[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA]

Investigations on Steroids. XXVI. Synthesis of 19-Hydroxy-Δ^4-androstene-3,17-dione[1,2]

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The transformation of strophanthidin, by way of the known 3β,19-diacetoxy-5-hydroxyetianic acid (XII), into 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII) is described. The preparation of the intermediates leading to XII has been improved and the scope of these reactions has been studied more fully. XII was converted into 3β,19-diacetoxy-21-diazopregnan-5-ol-20-one (XIII) which by treatment with concentrated hydriodic acid was transformed into 3β,19-diacetoxypregnan-5-ol-20-one (XV). Refluxing XV with acetic anhydride under strictly controlled conditions gave satisfactory yields of 3β,5,19-triacetoxypregnan-20-one (XVI). Deviations from these conditions tended to produce unsaturated compounds (XVII and XVIII). Oxidation with perbenzoic acid of XVI gave 3β,5,17β,19-tetraacetoxyetiocholane (XX) which by saponification was converted into etiocholane-3β,5,17β,19-tetrol (XXI). Oxidation of XXI with 2,4 equivalents of N-bromoacetamide and subsequent treatment of the reaction product (XXII) with Girard's reagent T gave 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII). The identity of XXIII with an adrenal-enzymatic hydroxylation product of Δ^4-androstene-3,17-dione[11] has been established. As a new synthesis of 19-hydroxyprogesterone (XXVIII), XV was saponified to pregnane-3β,5,19-triol-20-one (XIV) which was oxidized with N-bromoacetamide to pregnane-5,19-diol-3,20-dione (XXVII) which in turn by treatment with Girard's reagent T underwent dehydration, furnishing XXVIII. The new compounds have been characterized by suitable derivatives.

In previous publications from this laboratory[3-5] the synthesis of the 19-hydroxy and 19-oxo analogs of progesterone and 11-desoxycorticosterone was described. Thereafter, 19-hydroxy-11-desoxycorticosterone was identified as a component of beef adrenal extract.[6] Furthermore, it was shown in several laboratories[7-9] that appreciable quantities of this compound are formed on incubating 11-desoxycorticosterone with beef adrenal homogenates. 19-Hydroxy-11-desoxycorticosterone and, in addition, 17α,19-dihydroxy-11-desoxycorticosterone were isolated as products resulting from the perfusion of progesterone through bovine adrenal glands.[10] A. S. Meyer demonstrated[11] the formation of 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII) by incubating Δ^4-androstene-3,17-dione with beef adrenal homogenate. The same author also reported[12] conversion of the former compound (XXIII) to estrone by the action of endocrine tissue. Thus, the possibility has to be considered, that steroid hormones hydroxylated in the 19-position play a role as metabolic intermediates. In view of the demonstrated biological significance of steroids with an oxygen function in the 19-position, the synthetic preparation of additional members of this series appears to be indicated.

In the present paper, the synthesis from strophanthidin of 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII) is described. Although the basic steps of this procedure were published from this laboratory earlier, several simplifications have been introduced and the scope of some of the reactions has been studied more fully. It is considered justified, therefore, to include this additional information concerning previous work, especially as far as yields are concerned.

Strophanthidol diacetate (I) is accessible in excellent yield by selective reduction of strophanthidin and acetylation of the resulting strophanthi-

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(2) The findings of this paper were incorporated in reports given on July 22, 1955, at the XIVth International Congress of Pure and Applied Chemistry Covering Organic Chemistry in Zürich (M. R. Ehrenstein, *Analogs of Steroid Hormones Oxygenated in the 19-Position*, General Programme, p. 56) and on August 5, 1955, at the 3rd International Congress of Biochemistry in Brussels (*cf.* M. R. Ehrenstein, G. W. Barber, and M. Dünnenberger, *Analogs of Steroid Hormones Oxygenated in the 19-Position and Their Biological Significance*, Résumés des Communications, p. 4. [No. 1-26]). See also the presentation on April 20, 1956, before the American Society of Biological Chemists at the 40th annual meeting of the Federation of the American Societies for Experimental Biology in Atlantic City, N. J. [*cf.* Maximilian R. Ehrenstein and Max Dünnenberger, *Federation Proc.*, **15**, 246 (1956)].

(3) Barber and Ehrenstein, *J. Am. Chem. Soc.*, **76**, 2026 (1954).

(4) Barber and Ehrenstein, *J. Org. Chem.*, **19**, 1758 (1954).

(5) Barber and Ehrenstein, *J. Org. Chem.*, **20**, 1253 (1955).

(6) Mattox, *Proc. Staff Meetings Mayo Clinic*, **30**, 180 (1955).

(7) Hayano and Dorfman, *Arch. Biochem. and Biophys.*, **55**, 289 (1955).

(8) Zaffaroni, Troncoso, and Garcia, *Chemistry & Industry*, 534 (1955).

(9) Kahnt, Neher, and Wettstein, *Helv. Chim. Acta*, **38**, 1237 (1955).

(10) Levy and Kushinsky *Arch. Biochem. and Biophys.*, **55**, 290 (1955); **58**, 245 (1955).

(11) Meyer, *Experientia*, **11**, 99 (1955).

(12) Meyer, *Biochim. et Bioph. Acta*, **17**, 441 (1955).

dol.[13,14] An improved method of oxidizing I with potassium permanganate in acetone[15] gave yields of approx. 20% of the ketolactone III (3β,19-diacetoxy-5,14-dihydroxy-20-oxo-14β-pregnan-21-oic acid 21 ⟶ 14-lactone) and 44% of the free 3β,5,14,-19-tetrahydroxy-14β-etianic acid (IV).[16] Treatment of IV with 0.1 *N* ethanolic hydrogen chloride under controlled conditions resulted, in addition to esterification, mainly in selective dehydration affording a yield of at least 40% of ethyl 3β,5,19-trihydroxy-Δ^{14}-etienate (V).[17] As was shown previously, there are obtained small amounts (4–5%) of an isomer first[18] considered to be the corresponding $\Delta^{8,14}$-compound but later[19] interpreted to possess the structure of ethyl 3β,5-dihydroxy-8,19-epoxyetianate (VI). Furthermore, small quantities of a dianhydro product, *i.e.* ethyl 3β,19-dihydroxy-$\Delta^{5,14}$-etiadienate (VII), had been isolated.[19] Depending on the experimental conditions, a yield of 5–10% of another product was obtained. This was first[20] considered to be a lactone having the empirical formula $C_{20}H_{28}O_4$, but has now been recognized to possess the structure of the trianhydro product ethyl 19-hydroxy-$\Delta^{3,5,14}$-etiatrienate (VIII). It was characterized by its acetate (IX). The hydrogenation of V to ethyl 3β,5,19-trihydroxyetianate (X) proceeded in agreement with earlier observations.[21] On saponification of this ester, the yields of 3β,5,19-trihydroxyetianic acid (XI) were superior to those reported previously.[22] XI has served as the key intermediate in the syntheses of the 19-hydroxy and 19-oxo analogs of progesterone and 11-desoxycorticosterone.[3–5] Acetylation under well defined conditions gave uniform yields of crystalline 3β,19-diacetoxy-5-hydroxyetianic acid (XII).[23]

The acid chloride of XII was prepared by reaction of the sodium salt with oxalyl chloride according to the procedure of Wilds and Shunk.[24] Treatment of the acid chloride with diazomethane produced the crystalline 3β,19-diacetoxy-21-diazopregnan-5-ol-20-one (XIII). By reaction of XIII with concentrated hydriodic acid[25] 3β,19-diacetoxypregnan-5-ol-20-one (XV) resulted. Saponification of XV with aqueous methanolic potassium carbonate gave the free pregnane-3β,5,19-triol-20-one (XIV) which by acetylation under ordinary conditions (pyridine-acetic anhydride; room temperature) was reconverted into XV. Refluxing XV with acetic anhydride under strictly controlled conditions yielded appreciable amounts of 3β,5,19-triacetoxypregnan-20-one (XVI). The acetylation of the tertiary hydroxyl group apparently proceeds best in a relatively dilute solution. When, in a large scale experiment, a relatively concentrated solution of XV in acetic anhydride was heated under reflux, unfortunately side reactions occurred to an appreciable extent, leading to 3β,19-diacetoxy-Δ^5-pregnen-20-one (XVII)[26] and 19-acetoxy-$\Delta^{3,5}$-pregnadien-20-one (XVIII). Saponification of XVII with aqueous ethanolic potassium carbonate unexpectedly did not furnish Δ^5-pregnene-3β,19-diol-20-one, but instead 3β-acetoxy-Δ^5-pregnen-19-ol-20-one (XIX). When subjected to the Oppenauer reaction, XIX was recovered unchanged which is compatible with the assigned structure.[27] Saponification

(13) Ehrenstein and Johnson, *J. Org. Chem.*, 11, 823 (1946).

