, 79.3; H, 10.8. Found: C, 79.2; H, 10.7.

Hydrolysis of α-Keto-dihydrolanosterol Acetate.—A solution of 500 mg. of α-keto-dihydrolanosterol acetate, 30 cc. of ethyl alcohol and 300 mg. of potassium hydroxide was refluxed for thirty minutes. Water was added and the product was extracted with ether. After evaporation of the ether, the residue was crystallized from ethyl acetate–methyl alcohol. This crystallized in needles giving keto-dihydrolanosterol, m. p. 135°.

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.4; H, 11.4. Found: C, 81.9; H, 11.6.

On refluxing with acetic anhydride for one-half an hour, this compound gave β-keto-dihydrolanosteryl acetate m. p. 151°. Mixed with β-keto-dihydrolanosteryl acetate it gave no depression in melting point. Mixed with α-keto-dihydrolanosteryl acetate gave a depression in melting point of 12°. By this method the α-keto-dihydrolanosterol acetate is converted into β-keto-dihydrolanosteryl acetate.

Hydrolysis of β-Keto-dihydrolanosteryl Acetate.—Upon hydrolysis of β-keto-dihydrolanosteryl acetate using the same method as in the hydrolysis of the alpha compound, a product was obtained melting at 134°. Mixed melting point with keto-dihydrolanosterol prepared from the alpha acetate gave no depression.

Reduction of Keto-dihydrolanosteryl Acetate with Sodium in Isopropyl Alcohol.—To a solution of 400 mg. of keto-dihydrolanosterol in 35 cc. of boiling isopropyl alcohol was added 2 g. of sodium over a period of one hour. The solution was poured into water and the product was extracted with ether. The residue after evaporation of the ether was crystallized from 80% methyl alcohol. This crystallized in needles, m. p. 165°, and is a hydroxydihydrolanosterol.

Anal. Calcd. for $C_{30}H_{52}O_2$: C, 81.0; H, 11.8. Found: C, 81.1; H, 11.7.

Dihydroagnosteryl Acetate from Hydroxydihydrolanosterol.—Hydroxydihydrolanosterol was refluxed for one hour with an excess of acetic anhydride. The acetic anhydride was distilled *in vacuo* and the residue was crystallized from acetone–methyl alcohol. It crystallized in plates, m. p. 165°. A mixture with dihydroagnosteryl acetate prepared by reducing agnosteryl acetate which was isolated from "isocholesterol" showed no depression in melting point.

Anal. Calcd. for $C_{32}H_{52}O_3$: C, 82.0; H, 11.2. Found: C, 82.0; H, 11.3.

It was found later that dihydroagnosteryl acetate could be prepared much more conveniently by merely reducing the crude mixture of α-keto-dihydrolanosteryl acetate and β-keto-dihydrolanosteryl acetate with sodium in isopropyl alcohol. The crude reduction product when boiled with acetic anhydride gave dihydroagnosteryl acetate.

Dihydroagnosterol.—Hydrolysis of dihydroagnosterol acetate with alcoholic potassium hydroxide, and crystallization of the product from ethyl acetate–methyl alcohol gave dihydroagnosterol, m. p. 150°. A mixture with dihydroagnosterol prepared from natural agnosterol gave no depression in melting point.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.4; H, 11.8. Found: C, 84.3; H, 12.0.

Monoacetate of Hydroxydihydrolanosterol.—The mother liquors from the crystallization of dihydroagnosteryl acetate were evaporated to dryness and the residue

was crystallized from methyl alcohol–water. This crystallized in fine needles, m. p. 130°. This product analyzed for a monoacetate of hydroxydihydrolanosterol.

