[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XLVIII. Four Routes to Cortisone Acetate from Gentrogenin Acetate[2a,b]

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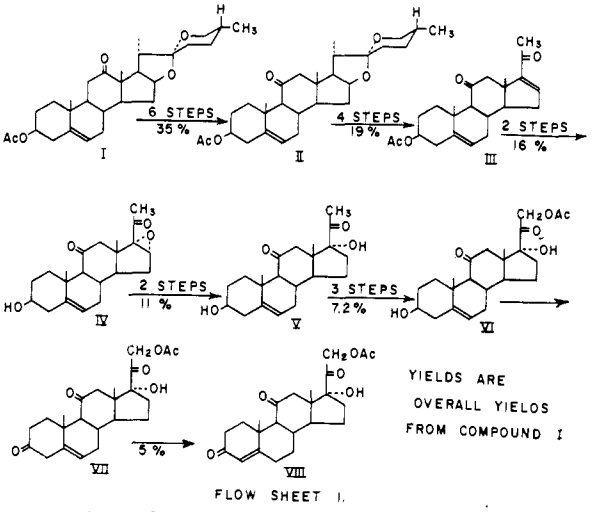
Side chain degradation of 11-keto diosgenin acetate (II) gave the key intermediate 3β-acetoxy-5,16-pregnadiene-11,20-dione (III), which was converted by conventional methods to 21-acetoxy-3β,17α-dihydroxy-5-pregnen-11,20-dione (VI). The latter compound after oxidation with chromium trioxide in acetone gave the Δ^5-3-ketone isomer of cortisone acetate which on equilibration with acid or base catalyst isomerized to cortisone acetate. Several alternate routes to cortisone acetate are also presented.

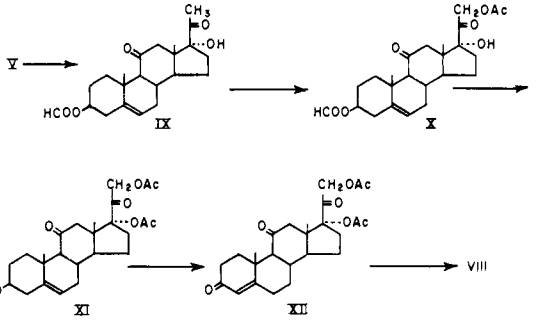
For several years we have been carrying out researches in the field of steroidal sapogenins, particularly the C-ring oxygenated sapogenins hecogenin and the more recently discovered gentrogenin, with the view in mind of testing their potential usefulness for cortisone synthesis.[3] In previous papers we have described the discovery,[4] isolation and properties of gentrogenin, certain of its reactions and degradation products[5] and the



FLOW SHEET I.

conversion of gentrogenin and its C-25 epimer to 11-ketodiosgenin and 11-ketoyamogenin.[6] In this paper we wish to present laboratory scale results showing two completed conversions of gentrogenin to cortisone and two formal syntheses carried to the 11α-hydroxyprogesterone and 16α,17α-epoxy-11α-hydroxyprogesterone stage.

The conversion of gentrogenin acetate (I) to 11-ketodiosgenin acetate (II) was effected in six steps in 35 to 48% yield as previously described.[6] The subsequent standard method side chain degradation[7] of the 11-keto sapogenin (II) required four further steps and gave a 55% yield of 3β-acetoxy-5,16-pregnadiene-11,20-dione (III) (19.3% yield from I). Introduction of the 17α-hydroxyl group by Julian's method of hydrogen peroxide epoxidation, hydrogen bromide oxirane opening and Raney nickel dehalogenation[8] led to a 69% yield (11% from I) of 3β,17α-dihydroxy-5-pregnene-11,20-dione (V).



FLOW SHEET 2

This compound was converted to cortisone acetate by two independent routes. The method we first tried (see flow sheet 2) paralleled the experiments of Ringold, *et al.*,[9] in the preparation of Reichstein's Substance S. This procedure involved formylation of the 3β-hydroxyl group, introduction of the 21-acetoxyl group by brominative procedures followed by the protective acetylation of the 17α-hydroxyl group. The 17α-hydroxyl acetylation reaction was necessary in order to stabilize the side chain and prevent D-homo rearrangement during Oppenauer oxidation of the 3-formate-5-ene system. In our hands this 17α-acetylation step worked badly and gave rise to much destruction of material and produc-

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) (a) Presented at the 134th National Meeting of the American Chemical Society, Chicago, Ill., September 7–12, 1958; (b) paper XLVII, Kenney, *et al.*, THIS JOURNAL, **80**, 5568 (1958).