(14) Ehrenstein, Johnson, Olmsted, Vivian, and Wagner, *J. Org. Chem.*, 15, 264 (1950).

(15) Former experiments *cf.* ref. 13, p. 833; ref. 14, p. 272; Barber and Ehrenstein, *J. Org. Chem.*, 16, 1615 (1951), p. 1619.

(16) Oxidation with potassium permanganate of acetylated cardiac aglycones and of acetylated bufogenins leads generally to the respective etianic acids and, in addition, to a 21 ⟶ 14 lactone [Lit. *cf. e.g.* Katz, *Helv. Chim. Acta*, 38, 1565 (1955), p. 1566; Pataki and Meyer, *Helv. Chim. Acta*, 38, 1631 (1955), p. 1634].

(17) Former experiments *cf.* ref. 13, footnote p. 828; 14, p. 273; Ehrenstein and Neumann, *J. Org. Chem.*, 16, 335 (1951), p. 340.

(18) Ref. 13, exper. *cf.* p. 844; ref. 14, exper. *cf.* p. 274.

(19) Ehrenstein and Neumann, *J. Org. Chem.*, 16, 335 (1951), exper. *cf*, p. 342.

(20) Ref. 13, exper. *cf.* p. 837; ref. 14, exper. *cf.* pp. 273, 274.

(21) Ref. 14, p. 274.

(22) Ref. 13, p. 845.

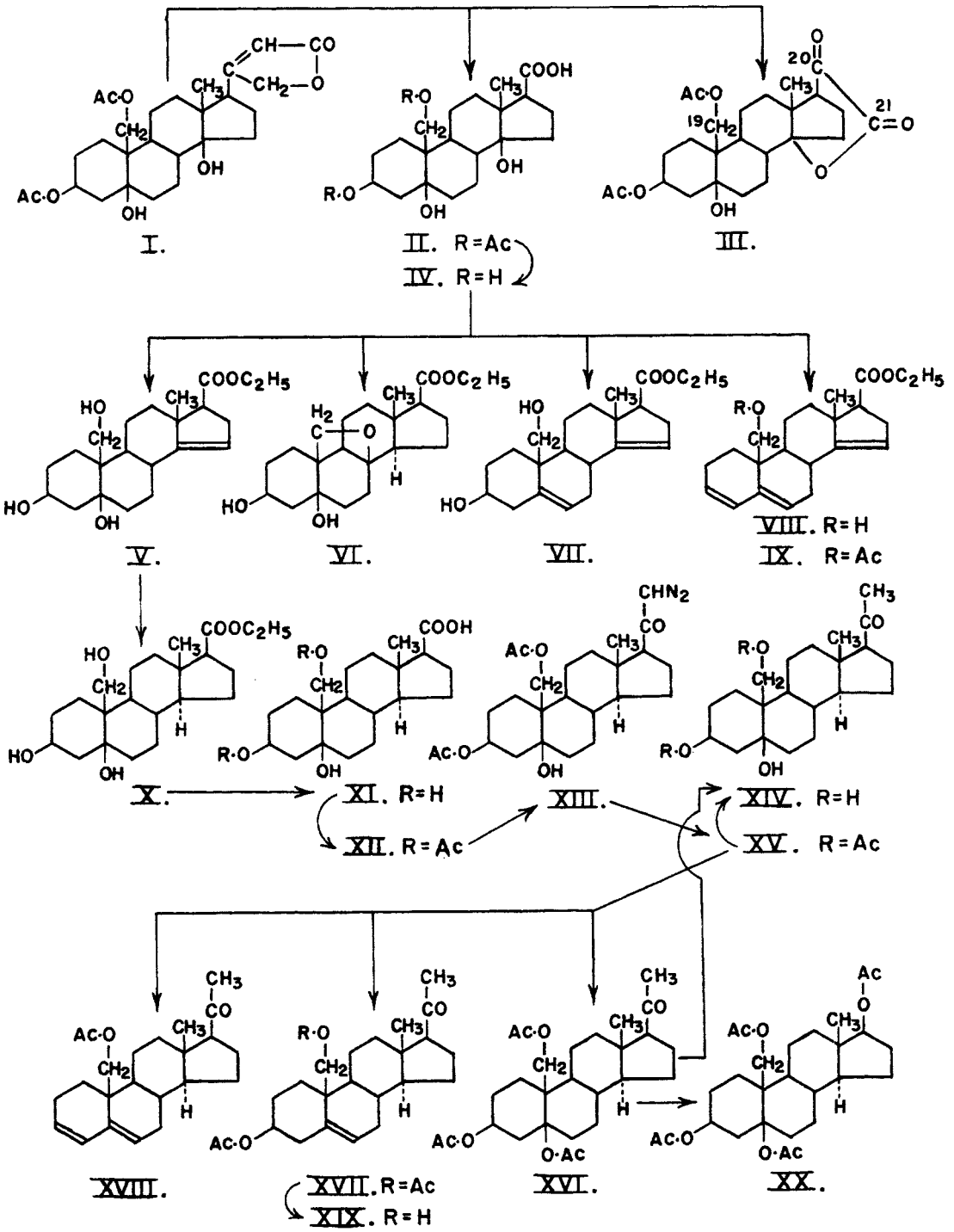
(23) Earlier experiments, *cf.* ref. 14, p. 275; Herzig and Ehrenstein, *J. Org. Chem.*, 17, 713 (1952), p. 717.

(24) Wilds and Shunk, *J. Am. Chem. Soc.*, 70, 2427 (1948).

(25) *Cf.* ref. 4; Heard and Ziegler, *J. Am. Chem. Soc.*, 72, 4328 (1950).

(26) In this connection an unpublished experiment by P. T. Herzig [*cf.* Herzig and Ehrenstein, *J. Org. Chem.*, 17, 713 (1952)] appears worth reporting. 3β,19-Diacetoxy-5-hydroxyetianic acid (XII) was fused in a high vacuum resulting in a weight loss of approx. 12%. After subsequently treating the material with thionyl chloride at −15° for one hour, the reagent was removed completely *in vacuo*. The residue, dissolved in dry benzene, was added to an ethereal solution of dimethylcadmium. The neutral fraction resulting from this reaction was chromatographed over alumina (activity III). Elution with petroleum ether and petroleum ether-benzene, 9:1, gave a product, m.p. 90–95°, which gave a yellow color with tetranitromethane; no ultraviolet absorption maximum in the range 220–300 mμ. $[\alpha]_D^{24}$ +194.1° (10.83 mg.; α +2.10°). *Anal.* Calc'd for $C_{23}H_{32}O_4$ (356.49): C, 77.49; H, 9.05. Found: C, 77.34; H, 9.60. Elution with petroleum ether-benzene, 2:3 and 1:4, furnished a compound, m.p. 105–105.5°, which is obviously identical with 3β,19-diacetoxy-Δ^5-pregnen-20-one (XVII); no depression of mixture m.p.; yellow color with tetranitromethane, $[\alpha]_D^{24}$ −20.5° (10.85 mg.; α −0.22°). The infrared spectrum was determined in carbon disulfide and in carbon tetrachloride solution. It is identical with that obtained with an authentic sample of XVII (*cf.* experimental part). *Anal.* Calc'd for $C_{25}H_{36}O_5$ (416.54): C, 72.08; H, 8.71. Found: C, 71.92; H, 8.78.

(27) The change in molecular rotation in passing from the monoacetate (XIX) to the diacetate (XVII) (+67°) is in agreement with this conclusion, since acetylation of a 3β-hydroxyl group in the presence of a 5,6-double bond is known to produce a shift in molecular rotation of approximately −35°; *cf.* Fieser and Fieser, *The Chemistry of Natural Products Related to Phenanthrene*, 3rd ed., Reinhold Publishing Corporation, New York, 1949, p. 208.



of XVI with methanolic potassium hydroxide at room temperature yielded the free pregnane-3β,5-19-triol-20-one (XIV).

Oxidation with perbenzoic acid has been used for replacing the methylketone side chain by an acetoxy group.[28–33] The yield apparently varies with the 20-ketopregnane used[28] and it seems essential to perform the reaction in a very concentrated solution.[28] Although the reaction has been successfully applied to a compound with a free hydroxyl group in the 3-position,[29] the danger of

(28) Sarett, *J. Am. Chem. Soc.*, **69**, 2899 (1947).

