# Oxidation of Steroidal α-Ketols to Glyoxals with Cupric Acetate[1]

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Catalytic amounts of copper acetate in methanol will convert the α-ketolic side chain of steroids to a glyoxal group. Oxidation is hastened by passing air over or through the solution, and retarded by addition of water. The reaction is general and gives high yields of steroidal glyoxals. In dry methylene chloride or benzene the glyoxals tend to polymerize; in aqueous ethyl acetate they are stable. With a ratio of copper acetate to ketolic steroid of 1:8, oxidation to the glyoxal is complete in an hour or less. With a longer reaction time the yield decreases because of a rearrangement of the glyoxal side chain to a methyl glycolate side chain. The glyoxals are characterized as hydrates, dimethyl acetals, and quinoxalines. The glyoxal from cortisone crystallizes from aqueous methanol as a stable 21-hemiacetal. The preparation of six 3α-hydroxy-5β-pregnanes with an α-ketolic function in the side chain and with variations in the molecule at C-9, C-11, and C-16 is described.

The finding that the α-ketolic side chain of steroids was altered in the presence of a trace of cupric ions[3] stimulated us to investigate this reaction in detail. One of the products of the reaction gave an immediate yellow color with the Porter–Silber[4] reagent, but did not reduce alkaline tetrazolium. These results indicated that the product contained a glyoxal (20-oxo-21-aldehyde) function. This oxidative process offered promise for the preparation of steroidal glyoxals which were needed for a study on the Porter–Silber reaction. The preparation of several α-ketolic steroids is described at the end of the discussion in this paper.

Various copper salts were tested for their ability to convert cortisone to the corresponding glyoxal. Of the salts investigated, copper acetate gave by far the most rapid and complete oxidation. Methanol was a satisfactory solvent for the reactants. At the time this work was done it was not known that Conbere[5] and Weijlard[6] had assigned patents to Merck and Company dealing with the preparation of steroidal glyoxals from α-ketols using copper acetate as the oxidant in both catalytic and stoichiometric amounts. Our results are presented since they are a confirmation and extension of the findings described in the patents.

3α,21-Dihydroxy-5β-pregnane-11,20-dione (I) was used as a model α-ketol in preliminary studies. The Porter–Silber reaction, given by the glyoxal (II) but not by the α-ketol (I), served as a convenient means of determining the extent of the oxidation.[7] The reaction can be terminated immediately by addition of EDTA (disodium ethylenedinitrilotetraacetate) to bind the copper; this reagent does not interfere with the Porter–Silber reaction.

The rate of oxidation of 3α,21-dihydroxy-5β-pregnane-11,20-dione (I) with methanolic cupric acetate at room temperature under conditions in which the ratio of copper to steroid varied from 1:2 to 1:100 is shown in Fig. 1. The catalytic nature of the process is illustrated by the fact that even with a ratio of 1:20 there is rapid and apparently complete conversion of the α-ketol to the glyoxal. When the copper to steroid ratio

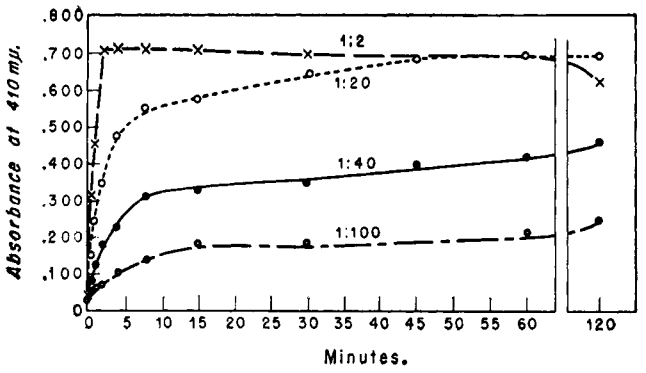


Fig. 1.—Rates of oxidation of 3α,21-dihydroxy-5β-pregnane-11,20-dione (I) with methanolic cupric acetate as indicated by increase in color produced in the Porter–Silber reaction. Molar ratios of copper to steroid were 1:2, 1:20, 1:40, and 1:100; steroid concentration was 0.00091 *M*.

was 1:40 or less, oxidation proceeded slowly and was incomplete after two hours. The presence of water lowers the rate of oxidation. With a copper to steroid ratio of 1:8 and a reaction time of one hour, absorbance readings in the Porter–Silber reaction for solutions containing 0, 5, 12.5, and 25% of water in methanol were 0.650, 0.655, 0.540, and 0.300, respectively.

Preparative scale experiments with a number of α-ketols were performed using copper to steroid ratios varying from 1:8 to 1:2 and oxidation times ranging from fifteen minutes to one hour. Oxidation rates were raised substantially by passing air into or over the surface of the reaction mixtures.

The optimal yield of glyoxal (II) from 3α,21-dihydroxy-5β-pregnane-11,20-dione (I) was obtained with a copper to steroid ratio of 1:8 and a reaction time of fifteen minutes. More prolonged reaction was attended by lower yields because of rearrangement of the glyoxal to a 20-epimeric mixture of methyl 3α,20-dihydroxy-11-oxo-5β-pregnan-21-oates.[8]

Almost immediately after mixing methanolic solutions of 3α,21-dihydroxy-5β-pregnane-11,20-dione (I) and cupric acetate, the originally blue-green solution became amethyst in color, then the solution became turbid, and the color changed from amethyst to yellow to green. The original clear blue-green color was restored in about ten minutes. In several experiments in which smaller amounts of copper acetate were used it was possible to regenerate repeatedly the blue-green from the amethyst color merely by swirling the flask or by bubbling air into the solution for a few seconds.

(1) Abridgement of thesis submitted by M. L. Lewbart to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry, June, 1961.

(2) This investigation was carried out during the tenure of a Fellowship from the Division of General Medical Sciences, U. S. Public Health Service.

(3) M. L. Lewbart and V. R. Mattox, *Nature*, **183**, 820 (1959).

(4) R. H. Silber and C. C. Porter, "Methods of Biochemical Analysis," Vol. 4, Interscience Publishers, New York, N. Y., 1957, pp. 139–169.

(5) J. P. Conbere, U. S. Patent 2,773,077 (1956).

(6) J. Weijlard, U. S. Patent 2,773,078 (1956).

(7) M. L. Lewbart and V. R. Mattox, *Anal. Chem.*, **33**, 559 (1961).

(8) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 1779 (1963).

