[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

Molecular Rearrangements of 17-Hydroxy-pregnane Compounds[1]

BY HOMER E. STAVELY

In the course of research dealing with the partial synthesis of compounds of the pregnane series, the transformation of Δ^5-17-ethynyl-androstendiol-3,17 (I) into the hydroxy-ketone II was studied independently by Ruzicka and Meldahl[2] and by Stavely.[3]

In the former case acetic acid was added to the acetylene bond and the resulting acetate saponified; in the latter, direct hydration was accomplished in the presence of mercuric salts. The same end-product was obtained by either method.

Degradation studies by Ruzicka, *et al.*,[4] have proved conclusively that this substance does not have the expected pregnane structure II, but that a molecular rearrangement must have occurred whose principal feature is the transformation of the five-membered ring D into a six-membered methylated ring. The position of the methyl group was fixed by dehydrogenation with selenium to form 1-methylchrysene, and the oxidation product obtained indicated that the carbonyl group was α to the methyl group, as in structure III. For the parent hydrocarbon of the new series the names chrysopregnane and D-homo-androstane[4(b)] have been proposed.

The authentic hydroxy-ketone II was prepared[5] by condensing the ethynyl compound I with aniline. After the total condensation product had been heated in aqueous methanol or dilute methanolic hydrochloric acid, a part could be recovered as the ketone II, and the remainder gave the correct analysis for the anil of this ketone. This was interpreted to mean that the splitting of the anil had attained equilibrium before complete hydrolysis occurred. It has now been found that the supposed anil, after purification, cannot be hydrolyzed. In view of the ease with which 17-hydroxy-pregnane derivatives rearrange it seems likely that the anil of II undergoes partial rearrangement under the experimental conditions to form a product in which the nitrogen is firmly bound, possibly in ring D. As expected, the yield of the hydroxy-ketone II was greatly increased when the aniline condensation was carried out in a two-phase benzene–water medium with constant stirring, thus affording an opportunity for the true anil to hydrolyze before rearrangement occurred.

The pregnane structure of II was proved by oxidation with chromic acid to dehydroisoandrosterone.[5] Further proof has been obtained by catalytic reduction of II to allopregnanetriol-3,17(α),20 and the isolation of isoandrosterone from the periodic acid oxidation products of the triol.

The hydroxy-ketone II undergoes quantitative rearrangement when heated with alcoholic potassium hydroxide to a product identical with the ketone III,[5] the substance obtained by direct hydration of the acetylene compound. When the hydroxy-ketone II (m. p. 174–176°, $[\alpha]_D$ −65°) is dissolved in benzene and chromatographed on Brockmann alumina, a quantitative rearrangement also takes place, but the ketone thus obtained (m. p. 180–182°, $[\alpha]_D$ −104°) is not identical with III. This unexpected fact came to light after an attempt had been made to prepare 17-(α)-hydroxyprogesterone (V) by oxidizing the ketone II with aluminum isopropylate. The partially crystalline product was chromatographed on Brockmann alumina for purification and the diketone VI was obtained. The hydroxy-ketone obtained by rearrangement on alumina (IV) was readily oxidized to the same diketone VI. Brockmann alumina is definitely alkaline in reaction, but since the two rearrangement products III and IV are dissimilar, the alumina must play a role in the rearrangement.

The two hydroxy-ketones, III and IV, are not dimorphic crystalline forms, since they cannot be interconverted. Moreover their acetates, 3-keto derivatives, and reduction products are markedly different. Both series of derivatives differ from that obtained from the pregnane hydroxy-ketone II.

When catalytically hydrogenated with platinum oxide the unsaturated ketone IV reacts with two moles of hydrogen to form a saturated triol.

