melting points indicated: benzoic anhydride, hexane, 43°; resublimed resorcinol, used directly, 108°; and *p*-hydroxybenzoic acid, used directly, 215°.

Apparatus and Method.—A schematic drawing of the apparatus is shown in Fig. 1. Constant temperature (±0.1°) was maintained by a stable refluxing liquid (*m*-diethylbenzene, 182°; *cis*-decalin, 193°; nitrobenzene, 212°; isopropyl benzoate, 220°; and *n*-propyl benzoate, 232°). The 3-l. flask was filled with approximately 1500 ml. of liquid. The temperature was measured with a mercury thermometer with 0.2° divisions, calibrated against a bureau of standards reference.

The solvent and catalyst were accurately weighed and transferred to the inner reaction chamber, bottom section 45 × 120 mm., top section 28 × 230 mm., and then placed in the refluxing liquid. The stability of the solvent could be checked by measuring the evolution of carbon dioxide at this point. When the temperature had equilibrated, the salicylic acid, pelletized for convenience, was added and the system was immediately closed.

A slow stream of purified nitrogen (approximately 100 ml./min.), line A, was bubbled through the solution to provide agitation and to carry the carbon dioxide from the reaction chamber. A fivefold change in flow rate did not significantly alter the calculated rate constant. Temperature readings, measured by a thermocouple inserted through the well sealed in the cap, indicated that the desired temperature of reaction was re-established within 5 min. after the addition of the salicylic acid. One hundred grams of solvent and the appropriate molar amounts of salicylic acid and catalyst were used with all concentrations expressed in moles per kilogram of solvent.

The gas emerging from the reactor was diverted to Dry Ice trap F, calcium chloride trap G, and Ascarite absorber H, by stopcock C. After exactly 10 min., stopcock C was turned to divert the gas to an identical adsorption train F′, G′, H′.

Stopcock D was turned to direct a second stream, B, of purified nitrogen (approximately 1.5 l./min.) through absorption system F, G, H. This was done to sweep all of the carbon dioxide produced during the first 10 min. into the Ascarite trap H. The lines leading from the reactor to stopcock C were kept as short as possible. After 18 min., stopcock E was turned to vent nitrogen from stream B to the atmosphere, trap H was removed and weighed to the nearest 0.1 mg. and immediately replaced in the system.

At exactly 20 min., stopcock C was turned toward absorption train F, G, H, then stopcock D was turned toward absorption train F′, G′, H′, and stopcock E was closed to begin sweeping train F′, G′, H′. The cycle was repeated. The increase in weight of the Ascarite absorber was recorded as the weight of carbon dioxide produced during the corresponding time interval.

A sensitive manometer (filled with Silicon 710 liquid) at the inlet side of nitrogen stream A indicated the development of back pressure in the system. A pressure difference when switching between the two absorption trains could not be tolerated. It could usually be avoided by the use of freshly prepared Ascarite traps.

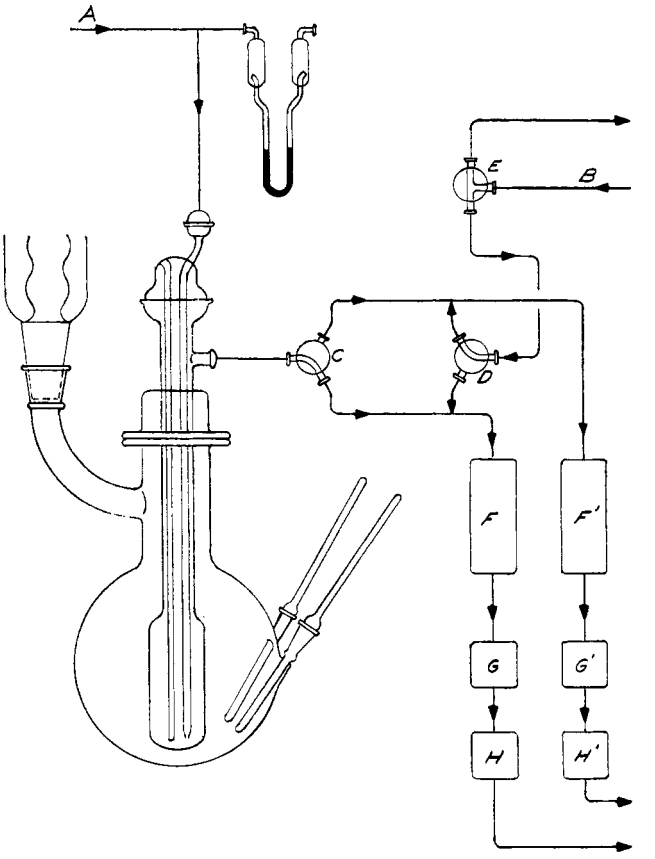

Figure 1.

Pseudo-first-order rate constants, k ($k = -\frac{1}{c}\frac{dc}{dt}$), were determined graphically by measuring the slope of the straight line obtained by plotting the logarithm of the salicylic acid concentration, c, *vs.* time, t.

Acknowledgment.—W. W. K. is indebted to Mrs. Veda Brink for assistance with the experimental work, to Dr. J. P. Surls and Mr. D. L. Bauer for making the run utilizing beryllium benzoate as the catalyst, and to Professor R. G. Rinker for stimulating discussions.

Glycolic Acid Metabolites of Desoxycorticosterone. Assignment of Configuration at C-20[1]

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For two epimeric glycolic (20-hydroxy-21-oic) acid metabolites of desoxycorticosterone assignment of configuration at C-20 cannot be made by measuring changes in molecular rotation after acetylation. Their absolute configurations have been determined by chemical and microbiological transformations which relate the configuration of one epimer both to 20β,21-dihydroxypregn-4-en-3-one and to two 11-oxygenated glycolic acids which possess a 20β-oxygen function. The preparation of a number of free and acetylated derivatives of the 20α- and 20β-epimers of 20,21-dihydroxypregn-4-en-3-one and 20-hydroxy-3-oxopregn-4-en-21-oic acid is described. The significance of differences in their molecular rotations is discussed.

Recently one of us reported that incubation of desoxycorticosterone with surviving guinea pig liver slices resulted in the formation of four acidic metabolites.[2] Two of these were the C-20 epimeric glycolic acids Va and Vb (Fig. 1), originally designated the polar and mobile acids, respectively. Their configurations at

(1) This work was supported by a research grant (AM 01255) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service.

(2) J. J. Schneider, "Proceedings of the First International Congress on Hormonal Steroids," Vol. I, Academic Press, New York, N. Y., 1964, pp. 127–135.

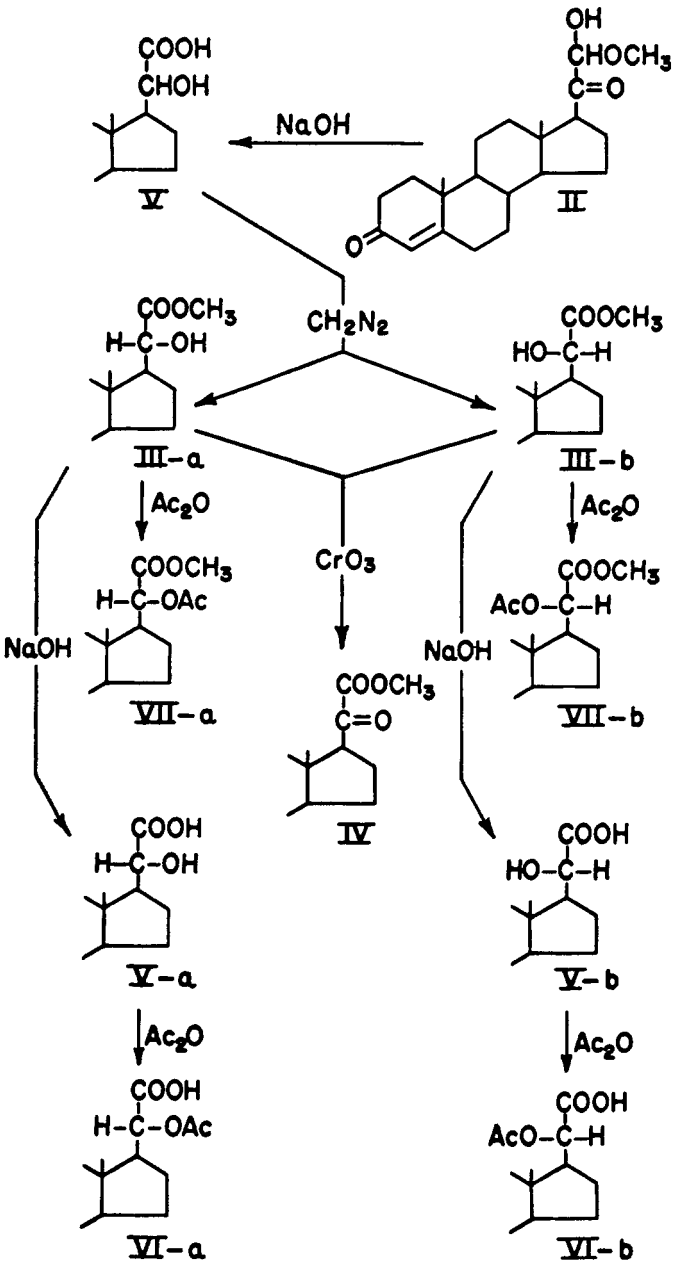

Figure 1.