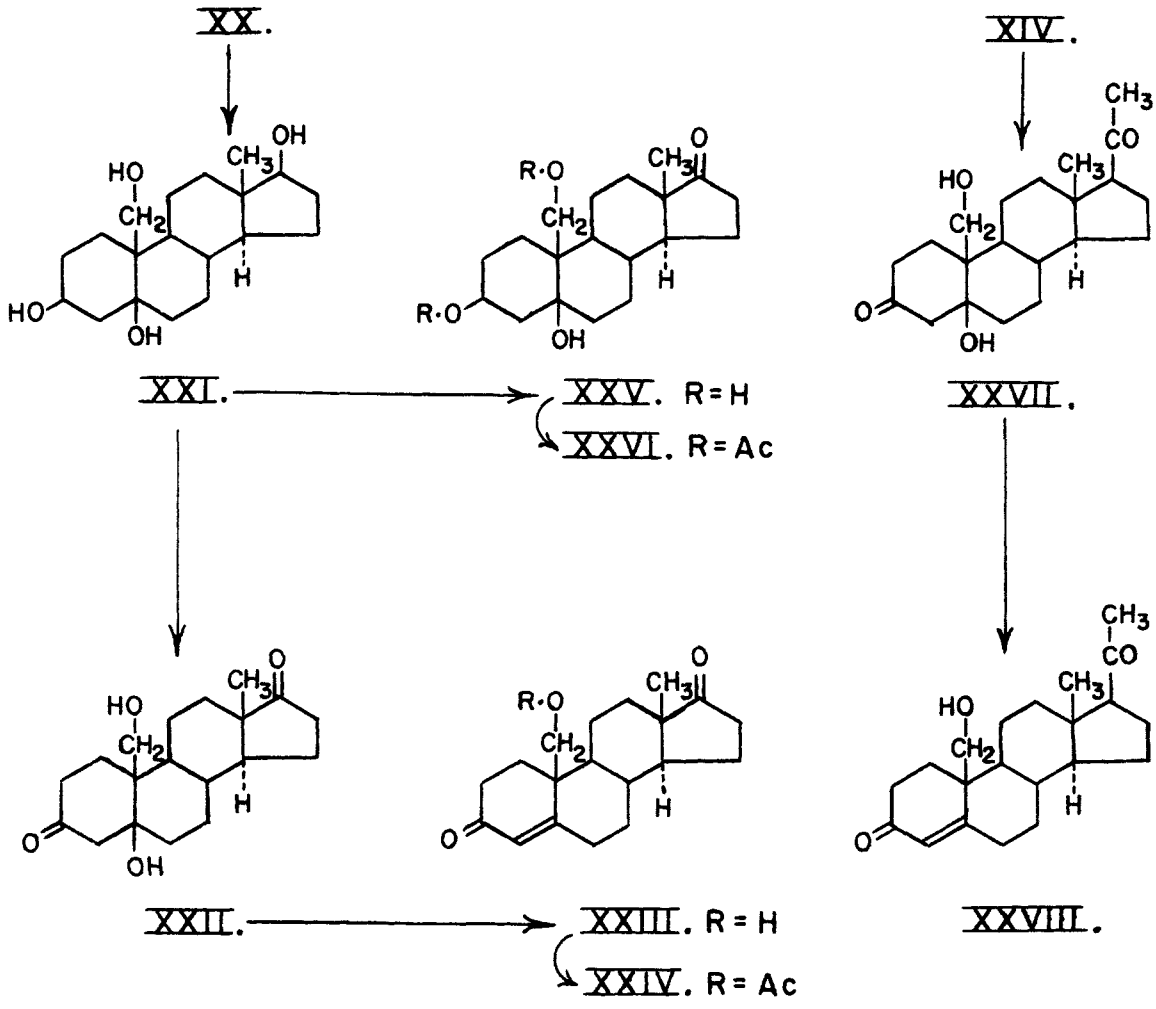
(29) Wieland and Miescher, *Helv. Chim. Acta*, **32**, 1768 (1949).

(30) Heusser, Eichenberger, and Kulkarni, *Helv. Chim. Acta*, **32**, 2145 (1949).

(31) Gallagher and Kritchevsky, *J. Am. Chem. Soc.*, **72**, 882 (1950).

(32) Fukushima and Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951).

(33) Hirschmann, Hirschmann, and Corcoran, *J. Org. Chem.*, **20**, 572 (1955).



side reactions has been pointed out.[30] Because of the availability of 3β,5,19-triacetoxypregnan-20-one (XVI), which possesses no free hydroxyl group, it was decided to subject this compound to treatment with perbenzoic acid. When carried out with 1.35 moles of oxidizing agent, the reaction yielded approximately 80% of 3β,5,17β,19-tetraacetoxyetiocholane (XX).[34] The β-configuration was assigned to the acetoxy group at carbon atom 17 on the basis of evidence presented in the literature, demonstrating that this type of reaction proceeds without inversion.[35] Saponification of XX with methanolic potassium hydroxide at room temperature gave etiocholane-3β,5,17β,19-tetrol (XXI).

Treatment of XXI with 2.4 equivalents of N-bromoacetamide resulted in oxidation of both secondary hydroxyl groups, yielding etiocholane-5,19-diol-3,17-dione (XXII) which was not obtained in a pure state. By treating the crude reaction product with Girard's reagent T, dehydration was achieved furnishing crude 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII). Chromatographic purification yielded, in addition to pure XXIII, a by-product of the oxidation, *viz.* etiocholane-3β,5,19-triol-17-one (XXV). The same compound (XXV) was obtained as the main reaction product on subjecting XXI to oxidation with 1.2 equivalents of N-bromoacetamide, followed by treatment of the material with Girard's reagent T. The monoketone (XXV) was characterized as the 3,19-diacetate (XXVI). The partial oxidation was originally undertaken with the intention of preparing etiocholane-5,17β,19-triol-3-one which by the action of Girard's reagent T should have been dehydrated to 19-hydroxytestosterone. As the experiment shows, preferential oxidation occurs at carbon atom 17 rather than at carbon atom 3. The characteristics of the synthetic 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII) (m. p. 172–173°. $[\alpha]_D^{21.5}$ + 188° λ_{max}^{alc} 242 mμ; ϵ_{max} 16,100) are in reasonable agreement with those reported for the product resulting from the action of adrenal homogenate upon Δ^4-androstene-3,17-dione (m.p. 168–170°. $[\alpha]_D^{30}$ + 178° ± 4°. λ_{max}^{MeOH} 242 mμ; ϵ_{max} 15,200).[11] In particular, the fingerprint re-

(34) In a preliminary experiment, stoichiometric ratios were used. In addition to XX, substantial amounts of starting material (XVI) were isolated.

(35) For the listing of the pertinent literature *c. f. e.g.* ref. 33.

gions (1150–800 cm.$^{-1}$) of the infrared spectra[36] are identical. Acetylation of synthetic XXIII gave 19-acetoxy-Δ^4-androstene-3,17-dione (XXIV) which resisted all attempts at crystallization. This agrees with the observations made on acetylating adreno-synthetic XXIII.[11]

The availability of pregnane-3β,5,19-triol-20-one (XIV) offered an alternate method for the preparation of 19-hydroxyprogesterone (XXVIII).[37] Oxidation of XIV with N-bromoacetamide gave pregnane-5,19-diol-3,20-dione (XXVII) which on treatment with Girard's reagent T underwent dehydration yielding 19-hydroxyprogesterone (XXVIII) (m.p. 173–174°. $[\alpha]_D^{21.5}$ + 184°) which proved to be identical with an authentic sample (m.p. 171–172°. $[\alpha]_D^{24}$ + 185°).[4]

EXPERIMENTAL

Melting points. The m.p.'s were determined with the Fisher-Johns melting point apparatus and are uncorrected.

Absorption spectra. Ultraviolet spectra were determined in 95% ethanol with a Beckman Model DU spectrophotometer. The infrared studies pertaining to this paper were carried out on a Perkin-Elmer Model 21 double beam spectrometer in the Division of Steroid Metabolism of the Sloan-Kettering Institute for Cancer Research through the courtesy of Dr. Thomas F. Gallagher. The interpretation was done by Friederike Herling. The correlations are based upon those summarized in the publication of Jones and Herling.[38]

Only those bands are mentioned which appear to have a direct bearing upon the structure of the particular compound. Details of other correlations between spectrum and structure will be summarized at a later time by the group at the Sloan-Kettering Institute.

