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).

Acknowledgment.—We wish to thank Dr. H. L. Mason for several helpful suggestions. We are indebted to Merck & Co., Inc., Rahway, New Jersey, for a generous supply of cortisone.

(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.

(23) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

(24) K. Dobriner, E. R. Katzenellenbogen, E. R. Jones, G. Roberts, and B. S. Gallagher, "Infrared Absorption Spectra of Steroids, an Atlas," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1958.

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.

(3) J. P. Conbere, U. S. Patent 2,733,077 (1956).

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

(5) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, in press.

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

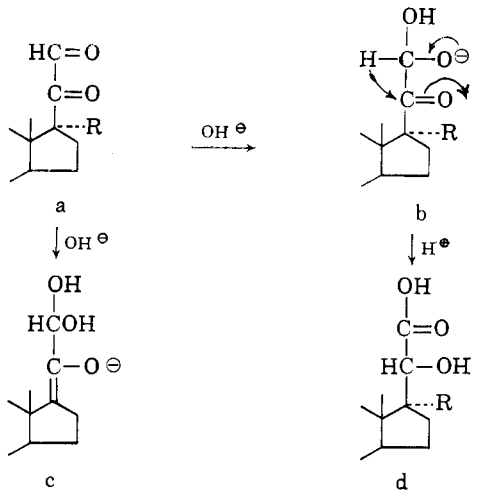

Fig. 1.—R = H or OH. 17-Hydroxy steroidal glyoxals cannot enolize to give structure c.

by this procedure it was found that the amount of glyoxal formed was maximal in fifteen to thirty minutes but decreased slowly at longer reaction periods. After seventy-two hours, only 15% of the glyoxal remained, as indicated by measurement[5] by the Porter–Silber procedure. A plot of the logarithm of the concentration of glyoxal against time gave a straight line. Approximate half-lives, when the methanolic solution was 0.02 *M* in glyoxal and the molar ratios of copper to steroid were 1:20, 1:8, 1:2, and 1:1, were fifty-seven, thirty, twenty-one, and twenty-one hours, respectively.

The major products of the reaction of 3α-hydroxy-11,-20-dioxo-5β-pregnan-21-al (II, Fig. 2) with copper acetate in methanol were the methyl esters (IIIa[7] and IIIb[7]) of the 20-epimeric 20-hydroxypregnan-21-oic-acids. Three by-products of unknown structure were obtained in low yield. This complex reaction mixture was separated readily by partition-type chromatography on a column of Celite.

That copper salts are capable of catalyzing, under neutral conditions, the rearrangement of α-keto aldehydes to α-hydroxy (glycolic) acids is not generally recognized. The only mention of this type of reaction that has been located in the literature is by Nef,[8] describing the conversion of acetol and benzoylcarbinol, in aqueous solutions, to lactic acid and mandelic acid, respectively, by treatment with aqueous cupric acetate or cupric sulfate. His presumption that the α-keto aldehydes were intermediates was confirmed by Evans[9] who showed that benzoylformaldehyde was convertible to mandelic acid under the same conditions.

The use of alkali or alkoxides to achieve this type of transformation is well known and, in effect, represents an intramolecular Cannizzaro reaction.[10] In the steroid field, Wendler and Graber[11] obtained a mixture of 20-epimeric glycolic acids (VIIa + VIIb) after treatment of 3α,17,21-trihydroxy-5β-pregnane-11,20-dione with dilute methanolic potassium hydroxide in an atmosphere of nitrogen. The diacetates of the corresponding methyl esters also were prepared, but the epimers were not separated. The amorphous 20-epimeric 11β,20-dihydroxy-3-oxopregna-1,4-dien-21-oic acids were obtained by Hirschmann, *et al.*,[12] from prednisolone as by-products in the reaction of 21-iodoprednisolone with silver dihydrogen phosphate.

Franzen[13] has shown that the rearrangement of methylglyoxal and phenylglyoxal to the glycolic acids is catalyzed by compounds which contain thiol and amino groups either in the same or in different molecules. By conducting the reaction in heavy water, he obtained evidence that a hydride ion transfer is involved in the rearrangement.