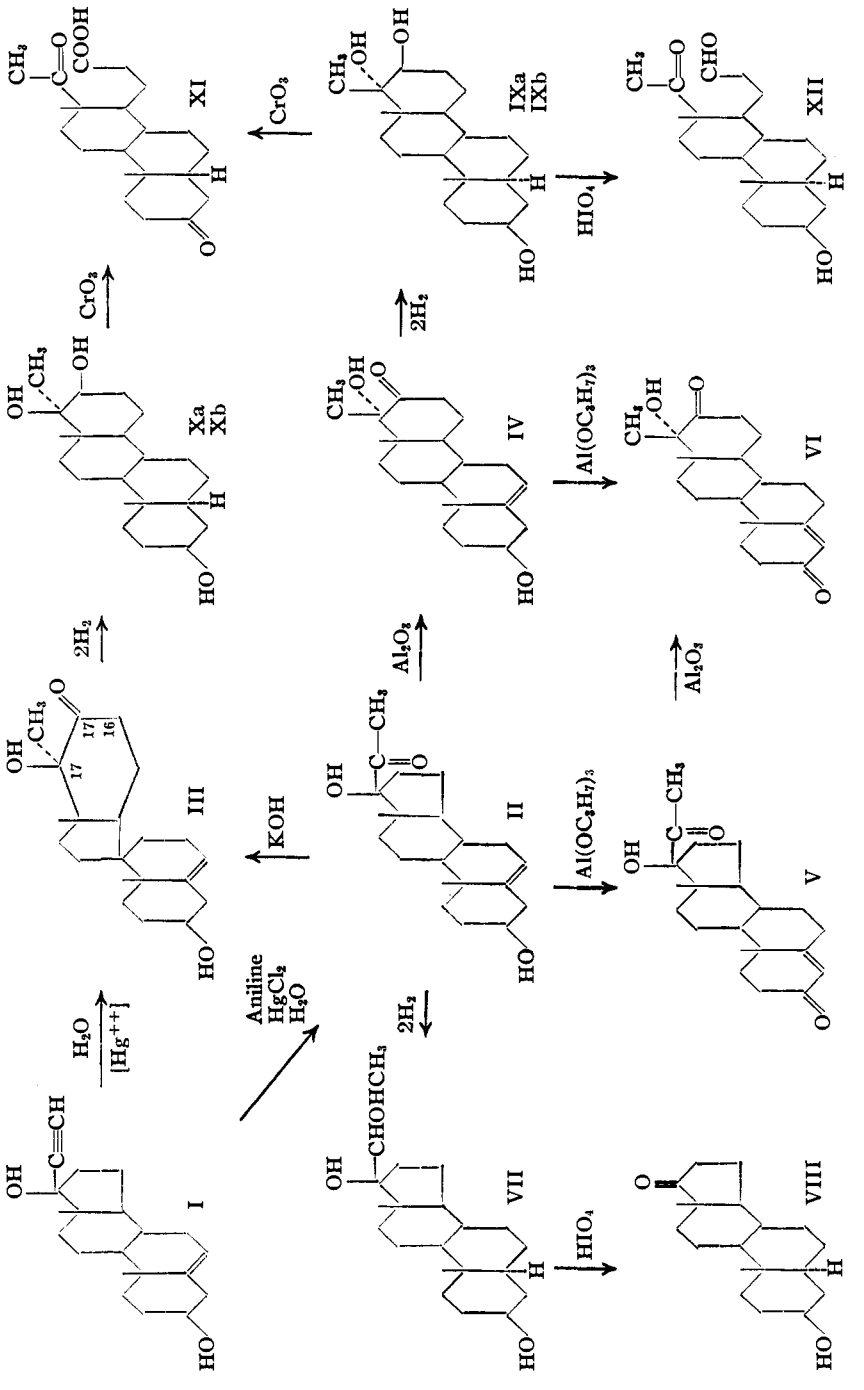
(1) Presented before the Division of Organic Chemistry at the St. Louis meeting of the American Chemical Society, April 8, 1941.

(2) Ruzicka and Meldahl, *Helv. Chim. Acta*, **21**, 1760 (1938).

(3) Stavely, THIS JOURNAL, **61**, 79 (1939).

(4) (a) Ruzicka, Gätzi and Reichstein, *Helv. Chim. Acta*,, **22**, 626 (1939); (b) Ruzicka and Meldahl, *ibid.*, **23**, 364 (1940); (c) Goldberg and Monnier, *ibid.*, **23**, 376 (1940).

(5) Stavely, THIS JOURNAL, **62**, 489 (1940).



Two isomeric triols can be made, IXa if the reduction is carried out in ethanol, and IXb if glacial acetic acid is used. Since 3-hydroxy steroids unsaturated in the 5,6 position always add hydrogen to give compounds of the cholestane type the isomerism must reside at the carbon atom holding the newly-formed hydroxyl group. Similarly the ketone III of known structure forms two isomeric triols Xa and Xb.[6] Although the triols IXa and Xa have almost the same melting point a mixture of the two melts 25° lower than either one alone.