Analyses. Unless stated otherwise, the microanalyses were performed by Dr. E. W. D. Huffman, Wheatridge, Colorado, on samples which were dried to constant weight *in vacuo* (P_2O_5; 80°) according to Milner and Sherman.[39] The percentage loss of weight on drying and gain of weight on exposure of the sample to the atmosphere are recorded.

Optical rotations. No corrections for crystal solvent have been made. Unless stated otherwise, the sample was dissolved in chloroform to make 2 cc. of solution and the rotation was determined in a 2-dm. semi-micro tube.

Chromatography. The alumina used as adsorbent for chromatography has been described.[4] Unless stated otherwise, activity III was used.

Oxidation of strophanthidol diacetate (I). To a solution of 9.07 g. of strophanthidol diacetate (I), m.p. 192–194°, in 500 cc. of freshly distilled acetone there was added in a 1 liter bottle 18 g. of finely ground potassium permanganate. The mixture was shaken until the purple color had disappeared (overnight) and, after the combination of three such batches (27.2 g. of I), the volume was reduced *in vacuo* to about 100 cc. Subsequent to the addition of 300 cc. of 10% sulfuric acid, the sludge was extracted with three portions of 600 cc. of ethyl acetate. The combined solutions were washed with water and were then extracted with three portions of *N* sodium carbonate. The carbonate phases were acidified with 10% sulfuric acid and were then re-extracted with ethyl acetate. Evaporation of the solvents gave 8.85 g. of a neutral and 16.95 g. of an acid fraction. In several experiments of the same kind a total of 81.35 g. of I furnished 26.85 g. of neutral and 47.35 g. of acidic material. The neutral material (26.85 g.) was divided into three equal parts which were reoxidized by dissolving in 500 cc. of acetone, adding 5 g. of potassium permanganate and shaking until the purple color had disappeared (overnight). The combined reaction mixtures (1500 cc.) were worked up as described above and thus furnished 19.65 g. of neutral and 5.30 g. of acidic material. Summary of yields, obtained from 81.35 g. of I, after two oxidations: neutral fraction, 19.65 g.; acid fractions, 47.35 g. (1st oxid.), 5.30 g. (2nd oxid.).

3β,19-Diacetoxy-5,14-dihydroxy-20-oxo-14β-pregnane-21-oic acid 21 ⟶ 14-lactone (III). Crystallization of the neutral fraction (19.65 g.; *v. supra*) from methanol-water gave 15.10 g. of stout needles, m.p. 211–213°; no depression of m.p. when mixed with authentic sample of III.[40] Yield of crystalline III, as obtained from I, 19.7%.

3β,5,14,19-Tetrahydroxy-14β-etianic acid (IV). To the acidic material resulting from the first oxidation (47.35 g.; *v. supra*) in 300 cc. of methanol was added a solution of 60 g. of potassium carbonate in 400 cc. of water. This mixture was allowed to stand under nitrogen at room temperature for a period of 16 hours. Then it was made acid to Congo Red by the addition of conc'd hydrochloric acid and was extracted with four portions of 800 cc. of ethyl acetate. After washing with water and drying over sodium sulfate evaporation of the solvent yielded 41.1 g. of a yellow residue. Crystallization from 60 cc. of methanol gave 24.25 g. of colorless cubes; m.p. 224–227°; no depression of m.p. with authentic sample of IV.[13] Additional amounts of IV may be present in the mother liquors. The acidic material resulting from the second oxidation (5.30 g.; *v. supra*) was hydrolyzed in analogous fashion and thus yielded, after crystallization from methanol 2.25 g. of cubes; m.p. 222–224°. Total yield of crystalline IV, as obtained from 81.35 g. of I, 26.50 g., *i.e.* 43.4%.

Dehydration of 3β,5,14,19-tetrahydroxy-14β-etianic acid (IV). Initially, the conditions applied were those of the method published earlier.[41] The yields of the "lactone $C_{20}H_{28}O_4$," now recognized to be ethyl 19-hydroxy-$\Delta^{3,5,14}$-etiatrienate (VIII), and of ethyl 3β,5,19-trihydroxy-Δ^{14}-etienate (V) were in agreement with previous observations, *i.e.* approx. 5% and 35% respectively. On varying the conditions by dissolving 1 part (wt.) of IV in 66 parts (vol.) of 0.1 *N* absolute alcoholic hydrogen chloride and concentrating by heating at atmospheric pressure to about 1/4 of the original volume over a period of 6 hours, the yields of VIII and V were 8.1–10.8% and 38.3–42.2% respectively. The amounts of IV invested in these modified dehydration experiments, varied between 6.4 and 12.7 grams. It is assumed that additional quantities of V and VIII, and also the previously reported compounds VI[18,19] and VII[19] are contained in the mother liquors.

Identification of the "lactone $C_{20}H_{28}O_4$": Ethyl 19-hydroxy-$\Delta^{3,5,14}$-etiatrienate (VIII). A 300 mg. sample of the crude compound (*v. supra*), m.p. 117–118°, was recrystallized five times from methanol-water, yielding 54 mg. of a felt-like mass of needles, m.p. 122–122.5°. No change of m.p. upon further recrystallization from ethanol-water. The compound gave an orange color with tetranitromethane. With conc'd sulfuric acid a brown color resulted which turned violet after a few seconds. $[\alpha]_D^{25}$ −95° (19.99 mg., α −1.90°). λ_{max}^{alc} 234 mμ; ϵ 17,130.

Anal. Calc'd for $C_{22}H_{30}O_3$ (342.46): C, 77.15; H, 8.83. Found: C, 76.93, 77.16; H, 8.87, 8.82; Residue, 0.17, 0; Weight loss, 5.41, 5.50.

(36) *Cf.* ref. 11, p. 99, footnote 1.

(37) Previous method *cf.* references 3 and 4.

(38) Jones and Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(39) Milner and Sherman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 427 (1936).

(40) Barber and Ehrenstein, *J. Org. Chem.*, **16**, 1615 (1951).

(41) Ref. 14, exper. *cf.* p. 273.

The infrared spectrum of VIII[42] was determined in carbon disulfide and in carbon tetrachloride solution. It shows O—H stretching bands at 3640 and 3590 cm.$^{-1}$ (CCl_4) which indicate the presence of the hydroxyl group at C_{19}. There are C—H stretching bands at 3050 cm.$^{-1}$ due to the Δ^{14}-double bond and at 3030 cm.$^{-1}$ (CCl_4) due to the $\Delta^{3,5}$-diene system. Strong absorption at 812 cm.$^{-1}$ (CS_2) is probably due to the $\Delta^{3,5}$-diene system. Carbonyl absorption at 1734 cm.$^{-1}$ (CCl_4) is due to C=O stretching vibration of the ethyl ester group. Bands at 1196 and 1156 cm.$^{-1}$ (CS_2) also indicate the presence of the ethyl ester group. Absorption at 1372 cm.$^{-1}$ (CCl_4) is due to C—H bending vibration of the angular C_{18}-methyl group.

Ethyl 19-acetoxy-$\Delta^{3,5,14}$-etiatrienate (IX). This compound was prepared by acetylation at room temperature of 40.0 mg. of VIII, m.p. 122–122.5°, with acetic anhydride and pyridine. After pouring into ice and working up, crystals of m.p. 134–135° resulted. Recrystallization from methanol-water gave 33 mg. of a felt-like mass of needles, m.p. 134.5–135°. Yield, after five additional recrystallizations, 22.2 mg.; m.p. 136.5–137°. $[\alpha]_D^{21}$ −120° (8.10 mg., α −0.97°). λ_{max}^{alc} 234 mμ; ϵ 20,240.

Anal. Calc'd for $C_{24}H_{34}O_4$ (384.50): C, 74.97; H, 8.39. Found: C, 74.92; H, 8.41.

The infrared spectrum of IX[42] was determined in carbon disulfide and in carbon tetrachloride solution. There is no hydroxyl absorption. The spectrum shows C—H stretching bands at 3050 cm.$^{-1}$ (Δ^{14}-double bond) and 3030 cm.$^{-1}$ ($\Delta^{3,5}$-diene). Carbonyl absorption at 1736 cm.$^{-1}$ (CCl_4) is due to the combined effect of the C=O stretching vibration of the acetate group and the C=O stretching vibration of the ethyl ester group. There are C—H bending bands at 1385 cm.$^{-1}$ due to the acetate methyl group and at 1372 cm.$^{-1}$ (CCl_4) due to the angular C_{18}-methyl group. C—O stretching vibration at 1235 cm.$^{-1}$ (CS_2) confirms the presence of the acetate group. Bands at 1198 and 1157 cm.$^{-1}$ (CS_2) indicate the presence of the ethyl ester function, and a strong band at 812 cm.$^{-1}$ (CS_2) is probably due to the $\Delta^{3,5}$-diene system.

