from chloroform (or dioxane). Product XII exhibited characteristic tosylate-group absorptions at 1362 and 1178 cm.$^{-1}$.

Anal. Calcd. for $C_{38}H_{44}O_{12}S_4$: C, 55.59; H, 5.40. Found: C, 55.29; H, 5.33.

1,2,4,5-Tetrakis(bromomethyl)cyclohexane (XI). (A) From Tetratosylate XII.—A glass-lined stainless steel bomb charged with 15.0 g. (0.0183 mole) of tetratosylate XII, 8.6 g. of lithium bromide, and 150 ml. of reagent acetone was heated at 110° for 14.5 hr. The resulting mixture, consisting of a fairly homogeneous solid phase and a discolored acetone phase, was filtered with suction. The filter cake, a mixture of product XI and tosylate salt, was triturated with four 100-ml. portions of carbon tetrachloride. The combined extracts were concentrated to give, in two crops (m.p. 207–208° and 205–207°), 3.0 g. (36%) of tetrabromide XI. The acetone filtrate from the original reaction mixture afforded no additional product. An analytical sample of XI, recrystallized from carbon tetrachloride, had m.p. 207–208°.

Anal. Calcd. for $C_{10}H_{16}Br_4$: C, 26.35; H, 3.54; Br, 70.12. Found: C, 26.56; H, 3.71; Br, 70.38.

(B) From Tetraol X.—Attempted bromination of X using phosphorus tribromide in carbon tetrachloride and carried out in the usual manner gave only 4% of tetrabromide XI as the sole isolable solid. This product was identical to XI derived from XII as described previously.

1,2,4,5-Tetrakis(iodomethyl)cyclohexane (XIII). (A) From Tosylate XII.[23]—A solution of 12.2 g. (0.0149 mole) of tosylate XII and 18.0 g. of dry sodium iodide in 80 ml. of reagent acetone was refluxed for 4 hr. During this time a white solid separated. The reaction mixture was cooled and filtered with suction. The filter cake, a mixture of product and sodium tosylate, was washed first with acetone, then thoroughly with water, and subsequently dried to give 95% crude iodide XIII, m.p. 244° dec. Product XIII is somewhat soluble in hot chloroform and moderately soluble in hot tetrahydrofuran.

An analytical sample, recrystallized from chloroform, had m.p. 245–246°.

Anal. Calcd. for $C_{10}H_{16}I_4$: C, 18.65; H, 2.50; I, 78.84. Found: C, 18.88; H, 2.54; I, 78.58.

(B) From Tetrabromide XI.—To a solution of 0.67 g. of XI (1.47 mmoles) in 75 ml. of hot acetone there was added, with stirring, 2.64 g. (17.6 mmoles) of dry sodium iodide. The resulting hot solution, initially clear, quickly became turbid and soon deposited a white solid. The mixture, after refluxing for 90 hr. (arbitrary), was cooled and filtered. To the filtrate was added an equal volume of chloroform and the resulting precipitate of inorganic salts was removed by filtration. The acetone–chloroform solution, hot, was used to triturate the filter cake from the original reaction mixture. The extract was concentrated to give, in two crops, 0.60 g. (63%) of tetraiodide XIII, m.p. 245–246° dec.

Acknowledgment.—The author is indebted to Dr. C. S. Marvel (National Science Foundation grant NSF-G-2626) with whose encouragement this work was initiated.

(23) The author is indebted to Mrs. C. L. Warren for initially carrying out this reaction.

Glycolic Acids and Esters From Cortisone[1]

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In methanolic cupric acetate the glyoxal from cortisone (20-keto-21-aldehyde) rearranges slowly to form the corresponding glycolic acids (20-hydroxy-21-acid) and methyl esters. Two 20-hydroxy acids (epimeric at C-20) and two epimeric 20-hydroxy esters are formed as main products. Reaction occurs slowly; 12% of starting material is present after two weeks. With alkali, rearrangement of the glyoxal to the same pair of epimeric 20-hydroxypregnenoic acids occurs rapidly. Acetylation of the epimeric acids under mild conditions gives the 17,20-diacetates whereas acetylation of the methyl esters under the same conditions gives the 20-monoacetates. Treatment of the methyl esters under vigorous acetylating conditions gives the 17,20-diacetates along with some of the corresponding C-3 enol acetates. The acetyl group is removed more readily from the 20α-acetate than from its 20β-epimer. The configurations at C-20 of the two series of epimers were determined.

In a previous paper it was shown that cupric acetate in methanol catalyzes the rearrangement of steroidal glyoxals to the methyl esters of steroidal glycolic acids.[3] From each glyoxal, two 20-epimeric 20-hydroxypregnan-21-oic esters were obtained. The rate of reaction of 17-hydroxy steroidal glyoxals was considerably less than that of the 17-deoxy analogs.

This paper describes the conversion of the glyoxal from cortisone into its 20-epimeric steroidal glycolates by catalysis with cupric acetate and with sodium hydroxide. Treatment of cortisone (I, Fig. 1) with methanolic cupric acetate for one hour[4] gave a good yield of glyoxal II. When this glyoxal was treated with methanolic cupric acetate at room temperature for two weeks, an 88% reduction in the Porter–Siber chromogenicity occurred. After the products of the reaction had been isolated, it was found that, in contrast to the analogous reaction with 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al,[3] there was a significant acidic fraction (11%). This fraction was shown, by paper chromatography, to consist chiefly of the free glycolic acids (VIIIa[5] and VIIIb[5]) together with a small amount of the corresponding etienic acid (17-hydroxy-3,11-dioxoeti-4-enic acid).

The neutral fraction was acetylated and, after separation of a small amount of the 20α-acetoxy ester (IVa) by crystallization, the product was fractionated by column chromatography.[3] Small amounts of three compounds of unknown structure were obtained. The principal products were the 20α- and 20β-acetoxy esters (IVa and IVb) which were obtained in 27 and 22% yield, respectively.

Alkaline rearrangement of glyoxal II occurred much more rapidly and gave a higher yield of crystallizable product than did the cupric acetate-catalyzed rearrangement. Treatment of an aqueous suspension of glyoxal II at 0° under nitrogen with 1.25 equivalents of sodium hydroxide for thirty minutes resulted in almost complete disappearance of the glyoxal. Successive esterification and acetylation of the product, followed by

(1) Abridgment 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.

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

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

(4) M. L. Lewbart and V. R. Mattox, *ibid.*, **28**, in press.

(5) "a" represents the 20 α-oxygen epimer; "b," the 20β-epimer.