C-20 were not determined because it had just been shown[3] that the accepted method of assigning configuration, based on the rules of Fieser and Fieser,[4] is not applicable to 20-acetoxypregnan-21-oic acids and their esters. The purpose of the present report is to detail the reactions and procedures employed to establish unequivocally the absolute configurations at C-20 of the glycolic acid metabolities.

The methyl esters IIIa and IIIb were prepared in quantities sufficient for characterization by alkaline rearrangement of the glyoxal hemiacetal II and subsequent esterification of the mixture of epimeric glycolic acids so obtained.[2] Their identity with the esterification products of the acidic metabolities has been shown. Although there is little difference in the paper chromatographic mobilities of the epimeric methyl esters (in the system—isooctane, 170; toluene, 30; methanol, 150; water, 50 ml.—IIIa and IIIb have R_f values of 0.26 and 0.32), their complete separation in this system was effected on a Celite column. Batchwise processing of a total of 3 g. of the glyoxal hemiacetal II using this method afforded the 20α- and 20β-hydroxy methyl esters in yields of 40 and 24%, respectively.

In order more firmly to establish the epimeric relationship of the methyl esters IIIa and IIIb, they were oxidized with chromic acid to a single product, namely methyl 3,20-dioxopregn-4-en-21-oate (IV).[2] Saponification of the methyl esters IIIa and IIIb provided the corresponding acids Va and Vb in good yields. Acetylation of the methyl esters and acids gave the acetoxy methyl esters VIIa and VIIb and the acetoxy acids VIa and VIb. The MD values for these compounds were determined and correlated (Table I). When the values for pairs 1, 2, 3, and 4 are examined, it is evident that the rotations of both free and acetylated compounds in the first epimeric series are uniformly greater than those in the second series. However, comparison of the acetylation increments shows no significant differences in the two series. It was therefore necessary to show configurational identity of the new epimeric glycolic acids with 20-hydroxysteroids for which configurations at C-20 definitely are established.

As outlined in Fig. 2, this aim was achieved by relating the configuration of one isolated ester (IIIb) both to the known 20β,21-diol XIb[5,6] and to two 11-oxygenated glycolic acids (XVIb[7] and XVIIIb[3]) which possess a 20β-oxygen function. A reaction described by Taub, *et al.*,[7] was utilized in preparing the 20β,21-diol XIb and relating it subsequently to the corresponding 21-oic acid. Treatment of desoxycorticosterone acetate (I) with sodium borohydride in aqueous dimethylformamide followed by column chromatography of the reaction mixture afforded the two glycol monoacetates VIIIb and IXb in a total yield of 44%. Solution of the more mobile, major, product IXb in 50% aqueous dimethylformamide containing 1% potassium bicarbonate[7] effected migration of the acetyl group from C-21 to C-20, furnishing the more polar, minor, product VIIIb in a yield of 75%. Since chromic acid oxidation of the more mobile monoacetate gave desoxycorticosterone acetate, it was designated the 21-acetate IXb. Similar oxidation of the more polar monoacetate afforded the acetoxy acid VIb. The minor product was therefore the 20-acetate VIIIb.

Both reduction products VIIIb and IXb could be converted to the diacetate Xb which was also prepared directly in 38% yield by reduction of desoxycorticosterone acetate as above followed by acetylation of the reaction mixture and column chromatography on silica gel. Treatment of the diacetate Xb with 1.25 equiv. of sodium hydroxide in aqueous *t*-butyl alcohol gave a mixture of products which readily was resolved on a Zaffaroni-type column of Celite. In addition to starting material (Xb), the two monoacetates VIIIb and IXb and the glycol XIb were isolated.

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

(4) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 614, 615. These authors state that (1) 20β-acetoxypregnanes are more dextrorotatory than their 20α-epimers, and (2) the MD (molecular rotation) increment for acetylation of a 20-ol is strongly positive for 20β-ols and negative or weakly positive for 20α-ols (Rule of Shift).

(5) H. J. Ringold, J. P. Ruelas, E. Batres, and C. Djerassi, *J. Am. Chem. Soc.*, **81**, 3710 (1959).

(6) M. Steiger and T. Reichstein, *Helv. Chim. Acta*, **21**, 171 (1938).

(7) D. Taub, R. D. Hoffsommer, and N. L. Wendler, *J. Am. Chem. Soc.*, **81**, 3291 (1959).

TABLE I

MOLECULAR ROTATIONS[a] OF PREGN-4-EN-3-ONES WITH SUBSTITUENTS AT C-11, C-20, AND C-21

Pair no.	Substituents C-11	C-20	C-21	Epimers 20α	20β	Increment α	β	Δ[b] α − β
1	H_2	OH	COOH	+277	+152			+125
2	H_2	OH	$COOCH_3$	+284	+169			+115
3	H_2	OAc	COOH	+345	+225	+68	+73	+120
4	H_2	OAc	$COOCH_3$	+390	+235	+106	+66	+155
5	=O	OAc	COOH	+618	+436			+182
6	H_2	OH	OH	+309	+315			−6
7	H_2	OH	OAc	+314	+292			+22
8	H_2	OAc	OH	+243	+475	−66	+150	−232
9	H_2	OAc	OAc	+270	+540	−44	+248	−270

[a] Molecular rotations, MD, are $[\alpha]D \times$ mol. wt./100. [b] $\Delta = MD\ 20\alpha - MD\ 20\beta$.

The constants for the glycol XIb are in excellent agreement with those reported[5,6] for 20β,21-dihydroxypregn-4-en-3-one. In order to provide further proof of configurational identity between the "b" and 20β-series, the glycol XIb was also prepared in high yield from the methyl ester IIIb by lithium aluminum hydride reduction of the 3-ethylene ketal XVIIb followed by acid hydrolysis. It is well established that reduction of α-hydroxy esters under these conditions proceeds without inversion of the asymetric center.[8]

This sequence of reactions clearly establishes that the stereochemical configuration at C-20 of one glycolic acid and the sodium borohydride reduction products of desoxycorticosterone acetate is the same. Although assignment of the 20β-configuration to these compounds can be made with a high degree of certainty, it seemed desirable to fix the structures of the glycolic acids derived metabolically from desoxycorticosterone in yet another manner. Conversion of these substances to 11-oxygenated analogs for which configurations recently have been established[9] appeared to offer the most promising approach.

Incubation of the 20-monoacetate VIIIb with the mold *Aspergillus ochraceus* NRRL 405 gave in 54% yield a product (XIIb) in which the acetyl group is retained.[10] That the microbiologically introduced hydroxyl group is in the 11α-position was shown by the following reactions. (1) Saponification of XIIb gave a triol (XIIIb) which was oxidized with chromic acid to an acid with the same paper chromatographic mobility as 3,11-dioxoeti-4-enic acid. (2) Vigorous chromic acid oxidation of XIIb afforded an acetoxy acid (XVIb) which was identical with 20β-acetoxy-3,11-dioxopregn-4-en-21-oic acid prepared from XVb by the procedure of Taub, *et al.*.[7] (3) Mild chromic acid oxidation of XIIb gave 11-dehydrocorticosterone acetate in low yield. The formation of this product necessarily involves migration of the acetyl group prior to oxidation at C-20.