Initially, the glyoxal (II) was recovered from the reaction mixture by addition of an excess of aqueous EDTA, concentration of the solution under reduced pressure to remove the methanol, and extraction with methylene chloride. When the methylene chloride solution was washed with water and taken almost to dryness, the residue was no longer completely soluble in methanol and a crystalline polymer[9] was obtained in low yield. When the residue was treated with benzene, the polymer was obtained in almost quantitative yield. In one experiment one-half of a methylene chloride extract of glyoxal was washed with both dilute sodium bicarbonate and water and the other half was washed with water alone. When the solvent was removed and the residues were dissolved in benzene, that fraction washed with water gave a heavy deposit of polymer in approximately one minute. The product from the bicarbonate-washed fraction deposited polymer to the same extent after about eighteen hours. These findings suggest that polymerization may be promoted by the small amounts of acetic or steroidal acid which are present.

Polymerization of the glyoxal (II) was avoided in the following manner. The oxidation mixture was diluted with water and extracted with ethyl acetate. After being washed with dilute sodium bicarbonate solution and water, the wet organic phase was taken to dryness. Crystallization from aqueous acetone gave the glyoxal hydrate in 94% yield.

A small amount of 3α-hydroxy-11-oxo-5β-etianic acid[10] (V) was obtained from the aqueous bicarbonate solution. This result indicates that during the oxidation, a small amount of steroid underwent cleavage between C-20 and C-21.

Successive treatment of both the glyoxal (II) and the polymer with dry methanolic hydrogen chloride followed by acetylation afforded the known acetoxy dimethyl acetal[11] (IV) in yields of 80 and 60%, respectively. The glyoxal (II) was convertible into a quinoxaline (III) by the method of Leanza, *et al.*[12]

nan-20-one (XIV) than with the 11-dehydro analog (I); the reaction time had to be extended to forty-five minutes. The corresponding glyoxals were obtained in 76 and 89% yield.

The rate of cupric acetate oxidation of 3α,21-dihydroxy-5β-pregn-16-ene-11,20-dione (IX) was slower than that of other α-ketols and a reaction time of one hour was employed. The glyoxal (X) was obtained in 82% yield. Of all the glyoxals studied this one showed the greatest tendency to undergo polymerization. However, if the product from the oxidation reaction was processed rapidly and crystallized immediately from aqueous acetone, the amount of methanol-insoluble material in the final product could be reduced to less than 1%.

Treatment of the $\Delta^{16}$-steroidal glyoxal (X) with methanolic hydrogen chloride followed by neutralization of the acid with potassium carbonate and acetylation of the product with acetic anhydride–pyridine gave the acetoxy dimethoxy derivative (XII) in 86% yield. In earlier experiments when the methanolic hydrogen chloride was neutralized by addition of an excess of potassium carbonate, the yield of XII was only 40%. By neutralization with an equivalent amount of alkali the yield was more than doubled. A possible explanation for these results stems from the work of Fukushima and Gallagher[13] who found that $\Delta^{16}$-20-keto steroids undergo a 1,4-attack by methanolic alkali to give saturated 16α-methoxy derivatives.

The quinoxaline of X could not be obtained from its precursor by the method of Leanza, *et al.*[14] Presumably addition of sodium bisulfite to the $\Delta^{16}$-steroidal glyoxal (X) occurs. The quinoxaline (XI) was obtained by refluxing an alcoholic solution of the glyoxal and *o*-phenylenediamine.

Treatment of cortisone (XVII) for one hour at room temperature with 0.5 molar equivalent of cupric acetate resulted in essentially complete oxidation to the corresponding 21-aldehyde (XVIII). In the work of Con-

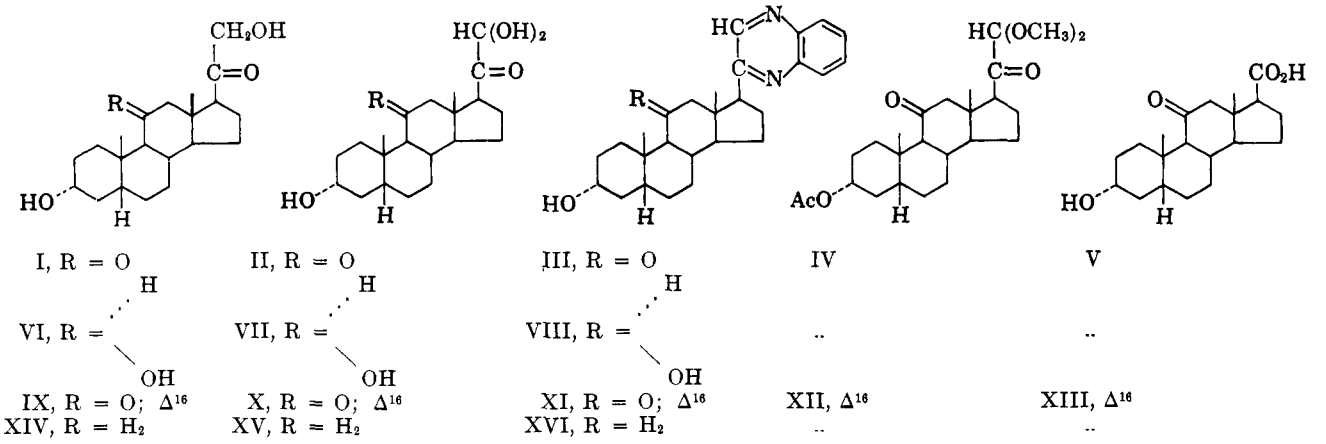


Figure 2

The sequence of color changes and the rate of oxidation occurred more slowly with 3α,11β,21-trihydroxy-5β-pregnan-20-one (VI) and 3α,21-dihydroxy-5β-pregnane[5] and Weijlard,[6] the copper to cortisone ratio was 1:30 and the reaction was carried out at 50–55° for fourteen hours in methanol which contained a small amount of water and acetic acid. Whereas they found that "any additional amount of catalyst (cupric ace-

(9) The tendency for this type of compound to polymerize is well known. H. Reich and T. Reichstein, *Helv. Chim. Acta*, **32**, 1124 (1949).

(10) J. von Euw, A. Lardon, and T. Reichstein, *ibid.*, **27**, 1287 (1944).

(11) V. R. Mattox, *J. Am. Chem. Soc.*, **74**, 4340 (1952).

(12) This procedure involves the initial formation of an aldehyde–bisulfite addition compound, followed by treatment with *o*-phenylenediamine.[14]

(13) D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951).

(14) W. J. Leanza, J. P. Conbere, E. F. Rogers, and K. Pfister, 3rd, *ibid.*, **76**, 1691 (1954).

tate) above about 3% will not increase the rate of reaction or the yield obtained," it is apparent from the present study that, under the specified conditions, larger amounts of cupric acetate bring about a greater rate of oxidation.