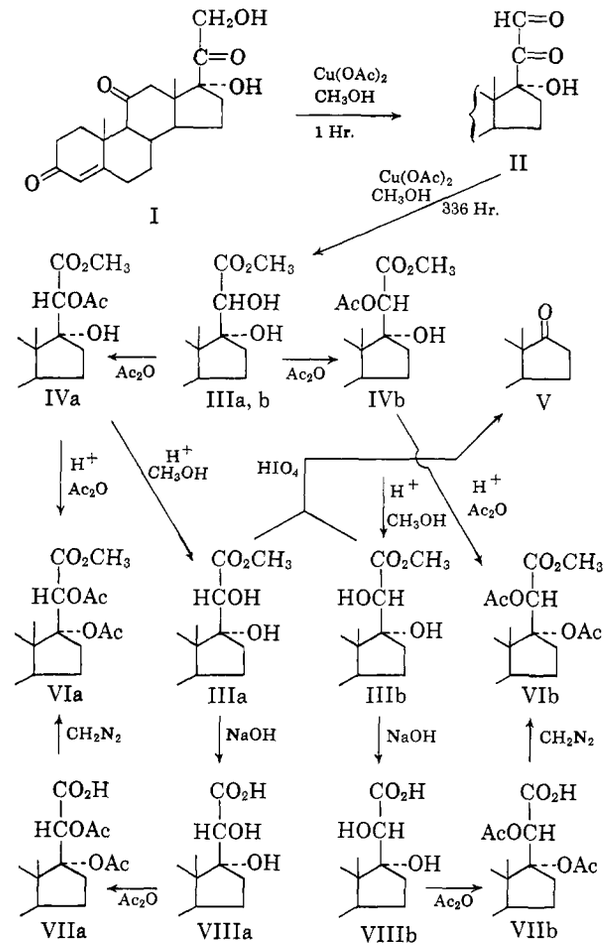

Figure 1

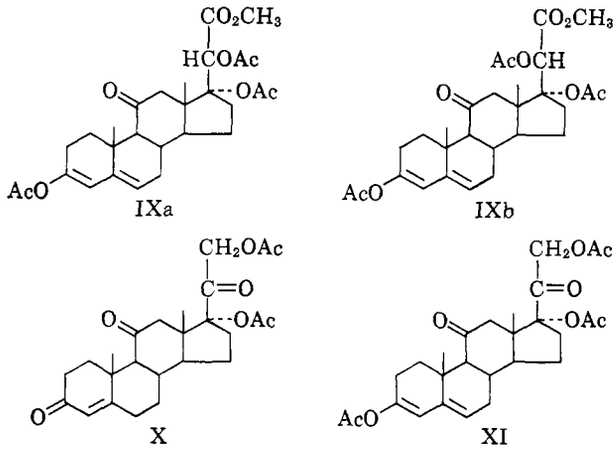

Figure 2

column chromatography, gave the 20α- and 20β-acetoxy methyl esters (IVa and IVb) in yields of 47 and 34%. These products were identical with the acetylated products obtained from reaction of the glyoxal II with methanolic cupric acetate.

The structures of IVa and IVb were anticipated from the studies on the glycolic acid derivates of 17-deoxy steroidal glyoxals[5] and were established from the transformations shown in Fig. 1. Treatment of the acetoxy esters (IVa and IVb) with methanolic hydrogen chloride removed the acetyl group from C-20 and gave the epimeric 20-hydroxy esters (IIIa and IIIb) in good yield. That these products contained vicinal hydroxyl groups was shown by their conversion to adrenosterone (V) by treatment with periodic acid. Attempts to convert the epimeric 20-hydroxy esters (IIIa and IIIb) to the corresponding 20-ketone by chromic acid oxidation under mild conditions were unsuccessful; adrenosterone was the only product detectable.

Saponification of the 20-hydroxy methyl esters (IIIa and IIIb) afforded the free 20-hydroxy acids (VIIIa and VIIIb) in about 80% yield. These acids had the same chromatographic mobility and gave the same color reactions as the major acidic artifact produced by the action of traces of copper on cortisone during paper chromatography.[6]

In an attempt to acetylate selectively the 20-hydroxyl group in the dihydroxy acids (VIIIa and VIIIb), these compounds were treated at room temperature with acetic anhydride and pyridine. The corresponding **17,20-diacetyl** derivatives (VIIa and VIIb) were obtained in yields of 51 and 44%, respectively. This result was unexpected since acetylation of 17-hydroxypregnanes ordinarily requires strenuous[7] conditions. It may have been due to formation of a mixed anhydride which served to bring an acetyl function into a position favorable for transfer to the 17-hydroxyl group by an intramolecular process.[8] From acetylation of corresponding dihydroxy esters (IIIa and IIIb) only the **20-monoacetates** (IVa and IVb) were obtained.

Proof of structure of the 17,20-diacetoxy acids (VIIa and VIIb) was obtained by esterification with diazomethane to give diacetoxy esters VIa and VIb. These products were prepared by acetylation at C-17 of the 20-acetyl methyl esters (IVa and IVb, respectively) with a mixture of acetic anhydride, acetic acid, and *p*-toluenesulfonic acid.[7]

From the acetylation at C-17 of each 20-acetyl methyl ester also was formed a less polar by-product. Only that product from the 20β-epimer could be obtained in crystalline form. Because of an ultraviolet absorption maximum at 234 mμ and the absence from the infrared spectrum of a band at 1669 cm.$^{-1}$ (characteristic for Δ^4-3-ketones), the substance was presumed to be the enol acetate (IXb, Fig. 2) of VIb (Fig. 1).

In describing the acylation at C-17 of 17-hydroxypregnanes, Turner[7] noted that, whereas yields from compounds saturated in ring A were nearly quantitative, those from Δ^4-3-ketones were less than 50%. Although Turner considered enol acetate formation as an explanation for the poor yields, he discounted such a possibility because of the absence in crude reaction mixtures of infrared absorption bands which are characteristic for such compounds.[9] Nevertheless, the crystalline by-product from forced acetylation of IVb was in fact the enol[10] acetate (IXb) as proven by elemental

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

(7) R. B. Turner, *J. Am. Chem. Soc.*, **75**, 3489 (1953).

(8) We are indebted to R. M. Dodson for this suggestion.

(9) R. N. Jones and K. Dobriner, "Infrared Spectrometry Applied to Steroid Structure and Metabolism in Vitamins and Hormones," Vol. 7, Academic Press, Inc., New York, N. Y., 1949, p. 323.

(10) Further confirmation of the general formation of enol acetates from Δ^4-3-ketones was obtained when Turner's conditions were applied to cortisone in this laboratory. The 17,21-diacetate (X) and the 3,17,21-triacetate (XI) were each isolated in 37% yield. The (MDXI)-(MDX) of -796 units was of the expected magnitude. In addition, Ringold, *et al.*,[12] have also isolated an enol acetate as a by-product in the acetylation of 6-methyl-17-hydroxyprogesterone under Turner's conditions. The (MD $\Delta^{3,5}$-3-acetate)—(MDΔ^4-3-ketone) was -879 units. It was noted that shortening the reaction time reduced enol acetate formation, and afforded the 17-acetates in much better yield. Our findings are in agreement with this observation.

analysis and correlation of optical rotatory values. As shown by Westphal,[11] conversion of a Δ^4-3-ketone to the corresponding $\Delta^{3,5}$-3-acetate is associated with a strongly negative shift in the optical rotation. For example (MD$\Delta^{3,5}$-3-acetate)-(MDΔ^4-3-ketone) values for compounds derived from cholestenone, progesterone, and testosterone are −789, −756, and −855 units, respectively. In agreement with these values is (MDIXb)-(MDVIb) of −940 units.