(3) C. Djerassi, H. J. Ringold and G. Rosenkranz, *ibid.*, **73**, 5513 (1951), have reported a cortisone synthesis from hecogenin by a route whose key step involved bismuth oxide oxidation of a C-ring ketol. More recently significant improvements in the utilization of hecogenin have been made by the Glaxo group. *Cf.* J. Elks, G. H. Phillipps, T. Walker and L. J. Wyman, *J. Chem. Soc.*, 4330 (1956); J. H. Chapman, J. Elks, G. H. Phillips and L. J. Wyman, *ibid.*, 4344 (1956); R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, A. G. Long, J. F. Oughton, L. Stephanson, T. Walker and B. M. Wilson, *ibid.*, 4356 (1956).

(4) H. A. Walens, S. Serota and M. E. Wall, THIS JOURNAL, **77**, 5196 (1955); *J. Org. Chem.*, **22**, 182 (1957); M. E. Wall, J. J. Willaman, T. Perlstein, D. S. Correll and H. S. Gentry, *J. Am. Pharm., Sci. Ed.*, **46**, 155 (1957).

(5) E. S. Rothman and M. E. Wall, *J. Org. Chem.*, **22**, 223 (1957).

(6) (a) E. S. Rothman and M. E. Wall, THIS JOURNAL, **79**, 3228 (1957). (b) The step of conversion of 5,6,11,23?-tetrabromohecogenin acetate to 11,23?-dibromogentrogenin acetate has been improved over that reported in reference 6a to give an 86% yield by modifying the work-up procedure. The *cooled* reaction mixture is diluted with an equal volume of water and the crystalline product is directly filtered off and washed with dilute sodium iodide and water.

(7) (a) R. E. Marker, *et al.*, *inter alia*, *ibid.*, **62**, 3350 (1940); (b) M. E. Wall, H. E. Kenney and E. S. Rothman, *ibid.*, **77**, 5665 (1955); (c) M. E. Wall and S. Serota, *ibid.*, **79**, 6481 (1957).

(8) P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller, *ibid.*, **72**, 5145 (1950).

(9) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *ibid.*, **78**, 820 (1956).

tion of highly colored products seriously affecting the eventual yield of cortisone.

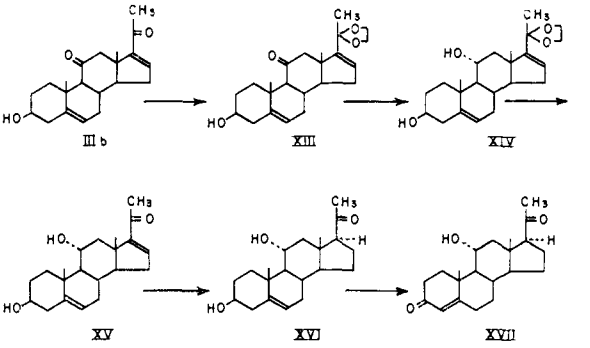
Our improved route from 3β,17α-dihydroxy-5-pregnene-11,20-dione (V), (see flow sheet 1) avoided this 17α-hydroxyl acetylation step and gave cortisone acetate in five steps in 35-55% yield (5 to 7% yield from I for nineteen reaction steps.) The 21-acetoxyl group was introduced as before by brominative procedures starting with the free 3β-hydroxyl compound V to give 21-acetoxy-3β,17α-dihydroxy-5-pregnene-11,20-dione (VI) in 55% yield for the three steps for bromination, reaction with sodium iodide and metathetical acetoxylation.

It was observed, after addition of the first molar equivalent of bromine, that infrared unsaturation bands near 830 cm.$^{-1}$ survived. This observation suggests that substitutive bromination at C-21 occurred, at least in part, prior to addition at C5-C6. These bands disappeared completely after addition of the second equivalent of bromine. In the preceding route where the corresponding 3-formate (IX), *vide supra*, was treated with a single molecular equivalent of bromine, isolation at a later stage of 21-acetoxylated material (X) again indicated that the substitution reaction occurred, at least in part, preferentially over the addition raection.

Djerassi, Engle and Bowers[10] had shown that the use of chromium trioxide–acetone–sulfuric acid reagent[11] converted Δ^5-3β-hydroxy steroids to Δ^5-3-ketones. These workers had not submitted cortical side chain steroids to oxidative attack by this reagent, but we found that cortisone acetate itself was completely unaffected when so treated. We did not observe any D-homo rearrangement, oxidative side chain fission or 21-aldehyde formation. On this basis we subjected 21-acetoxy-3β,-17α-dihydroxy-5-pregnene-11,20-dione (VI) to the chromium trioxide–acetone reagent and obtained a very high yield of a cortisone acetate unsaturation isomer, namely 21-acetoxy-17α-hydroxy-5-pregnene-3,11,20-trione (VII). Isomerization of this compound with a trace of ammonium hydroxide in methanol caused a shift of the double bond into conjugation with the 3-keto group to produce cortisone acetate.