The glyoxal from cortisone was characterized as a hydrate (XVIII) and a quinoxaline (XIX) which had properties in good agreement with those reported.[14] When the glyoxal was crystallized from aqueous methanol, it separated as a colorless substance which had a melting point different from that of the hydrate. Ele-

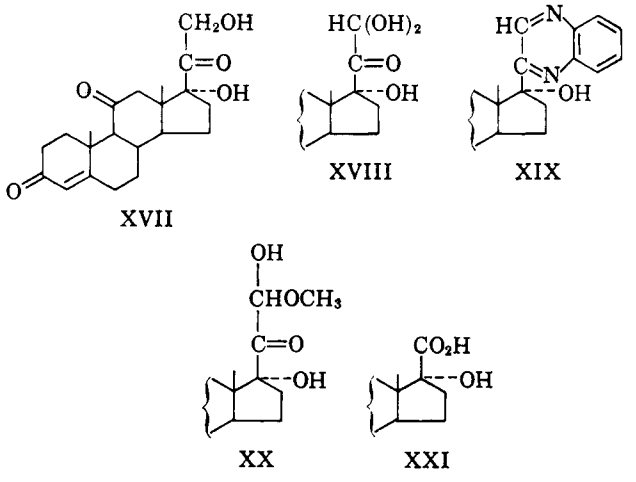


Figure 3

mental and Zeisel analyses showed that the glyoxal was associated with one molecule of methanol. This product is formulated as a hemiacetal (XX) rather than as a free glyoxal with methanol of crystallization. Recrystallization of the hemiacetal from aqueous acetone yielded the glyoxal hydrate (XVIII). Crystallization of the glyoxal from aqueous ethanol gave a compound different from the one obtained by crystallization from methanol. The infrared spectra in Nujol of the two hemiacetals and the glyoxal were different. After evaporation of benzene solutions of the hemiacetals and the glyoxal hydrate the residues had identical spectra in chloroform. The glyoxal hydrate is sparingly soluble in chloroform, but the hemiacetals dissolve readily. Solutions of this glyoxal and others described in the paper are colorless in methanol and aqueous methanol. Solutions of the glyoxals in chloroform are yellow and have maxima[15] at about 450 mμ with molecular extinction coefficients of less than 40. These properties suggest that the mole of solvent in the hydrates and alcoholates is combined with the aldehyde group at C-21.

Steps used in the preparation of several α-ketolic steroids are indicated in Fig. 4. Three of these compounds (C, E, and N) are metabolites of adrenocortical hormones. In reports dealing with their isolation,[16-18] identification has been made largely by comparison of infrared spectra with reference compounds supplied by other workers in the field. To the authors' knowledge,

(15) This spectral constant is typical of that for steroidal glyoxals.

(16) L. L. Engel, P. Carter, and L. L. Fielding, *J. Biol. Chem.*, **213**, 99 (1955).

(17) E. M. Richardson, J. C. Touchstone, and F. C. Dohan, *J. Clin. Invest.*, **34**, 285 (1955).

(18) W. R. Eberlein and A. M. Bongiovanni, *J. Biol. Chem.*, **223**, 85 (1956).

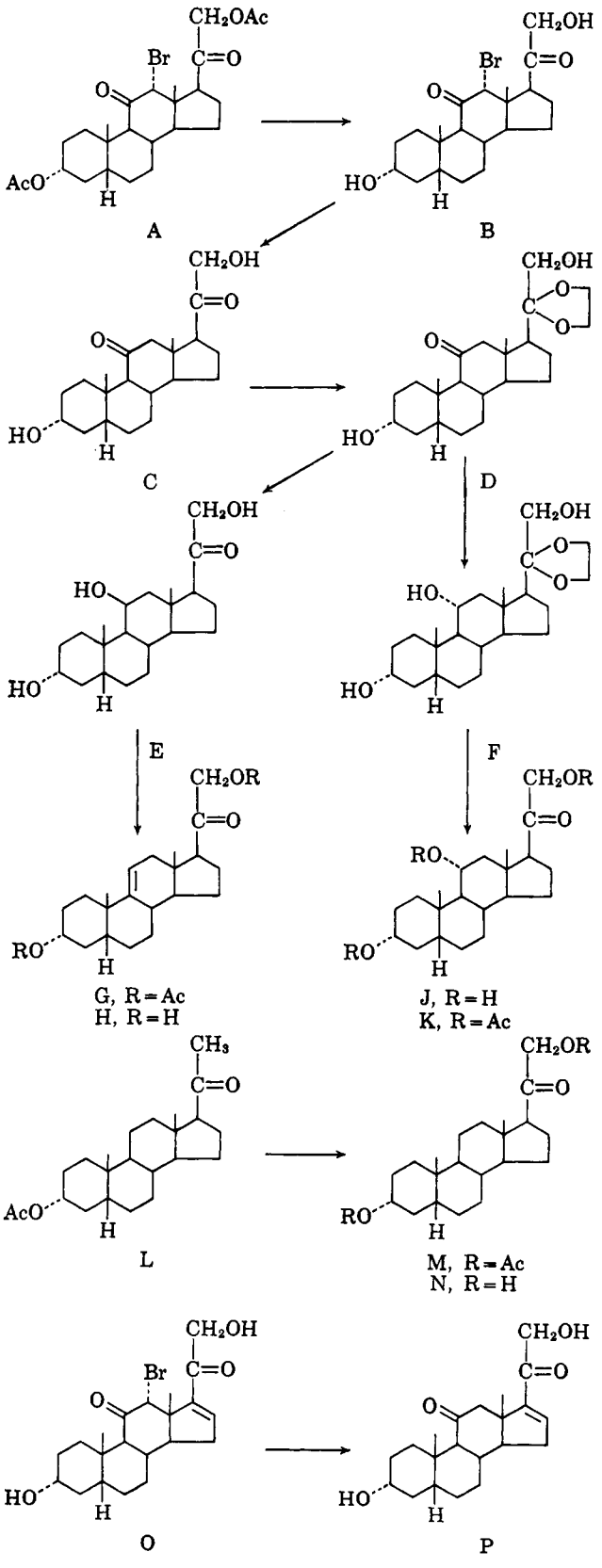


Figure 4

the physical constants and analyses for these substances have not been reported.

The presence of an α-ketolic group was deduced from the method of preparation and was verified by the rapid reduction of tetrazolium blue by the compounds in question. Several of the substances have been converted to the corresponding glyoxals. The double bond

in G and H is assigned $\Delta^{9(11)}$, rather than $\Delta^{11}$, because dehydration of 11β-hydroxy steroids with boron trifluoride gives $\Delta^{9(11)}$ derivatives.[19]

## Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured in methanol at a concentration of about 1% and at a temperature of 24 ± 2° unless otherwise designated. Analyses were by J. F. Alicino, Metuchen, N. J.