The 20-acetoxy acids (XIIa and XIIb, Fig. 3), unsuccessfully sought by acetylation of the 20-hydroxy acids, could be obtained in low yields by partial hydrolysis of the 20-acetoxy esters (IVa and IVb). Treatment of these esters with one equivalent of sodium hydroxide in aqueous ethanol for fifteen minutes at room temperature gave a mixture of neutral and acidic products. Column chromatography of the mixture from the 20α-epimer gave the 20α-hydroxy acid (VIIIa), the 20α-acetoxy acid (XIIa), and the 20α-hydroxy ethyl ester (XIIIa), all of which could be crystallized. From the 20β-epimer (IVb) was obtained the 20β-hydroxy acid (VIIIb), the 20β-acetoxy acid (XIIb), the 20β-hydroxy ethyl ester (XIIIb), the 20β-acetoxy ethyl ester (XIVb), and the 20β-acetoxy methyl ester (IVb, starting material). The structures of the ethyl esters were established by independent preparation of them by treatment of the respective acids with diazoethane.

The isolation of significant amounts of ethyl esters of the acids indicates that, under the conditions employed, transesterification occurs rapidly. Also, in considering the hydrolysis of IVa and IVb, it is apparent that the 20β-acetyl group is removed less readily than is the 20α-acetyl group.

Values for the optical rotation of various derivatives of the 20-hydroxy epimers are given in Table I. It is apparent that values for molecular rotations of one epimeric series of 17-hydroxy-20-*acetoxy* compounds (pairs 3, 4, and 6) are uniformly greater than those of the other series. However, the acetylation increments[13] which can be calculated for compounds derived from one C-20 epimeric series are not uniformly larger (or smaller) than those derived from the other epimeric series. This can be seen by subtracting 1 from 3, 2 from 4, and 5 from 6 in the two epimeric series in Table I. Consequently, assignment of configuration at C-20 cannot be made with confidence from optical rotatory values.

TABLE I

MOLECULAR ROTATIONS[a] OF 3,11-DIOXOPREGN-4-ENE DERIVATIVES WITH SUBSTITUENTS AT C-17, C-20, AND C-21

Pair	Substituents			Epimers		Δ^5
no.	C-17	C-20	C-21	20α	20β	α-β
1	α-OH	OH	O_2H	+463	+467	−4
2	α-OH	OH	O_2CH_3	+453	+465	−12
3	α-OH	OAc	O_2H	+561	+548	+13
4	α-OH	OAc	O_2CH_3	+562	+493	+69
5	α-OH	OH	$O_2C_2H_5$	+562	+449	+113
6	α-OH	OAc	$O_2C_2H_5$	+621	+580	+41
7	α-OAc	OAc	O_2H	+253	+437	−184
8	α-OAc	OAc	O_2CH_3	+313	+451	−138

[a] Molecular rotations, MD, are $[\alpha]D \times$ mol. wt./100. [b] Δ = MD$^{20}\alpha$ − MD$^{20}\beta$.

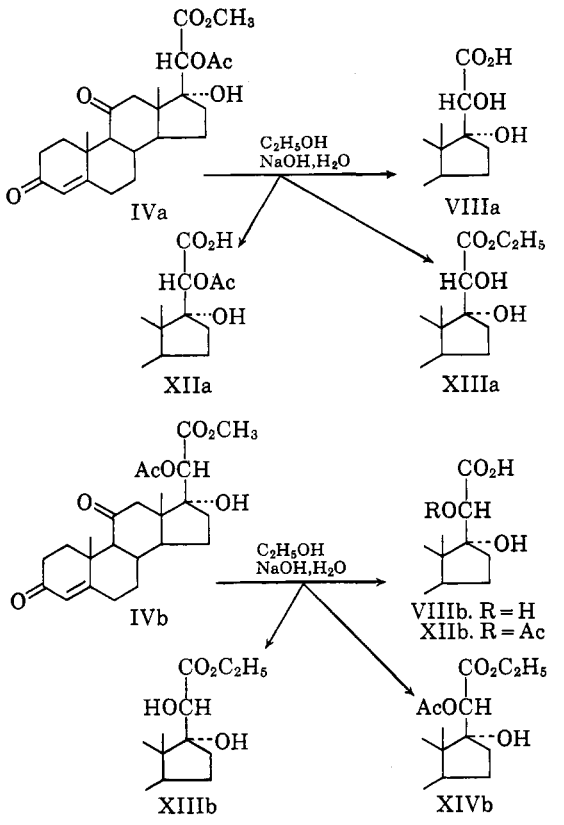

Figure 3

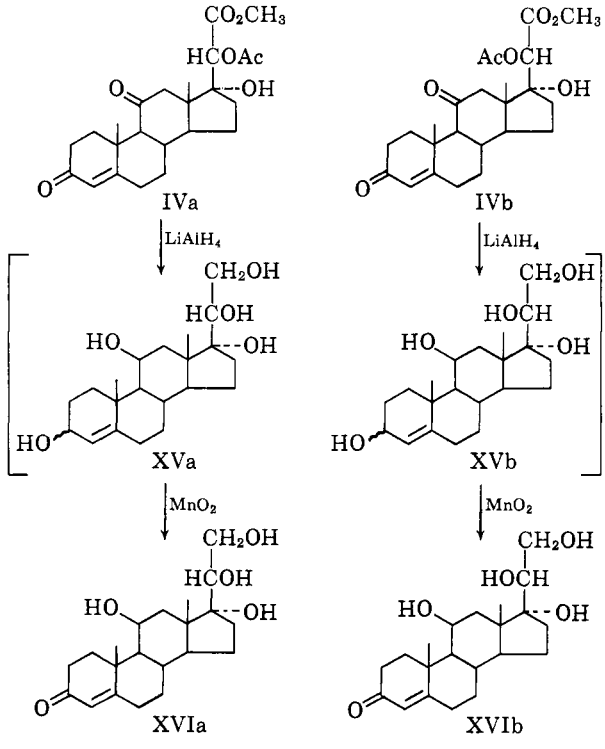

Figure 4

Configuration at C-20 was established definitively by lithium aluminum hydride reduction of the acetoxy methyl esters (IVa and IVb, Fig. 4) to their respective 3,11,17,20,21-pentols (XVa and XVb), followed by regeneration of the Δ^4-3-keto system with manganese

(11) U. Westphal, *Ber.*, **70**, 2128 (1937).

(12) H. J. Ringold, J. P. Ruelas, E. Batres, and Carl Djerassi, *J. Am. Chem. Soc.*, **81**, 3712 (1959).

(13) L. F. Fieser, and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959. pp. 612–618.

dioxide.[14] The configurations of the products, 11β,17,-20β,21 - tetrahydroxypregn - 4 - en - 3 - one[15,16] (XVIb) and its 20α-epimer[17] (XVIa), are known. Since lithium aluminum hydride reduction of α-hydroxy[18] acids proceeds without change of configuration[19] at the α-carbon[18] atom, this sequence of transformations establishes the configuration at C-20 in the two epimers (IVa and IVb).

The finding of greater dextrorotatory values for 17α-hydroxy-20α-acetoxy compounds than for the 17α-hydroxy-20β-acetoxy epimers (pairs 3, 4, and 6, Table I) parallels the findings[3] on six pairs of 20-epimeric 17-deoxy-20-hydroxy-5β-pregnan-21-oic acids and esters. However, when both C-17 and C-20 bear acetoxy groups (pairs 7 and 8, Table I) the 17α,20α-diacetoxy acid and ester are less dextrorotatory than the corresponding 17α,20β-diacetoxy acid and ester. From these findings, and others,[12] it is apparent that, in general, both the function at C-17 and the function at C-21 determine to a considerable extent whether an acetoxy group at C-20 is dextrorotatory or levorotatory in a particular configuration.