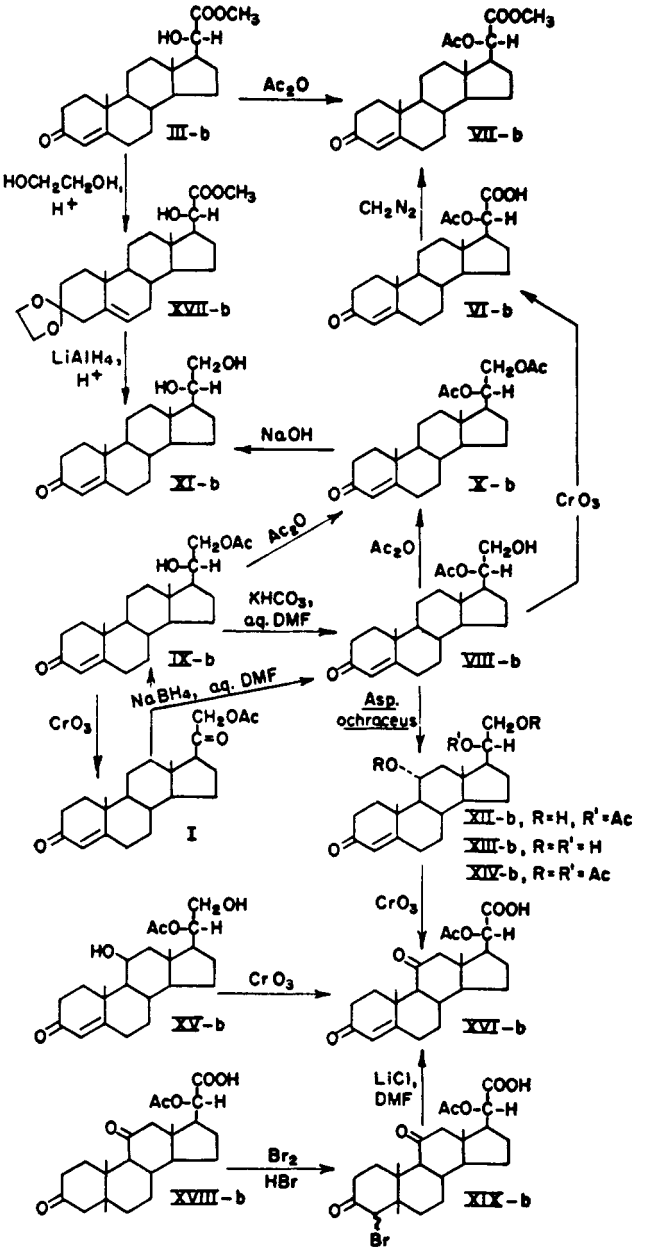

Figure 2.

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

(9) The C-20 configuration of the 11-oxygenated analogs was determined by Dr. Vernon R. Mattox who states, "The β-configuration of the 20-acetyl group in 3α,20-diacetoxy-11-oxo-5β-pregnan-21-oic acid[3] (m.p. 199–200°, $[\alpha]D$ +41°) was established by converting this substance into 5β-pregnane-3α,20β-diol, the absolute configuration of which is known. Ozonization of 21-benzylidene-3α,20β-diacetoxy-5β-pregnan-11-one gave the corresponding aldehyde which was converted into 3α,20β-diacetoxy-21-hydroxy-5β-pregnan-11-one (**1**) by treatment with Pt–H_2. Oxidation of **1** with chromic acid in acetic acid produced 3α,20β-diacetoxy-11-oxo-5β-pregnan-21-oic acid (m.p. 198.5–200.5°). Alkaline hydrolysis of **1** gave 3α,20β,21-trihydroxy-5β-pregnan-11-one. The 11-oxygen function was removed by the Wolff–Kishner procedure and the resulting 3α,20β,21-triol was converted into the 21-tosylate which furnished 5β-pregnane-3α,20β-diol when treated with lithium aluminum hydride." We would like to thank Dr. Mattox for allowing us to include this summary of his unpublished results and for his critical evaluation of the manuscript.

(10) As indicated in recent reviews [D. H. Peterson, "Biochemistry of Industrial Micro-organisms," C. Rainbow and A. H. Rose, Ed., Academic Press, New York, N. Y., 1963, p. 537; and Ch. Tamm, *Angew. Chem., Intern. Ed. Engl.*, **1**, 178 (1962)], molds rarely introduce but frequently remove acetyl groups from steroids. Our example is of interest both because the acetyl group remained intact and because 11α-hydroxylation was effected in a steroid bearing a glycol side chain, a type of substrate not previously utilized. We wish to thank Dr. C. W. Hesseltine for the *Aspergillus ochraceus* culture used in this work.

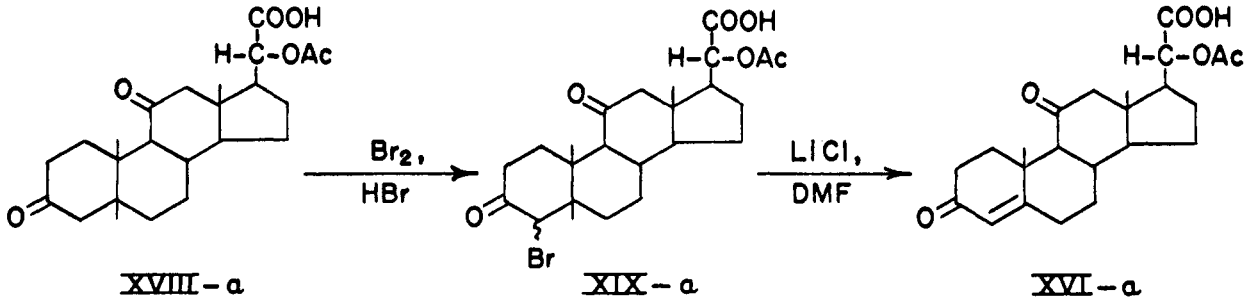

Figure 3.

(4) Acetylation of XIIb (λ_{max} 242 mμ) provided a triacetate (XIVb, λ_{max} 240 mμ). This hypsochromic shift is consistent with the conversion of a 11α-hydroxyl to an 11α-acetoxyl grouping.[11]

In order to relate the acetoxy acid XVIb to the glycolic acids derived from the glyoxals of 3α,21-dihydroxypregnane-11,20-dione and of 3α,11β,21-trihydroxypregnan-20-one, 20β-acetoxy-3,11-dioxopregnan-21-oic acid (XVIIIb[3]) was first converted to the 4-bromo intermediate XIXb by the procedure of Engel.[12] Dehydrobromination with lithium chloride in dimethylformamide[13] gave the same acetoxy acid (XVIb) as was obtained from both XIIb and from XVb by chromic acid oxidation.

By a sequence of reactions (Fig. 3) identical with those used in the preparation of XVIb from XVIIIb, 20α-acetoxy-3,11-dioxopregn-4-en-21-oic acid (XVIa) was synthesized from the saturated acetoxy acid XVIIIa.[3] As would be predicted, the 20α-acetoxy acid is more dextrorotatory than the 20β-acetoxy acid (pair 5, Table I).

For the preparation of the less accessible 20α,21-diol XIa, the methyl ester IIIa was first converted to the 3-ethylene ketal XVIIa (Fig. 4). Reduction with lithium aluminum hydride followed by hydrolysis of the ketal grouping provided 20α,21-dihydroxypregn-4-en-3-one (XIa) in an over-all yield of 60%.[14]

Acetylation of the glycol XIa under the usual conditions gave the diacetate Xa. Partial hydrolysis of Xa under the conditions employed in the preparation of the monoacetates VIIIb and IXb from Xb was not satisfactory for the preparation of VIIIa and IXa. Not only were the monoacetates formed in smaller amounts, but also their paper and column chromatographic separations were more difficult to achieve.[15] However, the monoacetates could be prepared by partial acetylation of the glycol XIa, affording IXa in 47% yield and VIIIa in 5.8% yield. That IXa is the 21-monoacetate followed from its ready oxidation to desoxycorticosterone acetate. Oxidation of VIIIa to the acetoxy acid VIa fixed the position of the acetyl group at C-20. Additional amounts of the 20-monoacetate could be prepared from XIa under conditions favoring acetyl migration. It is of interest that migration of the acetyl group is not limited to glycol monoacetates possessing a 20β-oxygen function.