**3α,21-Dihydroxy-12α-bromo-5β-pregnane-11,20-dione (B).**—To 153.6 g. (0.3 mole) of 3α,21-diacetoxy-12α-bromo-5β-pregnane-11,20-dione[20] in 800 ml. of chloroform and 2800 ml. of methanol was added 200 ml. of water and 338 ml. of concentrated hydrochloric acid. After 22 hr. at room temperature, 400 g. of sodium acetate trihydrate in 1 l. of water was added and most of the organic phase was evaporated under reduced pressure. The aqueous residue was extracted three times with a total of 2 l. of methylene chloride. The organic layer was washed with dilute sodium hydroxide and water, dried, and concentrated to dryness. Recrystallization from 1100 ml. of hot acetone gave 105 g. (m.p. 152.5–155.5°) of bromodiol (B) as a 1:1 solvate. The mother liquor yielded an additional 23.5 g. (m.p. 151–153°) of product. A sample, which had been recrystallized from acetone and air dried, lost 11.35% in 2 hr. at 100° and 0.1 mm.; calcd. for loss of 1 mole of acetone, 11.95%; m.p. 163–165°; $[\alpha]_D$ −1 ± 2° (chloroform).

*Anal.* Calcd. for $C_{21}H_{31}O_4Br$: C, 59.01; H, 7.31. Found: C, 59.34; H, 7.59.

**3α,21-Dihydroxy-5β-pregnane-11,20-dione (C).**—To an agitated solution of 29.2 g. (60 mmoles) of 3α,21-dihydroxy-12α-bromo-5β-pregnane-11,20-dione (containing 1 mole of acetone) in 500 ml. of glacial acetic acid was added 40 g. of powdered zinc in portions over a period of 1 hr. The temperature was maintained at 40–45° during the addition of zinc and for a subsequent 30-min. period. The reaction mixture was filtered into 2 l. of water and the zinc was washed with 50 ml. of acetic acid. The product was recovered by extraction with methylene chloride (1250 ml. in three portions). After successive washings with 300 ml. of water, 400 ml. of *N* sodium hydroxide, and 300 ml. of water, the organic phase was dried and concentrated to dryness. Three crops of cubes were obtained from methanol (14.9 g., m.p. 215–217°; 4.3 g., m.p. 213–215°; 1.0 g., m.p. 209–211°). A sample for analysis was recrystallized from methanol; m.p. 215–216°; $[\alpha]_D$ + 129 ± 2°.

*Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.51; H, 8.97.

**3α,21-Dihydroxy-5β-pregnane-11,20-dione 20-Ethylene Ketal (D).**—A suspension of 10.5 g. (30 mmoles) of 3α,21-dihydroxy-5β-pregnane-11,20-dione in 300 ml. of benzene and 15 ml. of ethylene glycol was dried by refluxing for 10 min. in an apparatus fitted with a water separator.[21] To the dried mixture was added 300 mg. of *p*-toluenesulfonic acid and refluxing was resumed for 3 hr. The reaction mixture was cooled, washed with dilute sodium bicarbonate and water, and concentrated to dryness. Crystallization from acetone afforded three crops of needles (6.7 g., m.p. 188.5–190.5°; 2.2 g., m.p. 187–189°; 0.4 g., m.p. 183–186°). A sample for analysis was recrystallized from methanol; m.p. 191.5–193.5°; $[\alpha]_D$ + 67 ± 2°.

*Anal.* Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.33; H, 9.09.

**3α,11β,21-Trihydroxy-5β-pregnan-20-one (E).**—To 7.84 g. (20 mmoles) of 3α,21-dihydroxy-5β-pregnane-11,20-dione 20-ethylene ketal in 250 ml. of methanol was added 15.2 g. (400 mmoles) of sodium borohydride in 75 ml. of water. After 36 hr. at room temperature, excess reducing agent was decomposed with acetic acid. The reaction mixture was added to 400 ml. of water and extracted with methylene chloride, which, after being washed with water, was dried, and concentrated to dryness. The 11β-hydroxy-20-ketal, which could not be obtained in crystalline form, was dissolved in a mixture of 250 ml. of methanol and 25 ml. of 8% (v./v.) sulfuric acid in water and the solution was refluxed for 50 min. The reaction mixture was diluted with 450 ml. of water and extracted with methylene chloride. Addition of 15 ml. of acetone and 30 ml. of ether to the residue from the methylene chloride extract brought about crystallization (5.15 g., m.p. 145–146.5°; 0.72 g., m.p. 144.5–146°). The sample for analysis was recrystallized from acetone–ether; m.p. 145.5–147°; $[\alpha]_D$ +133 ± 2°.

*Anal.* Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.98; H, 9.98.

**3α,11α,21-Trihydroxy-5β-pregnan-20-one 20-Ethylene Ketal (F).**—To 3.92 g. (10 mmoles) of 3,21-dihydroxy-5β-pregnane 11,20-dione 20-ethylene ketal in 400 ml. of *n*-propyl alcohol was added in one portion 40 g. of metallic sodium.[22] The mixture was allowed to reflux spontaneously for 1 hr. The precipitated sodium propoxide was dissolved by addition of 150 ml. of methanol and the unchanged sodium was decomposed by slow addition of 250 ml. of water. After evaporation of most of the organic solvents the aqueous residue was extracted with three 100-ml. portions of ethyl acetate. The extract was washed twice with water, dried, and concentrated. Crystals separated slowly (3.4 g., m.p. 200–201°; 0.24 g., m.p. 195.5–197.5°). Recrystallization from ethyl acetate gave the analytical sample; m.p. 200–201°; $[\alpha]_D$ +33± 1°.

*Anal.* Calcd. for $C_{23}H_{38}O_5$: C, 70.02; H, 9.70. Found: C, 70.77; H, 9.30.

**3α,21-Diacetoxy-5β-pregn-9(11)-en-20-one (G).**—To 700 mg. (2 mmoles) of 3α,11β,21-trihydroxy-5β-pregnan-20-one was added 2 ml. each of pyridine and acetic anhydride. After 10 hr. at room temperature the product was recovered in the usual manner. Since the resulting 3α,21-diacetoxy-11β-hydroxy-5β-pregnan-20-one could not be crystallized, it was dissolved in 50 ml. of glacial acetic acid and treated with 2 ml. of boron fluoride etherate for 20 hr. at room temperature. The product, recovered by dilution of the reaction mixture with water and extraction with methylene chloride, crystallized as needles and rosettes from ether (489 mg., m.p. 108–112°; 119 mg., m.p. 106–108°; 100 mg., m.p. 105–106.5°). On recrystallization from ether, prisms were obtained (673 mg.); m.p. 124–127°; $[\alpha]_D$ +134 ± 3°.

*Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.19; H, 8.81.

**3α,21-Dihydroxy-5β-pregn-9(11)-en-20-one (H).**—To 416 mg. (1 mmole) of 3α,21-diacetoxy-5β-pregn-9(11)-en-20-one in 25 ml. of methanol and 4 ml. of water was added 2 ml. of concentrated hydrochloric acid. After 20 hr. at room temperature, 2.5 g. of sodium acetate trihydrate in 10 ml. of water was added and the mixture was concentrated to turbidity. The aqueous residue was extracted with methylene chloride and the organic phase, after being washed with dilute sodium bicarbonate and water, was concentrated to dryness. Crystallization from ether gave needles (168 mg., m.p. 145.5–146.5°; 82 mg., m.p. 144–145°); $[\alpha]_D$ +121 ± 2°.

*Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.88; H, 9.76.

**3α,11α,21-Trihydroxy-5β-pregnan-20-one (J).**—A solution of 3α,11α,21-trihydroxy-5β-pregnan-20-one 20-ethylene ketal (3.4 g.) in methanol (100 ml.) and 8% (v./v.) sulfuric acid (10 ml.) was refluxed for 1 hr. The product, recovered by addition of the reaction mixture to 500 ml. of water and extraction with methylene chloride, crystallized from ethyl acetate as needles (2.5 g., m.p. 180.5–182.5°; 0.46 g., m.p. 180–181°; 0.05 g., m.p. 179–180°). A sample for analysis was recrystallized from acetone and dried for 3 hr. at 100° and 1–2 mm.; m.p. 180–181.5°; $[\alpha]_D$ +103 ± 2°.

*Anal.* Calcd. for $C_{21}H_{34}O_4 \cdot CH_3COCH_3$: C, 70.55; H, 9.87. Found: C, 70.98; H, 9.54.

**3α,11α,21-Triacetoxy-5β-pregnan-20-one (K).**—Acetylation of 3α,11α,21-trihydroxy-5β-pregnan-20-one (240 mg.) was carried out in 1 ml. each of pyridine and acetic anhydride for 11 hr. at room temperature. The product crystallized from acetone–petroleum ether (301 mg., 92.1%; m.p. 187–189°). Recrystallization from acetone–petroleum ether did not raise the melting point; $[\alpha]_D$ +88 ± 2°.

*Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46; $CH_3CO$, 27.01. Found: C, 67.81; H, 8.35; $CH_3CO$, 28.44.

**3α,21-Dihydroxy-5β-pregnan-20-one (N).**—A solution of 10.8

(19) H. Heyman and L. F. Fieser, *J. Am. Chem. Soc.*, **73**, 5252 (1951).

(20) The physical constants and analytical data (G. A. Fleisher, unpublished findings) are given. Constants: m.p. 162.5–163°; $[\alpha]_D$ +32 ± 2° (chloroform). *Anal.* Calcd. for $C_{25}H_{35}O_5Br$: C, 58.71; H, 6.90; Br, 15.63. Found: C, 58.93; H, 6.86; Br, 15.82.

(21) R. Antonucci, S. Bernstein, R. Littell, K. J. Sax, and J. H. Williams, *J. Org. Chem.*, **17**, 1341 (1952).

(22) H. L. Herzog, M. A. Jevnik, and E. B. Hershberg, *J. Am. Chem. Soc.*, **75**, 269 (1953).

g. (30 mmoles) of 3α-acetoxy-5β-pregnan-20-one[23] (m.p. 101–102°) in 400 ml. of glacial acetic acid and 10 ml. of acetic anhydride was maintained at 70–80° while being agitated with a magnetic stirring bar. To this solution was added 14.7 g. (33 mmoles) of lead tetraacetate[24] in portions over a 2-hr. period. After an additional 16 hr. at 70–80°, at which time a test with starch–iodide paper was negative, the reaction mixture was added to ice, diluted with 1500 ml. of water, and extracted three times with a total volume of 450 ml. of methylene chloride. The organic phase was washed with dilute sodium hydroxide and water, dried, and concentrated to dryness. Since only a small amount of poor quality diacetate (M) could be crystallized from the residue, it was hydrolyzed directly by treatment with a mixture of 350 ml. of methanol, 35 ml. of water, and 42 ml. of concentrated hydrochloric acid for 24 hr. at room temperature. After addition of 50 g. of sodium acetate trihydrate in 250 ml. of water, most of the methanol was removed under reduced pressure. The aqueous residue was extracted with 250 ml. of petroleum ether and, since the residue from the organic phase gave only a faint test with alkaline blue tetrazolium, it was discarded. The aqueous layer was extracted with methylene chloride and, after successive washings with dilute sodium hydroxide and water, the solvent was evaporated. The crude diolone (N) crystallized from benzene with 0.5 mole of solvent of crystallization (6.0 g., m.p. 138–143°; 1.8 g., m.p. 128–133°). Repeated crystallizations from ethyl acetate, aqueous methanol, and ether furnished 4.2 g. (42%, m.p. 153–154°) of pure 3α,21-dihydroxy-5β-pregnan-20-one. A sample for analysis was recrystallized from acetone; m.p. 152.5–153.5°; $[\alpha]_D$ +113 ± 2°.

*Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.44; H, 10.25. Found: C, 75.72; H, 10.15.

**3α,21-Diacetoxy-5β-pregnan-20-one (M).**—To 334 mg. (1 mmole) of 3α,21-dihydroxy-5β-pregnan-20-one was added 1 ml. each of pyridine and acetic anhydride. After 10 hr. at room temperature the product was recovered. Crystallization from ether–petroleum ether gave 309 mg. (74%, m.p. 94–96°) of needles. A sample, recrystallized from ether and dried at 65° and 0.1 mm., melted at 97–98°; $[\alpha]_D$ +148 ± 2°.

*Anal.* Calcd. for $C_{25}H_{38}O_5$: C, 71.73; H, 9.15. Found: C, 71.51; H, 9.28.