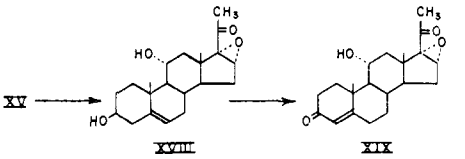
In addition to the above syntheses of cortisone from gentrogenin acetate, we have prepared from this source two 11α-hydroxylated derivatives of progesterone. These latter compounds had previously been prepared from C-ring desoxy steroids by microbiological[12a,b] or chemical[13] procedures and subsequently converted to cortisone.[14a,b,15]

In one such conversion, 3β-hydroxy-5,16-pregnadiene-11,20-dione (IIIb), m.p. 224–228°, $\alpha^{25}D$ −9° (flow sheet 3) was converted to the 20-dioxolane XIII. The 20-dioxolane XIII was reduced with sodium in ethanol to form 3β,11α-dihydroxy-5,16-pregnadiene-20-one 20-dioxolane[16] (XIV).



FLOW SHEET 3

Removal of the 20-dioxolane group of XIV produced 3β,11α-dihydroxy-5,16-pregnadiene-20-one (XV).[17] Palladium-catalyzed hydrogenation of XV to form the monoketone XVI followed by Oppenauer oxidation gave 11α-hydroxyprogesterone (XVII). This compound has been previously obtained by direct microbiological oxidation of progesterone[12a] or by chemical methods[13] and subsequently converted to cortisone.[14a,b] In a second such conversion (see flow sheet 4) 3β,11α-dihydroxy-5,16-pregnadiene-20-one (XV) was ep-



FLOW SHEET 4

oxidized with alkaline hydrogen peroxide to form 3β,11α - dihydroxy - 16α,17α - epoxy - 5 - pregnene-20-one (XVIII). Oppenauer oxidation of XVIII gave 16α,17α-epoxy-11α-hydroxy-4-pregnene-3,20-dione (XIX) ("11α-hydroxy-16α,17α-oxidoprogesterone"), identical with the material produced by microbiological hydroxylation of 16α,17α-epoxy-4-pregnene-3,20-dione[12b] and whose conversion to cortisone was described by Ercoli and Ruggieri.[15]

(10) C. Djerassi, R. R. Engle and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(11) This reagent was first used by the Manchester group for acetylenic carbinol and eburicoic acid oxidation studies. See, for example, K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946); P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch and G. W. Wood, *ibid.*, 2402 (1951); R. G. Curtis, I. Heilbron, E. R. H. Jones and G. F. Woods, *ibid.*, 457 (1953); A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *ibid.*, 2548 (1953); T. G. Halsall, R. Hodges and E. R. H. Jones, *ibid.*, 3019 (1953).

(12) (a) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, THIS JOURNAL, **74**, 5933 (1952); (b) D. H. Peterson, P. D. Meister, A. Weintraub, L. M. Reineke, S. H. Eppstein, H. C. Murray and H. M. Leigh Osborn, *ibid.*, **77**, 4428 (1955).

(13) O. Mancera, J. Romo, F. Sondheimer, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **17**, 1066 (1952).

(14) (a) O. Mancera, H. J. Ringold, C. Djerassi, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **75**, 1286 (1953); (b) J. A. Hogg, P. F. Beal, A. H. Nathan, F. H. Lincoln, W. P. Schneider, B. J. Magerlein, A. R. Hanze and R. W. Jackson, *ibid.*, **77**, 4436 (1955).

(15) A. Ercoli and P. de Ruggieri, *Gazz. chim. ital.*, **85**, 628, 1304 (1955).

(16) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953), used similar dioxolane protection of carbonyl groups to transform the 11-keto group of cortisone alcohol to Kendall's Compound F by means of lithium aluminum hydride reduction.

(17) The diacetate of this compound was identical with the product obtained by direct degradation of 11α-hydroxydiosgenin or 11α-hydroxyyamogenin.

Experimental

Melting points were determined on the Kofler block but are otherwise not corrected. Optical rotations were determined in chloroform, unless otherwise noted, in a 2-dm. tube using a solution of 25 mg. of steroid in 1.5 ml. of solvent. Infrared measurements were determined with a Perkin-Elmer model 21 instrument using 1.5 to 2.5 mg. of steroid in 0.35 g. of KBr; 10 mg. of steroid in 1 ml. of CS_2, 1-mm. cell; or 25 mg. of steroid in 1 ml. of $CHCl_3$, 0.5-mm. cell.