The triol IXa is readily oxidized by periodic acid, and analysis of the neutral product XII indicates that no carbon atoms were lost. In this case a carbon and hydrogen analysis is sufficient for deciding between the loss of no carbon atoms on the one hand, or one or two carbon atoms on the other, since the periodic acid oxidation products would differ by H_2, or by CH_4O or C_2H_6O, respectively. Hence the results of periodic acid oxidation established, first, that the triol IXa contained an α,β-diol group, and therefore the keto group of the rearrangement product IV is α to the hydroxyl group; and, second, that the rearrangement must have involved the incorporation of the $COCH_3$ side chain into ring D, as is the case in the rearrangement with alkali.

The keto-aldehyde XII reacts with only one mole of hydroxylamine to form a mono-oxime. Since a 17-keto-androstane derivative is excluded by the analytical figures for carbon and hydrogen, the formation of a mono- rather than of a dioxime is best explained by an intramolecular aldol condensation. Evidence for this was obtained by heating the keto-aldehyde XII with methanolic potassium hydroxide. This treatment changed the properties of the substance. However, the possibility remains that

(6) Triol Xb has been prepared by Ruzicka, *et al.*, ref. 4a.

one of the carbonyl groups in XII is hindered.

The most likely structure for the rearrangement ketone IV is that of the stereoisomer of the ketone III with the opposite configuration of carbon atom 17a. If the two rearrangement products were so related, periodic acid oxidation of the corresponding triols IXa and Xa should lead to the same product. An attempt was made to split the triol Xa with this reagent, but most of the compound was recovered unchanged. This difference in behavior between the two triols does not necessarily exclude the α,β-diol structure for Xa, but merely indicates that the hydroxyl groups in this case are *trans*, since *trans* ring diols are known to be resistant to periodic acid.

Ruzicka, *et al.*,[4a] prepared the diketo acid XI from the triol Xb with chromic oxide. If the triol IXb is indeed the C-17a epimer of Xb, the same acid oxidation product should be obtained from both. When the two triols were oxidized under the same conditions, the resulting acids and their methyl esters were found to be identical. It must be concluded that the molecular rearrangement products III and IV are stereoisomers differing only in the configuration of carbon atom 17a.

The hydroxy-ketone II has a configuration at carbon 17 designated α by Reichstein,[7] since all synthetic pregnane derivatives have a configuration opposite to that of the natural adrenal steroids (17β). Recently Prins and Reichstein[8] reported that the naturally occurring adrenal substance K underwent a rearrangement of the type described in this paper when heated with alcoholic hydrochloric acid. Therefore neither the $17(\alpha)$- nor $17(\beta)$-hydroxypregnane derivatives are immune to rearrangement.

It has been pointed out[9] that until recently steroid hormone physiological activity has been found to reside only in substances containing the perhydrocyclopentenophenanthrene ring system, except in the estrogenic group. On the basis of this specificity, one might expect that substances with the perhydrochrysene ring system would have no androgenic or progestational activity. However, Goldberg and Monnier[9] prepared the perhydrochrysene homologs of dihydrotestosterone and androsterone with a six-membered ring and found them to be fully as active as the natural hormones. The diketone VI was tested for progestational activity[10] and found to be active at a dosage of 12.5 mg.

(7) Reichstein and Gätzi, *Helv. Chim. Acta*, **21**, 1185 (1938).
(8) Prins and Reichstein, *ibid.*, **23**, 1491 (1940).
(9) Goldberg and Monnier, *ibid.*, **23**, 840 (1940).

Experimental

Δ^5-Pregnenediol-3,17-one-20 (II).—Four grams of Δ^5-17-ethynylandrostenediol-3,17, 7 g. of mercuric chloride, 1.2 ml. of aniline, 250 ml. of benzene, and 50 ml. of water were heated to 60° and stirred for twenty hours. The benzene and aniline were removed by steam distillation and hydrogen sulfide passed into the aqueous suspension. The precipitate was filtered and the organic material was removed by extraction with ether in a Soxhlet apparatus. The ether was washed with water, dried, and evaporated to dryness. The residue weighed 3.8 g. By means of Girard's ketone reagent T, a ketonic fraction amounting to 1.7 g. was isolated. After treatment with Norit and several crystallizations from aqueous methanol, the m. p. was 174–176°,[11] $[\alpha]^{24}D$ −65.5° (8.4 mg. in 1.0 ml. chloroform, 1-dm. tube, α^{24} −0.55).

Anal.[12] Calcd. for $C_{21}H_{32}O_4$: C, 75.85; H, 9.71. Found: C, 75.91; H, 9.87.