Experimental

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

A. Methyl 17-Hydroxy-20α(and 20β)-Acetoxy-3,11-dioxopregn-4-en-21-oates (IVa and IVb) and Two Unknown Compounds from Cortisone Glyoxal and Methanolic Cupric Acetate.—To a solution of 1.88 g. (4.82 mmoles) of cortisone glyoxal hemiacetal (17-hydroxy-3,11,20-trioxopregn-4-en-21-al 21-methyl hemiacetal)[4] in 125 ml. of methanol was added an equal volume of methanol containing 500 mg. (2.5 mmoles) of cupric acetate. After 14 days at room temperature, 11.6% of the original glyoxal remained. Disodium ethylenedinitrilotetraacetate (EDTA, 1 g.) in water (50 ml.) was added and the methanol was evaporated. The aqueous residue was extracted with methylene chloride which, after being washed with 5% sodium bicarbonate and water, was concentrated to dryness.

The combined aqueous washes were acidified with dilute hydrochloric acid and the resulting precipitate was extracted with ethyl acetate. The acidic residue from the ethyl acetate extract weighed 207 mg. (11.0%) and was shown by paper chromatography to consist of material with the same R_f and color reactions (periodate—Zimmermann,[20] ultraviolet absorption and sodium hydroxide-induced fluorescence) as the epimeric free 20-hydroxy acids (VIIIa and VIIIb, Fig. 1). A small amount of a substance with the mobility of **17-hydroxy-3,11-dioxoeti-4-enic acid** also was present.

After removal of residual glyoxal with sodium bisulfite,[4] the neutral fraction (methylene chloride extract) was treated with 3 ml. each of pyridine and acetic anhydride for 14 hr. at room temperature. The product gave **methyl 17-hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate** (IVa) as colorless plates (378 mg., m.p. 196–202°) from acetone. Recrystallization from acetone gave 300 mg. (14.4%, m.p. 205.5–207°).

The residue from the mother liquor was fractionated on a 6 × 40 cm. column of Celite (350 g.) impregnated with 157.5 ml. of the heavier phase of benzene (1800), cyclohexane (1200), formamide (200). The flow rate was 130 ml./hr.; 22-ml. fractions were collected. Prior to collection of fraction 1, 800 ml. of effluent was discarded. The absorbance at 238 mμ of the residues from aliquots of selected fractions was determined and fractions were pooled after construction and evaluation of an elution diagram. Several unknown compounds emerged before the 20-acetoxy methyl esters.

Fractions 1–30.—Not investigated.

Unknown from Fractions 30–80.—The residue gave 38 mg. (m.p. 227–229°) of crystals from ethyl acetate.

Unknown from Fractions 90–120.—Crystallization from acetone–petroleum ether gave 30 mg., m.p. 225–226°.

Methyl 17-Hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate (IVa). Fractions 124–170.—Crystallization from acetone–petroleum ether gave 257 mg. (m.p. 205–206°) and 11 mg. (m.p. 202–203°) of 20α-acetoxy methyl ester (IVa) for a total yield of 27%. A sample, recrystallized from acetone, melted at 208–209°; $[\alpha]_D$ +130° ± 1°; λ_{max}^{MeOH} 239 mμ, ε 15,800.

Anal. Calcd. for $C_{24}H_{32}O_7$: C, 66.62; H, 7.46; CH_3CO, 9.95; CH_3O, 7.17. Found: C, 66.44; H, 7.59; CH_3CO, 10.10; CH_3O, 7.33.

Methyl 17-Hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate (IVb). Fractions 176–230.—Crystallization from acetone–petroleum ether gave the 20β-acetoxy methyl ester IVb in 22% yield (369 mg., m.p. 193–195°; and 85 mg., m.p. 192.5–194°). The analytical sample was recrystallized from acetone–ether; m.p. 195.5–197.5°; $[\alpha]_D$ +114° ± 1°; λ_{max}^{MeOH} 239 mμ, ε 16,100.

Anal. Calcd. for $C_{24}H_{32}O_7$: C, 66.62; H, 7.46; CH_3CO, 9.95; CH_3O, 7.17. Found: C, 66.61; H, 7.42; CH_3CO, 10.28; CH_3O, 7.27.

B. Methyl 17-Hydroxy-20α(and 20β)-acetoxy-3,11-dioxopregn-4-en-21-oates (IVa and IVb) from Cortisone Glyoxal and Sodium Hydroxide.—To a suspension of 3.0 g. (7.7 mmoles) of cortisone glyoxal hemiacetal in 150 ml. of water, 4.8 ml. of 2.09 *N* sodium hydroxide (10 mmoles) was added slowly, while stirring rapidly at 0° under nitrogen. After 1 hr., the reaction mixture contained less than 5% of starting material as indicated by analysis of an aliquot by the Porter–Silber reaction. The mixture was extracted promptly with two 50-ml. volumes of ethyl acetate. The organic phase was washed with water and discarded. The combined aqueous phases were acidified with *N* hydrochloric acid and re-extracted with ethyl acetate. The ethyl acetate extract was washed twice with water, dried, and concentrated to dryness. The residue was dissolved in 30 ml. of methanol and treated with an excess of diazomethane. After removal of the solvent, the residue was treated with 5 ml. each of pyridine and acetic anhydride for 15.5 hr. at room temperature. Crystallization of the product from acetone gave 1200 mg. (m.p. 200–205°) of crude 20α-acetoxy methyl ester (IVa). Recrystallization from the same solvent gave 1057 mg. (32%, m.p. 206–208°) of pure material.

The mother liquor was evaporated to dryness and the residue was fractionated on a column identical with the one used for the mixture obtained with methanolic cupric acetate.

Methyl 17-Hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate (IVa). Fractions 166–221.—Crystallization from acetone–ether gave an additional 449 mg. (m.p. 206–208.5°) of product. The compound had an infrared spectrum identical with that of the more mobile acetylated epimer (IVa) obtained from cortisone glyoxal by reaction with methanolic cupric acetate.

Methyl 17-Hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate (IVb). Fractions 236–318.—Crystallization from acetone–ether gave three crops of product (873 mg., m.p. 195–196°; 184 mg., m.p. 193–194°; and 57 mg., m.p. 186–188°). Recrystallization of the second and third crops gave 198 mg. (m.p. 194–197°) of pure 20β-epimer. The infrared spectrum of this compound was identical with that of the less mobile epimer acetate obtained with methanolic cupric acetate.

The residues from the mother liquors of both major fractions were combined with the residue from fractions 222–235 and rechromatographed on a small column in the same system used for the original mixture. An additional 55 mg. (m.p. 205–206°) of IVa and 58 mg. (m.p. 195–198°) of IVb was recovered from this column. The total yield of 20α-acetoxy methyl ester (IVa) was 1561 mg. (47.0%) and of 20β-acetoxy methyl ester (IVb), 1129 mg. (33.8%).