Degradation of 11-Keto diosgenin Acetate; 3β-Acetoxy-5,16-pregnadiene-11,20-dione (III).—In a 250-ml. standard-taper, round-bottomed flask fitted with a wired-on gas-tight ground glass stopper[7a] were placed 14.5 g. of I, 37 ml. of acetic anhydride and a trace of acetic acid. The flask contents after heating for four hours at 180° were cooled, diluted with 74 ml. of acetic acid and 6 ml. of water. Thereafter the conditions of procedure A, ref. 7b, were followed to give III, 6.16 g., 49%, m.p. 183–186°, needles from hexane transforming to spicules on heating, $[\alpha]^{25}D$ −1.7°; λ_{max}^{MeOH} 234 mμ, log ε 3.93. The mother liquors from this preparation were also used since they gave an additional 6% of crystalline 16α,17α-epoxide IV when treated as below. The total yield was therefore considered to be 55%.

Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.36; H, 8.33.

16α,17α-Epoxy-3β-hydroxy-5-pregnene-11,20-dione (IV).—A 5.95-g. sample of the preceding preparation III was dissolved in 800 ml. of methanol at 10°, 5 ml. of 30% hydrogen peroxide and 2.3 ml. of 4 *N* sodium hydroxide were added and the mixture was stored overnight. A crystalline water-insoluble precipitate formed and was filtered off. The filtrate was diluted with an equal volume of water and a new crop of crystals formed and were filtered off. The filtrate was neutralized with hydrochloric acid and was well extracted with methylene chloride which was evaporated to give a glassy residue. To simplify the isolation procedures all solids were combined and converted to acetates by dissolving in 30 ml. of pyridine and 10 ml. of acetic anhydride and heating 0.75 hour on the steam-bath. On dilution with water and isolation by ether extraction, 5.1 g. of ethanol-recrystallized product was obtained (84% yield). The product was freed of 100 mg. of an unidentified extremely insoluble material (m.p. 318°) by solution in ethyl acetate, filtering and crystallizing by evaporation to dryness. The product, 3β-acetoxy-16α,17α-epoxy-5-pregnene-11,20-dione, melted from 205–206° forming dense polyhedra undergoing transition on the Kofler block to refractile, tetragonal prisms, $[\alpha]^{25}D$ +14.8.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.46; H, 8.08.

Saponification of the intermediate acetate with 5% methanolic potassium hydroxide gave 4.46 g. of 16α,17α-epoxy-3β-hydroxy-5-pregnene-11,20-dione (IV), m.p. 111° to about 114° when crystallized from acetone or ethanol. Perhaps the molecule thus crystallized is solvated but the lower indicated melting temperature is characteristic.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.98; H, 8.01.

3β,17α-Dihydroxy-5-pregnene-11,20-dione (V).—To a solution of 2.22 g. of 16α,17α-epoxy-5-pregnene-11,20-dione (IV) in 22 ml. of acetic acid was added 4.3 ml. of a 40% solution of hydrogen bromide in acetic acid. After standing for 25 minutes the reaction mixture was poured into 140 ml. of cold water and the precipitated bromohydrin was filtered off and air-dried. Ten grams of Raney nickel was heated with 100 ml. of refluxing acetone. Ten ml. of water, 2 ml. of acetic acid and the crude bromohydrin were added, and stirring and heating under reflux were maintained for 4 hours. The acetone solution was decanted and evaporated to dryness *in vacuo*. Crystallization of the residue from methylene chloride and from ethyl acetate gave 1.57 g. (71% yield) of 3β,17α-dihydroxy-5-pregnene-11,20-dione (V). The compound formed dense polyhedral crystals, m.p. 277–278° after transition over 220° to lance forms $[\alpha]^{25}D$ +6° (MeOH).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.41; H, 8.69.

3β-Formoxy-17α-hydroxy-5-pregnene-11,20-dione (IX).—The diol V of the preceding preparation, 1.45 g., was dissolved in 35 ml. of 90% formic acid and heated to 65° for two hours. The solution was poured into a large volume of cold water containing sodium chloride and sodium formate and the precipitated solids were filtered off and washed with water. Recrystallization from methanol gave 3β-formoxy-17α-hydroxy-5-pregnene-11,20-dione (IX), as thick scales. The compound melted from 255 to 260°, after transition on the Kofler block to overlapping thin scales covering the entire field. The yield was 80%. The infrared spectrum showed (KBr disk) bands at 3440 (17α-hydroxyl), 1693 (C-20-bonded carbonyl), 1710 (11-carbonyl), 1725 (formate ester carbonyl), 1180 (formate ester) and 813 cm.$^{-1}$ (C-5 olefin).