Ethyl 3β,5,19-trihydroxyetianate (X). Ethyl 3β,5,19-trihydroxy-Δ^{14}-etienate (V) was hydrogenated as described earlier.[21] The yield of recrystallized material, m.p. 184–186°, was approximately 80%.

3β,5,19-Trihydroxyetianic acid (XI).[43] The yields were invariably considerably better than reported earlier.[22] In a typical experiment, a solution of 840.4 mg. of pure ethyl 3β,5,19-trihydroxyetianate (X), m.p. 184–186°, in 100 cc. of *N* ethanolic potassium hydroxide was refluxed for 8 hours and then was kept at room temperature overnight (16 hours). After the evaporation of most of the ethanol *in vacuo* and the addition of 100 cc. of water, extracting with ethyl acetate furnished 27 mg. of neutral material. On acidifying the aqueous solution with 6 *N* hydrochloric acid, minute needles separated. After filtering and extracting the filtrate with ethyl acetate, the combined acid material was recrystallized by dissolving it in 20 cc. of methanol and then adding 20 cc. of boiling water; 774.0 mg. of colorless needles, m.p. 280–281°. (Yield: 99.5%). In analogous expts. with 500 mg., 500 mg., 1.257 g., and 350 mg. of X the yields of pure XI were: 89.3%, 95%, 88.4%, and 94.1% respectively.

3β,19-Diacetoxy-5-hydroxyetianic acid (XII). This compound was invariably obtained in pure crystalline form[23] when a procedure was followed in accordance with the following example: A solution of 1.965 g. of pure 3β,5,19-trihydroxyetianic acid (XI), m.p. 278–280°, in 20 cc. of pyridine and 20 cc. of acetic anhydride was kept at room temperature for 16 hours. After the addition of 200 cc. of *N* aqueous hydrochloric acid and 15 minutes' standing at room temperature, the mixture was extracted with ethyl acetate. The extract was washed quickly with 10% sulfuric acid saturated aqueous sodium bicarbonate (sufficient to neutralize mineral and acetic acid), and water. After evaporating the solvent *in vacuo*, the residue was dissolved in 10 cc. of glacial acetic acid and, after the addition of 5 cc. of water, the solution was heated on a water-bath for ½ hour. Finally, 20 cc. of water was added and the mixture was extracted with ethyl acetate. The extract was freed from acetic acid by treatment with a sufficient amount of saturated aqueous sodium bicarbonate and then was washed with water. After drying, the solvent was evaporated and the residue (2.15 g.) was crystallized from ethanol-water; wt. 1.765 g.; m.p. 163–165° (yield: 72.5%). Further recrystallization raised the m.p. to 167–168°. In analogous experiments with 2.490 g., 2.150 g., and 4.975 g. of XI, the yields of crystalline XII were 81.7%, 79.6%, and 82.9% respectively.

3β,19-Diacetoxy-21-diazopregnan-5-ol-20-one (XIII). A mixture of 1.015 g. of 3β,19-diacetoxy-5-hydroxyetianic acid (XII), m.p. 167–168.5°, in 20 cc. of absolute ethanol and of 0.227 g. of sodium bicarbonate in 10 cc. of water was brought to dryness *in vacuo* (30°). The residue was completely freed from moisture by treating it repeatedly, first with absolute ethanol and then with dry benzene (dried over sodium) and evaporating each time to dryness *in vacuo*. The material was suspended in 50 cc. of dry benzene and, after adding 30 drops of pyridine, 5 cc. of oxalyl chloride (Eastman) was poured into the chilled mixture (ice-salt bath). Subsequently the suspension was exposed to room temperature for exactly one minute and then was brought to dryness *in vacuo* (r.t.). The material was repeatedly treated with dry benzene followed by removal of the solvent *in vacuo*. Finally, the residue was taken up in 50 cc. of dry benzene and the suspension was filtered (anhydrous conditions; using pressure of nitrogen tank) through sintered glass dropwise and with continuous swirling into an ethereal solution of diazomethane (prepared from 17.5 g. of nitrosomethylurea and dried over sodium) at 0°. The reaction mixture was subsequently kept at room temperature for ½ hour and then was evaporated to dryness *in vacuo*. The residue (1.038 g.) was chromatographed over 30 g. of alumina (diam. of column: 1.8 cm.). Elution with benzene and benzene-ether, 3:1, gave a total of 0.912 g. of crystalline fractions; m.p. 141–143° (yield of crude XIII: 85.2%). Recrystallization from acetone-hexane furnished 0.815 g. of yellow needles; m.p. 147–147.5° (yield of pure XIII: 76.1%). In another run, 3.605 g. of XII was treated exactly with the proportions of ingredients and under the conditions of the experiment reported above. The yields of XIII were: Crude, 3.30 g., m.p. 141–142° (Yield: 86.8%), recrystallized, 2.83 g., m.p. 147–148° (Yield: 74.4%). Observance of the stated detailed instructions is considered essential. Orienting experiments under slightly different conditions had resulted in inferior yields. The analytical sample was recrystallized four times from methylene chloride-hexane; yellow prisms; m.p. 147–148.5°. $[\alpha]_D^{21}$ +135° (8.95 mg.; α +1.21°).

Anal. Calc'd for $C_{25}H_{36}O_6N_2$ (460.55): C, 65.19; H, 7.88. Found: C, 65.24; H, 7.98.

3β,19-Diacetoxypregnan-5-ol-20-one (XV). *A. From 3β,19-diacetoxy-21-diazopregnan-5-ol-20-one* (XIII). A solution of 445 mg. of XIII, m.p. 146.5–147.5°, in 100 cc. of chloroform was shaken with 22 cc. of 48% hydriodic acid (Baker's Analyzed Reagent) for 1½ minutes. After the addition of 400 cc. of chloroform, the organic layer was washed successively with a saturated solution of potassium iodide, with a solution of sodium thiosulfate and with water. After drying, evaporation of the solvent yielded 437 mg. of a product which was chromatographed over 10 g. of alumina (diam. of column: 1.0 cm.). Benzene (400 cc.) and benzene-ether, 9:1 (200 cc.), eluted 320 mg. of crystals, m.p. range 96–99°, representing crude 3β,19-diacetoxypregnan-5-ol-20-one (XV) (Yield: 76.2%). The later chromatographic fractions (ether, ether-methanol) gave 92 mg. of yellowish resin which did

(42) The fingerprint region is different from any spectrum in the collection of the Sloan-Kettering Institute.

(43) Experiments by Dr. G. Winston Barber.

not crystallize. Recrystallization of the crude XV from methylene chloride-hexane gave 295 mg. of pure XV; colorless needles; m.p. 104–106° (Yield: 70.3%). In subsequent experiments with 210 mg., 446 mg., 287 mg., and 3.25 g. of pure XIII, the yields of XV, m.p. range 102–104°, were 76.2%, 82.9%, 63.5%, and 79.3% respectively. The analytical sample was repeatedly recrystallized from acetone-water; stellate arrangement of needles; m.p. 106–108°. $[\alpha]_D^{21}$ +88° (5.00 mg.; α +0.44°).

Anal. Calc'd for $C_{28}H_{38}O_6$ (434.55): C, 69.09; H, 8.81. Found: C, 69.08; H, 8.69. Residue, 0.5 (Dried at 70°).

B. By acetylation of pregnane-3β,5,19-triol-20-one (XIV). A mixture of 102 mg. of XIV (m.p. 208–209°), 1 cc. of pyridine, and 2 cc. of acetic anhydride was kept at room temperature for 16 hours and then was worked up as usual. The crude reaction product, wt. 112 mg., was recrystallized from acetone-hexane; 101 mg. of needles; m.p. 104–106°. No depression of m.p. when mixed with the authentic sample described under *A*.

Pregnane-3β,5,19-triol-20-one (XIV). *A. By saponification of 3β,19-diacetoxy-5-ol-20-one* (XV). To 200 mg. of XV, m.p. 102–103°, in 30 cc. of methanol was added 30 cc. of aqueous *N* potassium carbonate. The mixture was kept at room temperature for 16 hours and then was extracted with ethyl acetate. The extract was washed with water and dried, and was finally evaporated to dryness *in vacuo*. Repeated recrystallization of the product from methylene chloride-hexane gave 139 mg. of pure pregnane-3β,5,19-triol-20-one (XIV) (Yield: 86.2%); needles and prisms; m.p. 208–209°. $[\alpha]_D^{21}$ +103° (12.4 mg.; α +1.28°).