On examination of the MD values which were obtained for the 20,21-diols and their derivatives (pairs 6, 7, 8, and 9 in Table I) it is apparent that the 20-acetates (pairs 8 and 9) of the second series are twice as dextrorotatory as those of the first series. Furthermore, the acetylation increments are strongly positive in the second series and moderately negative in the first series. On the basis of this perfect adherence to both tenets of the Fieser rule, the established configuration of the mold metabolite XIIb, and the known configuration of the 20,21-diol XIb, a 20β-oxygen function was assigned to the second series and a 20α-oxygen function to the first series as shown in Table I.[16] Assignment of the true configurations in the glycolic acid series follows.

Comparison of the MD values in the glycolic acid and glycol series shows that the nature of the substituent at C-21 influences greatly the effect of acetylation at C-20. In agreement with previous findings (ref. 3, Table III, p. 1783) the presence of a carboxyl or carboxymethyl function at C-21 causes 20α-acetoxy derivatives (pairs 3, 4, and 5) to be more dextrorotatory than their 20β-epimers. Another point of interest, not discussed in the earlier report, is that 20α-hydroxy-21-oic acids and esters consistently are more dextrorotatory than their 20β-epimers as evidenced by large Δ-values for pairs 1 and 2 in this report and for pairs 1, 2, and 3 in the earlier study.[3] In contrast, there are no significant differences in the MD values for 20,21-dihydroxy- or 20-hydroxy-21-acetoxypregnanes as evidenced by low Δ-values for pairs 6 and 7 in this paper and for other examples cited by Fieser and Fieser.[17]

It is also to be noted that, whereas acetylation increments in the 20α-series are uniformly greater than those in the 20β-series in the case of the glycolic acids acids derived from 11-oxygenated pregnanes, differences in acetylation increments calculated for the glycolic

(11) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell, and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953).

(12) C. R. Engel, *J. Am. Chem. Soc.*, **78**, 4727 (1956).

(13) R. P. Holysz, *ibid.*, **75**, 4432 (1953).

(14) It is well established that metal hydride reduction of 17-deoxy-α-ketols gives 20α-glycols in very low yields. Reduction with sodium in *n*-propyl alcohol affords 20α-ols primarily but is attended with considerable to marked reductive elimination of oxygen at C-21 as illustrated by recent experience in this laboratory. It is therefore proposed that, where applicable, rearrangement of 17-deoxyglyoxals to 20-hydroxy-21-oic acids followed by lithium aluminum hydride reduction of the pure 20α-hydroxy methyl ester represents a useful approach to the preparation of 17-deoxy-20α,21-diols. Additional examples of the synthesis of 20α-glycols from 20α-hydroxy-21-oates will be submitted in a future report.

(15) For example, in the system—isooctane, 140; toluene, 60; methanol, 150; water 50 ml.—VIIIb and IXb had R_f values of 0.26 and 0.45, respectively, while VIIIa and IXa had R_f values of 0.28 and 0.35.

(16) Recently [J. J. Schneider and M. L. Lewbart, *Tetrahedron*, **20**, 943 (1964)] we described the chromatography of five epimeric pairs of steroid 20,21-diols (including XIa and XIb) on paper impregnated with boric acid or borate buffers. There occurred uniformly a moderate to marked reduction in the mobilities of the 20α,21-diols and only a slight decrease in the R_f values of the 20β,21-diols as the imposed pH was increased. These results, which are in accord with the configurational assignments made in this paper for XIa and XIb, suggest yet another means of determining the stereochemistry at C-20 in 20,21-diols.

(17) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 616.

acids derived from desoxycorticosterone are too small to have meaning.

Experimental

Melting points were taken on a Fisher-Johns apparatus and are reported uncorrected. Optical rotations were determined in a Zeiss 0.05° polarimeter with a 2-dm. tube. Measurements were made in methanol at a concentration of about 0.5% and at a temperature of 27 ± 2°. Analyses were by E. Thommen, Basel, Switzerland. Thin layer chromatography (t.l.c.) was employed as described by Gamp, *et al.*[18] The adsorbent was Camag Kieselgel DF-5. Substances were detected by ultraviolet scanning or by visible or fluorescent colors produced by spraying the plate with 20% *p*-toluenesulfonic acid in ethanol and heating at 110° for 20 min. Columns of silica gel (Davison, 100–200 mesh) were prepared by adding a suspension of the adsorbent in the developing solvent and tapping to constant bed volume. Columns of grade 545 Celite with either Bush-[19] or Zaffaroni[20]-type solvent systems were prepared and assessed for evenness of packing as previously described.[3,21] All chromatography was carried out at room temperature (24 ± 2°).

Methyl 20α- and 20β-Hydroxy-3-oxopregn-4-en-21-oates (IIIa and IIIb) from II.—To a solution of 1080 mg. (3 mmoles) of 21-hydroxy-21-methoxypregn-4-ene-3,20-dione[2] in 30 ml. of methanol was added 1 l. of water and 15 ml. of 1 *N* sodium hydroxide. After 12 hr. at room temperature, the slightly turbid, yellow solution was acidified with dilute hydrochloric acid and extracted with ethyl acetate–methylene chloride (1:1). The organic phase was washed once with water and concentrated to dryness. The crude product was dissolved in methanol and treated with an excess of ethereal diazomethane.

The residue was dispersed in Celite and chromatographed on a 54 × 950 mm. column (310 ml. of stationary phase plus 620 g. of Celite) prepared with the system—isooctane, 170; toluene, 30; methanol, 150; water, 50 ml. (system 1). Fractions (12 ml.) were collected at 10-min. intervals.

Two additional 1080-mg. amounts of II were processed in the same manner and chromatographed successively on the same column, adding the mother liquors from the first two lots to the last. There was a slight but progressive increase in the mobilities of both products with succeeding lots.

Methyl 20β-Hydroxy-3-oxopregn-4-en-21-oate (IIIb). Fractions 360–460, 340–415, and 320–385.—Crystallization from ethyl acetate–*n*-hexane gave prismatic needles (748 mg., m.p. 177–179°; 25 mg., m.p. 176–177.5°) in a yield of 23.8%. The product did not depress the melting point of the previously prepared mobile methyl ester.[2]

Methyl 20α-Hydroxy-3-oxopregn-4-en-21-oate (IIIa). Fractions 440–550, 425–520, and 345–500.—Crystallization from ethyl acetate gave plates (1227 mg., m.p. 185.5–187°; 56 mg., m.p. 182–184°) in a yield of 39.6%. The product did not depress the melting point of the polar methyl ester previously prepared.[2]

Methyl 3,20-Dioxopregn-4-en-21-oate (IV) from IIIa and IIIb.—To separate solutions of 30-mg. each of methyl 20α-hydroxy-3-oxopregn-4-en-21-oate and methyl 20β-hydroxy-3-oxopregn-4-en-21-oate in 3 ml. of glacial acetic acid was added 20 mg. of chromic anhydride in 0.15 ml. of water. After 2.5 hr. at room temperature, excess oxidizing agent was reduced with methanol and the solvents were removed in a current of nitrogen. The residues were partitioned between ethyl acetate and water. The organic layer was filtered through anhydrous sodium sulfate and concentrated to dryness.

From IIIa.—Two crystallizations from ether gave 15 mg. of prismatic needles, m.p. 138.5–140.5°, $[\alpha]_D$ +181°. A mixture melting point with authentic methyl 3,20-dioxopregn-4-en-21-oate[2] (m.p. 138.5–141°, $[\alpha]_D$ +182°) was 137.5–140°.

From IIIb.—Two crystallizations from ether gave 18 mg. of prismatic needles, m.p. 138–140°, $[\alpha]_D$ +181°. A mixture melting point with the reference compound was 138–140.5°.

Reduction of IV with Sodium Borohydride in Dimethylformamide.—To 22 mg. of methyl 3,20-dioxopregn-4-en-21-oate in 0.5 ml. of dimethylformamide wasadded 2 mg. of sodium borohydride and 4 mg. of sodium bicarbonate, each in 0.025 ml. of water. After 4 hr. at room temperature the reaction mixture was diluted with water and extracted with ethyl acetate. Paper chromatography of an aliquot in system 1 showed as the major ultraviolet-absorbing product a substance with the same mobility (R_f 0.31) as methyl 20β-hydroxy-3-oxopregn-4-en-21-oate (IIIb). There was a trace only of a substance with the same mobility as methyl 20α-hydroxy-3-oxopregn-4-en-21-oate (IIIa). It is thus apparent that the presence of a carboxymethyl group at C-21 does not alter the usual steric course of reduction of the C-20 carbonyl group with sodium borohydride.