21-Acetoxy-3β-formoxy-17α-hydroxy-5-pregnene-11,20-dione (X).—The formate IX (1.253 g., 0.00334 mole) was dissolved in 25 ml. of dry methylene chloride and the solution was treated with a solution of bromine (0.00334 mole) in 13.32 ml. of methylene chloride. The volume was reduced by distillation *in vacuo* at 30°; the solution was washed with 1% sodium hydrogen carbonate solution; and the solvents removed *in vacuo*. The residue was dissolved in 25 ml. of acetone, and 2 g. of sodium iodide was added. The mixture was let stand overnight and was then poured into a cold solution of 2 g. of sodium thiosulfate in 70 ml. of water. The steroid was filtered off and dried *in vacuo* at room temperature. The crude iodo compound was dissolved in 25 ml. of dry acetone, 2.5 g. of potassium acetate was added and the mixture was heated under reflux and with stirring for 18 hours. The reaction mixture was cooled, poured into water and the steroid was isolated by extraction with ether. Evaporation of the ether *in vacuo* gave a colorless solid foam. Chromatography of this material with chloroform elution gave only 540 mg. of the desired crystalline 21-acetoxy-3β-formoxy-17α-hydroxy-5-pregnene-11,20-dione (X), m.p. 192.5–193.5°, red melt, $[\alpha]^{25}D$ +20.8°, but the colorless glassy residues eluted just after the crystalline material gave 100 mg. of the 17α-acetoxyl derivative XI when treated as below.

Anal. Calcd. for $C_{24}H_{32}O_7$: C, 66.65; H, 7.45. Found: C, 66.41; H, 7.44.

17α,21-Diacetoxy-3β-formoxy-5-pregnene-11,20-dione (XI).—The 17α-ol X, 99 mg., in 0.71 ml. of acetic anhydride was treated with 31.4 mg. of *p*-toluenesulfonic acid at room temperature overnight. On decomposing with water an amorphous solid was obtained which on chromatography on Florisil gave 80 mg. of crystallizable material. The earlier benzene-eluted 17α,21-diacetoxy-3β-formoxy-5-pregnene-11,20-dione (XI), $[\alpha]^{25}D$ +40° melts with decomposition from 228 to 229°, occasionally from 233 to 235°, and shows no hydroxyl band in the infrared spectrum, a broad multiple ketonic band and a series of ester bands at 1237, 1250 and 1260 cm.$^{-1}$.

Anal. Calcd. for $C_{26}H_{34}O_8$: C, 65.80; H, 7.22. Found: C, 65.50; H, 7.21.

The chloroform eluate gave 17α,21-diacetoxy-3β-hydroxy-5-pregnene-11,20-dione, m.p. 203–205°, $[\alpha]^{25}D$ +83° showing infrared hydroxyl bands at 3410 and 3500 cm.$^{-1}$ (KBr disk), broad ketone bands centered at 1705 and 1731 cm.$^{-1}$ and a strong, sharp ester band centered at 1258 cm.$^{-1}$ with a small band at 1245 cm.$^{-1}$. To augment the yield of cortisone diacetate non-crystallizable mother liquor residues were saved and carried through the Oppenauer oxidation reaction indicated in part b of the cortisone diacetate preparation.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 67.02; H, 7.60.

Cortisone 17α,21-Diacetate and Cortisone.—(a) Two hundred milligrams of crystalline, 17α,21-diacetoxy-3β-formoxy-5-pregnene-11,20-dione (XI) in 20 ml. of dry toluene, 200 mg. of aluminum isopropoxide and 2 ml. of cyclohexanone were refluxed for 45 minutes. Water, 100 ml., was added and the two-phase mixture was boiled until all organic solvents had been carried off in the vapors. The residue was collected as a pasty mass by decantation of the water. Crystallization from methanol gave a 75% yield of cortisone 17α,21-diacetate (XII).

(b) Eight hundred milligrams of the uncrystallized mother liquor residue from the above preparation consisting essentially of a mixture of the 3-hydroxy and 3-formoxyl compounds was dissolved in 25 ml. of dry toluene. Eight ml. of cyclohexanone and 840 mg. of aluminum isopropoxide were added and the mixture was refluxed for 0.75 hour, cooled and water was added. The contents of the flask were heated

until organic solvents were lost, and the gummy solid was collected by extraction with ether. Evaporation of the ether *in vacuo* and crystallization of the residue from methanol gave 400 mg. of cortisone 17α,21-diacetate (XII), m.p. 220–221°, λ_{max}^{MeOH} 238 mμ, ϵ 15,000, identical with an authentic specimen. Hydrolysis of cortisone diacetate in 5% sodium hydroxide in methanol gave an 80% yield of cortisone, identical with an authentic specimen.[18]