Anal. Calc'd for $C_{21}H_{34}O_4$ (350.48): C, 71.96; H, 9.78. Found: C, 71.93; H, 9.68.

B. By saponification of 3β,5,19-triacetoxypregnan-20-one (XVI). A solution of 12 mg. of XVI, m.p. 159–160°, in 5 cc. of 1% methanolic potassium hydroxide was allowed to stand at room temperature for 16 hours. After the addition of water, the product was extracted and isolated as usual (*cf.* under *A*); 7 mg. of colorless needles; m.p. 207–208°; no depression of m.p. when mixed with an authentic sample of pregnane-3β,5,19-triol-20-one (XIV) (*cf.* under *A*).

Acetylation of 3β,19-diacetoxypregnan-5-ol-20-one (XV): *A. 3β,5,19-Triacetoxypregnan-20-one* (XVI). A solution of 56 mg. of XV, m.p. 102–103.5°, in 10 cc. of acetic anhydride was gently refluxed under anhydrous conditions for 18 hours. Then it was evaporated to dryness *in vacuo* and the brown product was chromatographed over 3 g. of alumina (diam. of column: 0.8 cm.). Elution with petroleum ether-benzene (9:1, 3:1, and 1:1) furnished 54 mg. of crystalline fractions. Recrystallization from methylene chloride-hexane gave 48 mg. of pure 3β,5,19-triacetoxypregnan-20-one (XVI); needles; m.p. 160–161° (Yield: 78.2%). Results of additional experiments (18 hours reflux; the amounts of XV and acetic anhydride and the yields of XVI are recorded): 25 mg. (5 cc.), 22 mg. (80.2%); 210 mg. (20 cc.), 168 mg. (72.9%); 120 mg. (10 cc.), 72 mg. (54.7%); 172 mg. (10 cc.), 110 mg. (58.3%); 472 mg. (25 cc.), 280 mg. (54.1%); 349 mg. (15 cc.), 175 mg. (45.7%). The analytical sample of XVI was recrystallized twice from methylene chloride-hexane; m.p. 161.5–162°. $[\alpha]_D^{21}$ +75° (8.05 mg.; +0.60°).

The infrared spectrum[44] shows no hydroxyl absorption. Absorption at 1710 cm.$^{-1}$ (20-ketone group), 1740 cm.$^{-1}$ (acetate groups) (CCl_4).

Anal. Calc'd for $C_{27}H_{40}O_7$ (476.59): C, 68.04; H, 8.46. Found: C, 67.76; H, 8.53. Weight loss, 0.72.

B. 3β,5,19-Triacetoxypregnan-20-one (XVI) *and by-products [19-acetoxy-Δ^{3,5}-pregnadien-20-one* (XVIII) *and 3β,19-diacetoxy-Δ^5-pregnen-20-one* (XVII)]. A solution of 2.225 g. of XV, m.p. 102–103°, in 30 cc. of acetic anhydride was gently refluxed for 16 hours and was subsequently evaporated to dryness *in vacuo*. The crude product was chromatographed over 50 g. of alumina (diam. of column: 2.5 cm.; 100-cc. fractions). The following eluates were collected: (a) petroleum ether-benzene, 9:1 (1200 cc.); 0.765 g. of material, soluble in hexane. (b) petroleum ether-benzene, 3:1 (700 cc.); 0.440 g. of product which crystallized from methylene chloride-hexane, m.p. 155–157°. (c) petroleum ether-benzene, 1:1 (500 cc.); 0.315 g. of material which crystallized from methylene chloride-hexane, m.p. 153–155°. (d) benzene (800 cc.); 0.520 g. of yellowish resin which did not crystallize. (e) benzene-ether, 5:1 (500 cc.); 0.055 g. of compound which crystallized on spraying it with hexane; m.p. 99–100°; no depression of m.p. with starting material (XV). (f) ether and ether-methanol combinations; 100 mg. of deep yellow resin.

The combined fractions (b) and (c) (0.755 g.) were recrystallized twice from methylene chloride-hexane; 0.515 g. of colorless needles, m.p. 160.5–162°; identified as 3β,5,19-triacetoxypregnan-20-one (XVI) by determination of the mixture m.p. (Yield: 21.1%). Fraction (a) (0.765 g.) was rechromatographed over 20 g. of alumina (activity II–III; diam. of column: 1.8 cm.; 100-cc. fractions). The eluates were as follows: (a1) petroleum ether-benzene, 9:1 (500 cc.); 0.212 g. of product; m.p. range (100–105°). (a2) petroleum ether-benzene, 9:1 (500 cc.); 0.195 g. of material which resisted all attempts at crystallization. (a3) petroleum ether-benzene, 9:1 (700 cc.); 0.160 g. of colorless resin which crystallized slowly (12 hours); m.p. range 93–100°. (a4) petroleum ether-benzene, 5:1 (300 cc.); 0.168 g. of yellow resin which did not crystallize.

19-Acetoxy-Δ^{3,5}-pregnadien-20-one (XVIII). The product described under (a1) (0.212 g.) was recrystallized 5 times from ethanol-water; 78 mg. of colorless needles; m.p. 115–115.5°. The compound gave a deep orange color with tetranitromethane. $[\alpha]_D^{25}$ −83° (16.8 mg.; α −1.40°). λ_{max}^{alc} 234 mμ; ϵ 19,800.

Anal. Calc'd for $C_{23}H_{32}O_3$ (356.49): C, 77.49; H, 9.05. Found: C, 77.41; H, 9.02. Weight loss, 0.27. (Dried at 70°).

3β,19-Diacetoxy-Δ^5-pregnen-20-one (XVII).[26] The material isolated under (a3) (0.160 g.) was recrystallized 4 times from ethanol-water; 35 mg. of needles, m.p. 104.5–105°. With tetranitromethane a pale yellow color was obtained. No u.v. absorption maximum in the range 220–300 mμ. $[\alpha]_D^{25}$ −21° (11.5 mg.; α −0.24°).

The infrared spectrum of XVII[42] was determined in carbon disulfide and in carbon tetrachloride solution. There is no hydroxyl absorption. The spectrum shows a shoulder band at about 3040 cm.$^{-1}$ which suggests the presence of a double bond. Broad carbonyl absorption at 1745–1737 cm.$^{-1}$ (CCl_4) is due to the combined effect of the C=O stretching vibrations of the acetoxy groups at C_3 and C_{19}. A band at 1706 cm.$^{-1}$ (CCl_4) is due to the 20-ketone group, and a weak C=C stretching band at 1670 cm.$^{-1}$ suggests the presence of a Δ^5 double bond. There are C—H bending bands at 1387 cm.$^{-1}$ due to the angular C_{18}-methyl group and at 1367 cm.$^{-1}$ (CCl_4) due to the acetate methyl groups. A shoulder band at 1357 cm.$^{-1}$ is due to the C_{21}-methyl group in 20-ketosteroids, C—O stretching vibration at about 1232 cm.$^{-1}$ (broad band) (CS_2) confirms the presence of the acetate groups. Bands at 836 and 798 cm.$^{-1}$ (CS_2) indicate a Δ^5-steroid.

Anal. Calc'd for $C_{25}H_{36}O_5$ (416.54): C, 72.08; H, 8.71. Found: C, 71.90; H, 8.86. Residue, 0.13; weight loss, 0.41. (Dried at 60°).

Saponification of 3β,19-diacetoxy-Δ^5-pregnen-20-one (XVII): *3β-Acetoxy-Δ^5-pregnen-19-ol-20-one* (XIX). To 26 mg. of 3β,19-diacetoxy-Δ^5-pregnen-20-one (XVII), m.p. 104.5–105°, in 1 cc. of ethanol was added 5 cc. of aqueous *N* potassium carbonate. The mixture was kept under an atmosphere of nitrogen at room temperature for 16 hours. The saponification product was extracted and isolated in the usual fashion. The material, 21 mg., crystallized from

(44) This spectrum was kindly determined by Dr. Ulrich Schiedt, Department of Biochemistry, School of Medicine, University of Pennsylvania.

methanol-water; m.p. 137–139°. Five recrystallizations from methanol-water gave 11 mg. of needles, m.p. 144–145°. $[\alpha]_D^{25}$ −41° (7.1 mg.; α −0.29°).