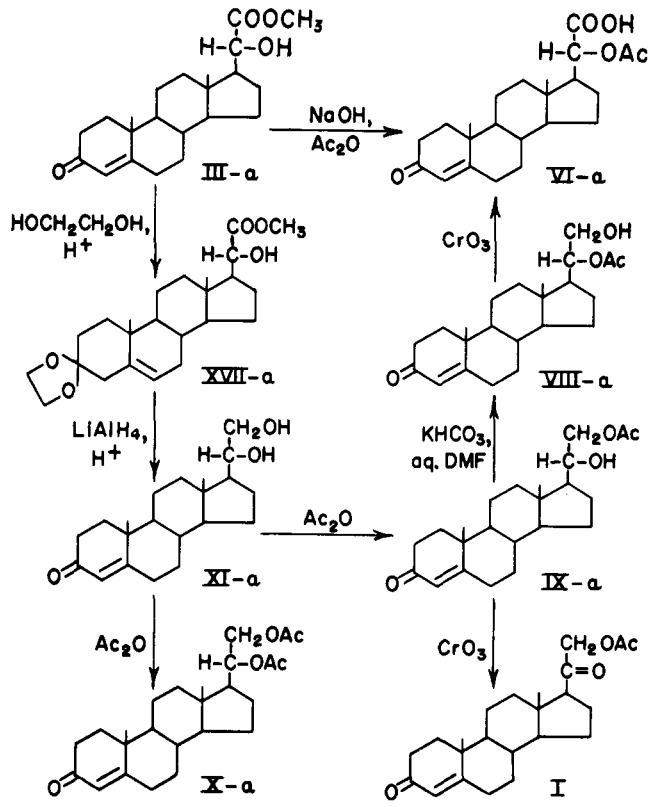

Figure 4.

20β-Hydroxy-3-oxopregn-4-en-21-oic Acid (Vb) from IIIb.—To 50 mg. of methyl 20β-hydroxy-3-oxopregn-4-en-21-oate in 5 ml. of methanol was added 5 ml. of water and 0.4 ml. of 1 *N* sodium hydroxide. After 15 min. at room temperature most of the methanol was removed with a current of nitrogen. The solution was diluted with water and acidified with 1 *N* hydrochloric acid; the precipitated acid was extracted with ethyl acetate. Crystallization from acetone gave elliptical prisms (38 mg., m.p. 212.5–215° dec.; 5 mg., m.p. 210.5–213.5° dec.) in a yield of 90%. The sample for analysis was recrystallized from acetone, m.p. 213–215° dec., $[\alpha]_D$ +44°, λ_{max}^{MeOH} 242 mμ (ε 16,800).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.66; H, 8.64.

20β-Acetoxy-3-oxopregn-4-en-21-oic Acid (VIb) from Vb.—Acetylation of 50 mg. of 20β-hydroxy-3-oxopregn-4-en-21-oic acid in pyridine and acetic anhydride at room temperature and crystallization from acetone–*n*-hexane gave 47.5 mg. of prisms, m.p. 202.5–203.5° dec. Recrystallization from the same solvent system did not alter the melting point. A sample, dried at 100° in high vacuum for 21 hr., lost 4.75% of its weight; calcd. for loss of one molecule of water, 4.43%. The dried analytical sample had $[\alpha]_D$ +58°, λ_{max}^{MeOH} 242 mμ (ε 16,700).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30; CH_3CO, 11.08. C, 70.88; H, 8.30; CH_3CO, 11.26.

Methyl 20β-Acetoxy-3-oxopregn-4-en-21-oate (VIIb) from IIIb.—Acetylation of 25 mg. of methyl 20β-hydroxy-3-oxopregn-4-en-21-oate with acetic anhydride and pyridine at room temperature and crystallization from ether gave 24.5 mg. of colorless needles, m.p. 155.5–156.5°. The sample for analysis was recrystallized from ether, m.p. 156–157°, $[\alpha]_D$ +58°, λ_{max}^{MeOH} 242 mμ (ε 17,100).

(18) A. Gamp, P. Studer, H. Linde, and K. Meyer, *Experientia*, **18**, 292 (1962).

(19) I. E. Bush, *Biochem. J.*, **50**, 370 (1952).

(20) A. Zaffaroni, R. B. Burton, and E. H. Keutmann, *Science*, **111**, 6 (1950).

(21) M. L. Lewbart, W. Wehrli, and T. Reichstein, *Helv. Chim. Acta*, **46**, 505 (1963).

Anal. Calcd. for $C_{24}H_{35}O_5$: C, 71.61; H, 8.51. Found: C, 71.65; H, 8.53.

21-Hydroxy-20β-acetoxypregn-4-en-3-one (VIIIb) and 20β-Hydroxy-21-acetoxypregn-4-en-3-one (IXb) from I.—To 2 g. of desoxycorticosterone acetate in 50 ml. of dimethylformamide was added in rapid succession 150 mg. of sodium borohydride and 300 mg. of sodium bicarbonate, each in 2.5 ml. of water. After 6 hr. at room temperature, excess reducing agent was decomposed with acetic acid, the solution was diluted with 250 ml. of water and extracted with ethyl acetate. The organic phase was washed with water, filtered through anhydrous sodium sulfate, and concentrated to dryness. A benzene-insoluble, ultraviolet-negative fraction (120 mg.) was separated and discarded.

The remainder was chromatographed with the system—benzene, 100; *n*-hexane, 150; formamide, 15 ml.—on a 46-mm.-diameter column packed to a height of 585 mm. with 250 g. of Celite containing 125 ml. of stationary phase. Each fraction contained 12.5 ml.; the flow rate was 50 ml./hr.

20β-Hydroxy-21-acetoxypregn-4-en-3-one (IXb). Fractions 93–135.—After removal of a less soluble contaminant by fractional crystallization from acetone, a total of 562 mg. (28%) of the 21-acetate (IXb) was obtained from acetone–*n*-hexane as colorless, cuboidal prisms, m.p. 153–155°, $[\alpha]_D$ +78°, λ_{max}^{MeOH} 242 mμ (ε 17,100).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15; CH_3CO, 11.49. Found: C, 74.00; H, 9.18; CH_3CO, 11.84.

Oxidation of IXb (37 mg.) with chromic anhydride (20 mg.) in 95% acetic acid (2 ml.) for 4.5 hr. at room temperature gave a crystalline product which reduced alkaline blue tetrazolium. Recrystallization from ethanol gave prismatic needles (21 mg., m.p. 156–157.5°; 8 mg., m.p. 154–155.5°) which did not depress the melting point of authentic **desoxycorticosterone acetate** (I).

21-Hydroxy-20β-acetoxypregn-4-en-3-one (VIIIb). Fractions 210–251.—Several crystallizations from acetone gave 322 mg. (16.1%) of prismatic needles, m.p. 185–188°, $[\alpha]_D$ +127°, λ_{max}^{MeOH} 242 mμ (ε 17,100).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15; CH_3CO, 11.49. Found: C, 73.59; H, 9.00; CH_3CO, 11.22.

Oxidation of VIIIb (66 mg.) in acetic acid (5 ml.) with chromic anhydride (60 mg.) in water (0.1 ml.) was carried out for 1 hr. at room temperature. The crude product was suspended in Tris buffer, pH 7, and extracted with ethyl acetate. The major component of this neutral fraction (18 mg.) reduced alkaline blue tetrazolium and had the same paper and thin layer chromatographic mobility as desoxycorticosterone acetate (I).

After acidification of the aqueous layer and extraction with ethyl acetate, an acidic fraction (54 mg.) was obtained. Crystallization from acetone–ether gave 39 mg. (57%) of **20β-acetoxy-3-oxopregn-4-en-21-oic acid** (VIb) as prisms, m.p. 200–203° dec. A mixture melting point with VIb, prepared by acetylation of Vb, was 202–203.5° dec.