The acetate was prepared and was identical with that reported previously.[5]

The non-ketonic fraction after purification was identical with the anil reported previously.

Oxidation of Δ^5-Pregnenediol-3,17-one-20 (II) with Aluminum Isopropylate.—Four hundred milligrams of Δ^5-pregnenediol-3,17-one-20, 600 mg. of freshly distilled aluminum isopropylate, 10 ml. of dry cyclohexanone, and 20 ml. of dry toluene were refluxed for one hour, then poured into 2 *N* hydrochloric acid, and the mixture extracted with ether. The ether residue was subjected to prolonged steam distillation to remove cyclohexanone and toluene. The aqueous mixture was again extracted with ether. The ether residue crystallized poorly from several solvents. The product was chromatographed on Brockmann alumina and 135 mg. of crystalline product was obtained. The substance was recrystallized from ether-hexane, m. p. 180°; $[\alpha]^{23}D$ 60° (8.4 mg. in 1.0 ml. chloroform, 1-dm. tube, α^{23} 0.50).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.39; H, 9.22.

The dioxime was prepared from the diketone in the usual manner, m. p. 255°.

Anal. Calcd. for $C_{21}H_{32}O_3N_2$: N, 7.77. Found: N, 8.05, 8.12.

Treatment of the Diketone VI with Methanolic Potassium Hydroxide.—Twenty milligrams of the diketone obtained from Δ^5-pregnenediol-3,17-one-20 was refluxed with 5% methanolic potassium hydroxide. The starting material was recovered unchanged.

Rearrangement of Δ^5-Pregnenediol-3,17-one-20 (II) on an Alumina Column.—Seventy-eight milligrams of Δ^5-pregnenediol-3,17-one-20 (m. p. 176°; $[\alpha]D$ −65°) dis-

(10) The test was carried out by Dr. H. B. van Dyke of the Division of Pharmacology of the Squibb Institute for Medical Research. When the test was made the diketone VI was thought to be $17(\alpha)$-hydroxyprogesterone.
(11) All melting points are uncorrected.
(12) Analyses herein reported are by J. F. Alicino, Fordham University.

solved in benzene was passed through a column of Brockmann alumina 15 × 1.5 cm., and the column developed with benzene. The eluant was collected in 100-cc. portions. The first five fractions yielded only 4 mg. of oily residue. A solution of 1% ethanol in benzene was then passed through the column and the next two fractions yielded 71 mg. of crystalline residue. After recrystallization from aqueous methanol the melting point was 180–182°; $[\alpha]^{24}D$ −104° (5.4 mg. in 1.0 cc. chloroform, 1-dm. tube, α^{24} −0.56°).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.71. Found: C, 76.15; H, 10.02.

The acetate was prepared with acetic anhydride in pyridine, m. p. 174–176°; $[\alpha]^{25}D$ −98° (5.45 mg. in 1.0 ml. chloroform, 1-dm. tube, α^{25} −0.53°).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.16. Found: C, 73.88; H, 9.17.

Oxidation of the Ketone IV with Aluminum Isopropylate.—One hundred thirty milligrams of the rearranged hydroxy-ketone IV was oxidized with aluminum isopropylate. The product was identical with the diketone obtained from Δ^5-pregnenediol-3,17-one-20; m. p. 179°; $[\alpha]D$ 60°.

Hydrogenation of Δ^5-Pregnenediol-3,17-one-20 (II).—Five hundred milligrams of Δ^5-pregnenediol-3,17-one-20 was catalytically reduced with 200 mg. of platinum oxide in absolute ethanol. Two moles of hydrogen was taken up. After several crystallizations from benzene–hexane the m. p. was constant at 215–216°.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 74.96; H, 10.78. Found: C, 75.16; H, 10.83.

A diacetate was prepared from the saturated triol with acetic anhydride in pyridine; m. p. 166–171°.

Anal. Calcd. for $C_{25}H_{40}O_5$: C, 71.39; H, 9.71. Found: C, 71.25; H, 9.60.

Isoandrosterone from Allopregnanetriol-3,17,20 (VII).—Fifty milligrams of the triol was dissolved in 8 ml. of methanol, and 75 mg. of periodic acid crystals in 2 ml. of water added. After standing for twenty-four hours at room temperature, water was added and the mixture extracted with ether and ethyl acetate. The combined solvents were washed with sodium carbonate solution, then with water, dried, and evaporated to dryness. The neutral residue weighed 33 mg. It was crystallized several times from ether–pentane; m. p. 169°; $[\alpha]^{24}D$ 90° (4.8 mg. in 1.0 ml. chloroform, 1-dm. tube, α^{24} 0.43°). Mixed with a sample of authentic isoandrosterone, there was no depression of the melting point.