21-Acetoxy-3β,17α-dihydroxy-5-pregnene-11,20-dione (VI).—Five grams of 3β,17α-dihydroxy-5-pregnene-11,20-dione (V) was dispersed in 300 ml. of chloroform and, during the course of 1.5 hours, one molar equivalent of bromine in 61.35 ml. of carbon tetrachloride was added. At this point the entire quantity of steroid had passed into solution. It was sometimes necessary to add a little more chloroform in reactions where reprecipitation occurred or where turbidity developed. A second molar equivalent of bromine was added although it was taken up with some reluctance. At the end of the bromination solvents were removed *in vacuo*. The residue was dissolved in a liter of acetone and treated with 20 g. of sodium iodide with stirring at room temperature overnight. The steroids were isolated with ether and freed of iodine by very cautious treatment with a minimum of sodium thiosulfate solution. The solvents were again removed *in vacuo* and the steroids dissolved in a liter of acetone containing 4 ml. of acetic acid and 20 g. of dry potassium acetate. The mixture was stirred at reflux for 12 hours and again steroidal matter was isolated with ether.[19] The product was recrystallized from aqueous acetone to give needles de-solvating at 97°, and showing a double m.p. 112°, 208–213°, $[\alpha]^{25}D$ +33°. The analytical results were indicative of a solvate.

Anal. Calcd. for $C_{23}H_{32}O_6 \cdot H_2O$: C, 65.38; H, 8.11. Found: C, 65.37; H, 8.02.

21-Acetoxy-17α-hydroxy-5-pregnene-3,11,20-trione (VII).—The 3β-ol VI, 1.35 g., was dissolved in 250 ml. of acetone (redistilled from potassium permanganate), cooled to 12° and treated with 1 ml. of an aqueous solution of 0.267 g. of chromium trioxide and 0.23 cc. of sulfuric acid. The reaction proceeded rapidly and within a minute a gray precipitate formed. After an additional two minutes passed the reaction was stopped by dilution with 600 ml. of saturated aqueous sodium chloride solution. The green solution gradually deposited a residue of pure-white flattened needles which were filtered off, washed with saline solution and with water. A sample was recrystallized from aqueous acetone to give 21-acetoxy-17α-hydroxy-5-pregnene-3,11,20-trione. The melting point depended somewhat on the rate of heating. Rapid determination on a preheated Kofler stage gave a value of 180–183°. A slower determination gave melting at 175° to a viscid mass that resolidified incompletely to short quadrangular forms reddening (decomposition) at 200° with final crystal disappearance at 220°. The sample was nearly transparent to ultraviolet 239 mμ radiation, ϵ 300 (methanol).

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.84; H, 7.13.

Isomerization to Cortisone Acetate.—A sample of 350 mg. of 21-acetoxy-17α-hydroxy-5-pregnene-3,11,20-trione (VII) was dissolved in 50 ml. of methanol and 0.4 ml. of concentrated ammonia water "28%" was added. The mixture was let stand 100 minutes and the steroid was precipitated by addition of saturated sodium chloride. The product after crystallization as flattened blades from hexane containing a very small amount of acetone was identical in every respect with authentic cortisone acetate.

(18) Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, THIS JOURNAL, **74**, 5394 (1952). Our thanks are due these authors for a comparison specimen.

(19) A careful adsorption chromatography of the reaction product on silica gel revealed the presence of two contaminants. One as yet unidentified, relatively non-polar component, 0.25 g., m.p. 208–214° showed a positive formazan reaction ν_{max}^{KBr} 3400 (OH), 1710 (C-11, ketone), 1725 (ketone and acetate), 1750 (acetate), 1250 (acetate) and characteristic sharp bands at 736 and 775 cm.$^{-1}$. A second somewhat more polar component was the 3-acetate of the desired compound, namely 3β,21-diacetoxy-17α-hydroxy-5-pregnene-11,20-dione, m.p. 187.5–188.5°, $[\alpha]^{25}D$ +21°, prismatic needles from ether. One sample showed the above melting point but underwent resolidification of the melt to refractile sheaves with a second melting point at 03°.

3β,11α-Dihydroxy-5,17-pregnadiene-20-one (XV).—A sample of XV was prepared from 3β-hydroxy-5,16-pregnadiene-11,20-dione (IIIb) using the general dioxolanation procedure[20] of Antonucci, *et al.* The dione IIIb, 1 g., 70 ml. of ethylene glycol, 35 ml. of toluene and 35 mg. of *p*-toluenesulfonic acid were refluxed for 4 hours under the usual water-removal conditions. The resulting solution was cooled and the mixture was diluted with dilute aqueous sodium bicarbonate. The steroid was isolated with ether and solvents were removed *in vacuo*. The residue was dissolved in 300 ml. of 1-propanol and 11 g. of sodium was added to the boiling solution.[21] The reduction product was isolated with ether and the protective dioxolane group was removed by heating on the steam-bath in 1:1 aqueous acetic acid solution. Needles of the product separated readily from the hydrolysis medium even before cooling. The product XV, recrystallized from ether and from ethyl acetate–*n*-hexane formed wedges with transition on the Kofler stage to forms with a rhombic profile, m.p. 210–218°, λ_{max}^{MeOH} 238 mμ, ϵ 8,230, $[\alpha]^{25}D$ −14.8°.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.49; H, 9.11.

