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.

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[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

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[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).

hour, diluted with water and extracted with ether. The residue after evaporation of the ether was sublimed in high vacuum at 130°, then crystallized from 50% acetone. After three crystallizations a constant melting point of 136° was obtained. This product does not precipitate with digitonin and does not absorb bromine. Mixed with pregnanol-20-one-3 it gives a depression of 21° in melting point.

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

Acetate of *epi*-Pregnanolone.—A solution of 100 mg. of *epi*-pregnanolone in 5 cc. of acetic anhydride was refluxed for thirty minutes. The excess acetic anhydride was evaporated and the residue was crystallized from 70% acetone; m. p. 99°.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.8; H, 10.1. Found: C, 76.5; H, 10.2.

Pregnandione from *epi*-Pregnanolone.—To 100 mg. of *epi*-pregnanolone in 15 cc. of acetic acid at room temperature was added 50 mg. of chromic oxide in 25 cc. of acetic acid. It was let stand at 15° for twelve hours, then at room temperature for three hours. Water was added and the product was extracted with ether and the ether solution was washed with sodium carbonate solution. The residue after evaporation of the ether was sublimed under high vacuum at 100°. The product was then crystallized from dilute acetone; m. p. 120°. No depression in melting point was obtained upon mixture with an authentic sample of pregnandione from pregnandiol.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.8; H, 10.5.

Reduction of *epi*-Pregnanolone.—To a solution of 100 mg. of *epi*-pregnanolone in 100 cc. of acetic acid was added 100 mg. of Adams platinum oxide catalyst. The solution was shaken with hydrogen at 45 pounds (3 atm.) pressure for three hours. The product was crystallized from alcohol and washed with ether, m. p. 230°. Mixed with pregnandiol of m. p. 242° it gave a depression in melting point to 215°. This differs from natural pregnandiol only in the configuration of the -OH group on C-20. It does not precipitate with digitonin, showing that the original -OH group was in the 3-position in the *epi*-form.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.8; H, 11.3. Found: C, 78.6; H, 11.3.

Summary

epi-Pregnanolone an isomer of *epi*-*allo*-pregnanolone has been isolated from human pregnancy urine, and its structure proved by oxidation to pregnandione and reduction to a pregnandiol. A monoacetate and a monosemicarbazone were formed.

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Attempts to Prepare Cerous Amide[1]

BY F. W. BERGSTROM

Some years previously[2] it was found impossible to prepare aluminum amide by mixing liquid ammonia solutions of ammonium bromide and potassium ammonoaluminate. Since the substance so precipitated was of variable composition ($AlN + xNH_3$, an "ammonous nitride"), it became of interest to synthesize amides of other trivalent elements which might exhibit a similar behavior.

The addition of potassium amide to a solution of cerous iodide in liquid ammonia gives a fine white crystalline precipitate of an ammonobasic iodide, instead of the expected cerous amide. Dried in a vacuum at 20°, the compound corresponds to the formula, $CeI_3 \cdot Ce(NH_2)_3 \cdot 10NH_3$; heated in a vacuum at 150–180°, the composition becomes, $CeI_3 \cdot Ce(=NH)NH_2$. It has not yet proven possible to prepare pure cerous amide by the action of potassium amide on cerous thiocyanate in liquid ammonia.

Experimental

Cerous iodide, as prepared by the action of ammonium iodide on an excess of chemically pure metallic cerium in liquid ammonia, forms two colorless liquid phases.[3] At 0°, in a sufficiently large volume of ammonia, the two phases merge into one so that the ammonobasic salt is best precipitated at this temperature by the slow addition of somewhat less than the theoretical quantity of potassium amide. Prior to analysis, the crystals are washed several times with liquid ammonia and dried in a vacuum of 1–2 mm. at room temperatures, and at 150–180°.[4] The heated substance was a brick-red. It was hydrolyzed with water vapor, then dissolved in dilute sulfuric acid. Cerium was determined as the dioxide, after previous precipitation as hydroxide or oxalate. Iodine was weighed as silver iodide, and nitrogen was estimated by distillation of an aliquot portion with alkali into standard acid.

Mercuric cyanide reacts with an excess of cerium to form a sparingly soluble precipitate, probably of a cerous cyanide, which could not be freed from metallic cerium or mercury for analysis. Sulfur and selenium slowly dissolve

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(1) Original manuscript received June 11, 1935.

(2) Bergstrom, *J. Phys. Chem.*, **32**, 434 (1928).

(3) Bergstrom, *ibid.*, **29**, 162 (1925). The light pink solution previously reported was doubtless due to impurities in the cerium used.

(4) Franklin, "The Nitrogen System of Compounds," Reinhold Publishing Corporation, New York, N. Y., 1935, pp. 317–330.