Hydrogenation of Ketone IV.—Four hundred milligrams of the hydroxy-ketone rearranged on alumina (m. p. 180–182°) was reduced catalytically with platinum oxide in ethanol. Two moles of hydrogen was taken up. The catalyst was filtered and the filtrate concentrated. The saturated triol (IXa) was recrystallized from ethanol; m. p. 259–261°.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.96; H, 10.78. Found: C, 75.31; H, 10.51.

On standing with acetic anhydride in pyridine, a monoacetate was formed. After recrystallizing several times, it sintered at 185° and melted at 190°.

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.97; H, 10.11. Found: C, 73.16; H, 9.96.

The triol formed a triacetate on boiling with acetic anhydride for two hours. It was recrystallized from acetone–hexane, m. p. 247–250°. Four hours of treatment with acetic anhydride in pyridine on the steam-bath led to the formation of only a monoacetate.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.84; H, 8.90.

When the hydrogenation was carried out in glacial acetic acid instead of ethanol an isomeric saturated triol (IXb) was obtained; m. p. 272–274°.

Hydrogenation of Ketone III.—Two hundred fifty milligrams of the hydroxy-ketone (m. p. 276–278°) was catalytically reduced with platinum oxide in ethanol. After two moles of hydrogen was taken up, the catalyst was filtered and the filtrate concentrated. The crystalline precipitate (Xa) was recrystallized from ethanol, m. p. 256–258°. A mixed melting point with the saturated triol of m. p. 261° obtained from ketone IV showed a depression of 25°.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.96; H, 10.78. Found: C, 75.41; H, 10.41.

On acetylation with acetic anhydride in pyridine at room temperature a mixture of mono- and diacetates was obtained. The mixture was subjected to further acetylation on the steam-bath for one hour. The product had a m. p. of 220–222° and analyzed for the diacetate.

Anal. Calcd. for $C_{25}H_{40}O_5$: C, 71.39; H, 9.58. Found: C, 71.51; H, 9.33.

The saturated triol formed a triacetate on boiling with acetic anhydride for two and one-half hours; m. p. 227°.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.51; H, 9.28.

When the hydrogenation was carried out in glacial acetic acid instead of ethanol, an isomeric saturated triol (Xb) was obtained, m. p. 280–282°. This is the triol reported by Ruzicka to possess a m. p. of 304° when taken on the Reichert melting apparatus. The triol herein reported melted at 298° on the Fisher–Johns apparatus. A mixed melting point with the triol IXb of m. p. 274° made from the ketone IV in acetic acid showed a 25° depression.

Periodic Acid Oxidation of the Triol IXa.—One hundred twenty milligrams of the saturated triol IXa made by hydrogenation in ethanol was dissolved in 15 ml. of methanol and 150 mg. of periodic acid in 2 ml. of water was added. After standing for twenty-four hours at room temperature, water was added and the mixture extracted with ether and ethyl acetate; the solvents were washed with sodium carbonate solution, then with water, and evaporated to dryness in a vacuum. One hundred ten milligrams of colorless residue was obtained in the neutral fraction. The residue was crystallized from aqueous methanol, then from benzene–hexane, m. p. 150–152°.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.42. Calcd. for $C_{21}H_{34}O_3$: C, 75.41; H, 10.18. Found: C, 75.69; H, 9.87.

Fifty milligrams of the oxidation product was refluxed with hydroxylamine hydrochloride and potassium acetate in ethanol for three hours. Water was added and the precipitate filtered and recrystallized several times from aqueous methanol, m. p. 188–191°.

Anal. Calcd. for $C_{19}H_{31}O_2N$: C, 74.71; H, 10.30; N, 4.40. Calcd. for $C_{21}H_{36}O_3N_2$: C, 69.30; H, 10.00; N, 7.7. Calcd. for $C_{21}H_{35}O_3N$: C, 72.16; H, 10.09; N, 4.01. Found: C, 72.45; H, 9.70; N, 4.72.[13]

The semicarbazone was prepared from 10 mg. of the oxidation product, m. p. 187°.

Anal. Calcd. for $C_{22}H_{37}O_3N_3$: N, 10.73. Found: N, 9.92.

Thirty milligrams of the oxidation product was refluxed with 5% methanolic potassium hydroxide for three hours, water was added and the precipitate filtered and crystallized from benzene-hexane, m. p. 181–187°.