Methyl 17,20α-Dihydroxy-3,11-dioxopregn-4-en-21-oate (IIIa) from IVa.—To a solution of 432 mg. (1 mmole) of methyl 17-hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate in 25 ml. of methanol was added 25 ml. of 1.59 *N* hydrogen chloride in dry methanol. After 24 hr. at room temperature, the solution was

(14) Franz Sondheimer, C. Amendolla, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 5930 (1953).

(15) L. F. Fieser and Mary Fieser "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 622.

(16) T. Reichstein and J. von Euw, *Helv. Chim. Acta*, **24**, 247E (1941).

(17) Shlomo Burstein and R. I. Dorfman, *J. Biol. Chem.*, **213**, 581 (1955).

(18) In this instance α denotes the position adjacent to the carboxyl group rather than its configuration.

(19) D. S. Noyce and D. B. Denney, *J. Am. Chem. Soc.*, **72**, 5743 (1950).

(20) Constance de Courcy, and J. J. Schneider, *J. Biol. Chem.*, **223**, 865 (1956).

diluted with an equal volume of methylene chloride and added to 100 ml. of water. After two additional extractions with the organic solvent, the combined methylene chloride extracts were washed with water, dried, and concentrated to dryness. Crystallization from acetone–ether gave two crops (295 mg., m.p. 195.5–197° and 43 mg., m.p. 192–194°) of dihydroxy ester (IIIa) for a yield of 86.7%. A sample, recrystallized from acetone–ether, melted at 197–198.5°; $[\alpha]_D$ +116° ± 2°; λ_{max}^{MeOH} 239 mμ, ε 16,100.

Anal. Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.86; H, 7.75.

Methyl 17,20β-Dihydroxy-3,11-dioxopregn-4-en-21-oate (IIIb) from IVb.—To a solution of 158 mg. (0.366 mmole) of methyl 17-hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate in 10 ml. of methanol was added 10 ml. of 1.59 *N* hydrogen chloride in dry methanol. Paper chromatography of an aliquot removed after 24 hr. at room temperature revealed the presence of approximately 10% of starting material. After an additional 24 hr., the product was recovered in the same manner as the 20α-epimer. Crystallization from acetone–ether gave two crops (108 mg., m.p. 208–210°; and 17 mg., m.p. 202–204°) of dihydroxy ester IIIb; yield 87.5%. A sample for analysis was obtained by recrystallization from acetone–ether; m.p. 212–214°; $[\alpha]_D$ +119° ±2 °; λ_{max}^{MeOH} 239 mμ, ε 15,800.

Anal. Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.91; H, 7.65.

Androst-4-ene-3,11,17-trione (V, Adrenosterone) from IIIa and IIIb.—To 50 mg. each of the epimeric diols (IIIa and IIIb) in 5 ml. of methanol and 2 ml. of water was added 3 ml. of 4% periodic acid in 0.2 *N* sulfuric acid. After 3.5 hr. at room temperature, the reaction mixtures were diluted with 50 ml. of water and extracted with methylene chloride. The methylene chloride extracts were washed with dilute sodium bicarbonate solution and then water, dried, and concentrated to dryness. Crystallization from ethyl acetate gave 26 and 21 mg. of plates (m.p. 220–223° in each case) from the 20α- and 20β-epimers, respectively. The products did not depress the melting point of authentic adrenosterone (V) and their infrared spectra were identical with that of the reference compound.

17,20α-Dihydroxy-3,11-dioxopregn-4-en-21-oic Acid (VIIIa) from IIIa.—To 156 mg. (0.4 mmole) of methyl 17,20α-dihydroxy-3,11-dioxopregn-4-en-21-oate in 2 ml. of methanol was added 10 ml. of water and 0.4 ml. of 2 *N* sodium hydroxide (0.8 mmole). The turbid solution became clear in a few minutes, and, after 10 min. at room temperature, it was diluted with water and extracted with ethyl acetate. The organic phase was discarded. The aqueous phase was acidified with *N* hydrochloric acid and re-extracted with ethyl acetate. The extract was washed with water, dried, and concentrated to dryness. Crystallization from acetone gave 117 mg. (78.0%, m.p. 244.5–245° dec.) of 20α-hydroxy acid (VIIIa). A sample, recrystallized from acetone, melted at 245–246° dec.; $[\alpha]_D$ +123° ± 2°; λ_{max}^{MeOH} 239 mμ, ε 15,700.

Anal. Calcd. for $C_{21}H_{28}O_6$: C, 67.00; H, 7.49. Found: C, 66.89; H, 7.48.

17,20β-Dihydroxy-3,11-dioxopregn-4-en-21-oic Acid (VIIIb) from IIIb.—Hydrolysis of 156 mg. (0.4 mmole) of methyl 17,20β-dihydroxy-3,11-dioxopregn-4-en-21-oate was performed in the same manner as for the 20α-epimer. Crystallization from acetone gave 117 mg. (78.0%, m.p. 223–225° dec.) of dihydroxy acid (VIIIb). For the analytical sample, m.p. 224–225° dec.; $[\alpha]_D$ +124° ± 2°; λ_{max}^{MeOH} 239 mμ, ε 15,700.

Anal. Calcd. for $C_{21}H_{28}O_6$: C, 67.00; H, 7.49. Found: C, 66.98; H, 7.66.

17,20α-Diacetoxy-3,11-dioxopregn-4-en-21-oic Acid (VIIa) from VIIIa.—Acetylation of 200 mg. of 17,20α-dihydroxy-3,11-dioxopregn-4-en-21-oic acid was performed with 1 ml. each of pyridine and acetic anhydride for 19 hr. at room temperature. The solution was mixed with ice and water, and the product was extracted with ethyl acetate. The extract was washed with *N* hydrochloric acid, with water until neutral, and then dried, and evaporated to dryness. The residue was dissolved in a minimal volume of methanol and the solution was diluted with water to give a milky suspension. The mixture was made slightly alkaline by addition of 5% sodium bicarbonate solution, and extracted with ethyl acetate. The ethyl acetate extract was washed with water and concentrated to dryness. This neutral fraction weighed 80 mg. (40%) and gave crystals from ethyl acetate–petroleum ether. The crystalline material had a wide melting range (120–140°) and was a mixture of three compounds—R_f 0.5, 0.83, and 0.88 in toluene (275), isooctane (225), methanol (400), water (100). Its composition was not determined.

The acidic fraction was recovered by addition to the aqueous layer of slightly more than one equivalent of hydrochloric acid followed by extraction with ethyl acetate. Crystallization of the residue from acetone–ether gave 116 mg. (48%, m.p. 202–204°) of the diacetoxy acid (VIIa) as rosettes. For the analytical sample; m.p. 203–204° dec.; $[\alpha]_D$ +55° ± 2°; λ_{max}^{MeOH} 238 mμ, ε 15,700.

Anal. Calcd. for $C_{25}H_{32}O_8$: C, 65.19; H, 7.01; CH_3CO, 18.68. Found: C, 65.45; H, 7.09; CH_3CO, 17.92.