21-Hydroxy-20β-acetoxypregn-4-en-3-one (VIIIb) from IXb.—To 374 mg. (1 mmole) of 20β-hydroxy-21-acetoxypregn-4-en-3-one in 13 ml. of dimethylformamide was added slowly 9.35 ml. of 2% aqueous potassium bicarbonate. After 15 min. at room temperature, crystals began to separate. After a total reaction time of 1 hr., an additional 3.65 ml. of potassium bicarbonate solution was introduced[22] and the reaction mixture was set aside for an additional hour. Dilution with 50 ml. of water, filtration, and drying *in vacuo* over anhydrous calcium chloride gave a product which weighed 326 mg. and which melted at 185–189°. Recrystallization from acetone afforded 281 mg. (75.3%) of purified product, m.p. 186–189°, which did not depress the melting point of the more polar monoacetate prepared directly from I.

20β,21-Diacetoxypregn-4-en-3-one (Xb) from VIIIb and IXb.—Treatment of 37 mg. each of 21-hydroxy-20β-acetoxypregn-4-en-3-one and 20β-hydroxy-21-acetoxypregn-4-en-3-one with acetic anhydride and pyridine and crystallization from acetone–*n*-hexane gave colorless, prismatic needles (m.p. 154.5–157°) which showed no depression on admixture. The sample for analysis was recrystallized from acetone–*n*-hexane, m.p. 155–156.5°, $[\alpha]_D$ +130°, λ_{max}^{MeOH} 242 mμ (ε 17,500).

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71; CH_3CO, 20.66. C, 71.95; H, 8.74; CH_3CO, 20.69.

Treatment of 20β,21-Diacetoxypregn-4-en-3-one (Xb) with 1.25 Equiv. of Sodium Hydroxide.—To a chilled solution of 500 mg. (1.2 mmoles) of the diacetate Xb in 40 ml. of *t*-butyl alcohol[23] plus 15 ml. of water was added 1.5 ml. (1.5 mmoles) of sodium hydroxide. After 1.5 hr. at 0°, 2 drops of glacial acetic acid was added and the solution was concentrated *in vacuo*. The largely aqueous concentrate was extracted with methylene chloride. The crude extract was fractionated on a 18 × 640 mm. column (35 ml. of stationary phase plus 70 g. of Celite) prepared with the system—benzene, 90; *n*-hexane, 90; formamide, 50 ml. Fractions (5 ml.) were collected at a rate of four per hour. Beginning with fraction 250, the mobile phase was changed to benzene saturated with formamide.

20β,21-Diacetoxypregn-4-en-3-one (Xb.) Fractions 16–20.—Crystallization from acetone–*n*-hexane gave 184 mg. (36.8%, m.p. 153–155°) of needles which did not depress the melting point of starting material.

20β-Hydroxy-21-acetoxypregn-4-en-3-one (IXb). Fractions 27–35.—Crystallization from acetone–*n*-hexane gave 56 mg. (12.5%, m.p. 153–155°) of prisms. A mixture melting point with the more mobile monoacetate prepared directly from I was 152.4–154.5°.

21-Hydroxy-20β-acetoxypregn-4-en-3-one (VIIIb) Fractions 50–65.—Crystallization from acetone gave 44 mg. (9.8%, m.p. 185–190°) of needles which did not depress the melting point of the more polar monoacetate obtained directly from I.

20β,21-Dihydroxypregn-4-en-3-one (XIb). Fractions 280–295.—Crystallization from acetone gave 114 mg. (28.6%, m.p. 168–169°) of rosettes, $[\alpha]_D$ +95°, λ_{max}^{MeOH} 242 mμ (ε 17,000); lit.[6] m.p. 166–167°, $[\alpha]_D$ +93° (ethanol); lit.[5] m.p. 169–170°, $[\alpha]_D$ +111° ($CHCl_3$).

11α,21-Dihydroxy-20β-acetoxypregn-4-en-3-one (XIIb) from VIIIb and *Aspergillus ochraceus*.—Two 2800-ml. Fernbach flasks, each containing 1 l. of Upjohn[24] medium, were heavily inoculated with submerged cultures of *Aspergillus ochraceus* NRRL 405 and actively agitated on a rotary shaker for 15 hr. at room temperature. To each flask was added 160 mg. of 21-hydroxy-20β-acetoxypregn-4-en-3-one in a minimum volume of dimethylformamide and shaking was continued for an additional 24 hr. The combined incubates were diluted with water and extracted with chloroform–ethyl acetate (2:1). The extract was washed with aqueous sodium carbonate and water and evaporated to dryness *in vacuo*. The crude, highly pigmented extract was dispersed in Celite and chromatographed on a 30 × 650 mm. column (75 ml. of stationary phase plus 150 g. of Celite) prepared with the system, benzene–chloroform, 2:1, saturated with formamide. Fractions (8 ml.) were collected at a rate of four per hour.

From fractions 186–220 were obtained 172 mg. (54%) of faintly pigmented needles. Several recrystallizations from acetone and from acetone–methanol gave the analytical sample, m.p. 185–187.5°, $[\alpha]_D$ +114°, λ_{max}^{MeOH} 242 mμ (ε 15,900).

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78; CH_3CO, 11.02. Found: C, 70.54; H, 8.78; CH_3CO, 10.24.

11α,20β,21-Trihydroxypregn-4-en-3-one (XIIIb) from XIIb.—To 50 mg. of 11α,21-dihydroxy-20β-acetoxypregn-4-en-3-one in 5 ml. of methanol was added 1 ml. of water and 0.2 ml. of 1 *N* sodium hydroxide. After 1 hr. at room temperature the solution was diluted with water and extracted with methylene chloride. Crystallization from acetone afforded rosettes (26 mg., m.p. 197–198°). Recrystallization of the product from acetone did not raise the melting point, $[\alpha]_D$ +70°, λ_{max}^{MeOH} 242 mμ (ε 15,500).

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.16; H, 9.26.

Treatment of the saponification product (6 mg.) in acetic acid (0.25 ml.) with chromic anhydride (5 mg.) in water (0.025 ml.) for 1.5 hr. at room temperature gave an acid (m.p. 249–251° dec.) which did not depress the melting point (250–252° dec.) of **3,11-dioxoeti-4-enic acid** obtained by oxidation of corticosterone with chromic anhydride. In addition, their paper chromatographic mobilities in the system—isooctane, 100; toluene, 100; methanol, 100; water, 70; acetic acid, 30 ml.—were identical (R_f 0.28).

11α,20β,21-Triacetoxypregn-4-en-3-one (XIVb) from XIIb.—Treatment of 11α,21-dihydroxy-20β-acetoxypregn-4-en-3-one with acetic anhydride and pyridine and crystallization from ether–

(22) If the total volume of aqueous alkali is added initially, starting material will precipitate.

(23) Reaction of Xb in aqueous methanol under the same conditions resulted in its rapid, complete hydrolysis to the glycol XIb presumably by a transesterification mechanism. The sterically hindered hydroxyl group of *t*-butyl alcohol cannot participate in such a reaction.

(24) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister, and H. M. Leigh, *J. Am. Chem. Soc.*, **74**, 5933 (1952).

n-hexane gave long, fine needles, m.p. 162–162.5°, $[\alpha]_D$ +97°, λ_{max}^{MeOH} 240 mμ (ε 16,400).

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07; CH_3CO, 27.18. Found: C, 68.28; H, 8.11; CH_3CO, 25.89.

20β-Acetoxy-3,11-dioxopregn-4-en-21-oic Acid (XVIb) from XIIb.—To 78 mg. (0.2 mmole) of 11α,21-dihydroxy-20β-acetoxypregn-4-en-3-one in 5 ml. of acetic acid was added 80 mg. (0.8 mmole) of chromic anhydride in 0.1 ml. of water. After 1 hr. at room temperature, methanol was added, and the solvents were removed. The residue was dissolved in methylene chloride and the solution was extracted three times with a total of 15 ml. of 2% aqueous sodium bicarbonate. The combined alkaline washes were acidified carefully and extracted with methylene chloride. Crystallization from acetone–ether gave 37 mg. of rosettes, m.p. 240–245° dec., $[\alpha]_D$ +127°; lit.[7] m.p. 240–246° dec., $[\alpha]_D$ +136° ($CHCl_3$), λ_{max}^{MeOH} 237.5 mμ (ε 15,300). 20β-Acetoxy-3,11-dioxopregn-4-en-21-oic acid prepared in this laboratory according to Taub[7] had m.p. 240–244.5° dec., $[\alpha]_D$ +133° ($CHCl_3$), $[\alpha]_D$ +126°, λ_{max}^{MeOH} 238 mμ (ε 15,300). A mixture melting point of the oxidation product and the reference compound was 239.5–244° dec.