The infrared spectrum of XIX[42] was determined in carbon disulfide and in carbon tetrachloride solution. It shows O—H stretching bands at about 3605 cm.$^{-1}$ which indicate the presence of the hydroxyl group at C_{19}. A shoulder band at about 3030 cm.$^{-1}$ suggests the presence of a double bond. Carbonyl absorption at 1745 cm.$^{-1}$ is due to the C=O stretching vibrations of the acetate group, and at 1706 cm.$^{-1}$ (CCl_4) due to the C=O stretching vibrations of the 20-ketone group. A weak C=C stretching band at 1670 cm.$^{-1}$ (CCl_4) suggests the presence of a Δ^5 double bond. C—H bending bands at 1387 cm.$^{-1}$ are due to the angular C_{18}-methyl group and at 1365 cm.$^{-1}$ (CCl_4) due to the acetate methyl group. A shoulder band at 1357 cm.$^{-1}$ is due to the C_{21}-methyl group (in 20-ketosteroids). A broad C—O stretching band at 1229 cm.$^{-1}$ (CS_2) confirms the presence of the acetate group. Bands at 835 and 798 cm.$^{-1}$ indicate a Δ^5 steroid.

Anal. Calc'd for $C_{21}H_{32}O_3$ (diolone) (332.47): C, 75.86; H, 9.70. Calc'd for $C_{23}H_{34}O_4$ (monoacetate) (374.50): C, 73.76; H, 9.15. Found: C, 73.89; H, 8.78.

In the original, mistaken, belief that this substance was Δ^5-pregnen-3β,19-diol-20-one, the product was subjected to the Oppenauer reaction (benzene, acetone, aluminum isopropoxide). The compound was recovered unchanged.

3β,5,17β,19-Tetraacetoxyetiocholane (XX). A total of 201 mg. (1.455 millimoles, *i.e.* 35% excess) of perbenzoic acid in 1.18 cc. of chloroform[45] was added to 515 mg. (1.081 millimoles) of 3β,5,19-triacetoxypregnan-20-one (XVI), m.p. 160.5–162°. The clear mixture was kept in the dark at room temperature for 12 days. After the addition of ethyl acetate, the solution was washed with *N* sodium carbonate and with water. Finally it was dried and the solvent was evaporated *in vacuo*. Crystallization of the obtained resin from methanol-water gave 415 mg. of pure 3β,5,17β,19-tetraacetoxyetiocholane (XX); needles; m.p. 136–137° (Yield: 78.0%). The mother liquor contains additional amounts of XX and also of starting material (XVI). This conclusion is based on a preliminary experiment in which the total reaction product was subjected to a separation by means of Girard's reagent T. In two other, analogous, experiments (duration of reaction: 10 days) with 95 mg. and 110 mg. of XVI, the yields of pure XX, as obtained by direct crystallization (*i.e.* without separation with Girard's reagent T), were 83 mg. (84.5%) and 81 mg. (71.2%) respectively. The analytical sample of XX was recrystallized several times from methanol-water; m.p. 136–138°. $[\alpha]_D^{23}$ +11° (12.5 mg.; α +0.14°).

Anal. Calc'd for $C_{27}H_{40}O_8$ (492.59): C, 65.83; H, 8.19. Found: C, 65.89; H, 8.22 (Dried at 70°).

Etiocholane-3β,5,17β,19-tetrol (XXI). A solution of 83 mg. of 3β,5,17β,19-tetraacetoxyetiocholane (XX), m.p. 135–137°, in 25 cc. of 1% methanolic potassium hydroxide was kept under an atmosphere of nitrogen at room temperature (33°) for 21 hours. Subsequently water was added, the methanol was evaporated *in vacuo* (30°), and the product was extracted with ethyl acetate. After washing the extract with water and drying, evaporation of the solvent gave 55.5 g. of crude etiocholane-3β,5,17β,19-tetrol (XXI); m.p. 203–206° (Yield: 100%). Recrystallization from acetone-hexane gave 48.2 mg. of pure XXI; m.p. 207–208° (Yield: 88.2%). In another saponification, 496 mg. of XX gave 260 mg. of pure XXI (Yield: 79.6%). For analysis the product was repeatedly recrystallized from acetone-hexane; small needles; m.p. 211–212°. $[\alpha]_D^{22}$ +38° (6.4 mg. in 2 cc. of chloroform containing 1 drop of ethanol; α +0.24°).

Anal. Calc'd for $C_{19}H_{32}O_4$ (324.45): C, 70.33; H, 9.94. Found: C, 70.44; H, 9.90.

(45) Gilman and Blatt, *Org. Syntheses*, Coll. Vol. 1, 2nd ed., 431 (1941).

19-Hydroxy-Δ^4-androstene-3,17-dione (XXIII). To 195 mg. of etiocholane-3β,5,17β,19-tetrol (XXI), m.p. 206–207°, in 5 cc. of redistilled *tert*-butanol was added 195 mg. (2.4 equivalents) of *N*-bromoacetamide (freshly recrystallized from chloroform; m.p. 108°) and 1 cc. of water. After keeping the solution at room temperature for 20 hours, 100 cc. of water and 400 mg. of sodium thiosulfate were added and the mixture was extracted with ethyl acetate. From the extract was isolated a crude product (185 mg.) which was crystallized from methylene chloride-hexane furnishing 88 mg. of crystals; m.p. 142–148° (a). Weight of the material contained in the mother liquor: 97 mg. (b).—Purification of the crystalline material (a) [consisting essentially of etiocholane-5,19-diol-3,17-dione (XXII)] by recrystallization failed (fluctuation of melting points). Therefore, the crystalline product (88 mg.) was dissolved in 6 cc. of absolute ethanol and, after the addition of 300 mg. of Girard's reagent T and 0.1 cc. of glacial acetic acid, the solution was refluxed for 1 hour. After the addition of ice, the mixture was extracted with ethyl acetate. The extract yielded 18.5 mg. of non-ketonic material. The aqueous phase was acidified to Congo Red with *N* hydrochloric acid and, after standing at room temperature for 1/2 hour, was extracted with ethyl acetate. Evaporation of the solvent gave 58 mg. of ketonic material.—In a like fashion the material obtained from the mother liquor (b) (97 mg.) was dissolved in 6 cc. of absolute ethanol and, after the addition of 300 mg. of Girard's reagent T and 0.1 cc. of glacial acetic acid, the mixture was treated and worked up as described above. This gave 23.5 mg. of non-ketonic, and 57 mg. of ketonic material.—The ketonic fractions isolated under (a) (58 mg.) and (b) (57 mg.) were separately subjected to chromatographic purification.

The ketonic material (a) (58 mg.) was chromatographed over 3 g. of alumina (diam. of column: 0.9 cm.; 50 cc. fractions). The following eluates were collected: (a1) benzene (200 cc.); 6.5 mg. of resin; soluble in hexane. (a2) benzene-ether combinations and ether eluted only traces of material. (a3) ether-methanol, 200:1 (600 cc.); 36.5 mg. of crystalline fractions; m.p. range 165–172°. (a4) ether-methanol, 100:1 (200 cc.); 9.5 mg. of resin. The combined fractions under (a3) (36.5 mg.) were recrystallized three times from acetone-hexane; 25.5 mg. of needles, representing pure 19-hydroxy-Δ^4-androstene-3,17-dione (XXIII); m.p. 172–173°. $[\alpha]_D^{21.5}$ +188° (14.4 mg.; α +2.71°). λ_{max}^{alc} 242 mμ; ϵ 16,100.

The infrared spectrum of XXIII was determined in chloroform solution. It shows O—H stretching bands at 3625 cm.$^{-1}$ and at 3450 cm.$^{-1}$ which indicate the presence of the hydroxyl group at C_{19}. Carbonyl absorption at 1736 cm.$^{-1}$ is due to the 17-ketone group, and bands at 1665 and 1617 cm.$^{-1}$ are due to the Δ^4-3-ketone system. The fingerprint region (1150–800 cm.$^{-1}$) is identical with the spectrum of the adrenosynthetic 19-hydroxy-Δ^4-androstene-3,17-dione reported by A. S. Meyer,[35] examined in chloroform solution by Dr. R. Norman Jones.

Anal. Calc'd for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.27; H, 9.03.