17,20β-Diacetoxy-3,11-dioxopregn-4-en-21-oic Acid (VIIb) from VIIIb.—Acetylation of 250 mg. of 17,20β-dihydroxy-3,11-dioxopregn-4-en-21-oic acid was carried out with 1 ml. each of pyridine and acetic anhydride for 21 hr. at room temperature. The product was recovered and separated into neutral and acidic fractions by the same procedure used for the 20α-epimer. The neutral fraction gave crystals from acetone–ether; this fraction was not homogeneous. Crystallization of the acidic fraction from acetone afforded 145.5 mg. (48%, m.p. 154–155°) of diacetoxy acid (VIIb) as well formed prisms. For the analytical sample, m.p. 154–155°; $[\alpha]_D$ +95° ● 2°; λ_{max}^{MeOH} 238 mμ, ε 15,900.

Anal. Calcd. for $C_{25}H_{32}O_8$: C, 65.19; H, 7.01; CH_3CO, 18.68. Found: C, 65.73; H, 6.84; CH_3CO, 18.15.

Methyl 17,20α-Diacetoxy-3,11-dioxopregn-4-en-21-oate (VIa) from IVa.—Acetylation of 250 mg. of methyl 17-hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate in a mixture of acetic anhydride (2 ml.) and glacial acetic acid (10 ml.) containing *p*-toluenesulfonic acid (200 mg.) was carried out in the manner described by Turner.[7] After 4 hr. at room temperature, the reaction mixture was diluted with methylene chloride and washed successively with 5% sodium bicarbonate solution and water, dried, and concentrated to dryness. The acidic fraction was not investigated. The residue from the neutral fraction was chromatographed on a 1.8 × 47 cm. column prepared by treating 50 g. of Celite with 22.5 ml. of formamide. The mobile phase was a 2:1 mixture of cyclohexane–benzene saturated with formamide. Five-milliliter fractions were collected after the first 50 ml. of effluent was discarded.

Methyl 3,17,20α-Triacetoxy-11-oxopregna-3,5-dien-21-oate (IXa). Fractions 5–11.—The residue (81 mg., 27%) could not be crystallized. It was provisionally identified as the $\Delta^{3,5}$-enol acetate (IXa, Fig. 2) by its intense ultraviolet absorption (λ_{max}^{MeOH} 234 mμ).

Methyl 17,20α-Diacetoxy-3,11-dioxopregn-4-en-21-oate (VIa). Fractions 22–36.—The residue gave prisms (93 mg., m.p. 197.5–199°; and 8 mg., m.p. 195–197°) from acetone and acetone–petroleum ether, respectively, in 36.9% yield. When the acetylation reaction went for 15 instead of 4 hr. at room temperature, the yield of diacetoxy methyl ester (VIa) was only 26.5%. Recrystallization from acetone–ether gave the analytical sample; m.p. 197–199.5°; $[\alpha]_D$ +66° ± 2°; λ_{max}^{MeOH} 238 mμ, ε 16,000.

Anal. Calcd. for $C_{26}H_{34}O_8$: C, 65.80; H, 7.22. Found: C, C, 65.85; H, 7.12.

Treatment of the 17,20α-diacetoxy acid (VIIa) with diazomethane gave a product which did not depress the melting point of VIa and which had an infrared spectrum identical with that of VIa.

Methyl 3,17,20β-Triacetoxy-11-oxopregna-3,5-dien-21-oate (IXb) from IVb.—Acetylation of 250 mg. of methyl 17-hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate was performed in the same manner as for the 20α-epimer. Direct crystallization from methanol gave needles (80 mg., m.p. 190–192°) of IXb. The residue from the mother liquor was fractionated on the same column and under the same conditions used for the 20α-epimer.

Fractions 5–11.—Crystallization from methanol gave 23 mg. (m.p. 189–190°) of material identical with that crystallized directly. The total yield of enol acetate was 103 mg. (34.6%). The yield after a reaction time of 15 hr. was 120.5 mg. (48.5%). A sample, recrystallized from methanol, melted at 190–192°; $[\alpha]_D$ −95° ± 2°; λ_{max}^{MeOH} 234 mμ, ε 19,300.

Anal. Calcd. for $C_{28}H_{36}O_9$: C, 65.09; H, 7.03. Found: C, 65.04; H, 7.14.

Methyl 17,20β-Diacetoxy-3,11-dioxopregn-4-en-21-oate (VIb) from IVb. Fractions 21–40.—Crystallization from acetone–ether gave fine needles (77 mg., m.p. 190–191°; and 19 mg., m.p. 189.5–191°) in 35% yield. The analytical sample was

recrystallized from acetone–petroleum ether; m.p. 191–191.5°; $[\alpha]_D$ +95° ± 1°; λ_{max}^{MeOH} 238 mμ, ε 16,200.

Anal. Calcd. for $C_{26}H_{34}O_5$: C, 65.80; H, 7.22. Found: C, 65.87; H, 7.19.

Treatment of the 17,20β-diacetoxy acid (VIIb) with diazomethane gave a product with an infrared spectrum identical with that of VIb.

17,21-Diacetoxypregn-4-ene-3,11,20-trione (X) and 3,17,21-Triacetoxypregna-3,5-diene-11,20-dione (XI) from Cortisone.—Acetylation of 250 mg. of cortisone for 18 hr. was carried out under the same conditions used for acetylation of IVa and IVb. Paper chromatography of an aliquot from the reaction mixture was carried out in isooctane (140), toluene (60), methanol (160), water (40). Two ultraviolet-absorbing compounds (R_f 0.32 and 0.66) were present in approximately equal amounts. The mixture was fractionated on a 1.8 × 38 cm. column prepared by treating 40 g. of Celite with 20 ml. of stationary phase from the system cyclohexane (480), benzene (320), formamide (25). Numbering of the 5-ml. fractions was begun after 40 ml. of effluent had been discarded.

3,17,21-Triacetoxypregna-3,5-diene-11,20-dione (XI). Fractions 5–9.—Precipitation from methanol solution with water gave 125 mg. (37%, m.p. 113–115°) of a white solid. A sample for analysis was reprecipitated from aqueous methanol and dried for 2.5 hr. at 78° and 0.1 mm. over P_2O_5; m.p. 115–120°; $[\alpha]_D$ −57° ± 2°; λ_{max}^{MeOH} 234 mμ, ε, 17,200.

Anal. Calcd. for $C_{27}H_{34}O_5 \cdot {}^1/_2H_2O$: C, 65.44; H, 7.12. Found: C, 65.23; H, 6.44.

17,21-Diacetoxypregn-4-ene-3,11,20-trione (X). Fractions 49–61.—Crystallization from methanol gave X (97 mg., m.p. 224.5–226.5°; 15 mg., m.p. 222–223°; and 2.5 mg., m.p. 212–215°) in a yield of 37%. For the analytical sample, m.p. 222–223°; $[\alpha]_D$ +117° ± 2°; λ_{max}^{MeOH} 238 mμ, ε 16,400. Reported[7] m.p. 223.5–224.5°; $[\alpha]_D$ +113° (dioxane).

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.54; H, 7.25. Found: C, 67.45; H, 6.86.