Although we have not investigated the mechanism of the conversion of glyoxals to methyl glycolates by copper acetate in methanol, we present the following speculation. The yellow anhydrous glyoxal II becomes colorless when it is dissolved in methanol. It is assumed that a hemiacetal is formed since the methyl and ethyl hemiacetals[5] of the aldehyde from cortisone have been crystallized from aqueous solutions of the corresponding alcohols. If the rearrangement of the glyoxal hemiacetal involves a transfer of a hydride ion from C-21 to C-20, which seems probable, any process which would increase the electron density at C-21 or tend to produce a positive charge on C-20 would promote the reaction.[14]

Copper acetate is a Lewis-type acid and is known to form complexes with many substances. It is postulated that the hemiacetal of the glyoxal forms a complex with copper acetate involving the carbonyl oxygen at C-20 and the hydroxyl oxygen at C-21. It is assumed that copper acetate acts as an acceptor for a pair of electrons from the carbonyl oxygen at C-20, and that the positive charge so induced on C-20 provides the driving force for the reaction. After the rearrangement occurs, the regenerated catalyst is available for reaction with another molecule of glyoxal hemiacetal.

In methanolic cupric acetate, steroidal glyoxals with a hydroxyl group at C-17 rearrange much more slowly than do 17-deoxy glyoxals. Whether this phenomenon is due to inhibition of formation of a reactive complex with cupric acetate because of hindrance by or combination with the hydroxyl group at C-17 or to an electronic effect exerted on C-20 by the hydroxyl group at C-17 is not known. It is noteworthy that no significant amount of acidic material[15] is obtained from the 17-deoxy steroidal glyoxals whereas the acidic fraction from a 17-hydroxy glyoxal[16] (derived from cortisone) amounts to 11%.

Treatment of both the 17-deoxy glyoxal II and the 17-hydroxy glyoxal from cortisone with alkali gives mixtures of the corresponding glycolic acids. With glyoxal II in 4 *N* alkali, about eight hours is required for complete reaction, whereas with the glyoxal from cortisone in 1.25 *N* alkali, the reaction is complete within thirty minutes.[16] If the rearrangement occurs by the type of mechanism[13] which has been suggested for this process (a → b → d for 20α-epimer in Fig. 1), the slower reaction of the 17-deoxy glyoxal may be due to 17(20)-enolization to give intermediate c, or the equivalent, which competes with the reaction that leads to the gly-

(7) "a" represents the 20α-oxygen epimer; "b," the 20β-oxygen epimer.
(8) J. U. Nef, *Ann. Chem.*, **335**, 247 (1904).
(9) W. L. Evans, *Am. Chem. J.*, **35**, 115 (1906).
(10) T. A. Geissman, *Org. Reactions*, **2**, 94 (1944).
(11) N. L. Wendler and R. P. Graber, *Chem. Ind.* (London), 549 (1956).
(12) R. Hirschmann, G. Bailey, and J. M. Chemerda, *ibid.*, 682 (1958).
(13) V. Franzen, *Chem. Ber.*, **88**, 1361 (1955); **89**, 1020 (1956).
(14) N. C. Deno, H. J. Peterson, and G. S. Saines, *Chem. Rev.*, **60**, 7 (1960).
(15) A small amount of acidic product is formed when glycolic esters IIIa and IIIb are prepared by treatment of ketol I with cupric acetate. The acidic material is formed during oxidation of ketol I rather than during rearrangement of glyoxal II.
(16) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 1773 (1963).

colic acid mixture. A 17-hydroxyl group precludes a structure of type c. The Cannizzaro reaction ordinarily is associated with substances that have no enolizable hydrogen in the function undergoing reaction.

To study the scope and rate of this rearrangement, the steroidal glyoxals listed in Table I were treated with copper acetate in methanol. The half-lives of the glyoxals, as indicated by rate of disappearance of Porter–Silber chromogenicity, were determined.

TABLE I

RATE OF DISAPPEARANCE OF GLYOXALS IN 0.01 *M* METHANOLIC CUPRIC ACETATE[a]

Glyoxal from	Half-life[b]
3α,21-Dihydroxy-5β-pregnane-11,20-dione	17
3α-21-Dihydroxy-5β-pregnan-20-one	19
3α,21-Dihydroxy-16α,17α-epoxy-5β-pregnane-11,20-dione	20
3α,11β,21-Trihydroxy-5β-pregnan-20-one	23
11-Deoxycorticosterone	23
Corticosterone	30
Cortisone	128
3α,17,21-Trihydroxy-5β-pregnane-11,20-dione	170

[a] Molar ratio of copper to steroid, 1:2. [b] Half-life refers to time in hours required for a 50% decrease of absorbance in the Porter–Silber reaction. Initial absorbancies were obtained after treatment of the respective α-ketols for one hour.