11-Dehydrocorticosterone Acetate from XIIb.—To 78 mg. (0.2 mmole) of 11α,21-dihydroxy-20β-acetoxypregn-4-en-3-one in 4.5 ml. of acetic acid was added 40 mg. (0.4 mmole) of chromic anhydride in 0.5 ml. of water. After 19 hr. at room temperature t.l.c. of an aliquot using ethyl acetate as the developing solvent showed primarily starting material plus a second, more mobile, blue tetrazolium-negative compound. There was a trace only of a blue tetrazolium-positive compound with the same mobility as 11-dehydrocorticosterone acetate. An additional 40 mg. of chromic anhydride was introduced and the reaction mixture was allowed to stand for an additional 23 hr. at room temperature. Dilution with water and extraction with methylene chloride gave a combined acidic and neutral fraction which weighed 40 mg. The extract was applied to two 19 × 55 cm. sheets of Whatman No. 1 filter paper impregnated with 30% formamide in acetone and chromatographed for 2.5 hr. using benzene–chloroform (2:1) saturated with formamide as the mobile phase. The most mobile, ultraviolet-absorbing band was eluted in the manner previously described.[25] Crystallization from ethanol gave needles (5 mg., m.p. 178.5–180.5°; 1.2 mg., m.p. 176.5–178.5°). Recrystallization from the same solvent gave 4.1 mg., m.p. 180–182°. A mixture melting point with authentic 11-dehydrocorticosterone acetate was 179–182°. The infrared spectrum of the oxidation product was identical with that of the reference compound.[26]

Methyl 20β-Hydroxy-3-oxopregn-4-en-21-oate 3-Ethylene Ketal (XVIIb) from IIIb.—A solution of 200 mg. of methyl 20β-hydroxy-3-oxopregn-4-en-21-oate in 60 ml. of benzene and 2 ml. of ethylene glycol was dried by refluxing for 10 min. in an apparatus fitted with a water separator.[27] After addition of 6 mg. of *p*-toluenesulfonic acid, refluxing with vigorous stirring was continued for 8 hr. The cooled reaction mixture was washed with dilute sodium carbonate and water and concentrated to dryness. Crystallization from methanol gave needles (135 mg., m.p. 182–185°; 24 mg., m.p. 177–179°) in a yield of 71%. A sample for analysis was recrystallized from methanol, m.p. 186–188.5°, $[\alpha]_D$ −36°.

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.48; H, 8.99.

20β,21-Dihydroxypregn-4-en-3-one (XIb) from XVIIb.—A solution of methyl 20β-hydroxy-3-oxopregn-5-en-21-oate 3-ethylene ketal (100 mg.) and lithium aluminum hydride (76 mg.) in ether (35 ml.) was refluxed for 4 hr. The reaction mixture was diluted well with ethyl acetate and washed successively with 5 *N* hydrochloric acid, 2 *N* sodium hydroxide, and water. Thin layer chromatography of the crude product showed that extensive cleavage of the ketal grouping occurred during the work-up. To complete hydrolysis, the residue in 10 ml. of methanol was treated with 2 ml. of 1 *N* sulfuric acid for 60 hr. at room temperature. Crystallization of the recovered product from acetone gave needles (40 mg., m.p. 168–168.5°). A mixture melting point with XIb, prepared by hydrolysis of the diacetate Xb, was 168.5–169.5°. The mother liquor was chromatographed on a 12 × 280 mm. column of silica gel using ethyl acetate as the developing solvent. Fractions of 1.5-ml. size were collected at a rate of 6 ml./hr. From **fractions 42–68** was obtained 25.5 mg. (m.p. 168.5–169.5°) of glycol. The total yield was 80%.

20β-Acetoxy-4-bromo-3,11-dioxopregnan-21-oic Acid (XIXb) from XVIIIb.—20β-Acetoxy-3,11-dioxopregnan-21-oic acid (XVIIIb) was prepared in four steps from methyl 3α,20β-dihydroxy-11-oxopregnan-21-oate as previously reported[3] in an overall yield of 48.5%. To 539 mg. of XVIIIb (1.17 mmoles as the acetone solvate) in 10 ml. of glacial acetic acid was added 1 drop of 30% hydrobromic acid in acetic acid followed by 200 mg. (1.25 mmoles) of bromine in 6.58 ml. of acetic acid which was added slowly, with stirring, over a 15-min. period. Dilution with brine gave a crystalline precipitate (528 mg., m.p. 216–218° dec.). Several recrystallizations from acetone afforded 461 mg. of multifaceted prisms, m.p. 212–215° dec. The analytical sample melted at 212–214° dec., $[\alpha]_D$ +36° ($CHCl_3$).

Anal. Calcd. for $C_{23}H_{31}BrO_6$: Br, 16.53; CH_3CO, 8.90. Found: Br, 17.69; CH_3CO, 9.79.

20β-Acetoxy-3,11-dioxopregn-4-en-21-oic Acid (XVIb) from XIXb.—A solution of 20β-acetoxy-4-bromo-3,11-dioxopregnan-21-oic acid (362 mg.) and anhydrous lithium chloride (90 mg.) in dimethylformamide (3.75 ml.) was heated for 2 hr. at 100°. Addition of water to the cooled solution gave a crystalline precipitate. After drying and recrystallization from acetone–ether, colorless prisms (180 mg., m.p. 240–244°; 86 mg., m.p. 238–241°) were obtained in a yield of 88%.

A purified sample, recrystallized from acetone (m.p. 242–244.5°, $[\alpha]_D$ +114°) and dried for 12 hr. at 100° and 0.1 mm. over phosphorus pentoxide, lost 13.03% [m.p. 242–245°, $[\alpha]_D$ +131°, λ_{max}^{MeOH} 238 mμ (ε 15,300)]; calculated for loss of 1 mole of acetone, 12.60%. The dried product did not depress the melting point of XVIb derived from XVb.

Methyl 20β-Acetoxy-3,11-dioxopregn-4-en-21-oate from XVIb. A. Derived from XVIIIb.—Treatment of 25 mg. in methanol with excess ethereal diazomethane gave prismatic needles (16 mg., m.p. 207–209°) from acetone, $[\alpha]_D$ +118°.

B. Derived from XVb.—A 25-mg. sample treated as in A gave 19 mg. of product, m.p. 206.5–208°, $[\alpha]_D$ +117°.

C. Derived from XIIb.—A 10-mg. sample treated as in A gave 6.6 mg. of product, m.p. 207.5–209.5°, $[\alpha]_D$ +118°.

Mixture melting points of the three products showed no depression. The analytical sample had λ_{max}^{MeOH} 238 mμ (ε 16,100).

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.47; H, 7.89.

20α-Hydroxy-3-oxopregn-4-en-21-oic Acid (Va) from IIIa.—To 60 mg. of methyl 20α-hydroxy-3-oxopregn-4-en-21-oate in 7 ml. of methanol was added 5 ml. of water and 0.5 ml. of 1 *N* sodium hydroxide. After 15 min. at room temperature, the product was recovered as in the preparation of Vb from IIIb. Crystallization from acetone gave cuboidal prisms (51 mg., m.p. 213–215° dec.). The analytical sample had m.p. 214–216° dec., $[\alpha]_D$ +80°, λ_{max}^{MeOH} 242 mμ (ε 16,700).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 73.06; H, 8.69.

20α-Acetoxy-3-oxopregn-4-en-21-oic Acid (VIa) from Va.—Acetylation of 25 mg. of 20α-hydroxy-3-oxopregn-4-en-21-oic acid with acetic anhydride and pyridine and crystallization from ethyl acetate–*n*-hexane gave rectangular prisms (23 mg., m.p. 183.5–185.5° dec.; 4 mg., m.p. 181–185° dec.). The analytical sample, crystallized from acetone–*n*-hexane, melted at 184.5–186.5° dec., $[\alpha]_D$ +89°. When a portion was dried at 100° and 0.1 mm. for 17 hr., it lost 4.40%; calculated for loss of 1 molecule of water, 4.43%; λ_{max}^{MeOH} 242 mμ (ε 17,000) (corrected for water of crystallization).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.94; H, 8.33.