Periodic Acid Oxidation of the Triol Xa.—Seventy-five milligrams of the triol made by hydrogenation in ethanol was placed with 100 mg. of periodic acid in aqueous methanol. Even after standing for forty-eight hours, no oxidation product could be isolated. The triol was recovered unchanged.

Chromic Trioxide Oxidation of Triol Xb.—Sixty milligrams of the triol made by hydrogenation of the ketone III in glacial acetic acid was dissolved in 2 ml. of glacial acetic acid and placed in an ice-bath. Chromic trioxide, 40 mg., in 2 ml. of acetic acid was added dropwise with shaking. After standing for twenty hours at room temperature, the acetic acid was evaporated at a temperature below 30° in a vacuum, sodium carbonate solution was added, and the mixture extracted with ether. The neutral ether residue amounted to 2 mg. The sodium carbonate solution was acidified with hydrochloric acid and extracted with ether. The ether was washed with water, dried, and evaporated, the acid residue weighing 50 mg. It was recrystallized several times from ether–hexane, m. p. 214-216°. The melting point on the Fisher–Johns apparatus was 222–225°, agreeing with that given by Ruzicka, $[\alpha]D \pm 0.0$ (5.5 mg. in 1.0 ml. chloroform, 1-dm. tube, $[\alpha] \pm 0.01$).

The methyl ester was prepared by treatment with diazomethane and recrystallized from ether–pentane, m. p. 103–105°.

Chromic Trioxide Oxidation of Triol IXb.—Sixty milligrams of the triol made by hydrogenation of the ketone IV in acetic acid was dissolved in 2 ml. of acetic acid and oxidized with 40 mg. of chromic oxide exactly as in the case of the triol Xb. The neutral product amounted to 30 mg. and the acid products 25 mg. The acid oxidation product was recrystallized from ether–pentane, m. p. 212–215°. The melting point of a mixture with the acid obtained from triol Xb showed no depression: $[\alpha]D \pm 0.0°$ (3.8 mg. in 1.0 ml. chloroform, 1-dm. tube, $\alpha D \pm 0.01$).

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.02; H, 9.10.

The methyl ester was prepared by treatment with diazomethane, recrystallized from ether–pentane, m. p. 102–104°. Mixed with the methyl ester of the acid obtained from triol Xb there was no depression of the melting point.

(13) Oximes containing several methyl groups often give high values for N by the Dumas method, according to Hans Meyer, "Analyse und Konstitutionsermittlung organischer Verbindungen," Julius Springer, Berlin, 1931, p. 121.

Summary

In the presence of aniline, mercuric salts, and water, Δ^5-17-ethynyl-androstenediol-3,17 is partially changed into Δ^5-pregnenediol-3,17(α)-one-20, and the rest into a substance which contains firmly bound nitrogen and which gives the correct analytical values for the C_{20} anil of Δ^5-pregnenediol-3,17(α)-one-20. This is probably a rearrangement product. The pregnane structure of the ketodiol was confirmed by catalytic reduction to a pregnanetriol, and oxidation of the latter with periodic acid to isoandrosterone.

Δ^5-Pregnenediol-3,17(α)-one-20 undergoes molecular rearrangement when heated with methanolic alkali. The structure of the product has been elucidated by Ruzicka, *et al.*[4] A rearrangement also occurs when Δ^5-pregnenediol-3,17(α)-one-20 is chromatographed on alumina; but the product so obtained differs from, and is isomeric with, the alkali rearrangement product.

Both rearrangement products have been reduced to saturated triols. In each case hydrogenation in acetic acid leads to the formation of a saturated triol isomeric with that obtained when the reduction is carried out in ethanol. Thus four different isomeric saturated triols have been prepared.

Oxidation of the triols with periodic acid and chromic acid has proved that the two rearrangement products are stereoisomers, differing in the configuration of the carbon atom 17a holding methyl and hydroxyl groups.

Since Reichstein[8] has shown that his substance K from adrenal extracts undergoes the same type of rearrangement, 17(α)- and 17(β)-hydroxypregnane derivatives do not differ in this respect.

The 3-keto derivative of the alumina rearrangement product exhibits slight but definite progestational activity. This is the first substance not containing the perhydrocyclopentenophenanthrene ring system found to possess the physiological properties of the corpus luteum hormone.

NEW BRUNSWICK, N. J. RECEIVED AUGUST 16, 1941