The approximate half-life of 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al in various alcohols, with a copper to steroid ratio of 1:2, is shown in Table II. The reaction was more rapid in *n*-propyl alcohol than in methyl alcohol, but in *t*-butyl alcohol, was considerably slower than in methyl alcohol. That different products were formed from the glyoxal with the different alcohols was shown by the fact that paper chromatography gave R_f values varying from 0.38 for methyl alcohol to 0.62 for *t*-butyl alcohol, as shown in Table II. The half-lives of the glyoxal derivatives from cortisone in methyl, ethyl, propyl and butyl alcohols containing copper acetate also are given in the table.

TABLE II

RATE OF DISAPPEARANCE OF GLYOXALS IN 0.01 *M* CUPRIC ACETATE[a] IN VARIOUS ALCOHOLS

	Glyoxal from 3α,21-dihydroxy-5β-pregnane-11,20-dione			Glyoxal from cortisone	
Alcohol	Half-life[b]	R_fa[c]	R_fb[c]	Half-life[b]	R_fa[c]
CH_3OH	17.5	0.38	..	128	0.20
C_2H_5OH	11.0	.52	..	170	.42
n-C_3H_7OH	8.5	.65	0.35	144	.58
n-C_4H_9OH	10.0	.74	.48	143	.71
n-$C_5H_{11}OH$	10.5	..	.59		
n-$C_6H_{13}OH$	9.0	..	.65		
n-$C_7H_{15}OH$	10.0	..	.71		
i-C_3H_7OH	19.0	0.52	..		
i-C_4H_9OH	21.5	.58	..		
sec-C_4H_9OH	18.0	.64	..		
t-C_4H_9OH	31.0	.62	..		

[a] Copper to steroid ratio, 1:2. [b] Half-life denotes time in hours required for a 50% reduction of the original color in the Porter–Silber reaction. [c] R_f, ratio of movement of major spot to movement of solvent front; a, isooctane–toluene–methanol–water, 90:110:160:40; and b, isooctane–toluene–methanol–water, 140:60:160:40.

The structures of the two main products (IIIa and IIIb) from the reaction between methanolic cupric acetate and 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al (II) were deduced from the following observations. Substances IIIa and IIIb were neutral and had the same per cent composition. They contained no carbonyl groups active toward 2,4-dinitrophenylhydrazine. Oxidation of IIIa and IIIb with chromic acid gave a single compound (VI). Substances IIIa and IIIb contained two acylable hydroxyl groups, as shown by formation of the isomeric diacetoxy esters (IVa and IVb). Hydrolysis of dihydroxy esters IIIa and IIIb gave two dihydroxy acids (VIIa and VIIb) which were convertible to 3α-hydroxy-11-oxo-5β-etianic acid (V) by treatment with potassium permanganate in acetic acid. Neither the esters (IIIa and IIIb) nor the free acids (VIIa and VIIb) were altered by treatment with periodic acid. From these results it follows that substances IIIa and IIIb are 20-epimeric 20-hydroxypregnan-21-oic esters.