Treatment of Methyl 17-Hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate (IVa) with One Equivalent of Sodium Hydroxide.—To a solution of 432 mg. (1 mmole) of acetoxy ester (IVa) in 40 ml. of 95% ethanol was added 20 ml. of water and 0.5 ml. of 2.03 *N* sodium hydroxide (1 mmole). After 20 min. at room temperature, the clear solution was carefully acidified with slightly more than one equivalent of hydrochloric acid, added to 250 ml. of water, and extracted with 100 ml. of ethyl acetate. The extract was washed twice with water, dried, and concentrated to dryness. The residue, which consisted of both neutral and acidic products, was fractionated on a 1.8 × 36 cm. column of Celite (50 g.) impregnated with 25 ml. of the heavier phase of benzene (250), cyclohexane (250), methanol (250), acetic acid (75), water (175). After 44 ml. of effluent had been discarded, 5-ml. fractions were collected. Beginning with fraction no. 83, the top phase of benzene (500), methanol (250), acetic acid (75), water (175) was used as the mobile phase. The absorbance of selected fractions at 238 mμ was used to determine the elution diagram and indicate which fractions were to be pooled.

Ethyl 17,20α-Dihydroxy-3,11-dioxopregn-4-en-21-oate (XIIIa, Fig. 3). Fractions 32–55.—Crystallization from ether gave rosettes in two crops (85 mg., m.p. 155.5–157° with softening at 106°; and 3 mg., m.p. 152–154°); yield was 22%.

Treatment of 100 mg. of 17,20α-dihydroxy-3,11-dioxopregn-4-en-21-oic acid (VIIIa) with diazoethane[21] gave 96 mg. (89%; m.p. 155–157°) of crystals from ether. The product was proven identical with the compound from fractions 32–55 by a mixture melting point determination and by comparison of their infrared spectra. The analytical sample, recrystallized from ether, melted at 156–156.5°; $[\alpha]_D$ +139° ± 2°; λ_{max}^{MeOH} 238 mμ, ε 15,900.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.36; H, 7.88.

Ethyl 17-Hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate.—Ethyl 17,20α-dihydroxy-3,11-dioxopregn-4-en-21-oate (XIIIa) (50 mg.) was dissolved in 0.2 ml. each of pyridine and acetic anhydride. After 12 hr. at room temperature, the solvent was removed in a stream of nitrogen. Crystals (46 mg., m.p. 175.5–176°; and 3.5 mg., m.p. 174–175°) were obtained from ether; $[\alpha]_D$ +139° ± 2°; λ_{max}^{MeOH} 238 mμ, ε 16,100.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67. Found: C, 67.20; H, 7.38.

17-Hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oic Acid (XIIa). Fractions 106–130.—Crystallization from acetone afforded XIIa in 18% yield (65.5 mg., m.p. 187–188° dec.; and 10 mg., m.p. 185–186° dec.). A sample was recrystallized from ethyl acetate and dried in air. On further drying for 2.5 hr. at 100° and 0.2 mm., it lost 8.25%; calcd. for loss of 0.5 mole of ethyl acetate, 9.5%; m.p. 190° dec.; $[\alpha]_D$ +134° ± 2°; λ_{max}^{MeOH} 238 mμ ε 16,000.

On exposure to air the sample rapidly gained weight. When redried to constant weight at 100° and 0.2 mm. it lost 4.10%; calcd. for loss of 1 mole of water, 4.12%. On re-exposure to air the sample gained 4.21% after 41 hr.

Anal. Calcd. for $C_{23}H_{30}O_7 \cdot H_2O$: C, 63.29; H, 7.39. Found: C, 63.39, 63.41; H, 7.06, 7.11.

Treatment of the compound with diazomethane yielded a product which did not depress the melting point of the 20α-acetoxy methyl ester (IVa).

17,20α-Dihydroxy-3,11-dioxopregn-4-en-21-oic Acid (VIIIa). Fractions 180–223.—Crystallization from acetone gave 80 mg. (21.3%, m.p. 245.5–246° dec.) of a product which did not depress the melting point of the 20α-hydroxy acid (VIIIa) prepared directly from IIIa.

Treatment of Methyl 17-Hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate (IVb) with One Equivalent of Sodium Hydroxide.—Treatment of 432 mg. (1 mmole) of IVb with aqueous ethanolic sodium hydroxide was carried out in exactly the same manner as for the 20α-epimer (IVa). The mixture of neutral and acidic products, similarly obtained, was fractionated by chromatography in benzene (300), cyclohexane (200), methanol (250), acetic acid (75), water (175) on 50 g. of Celite plus 25 ml. of lower phase in a 1.8 × 3.6 cm. column. Fraction 1 (5 ml./fraction) was collected after 45 ml. of effluent had been discarded. After fraction 56 had been collected, the mobile phase was changed to the top phase of benzene (500), methanol (250), acetic acid (75), water (175). From appropriate fractions 100-μl. aliquots were removed and taken to dryness. The residues were dissolved in 3.00 ml. of methanol and the absorbance at 238 mμ was determined and plotted against fraction number to determine the elution pattern.

Ethyl 17-Hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate (XIVb). Fractions 8–14.—The residue gave needles from acetone–petroleum ether (29 mg., 6.5%, m.p. 166–168°). A purified sample had m.p. 169–170°; $[\alpha]_D$ +130° ± 1°; λ_{max}^{MeOH} 238 mμ, ε 16,200.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67. Found: C, 67.15; H, 7.49.

Treatment of 50 mg. of the 20β-acetoxy acid (XIIb) with diazoethane and crystallization from acetone–petroleum ether gave needles of XIVb (45 mg., 85%, m.p. 169.5–170.5°). This product did not depress the melting point of the chromatographed sample of XIVb.

Fractions 15–35.—This fraction consisted of a mixture of 20β-acetoxy methyl (IVb) and 20β-hydroxy ethyl (XIIIb) esters. The mixture was rechromatographed in a less polar system consisting of benzene (500), cyclohexane (200), formamide (30). Fifty grams of Celite plus 22.5 ml. of stationary phase was packed in a glass cylinder to give a 1.8 × 47 cm. column. Fifty milliliters of effluent was discarded prior to collection of fraction 1 (5 ml./fraction).

Methyl 17-Hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate (IVb). Fractions 55–81.—Crystallization from acetone–ether gave needles (26 mg., 6.0%, m.p. 197–199°) which did not depress the melting point of starting material (IVb).

Ethyl 17,20β-Dihydroxy-3,11-dioxopregn-4-en-21-oate (XIIIb). Fractions 130–170.—Crystallization from acetone–ether gave plates (60 mg., 14.9%, m.p. 193–195°). Recrystallization from ether gave the analytical sample; m.p. 195–196°; $[\alpha]_D$ +111° ±1°; λ_{max}^{MeOH} 238 mμ, ε 16,000.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.19; H, 7.79.

Treatment of 40 mg. of the 20β-hydroxy acid (VIIIb) with diazoethane afforded 35 mg. (84%, m.p. 193–195.5°) of plates from acetone–ether. The product was identical with the chromatographed compound (XIIIb) as shown by a mixture melting point determination and by comparison of their infrared spectra.

Methyl 17,20β-Dihydroxy-3,11-dioxopregn-4-en-21-oate IIIb). Fractions 46–67.—The compound in this fraction had the same R_f value and gave the same color reactions as the dihydroxy ester

(21) Prepared from N-ethyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co., Inc. Milwaukee, Wis.). *Cf.* A. F. McKay, W. L. Ott, G. W. Taylor, M. N. Buchanan, and J. F. Crooker, *Can. J. Res., Sect. B*, **28**, 683 (1950).