**3α,21-Dihydroxy-5β-pregn-16-ene-11,20-dione (P).**—A stirred solution of 4.5 g. of 3α,21-dihydroxy-12α-bromo-5β-pregn-16-ene-11,20-dione[25] (O) in 150 ml. of methanol was treated under nitrogen with excess chromous chloride, prepared by percolating 15.9 g. of $CrCl_3 \cdot 6H_2O$ in 100 ml. of methanol and 1 ml. of concentrated hydrochloric acid through a 1 × 13 cm. column of amalgamated zinc.[26] The addition of chromous chloride took 30 min. After an additional 15-min. period the reaction mixture was added to 1 l. of water and the solution was extracted with methylene chloride. The extract was washed with dilute sodium hydroxide and water, dried, and concentrated to dryness. Crystallization from ethyl acetate gave needles (2.67 g., m.p. 181.5–182.5°; 0.12 g., m.p. 178.5–180°). The sample for analysis was prepared by recrystallization from acetone; m.p. 183–185°; $[\alpha]_D$ +88 ± 2°; $\lambda_{max}^{MeOH}$ 238 mμ, ε 8400.

*Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.79; H, 8.73. Found: C, 72.59; H, 8.69.

**3α,21,21-Trihydroxy-5β-pregnane-11,20-dione (II) and 3α-Hydroxy-11-oxo-5β-etianic Acid (V).**—To 3.48 g. (10 mmoles) of 3α,21-dihydroxy-5β-pregnane-11,20-dione in 100 ml. of methanol was added 50 ml. of 0.025 *M* (1.25 mmoles) methanolic cupric acetate. After 20 sec. the color changed from blue to amethyst. Air was bubbled into the solution rapidly to hasten the oxidation. A sequence of further color changes ensued, but after approximately 10 min. the reaction mixture was blue once more. After 15 min. at room temperature the solution was added to 1 l. of water and the mixture was extracted twice with 200 ml. of ethyl acetate. The extract was washed with dilute sodium bicarbonate and water, filtered, and concentrated to dryness.

The combined aqueous washes were acidified with dilute hydrochloric acid and extracted with ethyl acetate. The extract yielded a crystalline product (86 mg., 2.5%, m.p. 291–293° dec.). Mixture melting point with authentic **3α-hydroxy-11-oxo-5β-etianic acid**[10] (V) (m.p. 292–294° dec.) showed no depression. The acetylation products of both authentic (m.p. 217.5–219.5°) and copper acetate-derived (m.p. 218–220°) acids showed no depression of melting point on admixture.

The residue from the neutral fraction gave crystals from aqueous acetone (3.14 g., m.p. 154–155°; 0.27 g., m.p. 153–155°) in a yield of 94%. The sample for analysis was recrystallized from acetone and dried at room temperature to constant weight under reduced pressure over anhydrous calcium chloride; m.p. 153.5–155°; $[\alpha]_D$ +109 ± 1°.

*Anal.* Calcd. for $C_{21}H_{32}O_5$: C, 69.28; H, 8.78. Found: C, 69.32; H, 9.18.

A sample of the glyoxal hydrate was dried for 18 hr. at 1–2 mm. and 125° in order to remove water of hydration; $\lambda_{max}^{CHCl_3}$ 450 mμ, ε 23.[15]

**Formation of Polymer from 3α,21,21-Trihydroxy-5β-pregnane-11,20-dione.**—In another preparation of II from 3.48 g. of I the reaction mixture was extracted with methylene chloride; the extract was washed with water and taken to dryness. When the residue was dissolved in benzene, a fine, white solid separated slowly. By repeated filtration of the white precipitate and concentration of the yellow mother liquor, a total of 3.39 g. (m.p. 200.5–201.5°) of product was obtained. The compound was poorly soluble in benzene, methylene chloride, acetone, methanol, and ethyl acetate. It gave a positive Porter–Silber reaction and, on successive treatment with dry methanolic hydrogen chloride and pyridine–acetic anhydride in the manner described later, afforded **3α-acetoxy-21,21-dimethoxy-5β-pregnane-11,20-dione**[11] (IV) in 60% yield.

**3α-Acetoxy-21,21-dimethoxy-5β-pregnane-11,20-dione (IV).**—3α,21-Dihydroxy-5β-pregnane-11,20-dione (3.48 g., 10 mmoles) was oxidized to the glyoxal in the manner described earlier. The amorphous yellow product was dissolved in 130 ml. of methanol and 30 ml. of 1.35 *N* hydrogen chloride in dry methanol was added. The colorless solution was refluxed for 2 hr. and, following addition of potassium carbonate (10 g. in 25 ml. of water), it was concentrated to remove the organic solvent. The aqueous residue was extracted with methylene chloride. The organic layer was washed with water, filtered through anhydrous sodium sulfate, and concentrated to dryness. The residue was treated with 10 ml. each of pyridine and acetic anhydride for 10 hr. at room temperature. The product (IV) crystallized from ether–petroleum ether to give 2.78 g. (64.0%) of rosettes, m.p. 104.5–105°; $[\alpha]_D$ +132 ± 2° (chloroform); reported[11] m.p. 106–107°; $[\alpha]_D$ +131 ± 2° (chloroform). The mother liquor gave successive crops (0.37 g., m.p. 101–102°; 0.31 g., m.p. 91–92°) of crystals. The infrared spectra of the purified product and of authentic dimethyl acetal (IV) were identical.

**2-(3α-Hydroxy-11-oxo-5β-androstan-17β-yl)quinoxaline (III).**—To a solution of 364 mg. (1 mmole) of 3α,21,21-trihydroxy-5β-pregnane-11,20-dione in 2 ml. of methanol was added 160 mg. of sodium bisulfite in 10 ml. of water. The mixture was heated on a steam bath for 5 min., the methanol was removed, and the residue in 10 ml. of water was treated with 160 mg. of *o*-phenylenediamine in 5 ml. of hot water for 30 min. on a steam bath. The product was crystallized from methanol–ether to give yellow needles (270 mg., 65%, m.p. 181.5–182.5°). Additional crops of product brought the total yield to 91%. The analytical sample had m.p. 180.5–181.5°; $[\alpha]_D$ +111 ± 1°; $\lambda_{max1}^{MeOH}$ 237 mμ, ε 31,000; $\lambda_{max\,2}^{MeOH}$ 319 mμ, ε 8000.

*Anal.* Calcd. for $C_{27}H_{34}O_2N_2$: C, 77.60; H, 8.13; N, 6.70. Found: C, 77.52; H, 8.04; N, 6.84.