Methyl 20α-Acetoxy-3-oxopregn-4-en-21-oate (VIIa) from IIIa.—Acetylation of methyl 20α-hydroxy-3-oxopregn-4-en-21-oate in the usual manner gave long needles from aqueous methanol, m.p. 86–89°, $[\alpha]_D$ +97°, λ_{max}^{MeOH} 242 mμ (ε 16,200).

Anal. Calcd. for $C_{25}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.56; H, 8.56.

Methyl 20α-Hydroxy-3-oxopregn-5-en-21-oate 3-Ethylene Ketal (XVIIa) from IIIa.—Treatment of methyl 20α-hydroxy-3-oxopregn-4-en-21-oate (720 mg., 2 mmoles) in benzene (150 ml.) and ethylene glycol (6 ml.) with *p*-toluenesulfonic acid (18 mg.) for 6 hr. as in the preparation of XVIIb from IIIb gave leaflets from methanol (515 mg., m.p. 188–192°; 100 mg. 183–185°) in a yield of 76%. Several recrystallizations from methanol gave the analytical sample, m.p. 194–197.5°, $[\alpha]_D$ −21°.

(25) J. J. Schneider, *Arch. Biochem. Biophys.*, **98**, 249 (1960).

(26) We are indebted to Dr. Seymour Lieberman for determining and comparing these spectra.

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

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.48; H, 9.01.

20α,21-Dihydroxypregn-4-en-3-one (XIa) from XVIIa.—A solution of methyl 20α-hydroxy-3-oxopregn-5-en-21-oate 3-ethylene ketal (545 mg., 1.45 mmoles) and lithium aluminum hydride (400 mg.) in ether (200 ml.) was refluxed for 4 hr. Recovery of the product as in the preparation of XIb from XVIIb and crystallization from acetone afforded 267 mg. of plates, m.p. 190–192°. The residue from the mother liquor was chromatographed on a 31 × 680 mm. column of silica gel using ethyl acetate as the developing solvent. Fractions (5 ml.) were collected at a rate of six per hour. From **fractions 123–190** were obtained polygonal prisms (114 mg., m.p. 193.5–194.5°; 7 mg., m.p. 185–187°). The total yield of glycol was 80.8%. The analytical sample, recrystallized from acetone, melted at 196.5–198.5°, $[\alpha]_D$ +93°, λ_{max}^{MeOH} 241 mμ (ε 17,300); lit.[28] m.p. 192.5–194.5°, $[\alpha]_D$ +97° ($CHCl_3$).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.99; H, 9.81.

Treatment of 20α,21-Dihydroxypregn-4-en-3-one (XIa) with 1.7 Equiv. of Acetic Anhydride.—To 310 mg. (0.93 mmole) of XIa in 10 ml. of pyridine were added, at 15-min. intervals, five 0.3-ml. volumes of a 1:10 dilution of acetic anhydride in pyridine (equivalent to 1.58 mmoles). After 4 hr. at room temperature, the solution was diluted with water and extracted with methylene chloride. The organic layer was washed with dilute hydrochloric acid and water, filtered through anhydrous sodium sulfate, and concentrated to dryness. The crude product was fractionated on a 30 × 650 mm. column of silica gel using ethyl acetate–benzene (2:1) as the developing system. Fractions (6 ml.) were collected at a rate of five per hour. At fraction 230 the system was changed to 10% ethanol in ethyl acetate.

20α,21-Diacetoxypregn-4-en-3-one (Xa). Fractions 52–75.—Crystallization from ether–*n*-hexane gave needles (30 mg., m.p. 101–102°; 13 mg., m.p. 90–95°). The yield was 11.1%. The analytical sample melted at 101–102.5°, $[\alpha]_D$ +65°, λ_{max}^{MeOH} 241 mμ (ε 17,100).

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71; CH_3CO, 20.66. Found: C, 71.91; H, 8.70; CH_3CO, 19.98.

20α-Hydroxy-21-acetoxypregn-4-en-3-one (IXa). Fractions 111–151.—Crystallization from acetone and from acetone–*n*-hexane gave prisms (146 mg., m.p. 180–182°; recrystallization from acetone did not raise the melting point), $[\alpha]_D$ +84°, λ_{max}^{MeOH} 241 mμ (ε 17,200).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15; CH_3CO, 11.49. Found: C, 73.96; H, 9.40; CH_3CO, 11.21.

Oxidation of a 15-mg. sample of IXa with chromic anhydride (15 mg.) in 98% acetic acid (1.53 ml.) for 1 hr. at room temperature followed by crystallization of the product from ethanol gave prismatic needles (6 mg., m.p. 154–156°) which did not depress the melting point of **desoxycorticosterone acetate.**

Fractions 152–220.—The residue from the pooled fractions weighed 63 mg. and consisted of a mixture of 20α-hydroxy-21-acetoxypregn-4-en-3-one (IXa) and 21-hydroxy-20α-acetoxypregn-4-en-3-one (VIIIa). Preparative paper chromatography was carried out on ten 19 × 55 cm. sheets of Whatman No. 1 filter paper impregnated with 40% formamide in acetone. The mobile phase was *n*-hexane–benzene (3:2) saturated with formamide, and a 7-hr. development period was allowed. The two main ultraviolet-absorbing bands were eluted by the usual procedure.

20α-Hydroxy-21-acetoxypregn-4-en-3-one (IXa).—Crystallization of the more mobile component from acetone–*n*-hexane gave rosettes (12.2 mg., m.p. 178.5–180.5°; 5.4 mg., m.p. 176.5–178.5°). The total yield of the 21-monoacetate was thus raised to 164 mg. (47.2%).

21-Hydroxy-20α-acetoxypregn-4-en-3-one (VIIIa).—Crystallization of the more polar component from acetone–*n*-hexane afforded rosettes (12.2 mg., m.p. 156–158°; 7.9 mg., m.p. 154.5–156.5°) for a total yield of 5.8%. Recrystallization from acetone–*n*-hexane gave the analytical sample, m.p. 155.5–158°, $[\alpha]_D$ +65°, λ_{max}^{MeOH} 241 mμ (ε 16,900).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15; CH_3CO, 11.49. Found: C, 73.53; H, 9.00; CH_3CO, 12.15.

When a 15-mg. sample of VIIIa was oxidized with chromic acid as above, the resulting acidic product (giving 7.4 mg. of prisms, m.p. 185.5–188°, from acetone–*n*-hexane) did not depress the melting point of 20α-acetoxy-3-oxopregn-4-en-21-oic acid (VIa).

20α,21-Dihydroxypregn-4-en-3-one (XIa). Fractions 246–275.—Crystallization from acetone gave 44 mg. (14.2%) of prisms, m.p. 194–196°, which did not depress the melting point of starting material.

20α-Acetoxy-4-bromo-3,11-dioxopregnan-21-oic Acid (XIXa) from XVIIIa.—To 613 mg. (1.36 mmoles as the ethanol solvate) of 20α-acetoxy-3,11-dioxopregnan-21-oic acid[3] in 5 ml. of acetic acid was added successively 1 drop of 30% hydrobromic acid in acetic acid and 240 mg. of bromine in 7.7 ml. of acetic acid. The product was recovered as in the preparation of XIXb from XVIIIb. Several recrystallizations from acetone provided 513 mg. (78.2%) of long needles, m.p. 130–132°, $[\alpha]_D$ +66°.

Anal. Calcd. for $C_{23}H_{31}BrO_6$: Br, 16.53; CH_3CO, 8.90. Found: Br, 17.78; CH_3CO, 8.35.

20α-Acetoxy-3,11-dioxopregn-4-en-21-oic Acid (XVIa) from XIXa.—A solution of 20α-acetoxy-4-bromo-3,11-dioxopregnan-21-oic acid (435 mg., 0.9 mmole) and anhydrous lithium chloride (108 mg.) in dimethylformamide (5 ml.) was heated at 100° for 2 hr. Addition of water to the cooled solution gave a fine, white solid mixed with a yellow gum. Recrystallization from acetone–ether gave 198 mg. (55%) of prisms, m.p. 210–215° dec. The analytical sample had m.p. 214–218° dec., $[\alpha]_D$ +160°, λ_{max}^{MeOH} 238 mμ (ε 15,400).

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.80; H, 7.65.

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