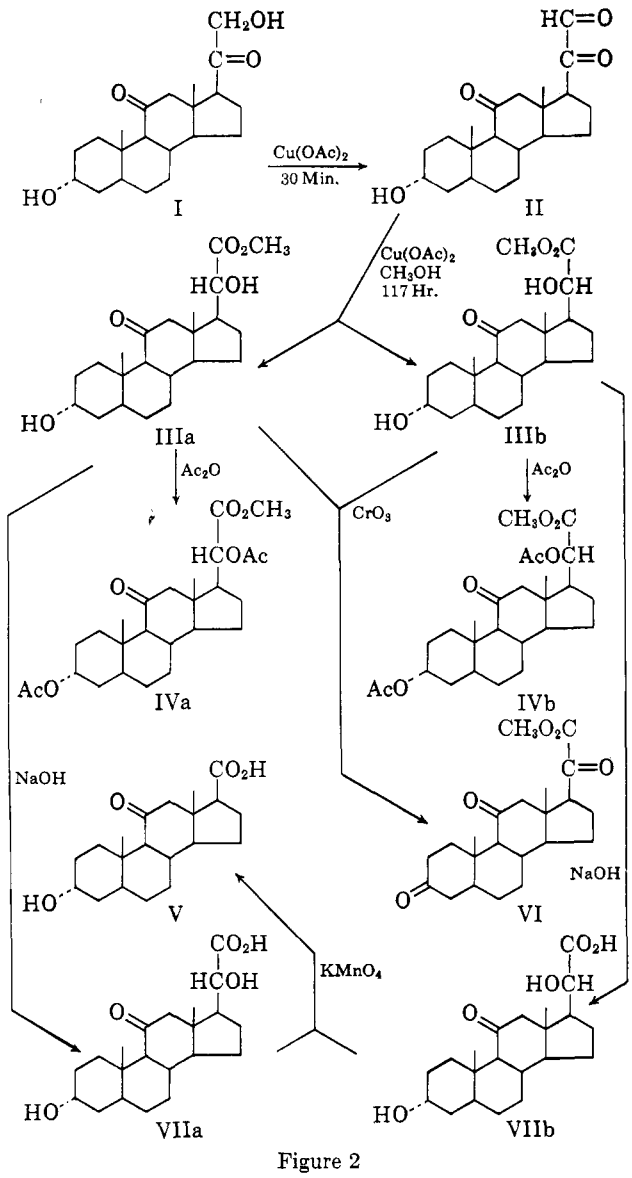

Figure 2

Acetylation of the dihydroxy acids (VIIa and VIIb) gave the corresponding diacetoxy acids (VIIIa and VIIIb, Fig. 3). The 3α-acetyl group was selectively removed from each of these compounds by mild treatment of the diacetoxy acids (VIIIa and VIIIb) with a slight excess of alkali. More vigorous treatment of the diacetates (VIIIa and VIIIb) produced the dihydroxy acids (VIIa and VIIb).