3β,11α-Dihydroxy-5,16-pregnadiene-20-one 11α-Monoacetate and 3β,11α-Diacetate.—A sample of 11α-acetoxydiosgenin acetate was carried through our usual degradation procedure.[7b,c] After the two-phase *t*-butyl alcohol–water–potassium hydroxide, saponification stage it was found that the product was 11α-acetoxy-3β-hydroxy-5,16-pregnadiene-20-one, m.p. 183–185°, λ_{max}^{MeOH} 235 mμ, ϵ 8,180, $[\alpha]^{25}D$ −44.5°. The infrared spectrum showed the presence of both hydroxyl (3640 and 3430 cm.$^{-1}$) and acetoxyl groups (1735 cm.$^{-1}$).

Anal. Calcd. for $C_{23}H_{33}O_4$: C, 74.16; H, 8.66. Found: C, 74.01; H, 8.53.

Acetylation with acetic anhydride–pyridine overnight at room temperature gave 3β,11α-diacetoxy-5,16-pregnadiene-20-one, m.p. 144–145°, λ_{max}^{MeOH} 234 mμ, ϵ 8,875, $[\alpha]^{25}D$ −40.3°. The infrared spectrum showed no hydroxyl bands, strong 1730 cm.$^{-1}$ and 1235–1255 cm.$^{-1}$ band of relative intensities consistent with two ester functions, 1679 cm.$^{-1}$ (unsaturated ketone) and 807,825 cm.$^{-1}$ bands (olefin).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.60, H, 8.02. Both the mono- and diacetate resisted saponification of the 11α-acetoxyl group, the infrared spectrum showing persistent acetate bands after overnight, room temperature reaction in the above-indicated butanolic caustic solution. Elevation of temperature resulted in resinification.

3β,11α-Dihydroxy-5-pregnene-20-one (XVI).—The diene XV, 200 mg. in 100 ml. of ether, was shaken with 1.5 g. of palladium-on-barium sulfate catalyst with hydrogen at 3 atmospheres pressure. The catalyst was filtered off, and the product was obtained as fibrous filaments on concentration of the ether, m.p. 181.5–182°, $[\alpha]^{25}D$ +70°. The product was transparent to ultraviolet radiation in the 230–245 mμ region, and in CS_2 solution showed a single strong band in the ketone region at 1712 cm.$^{-1}$. In KBr, in addition to a strong peak at 1710 cm.$^{-1}$, a weaker peak appeared at 1683 cm.$^{-1}$.

11α-Hydroxyprogesterone (XVII).—Oppenauer oxidation of 200 mg. of the diol-one XVI in 20 ml. of dry toluene with 3 ml. of cyclohexanone and 600 mg. of aluminum isopropoxide during a 0.75-hour reflux period gave after steam distillation of solvents and recrystallization from aqueous methanol 180 mg. of 11α-hydroxyprogesterone identical in every respect with an authentic specimen obtained by microbiological hydroxlation of progesterone.

3β,11α-Dihydroxy-16α,17α-epoxy-5-pregnene-20-one (XVIII).—The Δ^{16}-20-ketone XV, 3 g. in 100 ml. of methanol at 10°, was treated with 26 ml. of 30% hydrogen peroxide and 12 ml. of 4 *N* sodium hydroxide. After standing overnight at 10° 100 ml. of water was added and the volume was reduced in half by concentration *in vacuo* at 25°. The mixture was extracted with ether; the organic layer was washed

(20) R. Antonucci, S. Bernstein, R. Lenhard, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1369 (1952).

(21) *Cf.* H. Heusser, R. Anliker, and O. Jeger, *Helv. Chim. Acta.*, **35**, 1537 (1952).

with water, dried, and evaporated to a small volume, whereupon 2.75 g. of a crystalline product, m.p. 196–202°, separated. The analytical sample recrystallized from ether as prisms, m.p. 203–204°, $[\alpha]^{25}D$ −18.4°, transparent to ultraviolet.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.61; H, 8.62.

16α,17α-Epoxy-11α-hydroxy-4-pregnene-3,20-dione (16α,17α-Epoxy-11α-hydroxy-progesterone) (XIX).—The preceding preparation was submitted to Oppenauer oxidation under the same conditions used to prepare XVII. The product was XIX identical in all respects with the product obtained by microbiological fermentation.[12b]

PHILADELPHIA 18, PENNA.