The ketonic material (b) (57 mg.) was chromatographed under the conditions given in the preceding chromatogram. Eluates: (b1) benzene (200 cc.); 5.5 mg.; soluble in hexane. (b2) benzene-ether and ether gave practically empty eluates. (b3) ether-methanol, 200:1 (550 cc.); 38.5 mg. of crystalline fractions, m.p. range 166–172°. (b4) ether-methanol, 100:1 (100 cc.); 3.5 mg.; yellowish resin; no crystallization. (b5) ether-methanol, 100:1 (150 cc.); 9.3 mg.; yellowish resin; from methylene chloride-hexane: needles, m.p. range 199–203°. (b6) ether-methanol, 100:1 (50 cc.); yellowish resin; no recrystallization. The combined fractions under (b3) (38.5 mg.) were recrystallized from methylene chloride-hexane; 30.5 mg. of needles; m.p. 170–172°; identified as XXIII (mixture m.p. with analytical sample).

The non-ketonic material isolated under (a) and (b) (total: 42 mg.), which should have consisted largely of unchanged XXI, was re-oxidized with 2.4 equivalents of N-

bromoacetamide. After treating the reaction product with Girard's reagent T and working up as described, unexpectedly only 1.6 mg. of XXIII was isolated.

The total yield of fairly pure XXIII (m.p. range 165–172°) from 195 mg. of XXI was 76.6 mg. (42.2%).

The material under (b5) (9.3 mg.) was recrystallized from methylene chloride-hexane; 3.5 mg. of needles; m.p. 208–210°; identified as etiocholane-3β,5,19-triol-17-one (XXV) (mixture m.p. with authentic sample; *v. infra*).

19-Acetoxy-Δ⁴-androstene-3,17-dione (XXIV). A solution of 17.5 mg. of synthetic 19-hydroxy-Δ⁴-androstene-3,17-dione (XXIII), m.p. 172–173°, in 0.5 cc. of pyridine and 1 cc. of acetic anhydride was kept at room temperature overnight and then was evaporated to dryness *in vacuo* (40°). The product was chromatographed[43] over 2 g. of alumina (activity I–II; diam. of column: 0.8 cm.; 50-cc. fractions.) The major part of the material (13.0 mg.) was eluted with benzene; wt. of the peak fractions 6,7, and 8: 3.2 mg., 2.0 mg., and 2.3 mg. respectively. These fractions resisted all attempts at crystallization. λ_{max}^{alc} (fraction 6) 237.5 mμ. (A. S. Meyer[11] reports for the acetylation product of adrenosynthetic XXIII: λ_{max}^{alc} 239 mμ).

The infrared spectra of fractions 6 and 7 were determined in carbon disulfide and in carbon tetrachloride solution. The two samples show identical spectra.[42] There is no hydroxyl absorption. The spectra show a C—H stretching band at 3020 cm.$^{-1}$ (CS_2) which is due to the presence of the double bond. Carbonyl absorption at 1744 cm.$^{-1}$ (CCl_4) is due to the combined effect of the C=O stretching vibrations of the acetate group and the 17-ketone group, and at 1680 cm.$^{-1}$ due to the carbonyl group of the Δ⁴-3-ketone system. A C=C stretching band at 1623 cm.$^{-1}$ (CCl_4) is evidence for the Δ⁴ double bond as seen in Δ⁴-3-ketone systems. Absorption at 1418 cm.$^{-1}$ (CCl_4) is due to the scissoring vibrations of the unsubstituted C_2-methylene group next to the 3-ketone group and a band at 1407 cm.$^{-1}$ is due to the C_{16}-methylene group adjacent to the 17-ketone group. A broad C—O stretching band at 1230 cm.$^{-1}$ (CS_2) confirms the presence of the acetate group.

Etiocholane-3β,5,19-triol-17-one (XXV). To 65 mg. of etiocholane-3β,5,17β,19-tetrol (XXI), m.p. 206–207°, in 1.5 cc. of redistilled *tert*-butanol was added 32 mg. (1.2 equivalents) of N-bromoacetamide (freshly recrystallized) and 0.3 cc. of water. After 3 hours' standing at room temperature (25°), the reaction mixture was worked up in the usual fashion (*cf.* preceding expt.). The crude product, 59 mg., was separated into non-ketonic and ketonic material (2.5 cc. of ethanol, 125 mg. of Girard's reagent T, 0.04 cc. of glacial acetic acid). Result: non-ketonic, 12 mg.; ketonic, 43 mg. The ketonic part crystallized from methylene chloride-hexane; 31 mg. of needles, representing etiocholane-3β,5,19-triol-17-one (XXV); m.p. 205–208° (Yield: 48.0%). Three recrystallizations gave 23 mg. of needles, m.p. 208–210°. $[\alpha]_D^{21.5}$ +108° (8.5 mg.; +0.92°). No ultraviolet absorption (220–300 mμ) before and after heating substance with glacial acetic acid on the water-bath for ½ hour.

The infrared spectrum of XXV[42] was determined in chloroform solution. It shows O—H stretching bands at 3610 cm.$^{-1}$ and broad bands at about 3400 cm.$^{-1}$ which are due to the presence of associated hydroxyl groups. Carbonyl absorption at 1736 cm.$^{-1}$ is evidence for the presence of a 17-ketone group.

Anal. Calc'd for $C_{19}H_{30}O_4$ (322.43): C, 70.77; H, 9.37. Found: C, 70.71; H, 9.39.

3β,19-Diacetoxyetiocholan-5-ol-17-one (XXVI). A solution of 9.8 mg. of etiocholane-3β,5,19-triol-17-one (XXV), m.p. 208–210°, in 0.5 cc. of pyridine and 1 cc. of acetic anhydride was kept at room temperature overnight. After evaporation to dryness *in vacuo* (40°), the crude product was recrystallized twice from methylene chloride-hexane; stellate arrangement of needles (8.2 mg.); m.p. 180–182°. $[\alpha]_D^{21.5}$ +89° (8.0 mg.; α +0.71°).

The infrared spectrum of XXVI[42] was determined in carbon disulfide and in carbon tetrachloride solution. It shows O—H stretching bands at 3590 and 3460 cm.$^{-1}$ (weak band). Absorption at 1744 cm.$^{-1}$ (CCl_4) is due to the combined effects of the C=O stretching vibrations of the 17-ketone group and the acetate groups. A C—H bending band at 1407 cm.$^{-1}$ (CCl_4) is due to scissoring vibrations of the unsubstituted C_{16}-methylene group adjacent to the 17-ketone group. C—O stretching bands at 1239 and 1222 cm.$^{-1}$ (CS_2) confirm the presence of the acetate groups.

Anal. Calc'd for $C_{23}H_{34}O_6$ (406.50): C, 67.95; H, 8.43. Found: C, 68.59; H, 8.72. Weight loss, 0.64.

Pregnane-5,19-diol-3,20-dione (XXVII). To 139 mg. of pregnane-3β,5,19-triol-20-one (XIV), m.p. 208–209°, in 4 cc. of redistilled *tert*-butanol was added 80 mg. (1.6 equivalents) of N-bromoacetamide (freshly recrystallized) and 0.5 cc. of water. After keeping the mixture at room temperature for 16 hours, there was added water and an amount of solid sodium thiosulfate sufficient to cause decolorization. The product was extracted with ethyl acetate and isolated as usual. Repeated recrystallization of the crude material (135 mg.) from methylene chloride-hexane gave 105 mg. of pregnane-5,19-diol-3,20-dione (Yield: 76.0%); needles; m.p. 198–201° (sintering above 192°). $[\alpha]_D^{21}$ +107° (10.3 mg.; α +1.10°).

Anal. Calc'd for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26. Found: C, 72.07; H, 9.39. Weight loss, 0.31.

19-Hydroxyprogesterone (XXVIII). To 84 mg. of pregnane-5,19-diol-3,20-dione (XXVII), m.p. 198–201°, in 3 cc. of absolute ethanol was added 150 mg. of Girard's reagent T and 0.1 cc. of glacial acetic acid. After refluxing the solution for 1 hour, 10 cc. of *N* hydrochloric acid was added and the mixture was kept at room temperature for 40 minutes. Subsequent extraction with ethyl acetate gave 79 mg. of crude 19-hydroxyprogesterone (XXVIII), m.p. 168–169°. Recrystallization from methylene chloride-hexane furnished 57 mg. of needles; m.p. 170.5–171.5° (Yield: 71.6%). The analytical sample was recrystallized 3 times from acetone-water; shiny plates; m.p. 173–174°. No depression of mixture m.p. with an authentic sample.[4] $[\alpha]_D^{21.5}$ +184° (10.0 mg.; α +1.84°).

Anal. Calc'd for $C_{21}H_{30}O_3$ (330.45): C, 76.32; H, 9.15. Found: C, 76.71; H, 9.54. Weight loss, 0.33.

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