IIIb previously described. Because of the small amount present, no attempt was made to recover it in crystalline form.

17-Hydroxy-20β-acetoxy-3-,11-dioxopregn-4-en-21-oic Acid (XIIb). Fractions 83–110.—Crystallization from acetone–ether gave 125 mg. (30%, m.p. 194.5–196° dec.) of acetoxy acid. A purified sample melted at 198–198.5° dec.; $[\alpha]_D$ +131° ± 2°; λ_{max}^{MeOH} 238 mμ, ε 16,000.

Anal. Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 65.69; H, 7.30.

Treatment of the acid with diazomethane gave a product which did not depress the melting point of starting material (IVb).

17,20β-Dihydroxy-3,11-dioxopregn-4-en-21-oic Acid (VIIIb). Fractions 150–215.—Crystallization from acetone–ether gave two crops (38.5 mg., m.p. 225–226° dec.; and 6 mg., m.p. 222.5–223° dec.) of product which did not depress the melting point of the dihydroxy acid (VIIIb) prepared directly from IIIb. The yield was 12.0%.

Proof of Configuration at C-20 in the Epimeric 20-Hydroxy-21-oic Acids Derived from Cortisone Glyoxal. 11β,17,20α,21-Tetrahydroxypregn-4-en-3-one (XVIa, Fig. 4) from IVa.—To a solution of 216 mg. (0.5 mmole) of methyl 17-hydroxy-20α-acetoxy-3,11-dioxopregn-4-en-21-oate in 5 ml. of tetrahydrofuran (redistilled from lithium aluminum hydride) was added 190 mg. (5 mmoles) of lithium aluminum hydride in 20 ml. of tetrahydrofuran. After being refluxed for 30 min., the mixture was cooled and the excess reducing agent was decomposed with ethyl acetate. After the addition of a small volume of concentrated sodium sulfate solution and 10 g. of solid sodium sulfate, the mixture was filtered and the precipitate was washed well with tetrahydrofuran. Paper chromatography of an aliquot in toluene (120), ethyl acetate (80), methanol (100), water (100) showed the presence of six or seven compounds, all of which gave pink spots[22] with 10% phosphomolybdic acid in methanol at room temperature.

The tetrahydrofuran was evaporated and the residue was dissolved in 25 ml. of ethyl acetate. After addition of 2.5 g. of manganese dioxide, prepared according to Mancera, *et al.*,[23] the mixture was agitated on a mechanical shaker for 48 hr. at room temperature. The manganese dioxide was filtered off and washed repeatedly with hot methanol. The combined filtrates were concentrated to dryness. The absorption by the residue at 242 mμ was equivalent to 49 mg. of 11β,17,20α,21-tetrahydroxypregn-4-en-3-one (XVIa).

The mixture was chromatographed on a 1.8 × 48 cm. column prepared by treating 50 g. of Celite with 25 ml. of lower phase from the system benzene (375), ethyl acetate (125), methanol (250), water (250). Numbering of the 5-ml. fractions was begun after 65 ml. of effluent had been discarded. The fractions were analyzed by measurement of ultraviolet absorption at 242 mμ. The residue from fractions 25–42 crystallized from ethyl acetate as rosettes (16 mg., m.p. 242–246°). The reported[17] melting point for **11β,17,20α,21-tetra-hydroxypregn-4-en-3-one (XVIa)** is 239–243°.

11β,17-Dihydroxy-20α,21-diacetoxypregn-4-en-3-one from XVIa.—The residue from the mother liquor (7.6 mg.) plus 9.7 mg. of the crystalline material was treated with 0.1 ml. each of pyridine and acetic anhydride for 4 hr. at room temperature. The product crystallized as needles (10 mg., m.p. 204.5–205.5°) from petroleum ether. The infrared spectrum in chloroform was identical with that for authentic 11β,17-dihydroxy-20α,21-diacetoxypregn-4-en-3-one.[24]

11β,17,20β,21-Tetrahydroxypregn-4-en-3-one (XVIb) from IVb.—Methyl 17-hydroxy-20β-acetoxy-3,11-dioxopregn-4-en-21-oate (216 mg., 0.5 mmole) was reduced and selectively re-oxidized in the same manner as its 20α-epimer. The product of the final reaction mixture weighed 149 mg. The extinction at 242 mμ indicated the equivalent of 67 mg. of Δ⁴-3-ketopregnenetetrol (XVIb). The product was chromatographed under the same conditions used for the reaction mixture from the 20α-epimer. Fractions 20–47 gave 47 mg. of rosettes (m.p. 120–126°) from ethyl acetate. This product did not depress the melting point (m.p. 122–126°) of an authentic sample of 11β,17,20β,21-tetrahydroxypregn-4-en-3-one (XVIb).

11β,17-Dihydroxy-20β,21-diacetoxypregn-4-en-3-one from XVIb.—Acetylation of XVIb (17 mg.) gave 13.5 mg. (m.p. 232.5–233.5°) of needles from ethyl acetate. The infrared spectrum of this compound in chloroform was identical with that of an authentic sample of 11β,17-dihydroxy-20β,21-diacetoxypregn-4-en-3-one (Reichstein's substance E diacetate).

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(22) This color reaction was found to be characteristic of steroids with a Δ⁴-3-hydroxy grouping. Steroids with an analogous system (Δ⁵-7-hydroxy) in ring B give a bright blue color under the same conditions.

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Conversion of Steroid-17-yl Glyoxals to Epimeric Glycolic Esters[1]

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Methanolic cupric acetate catalyzes the rearrangement of a steroidal glyoxal (20-keto-21-aldehyde) to the corresponding methyl glycolate (20-hydroxy-21-acid ester). The reaction is general for steroidal glyoxals and may be conducted in various alcohols, each alcohol giving a different ester. Glyoxals from 17-deoxy steroids react much more rapidly than do those from 17-hydroxy analogs. The presence of water retards the rearrangement. The principal products from the reaction of methanolic cupric acetate and 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al are the 20α- and 20β-epimers of methyl 3α,20-dihydroxy-11-oxo-5β-pregnan-21-oate. Hydrolysis of these esters gives the corresponding 20-hydroxypregnan-21-oic acids. This same pair of epimeric 20-hydroxy acids also is obtained by treatment of 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al with aqueous sodium hydroxide. The mono- and diacetates of both epimeric acids and both esters were made and the absolute configuration at C-20 was established by comparison with a substance of known configuration. Optical rotations of the various derivatives were determined and correlated. In every instance, the compound with a 20α-oxygen function was more dextrorotatory than its 20β-epimer. This finding indicates that the rule which states that a 20β-acetoxypregnane is more dextrorotatory than its 20α-epimer is not applicable to 20-acetoxy-5β-pregnan-21-oic acids and esters.

The 21-hydroxyl group of an α-ketolic steroid can be oxidized to an aldehyde by treatment with methanolic cupric acetate.[3–6] During studies on the preparation[5] of a glyoxal (3α-hydroxy-11,20-dioxo-5β-pregnan-21-al)

(1) Abridgment 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.

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

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