**3α,11β,21,21-Tetrahydroxy-5β-pregnan-20-one (VII).**—To a solution of 350 mg. (1 mmole) of 3α,11β,21-trihydroxy-5β-pregnan-20-one in 25 ml. of methanol was added 25 ml. of 0.005 *M* (0.125 mmole) methanolic cupric acetate. Air was bubbled into the solution for 45 min. and, following the addition of 50 mg. of EDTA (disodium ethylenedinitrilotetraacetate) in 3 ml. of water, the methanol was removed. The residue was diluted with water and extracted with methylene chloride. The yellow extract, after being washed with dilute sodium bicarbonate and water, was concentrated to dryness. Yellow crystals were obtained from aqueous acetic acid (240 mg., m.p. 154.4–155.5°; 25 mg., m.p. 152–154.5°). The sample for analysis was dried to constant weight at room temperature and 1–2 mm. over anhydrous calcium chloride; m.p. 154.5–155.5°; $[\alpha]_D$ +120 ± 2°.

*Anal.* Calcd. for $C_{21}H_{32}O_4 \cdot 1.5\ H_2O$: C, 67.19; H, 9.39. Found: C, 66.91, 67.27; H, 10.02, 9.38.

A portion of the analytical sample, dried for 2 hr. at 5–10 μ

(23) Prepared by acetylation of 3α-hydroxy-5β-pregnan-20-one (Canada Packers, Ltd., Toronto, Canada) with pyridine and acetic anhydride.

(24) F. Sondheimer, G. Rosenkranz, O. Mancera, and C. Djerassi, *J. Am. Chem. Soc.*, **75**, 2601 (1953).

(25) F. C. Colton, W. R. Nes, D. A. Van Dorp, H. L. Mason, and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

(26) W. F. McGuckin and H. L. Mason, *J. Am. Chem. Soc.*, **77**, 1822 (1955).

mercury and 100° lost 7.22%; calcd. for loss of 1.5 moles of water, 7.20%.

**2-(3α,11β-Dihydroxy-5β-androstan-17β-yl)quinoxaline (VIII).**—Conversion of 183 mg. (0.5 mmole) of 3α,11β,21,21-tetrahydroxy-5β-pregnan-20-one to its quinoxaline derivative was accomplished by the procedure used for the preparation of III from II. The crude product crystallized from acetone to give 189 mg. of yellow needles, m.p. 118–127°. A sample for analysis was crystallized from acetone and dried to constant weight under reduced pressure at room temperature.

*Anal.* Calcd. for $C_{27}H_{36}O_2N_2 \cdot CH_3COCH_3$: C, 75.27; H, 8.84; N, 5.86. Found: C, 75.37; H, 8.74; N, 6.42.

Another sample, which had been recrystallized from acetone and dried to constant weight under reduced pressure at room temperature, was dried for a further 46 hr. at 1–2 mm. and 100°. The weight loss was 11.25%; calcd. for loss of 1 mole of acetone, 12.15%; m.p. 127–129° $[\alpha]D$ +127 ± 1°; $\lambda_{max1}^{MeOH}$ 237 mμ, ε 28,800; $\lambda_{max2}^{MeOH}$ 319 mμ, ε 8000.

*Anal.* Calcd. for $C_{27}H_{36}O_2N_2$: C, 77.10; H, 8.56; N, 6.67. Found: C, 76.51; H, 8.85; N, 6.47.

**3α,21,21-Trihydroxy-5β-pregn-16-ene-11,20-dione (X) and 3α-Hydroxy-11-oxo-5β-eti-16-enic Acid (XIII).**—To 1.73 g. (5 mmoles) of 3α,21-dihydroxy-5β-pregn-16-ene-11,20-dione in 125 ml. of methanol was added an equal volume of 0.005 *M* methanolic cupric acetate. After 1 hr., during which time the solution was aerated, EDTA (250 mg.) in water (10 ml.) was added and the methanol was evaporated. The aqueous residue was diluted with 50 ml. of water and extracted with methylene chloride. The organic layer was washed with dilute sodium bicarbonate and water and concentrated to dryness.

The acidic fraction recovered from the aqueous washings was recrystallized from acetone to give 25 mg. of needles, m.p. 267.5–268.5° dec. Product, recrystallized from ethyl acetate while solution was hot, gave prisms (m.p. 276–278° dec.; $\lambda_{max}^{MeOH}$ 218 mμ, ε 7600) which did not depress melting point of the acid obtained by treatment of 3α,21-dihydroxy-5β-pregn-16-ene-11,20-dione with periodic acid. The infrared spectra of the two samples of XIII in Nujol were identical. Crystals separated from cold ethyl acetate as long needles; in Nujol the infrared spectrum of the long needles was markedly different from that of the prisms. Neither the prisms nor the needles was solvated.

Treatment of 200 mg. of 3α,21-dihydroxy-5β-pregn-16-ene-11,-20-dione in 10 ml. of methanol with 400 mg. of $H_2IO_6$ in 2 ml. of water at room temperature for 5 hr. followed by separation of the organic acid and crystallization gave 54 mg. of **3α-hydroxy-11-oxo-5β-eti-16-enic acid**, m.p. 276–279°. The product crystallized from hot ethyl acetate as prisms, m.p. 276–278°.

*Anal.* Calcd. for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 72.41; H, 8.51.

The neutral fraction from the cupric acetate oxidation was dissolved in 25 ml. of 80% aqueous acetone and water was added to the point of turbidity. After removal of a small amount of insoluble material, the solution was concentrated until crystals began to form. The yield of air-dried product was 1.48 g. (82%). The compound exhibited unusual melting behavior. When placed on the stage at or above 170°, it melted immediately. After being subjected to initial temperatures below 160° for a brief period, the crystals melted only partially even when the temperature was raised to over 250°. The sample for analysis was crystallized from aqueous acetone and dried to constant weight at room temperature and 1–2 mm. over anhydrous calcium chloride; $[\alpha]D$ +63 ± 2°; $\lambda_{max}^{MeOH}$ 242 mμ, ε 8500.

*Anal.* Calcd. for $C_{21}H_{30}O_5 \cdot H_2O$: C, 66.29; H, 8.46. Found: C, 65.79; H, 8.53.

**3α-Acetoxy-21,21-dimethoxy-5β-pregn-16-ene-11,20-dione (XII).**—To a solution of 362 mg. (1 mmole) of 3α,21,21-trihydroxy-5β-pregn-16-ene-11,20-dione (X) in 25 ml. of methanol was added 5 ml. of 1.35 *N* hydrogen chloride in dry methanol. After 15 hr. at room temperature the reaction mixture was neutralized by the slow addition of 4.5 ml. of 5% sodium carbonate plus 100 ml. of water, and extracted with methylene chloride. The extract was washed with water and concentrated to dryness. The residue was treated with 1 ml. each of pyridine and acetic anhydride for 5 hr. at room temperature. The acetylated product was crystallized from ether–petroleum ether (352 mg., m.p. 149–151°; 20 mg., m.p. 147–149°). After being stored several weeks at room temperature the originally colorless compound had become yellow and its melting point had decreased by approximately 10°. Recrystallization from aqueous methanol gave the analytical sample; m.p. 153.5–155°; $[\alpha]D$ +75 ± 2°; $\lambda_{max}^{MeOH}$ 245 mμ, ε 8650.