Anal. Calcd. for $C_{32}H_{54}O_3$: C, 78.9; H, 11.2. Found: C, 78.6; H, 11.1

Summary

Both lanosteryl acetate and α-dihydrolanosteryl acetate upon vigorous oxidation lose five carbon atoms containing the acetate groups, giving the same acid $C_{25}H_{46}O_2$. α-Dihydrolanosteryl acetate isomerizes into a β-derivative with hydrochloric acid in chloroform. Mild oxidation of α-dihydrolanosteryl acetate gives a mixture of two keto-dihydrolanosterol acetates. Both of these upon hydrolysis give the same keto-dihydrolanosterol, which upon reduction with sodium in alcohol gives hydroxydihydrolanosterol. One –OH group of this product is removed by dehydration on refluxing with acetic anhydride to give dihydroagnosteryl acetate. This is identical with dihydroagnosteryl acetate prepared by catalytic reduction of natural agnosteryl acetate.

STATE COLLEGE, PENNA. RECEIVED APRIL 8, 1937

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE, AND THE PARKE, DAVIS & COMPANY RESEARCH LABORATORIES]

Sterols. XVII. Isolation of Pregnanolone from Human Pregnancy Urine

BY RUSSELL E. MARKER AND OLIVER KAMM

Recently the isolation of *epi-allo*-pregnanolone[1] an androgenic principle from human pregnancy urine, and later its synthetic preparation from chloro-*allo*-cholanic acid[2] were reported from these laboratories. This product was obtained from human pregnancy urine after the removal of the pregnandiols, theelin and theelol. Upon working up the residues from the crystallization of its semicarbazone, a new product was obtained which was identified as pregnanolone, an isomer of *epi-allo*-pregnanolone.

In our previous communication it was suggested that *epi-allo*-pregnanolone was an intermediate hydrogenation product of progesterone in its transformation to *allo*-pregnandiol. As pregnandiol is also a reduction product of progesterone it was suggested that possibly pregnanolone, its intermediate hydrogenation product, would also be present in human pregnancy urine. This product has now been isolated from the residues.

Butenandt[3] has shown that when progesterone is reduced in the laboratory, and the resulting product oxidized, a mixture of pregnandione and *allo*-pregnandione is obtained, showing the formation of *allo*-pregnandiol and pregnandiol by this reduction. As these products occur naturally in human pregnancy urine, it is apparent that their source is progesterone, which when used as a hormone by the pregnant woman is eliminated in the urine as its reduction products, of which the pregnanolones are the intermediate products. The structure of pregnanolone has been proved by a method similar to that of *epi-allo*-pregnanolone.

The compound forms a monoacetate and a monosemicarbazone. Upon oxidation it gave pregnandione. It did not precipitate with digitonin, showing that either the –OH group was of the epi-form in the 3-position or in the 20-position. Upon reduction of the ketone by platinum oxide in acetic acid solution, it gave a pregnandiol which did not precipitate with digitonin. If the ketone were in the 3-position, reduction under these conditions would give an –OH group of the *trans* type which would precipitate with digitonin. This proves that the –OH group was in the 3-position in the epi-form and the ketone in the 20-position.

Experimental

Semicarbazone of *epi*-Pregnanolone.—In an attempt to obtain an additional amount of *epi-allo*-pregnanolone, the crude mother liquors from the previous crystallization[1] were concentrated to a small volume and cooled. The crystalline product was filtered and crystallized from alcohol to a constant melting point of 248°. This product gave a depression in melting point of 15° when mixed with the semicarbazone of *epi-allo*-pregnanolone.

Anal. Calcd. for $C_{22}H_{37}N_3O_2$: C, 70.3; H, 9.9. Found: C, 70.5; H, 10.0.

***epi*-Pregnanolone.**—To a solution of 3 g. of the semicarbazone in 150 cc. of alcohol was added 15 cc. of sulfuric acid in 30 cc. of water. The mixture was refluxed for one

(1) Marker, Kamm and McGrew, THIS JOURNAL, **59**, 616 (1937).
(2) Marker, Kamm, Jones, Wittle, Oakwood and Crooks, *ibid.*, **59**, 768 (1937).
(3) Butenandt, *Ber.*, **68**, 2094 (1935).