[CONTRIBUTION FROM THE HORMONE RESEARCH LABORATORY AND THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Corticotropins (ACTH). XII. Acid–Base Equilibria of α-Corticotropin and Bovine Corticotropin[1]

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Titration curves for α-corticotropin and bovine corticotropin have been obtained in 0.1 *M* KCl solutions at 24.5°. The degree of ionization of phenolic groups has also been determined spectrophometrically. From these data, intrinsic ionization constants (pK_i) of all acidic and basic groups in the corticotropins, as well as values for the electrostatic parameter (w), have been estimated. The relationship of the amino acid sequence in the corticotropin molecule to the estimated values for pK_i and w is discussed.

α-Corticotropin from sheep glands,[3] which has been characterized as a polypeptide of relatively low molecular weight[4] and whose structure has been established,[5] has recently been compared with a seemingly identical compound isolated in pure form from bovine glands.[6] Titration curves for both the ovine and bovine hormones, together with details of an investigation of the ionization of phenolic hydroxyl groups, will be presented here.

Experimental

Titration Assembly.—The cell used for the acid–base titration is shown in Fig. 1; its design permitted the investigation of the rather limited amount of material available. The compact combination glass and reference electrode is supported by the cork stopper of the cell. Standard acid or base is delivered from an Agla micrometer syringe, the tip of the needle (glass, or stainless steel) being immersed in the sample only during the time required for additions. Stirring of the sample is performed by a small magnetic stirrer, rotating simultaneously with the main magnetic stirrer of the thermostatic bath. Proper precautions are applied to exclude carbon dioxide while the sample is being dissolved or transferred to the cell, a slow flow of purified nitrogen being maintained above the solution while the titration is being performed; a saturator, inserted in front of the cell, prevents changes in the volume of the sample resulting from evaporation. In the course of these experiments, each sample was titrated throughout the entire *p*H range under investigation, in a semi-continuous fashion.

Measurement of the *p*H.—The electrode train used was a combination glass electrode/Ag–AgCl reference electrode, of the type described by Cannon[7]; its small size and excellent concentric shielding make it especially suitable for semi-continuous titration of small volumes.[8] The *p*H measurements were made with a Beckman model G *p*H meter.

In order to standardize the *p*H scale, five reference buffers were prepared according to directions given by Bates[9] (0.05 *M* K tetroxalate; saturated KH tartrate; 0.05 *M* KH biphthalate; 0.025 *M* KH_2PO_4, 0.025 *M* Na_2HPO_4; 0.01 *M* borax). During calibration of the Beckman instrument, all these buffers afforded very consistent readings (among all 5 buffers, discrepancies higher than 0.01 of a *p*H unit were never observed). Since the *p*H readings of the buffers showed no drift over a period of several hours, it would appear that no significant leakage of saturated KCl from the liquid junction of the electrode occurred.

After each addition of acid or base during a titration experiment, equilibration of the sample to constant *p*H was usually reached within 2–3 minutes; only when some insoluble material separated out was it necessary to allow longer equilibration, although the time interval never had to be extended beyond 10 minutes.

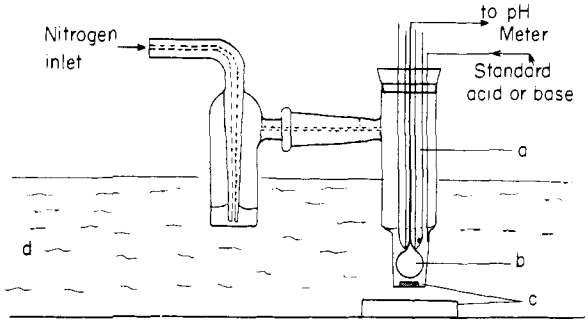

Fig. 1.—Titration cell: a, reference electrode; b, glass electrode; c, magnetic stirrer; d, thermostatic bath.

Acid–Base Titration Curves.—All titrations were carried out at 24.5°, with samples of 8–10 mg. (about 2 micromoles of α-corticotropin in 1 ml. of CO_2-free 0.1 *N* KCl. Standard 0.2 *M* HCl was added first, and the *p*H was read after each addition. When a *p*H around 2 had been reached, the sample was titrated in the other direction with standard CO_2-free 0.2 *M* KOH; finally, it was titrated again from a *p*H around 12 back to the initial *p*H value. By this time, the total volume in the cell has increased by approximately one half; however, the mutual neutralization of acid and base has restored the concentration of KCl to what it was at the start.

The protein concentration was averaged from the results of two types of analysis: namely, determination of total nitrogen and measurement of the ultraviolet absorption spectrum. The correction for free acid or base at extreme *p*H

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(2) Fulbright Grantee 1955–1957, on leave of absence from the University of Brussels, Belgium.

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(5) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

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(7) M. D. Cannon, *ibid.*, **106**, 597 (1947).

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(9) R. G. Bates, "Electrometric *p*H Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954.