*Anal.* Calcd. for $C_{25}H_{36}O_6$: $CH_3O$, 14.37. Found: $CH_3O$, 14.75.

**2-(3α-Hydroxy-11-oxo-5β-androst-16-en-17β-yl)quinoxaline (XI).**—To 181 mg. (0.5 mmole) of 3α,21,21-trihydroxy-5β-pregn-16-ene-11,20-dione in 5 ml. of absolute ethanol was added 80 mg. of *o*-phenylenediamine. After being refluxed for 1 hr., the solvent was evaporated and the residue crystallized from acetone (134 mg., 65%). Two crystal forms were present; one melted at 202–203°, and the other at 225–227°. When the temperature of a sample on the stage was raised above 228° and then cooled, all of the resulting crystals melted at 226.5–227.5°; $[\alpha]D$ +129 ± 1°; $\lambda_{max1}^{MeOH}$ 213 mμ, ε 21,900; $\lambda_{max2}^{MeOH}$ 260 mμ, ε 23,400; $\lambda_{max3}^{MeOH}$ 338 mμ, ε 9600.

*Anal.* Calcd. for $C_{27}H_{32}O_2N_2$: C, 77.86; H, 7.75; N, 6.73. Found: C, 77.46; H, 7.88; N, 6.94.

**3α,21,21-Trihydroxy-5β-pregnan-20-one (XV).**—3α,21-Dihydroxy-5β-pregnan-20-one (334 mg., 1 mmole) was converted to its glyoxal by the procedure used for the preparation of VII from VI. Crystallization from aqueous acetone gave a light yellow product (310 mg., 89%, m.p. 128–130°). A sample for analysis was crystallized from aqueous acetic acid and dried to constant weight under reduced pressure over anhydrous calcium chloride; m.p. 128.5–130°; $[\alpha]D$ +99 ± 2°.

*Anal.* Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 72.08; H, 9.89.

**2-(3α-Hydroxy-5β-androstan-17β-yl)quinoxaline (XVI).**—Conversion of 3α,21,21-trihydroxy-5β-pregnan-20-one (175 mg., 0.5 mmole) to its quinoxaline derivative was effected by the procedure used for the preparation of III from II. Crystallization from acetone gave yellow needles (112 mg., m.p. 198–201°; 22 mg., m.p. 195–200°). The analytical sample had m.p. 205–205.5°; $[\alpha]D$ +140 ± 2°; $\lambda_{max1}^{MeOH}$ 238 mμ, ε 31,300; $\lambda_{max2}^{MeOH}$ 319 mμ, ε 8200.

*Anal.* Calcd. for $C_{27}H_{36}ON_2$: C, 80.15; H, 8.97; N, 6.92. Found: C, 79.86; H, 8.88; N, 6.88.

**17,21-Dihydroxy-21-methoxypregn-4-ene-3,11,20-trione (XX) and 17-Hydroxy-3,11-dioxo-4-etienic Acid (XXI).**—To a solution of 3.60 g. (10 mmoles) of cortisone[27] in 250 ml. of methanol was added 250 ml. of 0.01 *M* methanolic cupric acetate. Air was bubbled into the solution for 1 hr. After addition of 500 mg. of EDTA in 50 ml. of water, the methanol was evaporated. The aqueous residue was extracted with methylene chloride and, after being washed with dilute sodium bicarbonate and water, the yellow solution was concentrated to dryness.

The acidic fraction yielded 16 mg. of needles (m.p. 251–258° dec.) from acetone. A mixture melting point with an authentic sample of 17-hydroxy-3,11-dioxo-4-etienic acid showed no depression.

Crystallization of the neutral fraction from aqueous methanol gave 2.93 g. (75%) of hemiacetal (XX), m.p. 125–126° (on melting point stage at 120°), and 0.52 g., m.p. 123–126°. A sample for analysis was recrystallized from aqueous methanol and dried for 14 hr. at room temperature and 0.1 mm. over phosphorus pentoxide; m.p. 113–115° (on melting point stage at 100°); $[\alpha]D$ +179 ± 2°; $\lambda_{max}^{MeOH}$ 238 mμ, ε 15,900.

*Anal.* Calcd. for $C_{22}H_{30}O_6$: C, 67.66; H, 7.74; $CH_3O$, 7.94. Found: C, 67.50; H, 7.77; $CH_3O$, 7.44.

**17,21,21-Trihydroxypregn-4-ene-3,11,20-trione (XVIII).**—The hemiacetal (XX) was recrystallized from aqueous acetone to yield the hydrate (XVIII) which was dried for 14 hr. at room temperature and 0.1 mm. over phosphorus pentoxide; m.p. 140–145° (on stage at 130°); $[\alpha]D$ +182 ± 2°; $\lambda_{max}^{MeOH}$ 238 mμ, ε 15,800; reported[14] m.p. 170–190°; $[\alpha]D$ +182° (*c* 2, methanol); $\lambda_{max}^{MeOH}$ 238 mμ, $E_{1\ cm}^{1\%}$ 418 (which corresponds to ε 15,700).

*Anal.* Calcd. for $C_{21}H_{28}O_6$: C, 67.01; H, 7.49. Found: C, 67.02; H, 7.65.

**2-(17-Hydroxy-3,11-dioxopregn-4-en-17β-yl)quinoxaline (XIX).**—Conversion of 17,21-dihydroxy-21-methoxypregn-4-ene-3,11,-20-trione (200 mg.) to its quinoxalyl derivative was effected in the usual manner.[14] The crude product (203 mg., 92%, m.p. 231–234°) gave needles (173 mg., m.p. 243–244.5°) from methanol. A sample, recrystallized from methanol and air dried, lost 7.06% when dried for 3 hr. at 100° and 1–2 mm. over phosphorus pentoxide; calcd. for loss of 1 mole of methanol, 6.92%; m.p. 245–246°; $\lambda_{max1}^{MeOH}$ 237 mμ, ε 47,500; $\lambda_{max2}^{MeOH}$ 319 mμ, ε 8150. The reported constants are m.p. 242–243°; $\lambda_{max1}^{MeOH}$ 238 mμ, $E_{1\ cm}^{1\%}$ 1045 (which corresponds to ε 44,900); $\lambda_{max2}^{MeOH}$ 319 mμ, $E_{1\ cm}^{1\%}$ 185 (which corresponds to ε 7950).

(27) The cortisone was kindly donated by Merck and Co., Rahway, N. J.