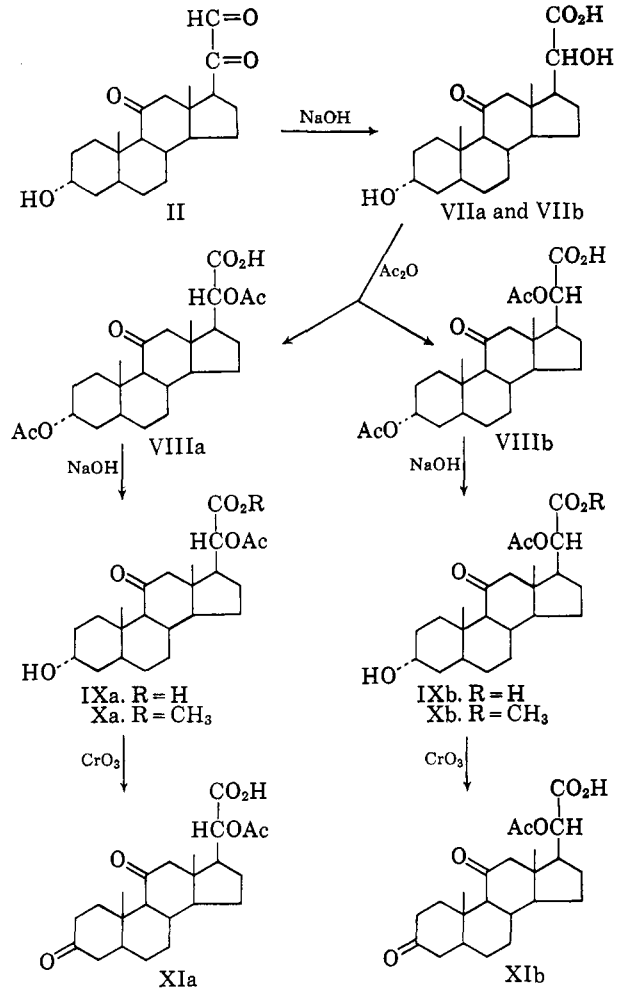

Figure 3

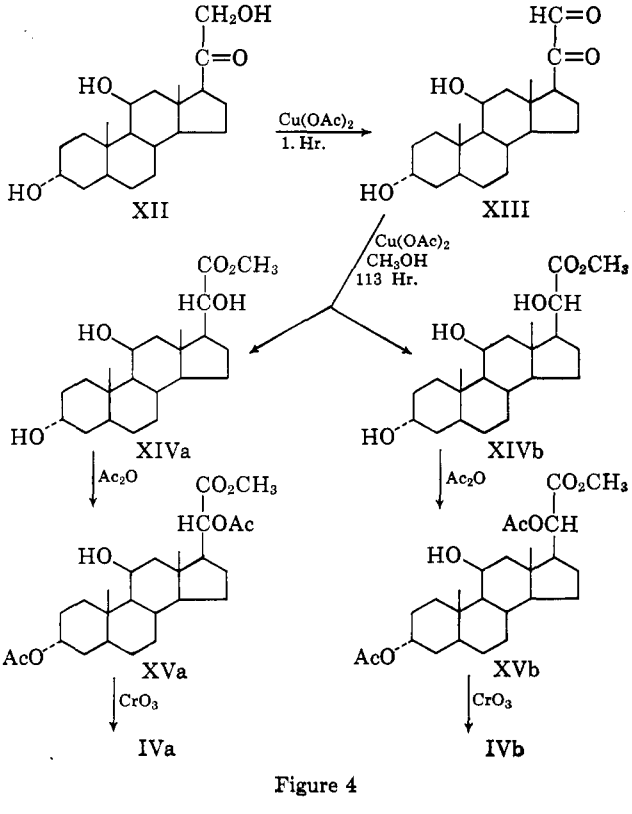

Figure 4

The 20-acetyl group is removed more easily from the 20α-acetate than from its 20β-epimer. This fact was demonstrated by treating the diacetoxy epimers (VIIIa and VIIIb) with four equivalents of sodium hydroxide under identical conditions and analyzing, by paper chromatography,[17] serial aliquots of the mixtures. After forty minutes at room temperature the 20β-acetoxy acid (IXb) was essentially the only product present in the reaction mixture from the 20β-epimer (VIIIb). From the 20α-epimer (VIIIa), a 1:1 mixture of 20α-acetoxy acid (IXa) and 3α,20α-dihydroxy acid (VIIa) was obtained. Esterification of the 20-acetoxy acids (IXa and IXb) with diazomethane produced the corresponding methyl esters (Xa and Xb). Oxidation of the 3α-hydroxy-20α- and 20β-acetoxy acids (IXa and IXb) with chromic acid yielded the corresponding 3-keto compounds (XIa and XIb).

The conversion of 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al (II) to the mixture of glycolic acids (VIIa and VIIb) could be obtained more quickly by treatment with alkali than the corresponding esters could be obtained by treatment with cupric acetate (eight *vs.* 117 hours). After acetylation of the mixture of acids, the 20α- and 20β-acetoxy compounds (VIIIa) and VIIIb) could be obtained in 42 and 34% yield by fractional crystallization from ethyl acetate.

Treatment of 3α,11β-dihydroxy-20-oxo-5β-pregnan-21-al (XIII, Fig. 4) with methanolic cupric acetate gave the 20α- and 20β-epimers of the glycolic acid methyl esters (XIVa and XIVb) in yields of 26 and 35%, respectively. Mild acetylation of these products gave the corresponding diacetates (XVa and XVb). Proof of structure of these products was established by chromic acid oxidation of the 11β-hydroxyl group to give corresponding 11-ketones (IVa and IVb).

The configuration at C-20 of a pair of epimeric 20-acetoxy pregnanes can be established by comparing their molecular optical rotations. A simple rule, formulated by Fieser and Fieser,[18] states that a 20β-acetoxy compound of any type is more dextrorotatory than its 20α-acetoxy epimer. The rule holds for pregnan-20-ols which are unsubstituted at C-17 and C-21 as well as for pregnane-20,21-diols, pregnane-17,20-diols and pregnane-17,20,21-triols.[19]

This rule was considered in assigning configurations[20] to the 20-epimeric 20-hydroxypregnan-21-oic derivatives listed in Table III. Its application would designate those compounds with the more positive rotations as having a 20β-oxygen function. However, on this basis the 20α-acetate should be the more difficult epimer to hydrolyze; in the acetylated glycolic acids from cortisone, the 20β-acetate[16] is more difficult to hydrolyze than is its 20α-epimer. Furthermore, with the 20-epimeric 17,20-dihydroxypregnen-21-oic derivatives

(17) Solvent system: isooctane (100), toluene (100), methanol (100), acetic acid (30), water (70). R_f values of the diacetoxy, monoacetoxy, and dihydroxy acids were 0.7, 0.11, and 0.01. There was no significant difference in the mobilities of the 20α- and 20β-epimer pairs.

(18) L. F. Fieser, and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 612.

(19) L. H. Sarett, *J. Am. Chem. Soc.*, **71**, 1165 (1949).

(20) Data in Table III are sufficient for determining C-20 acetylation increments for only two pairs of epimers (pairs 7 and 8 in Table III). The acetylation increments of the 20α-hydroxy compounds are positive and those of the 20β-hydroxy epimers are negative. This finding is at variance with results from acetylation of epimeric 20-hydroxy-pregnanes which are unsubstituted at C-21 or bear a hydroxyl group. With these compounds, acetylation increments for 20β-ols are strongly positive and those for 20α-ols are negative or very slightly positive.

from cortisone, neither the acetylation increments[18] nor the optical rotations of the 20-acetates of one series were consistently greater than those of the other.[16] Thus it was obvious that the generalizations concerning rotation and configuration at C-20 were not valid[21] for assigning C-20 configuration to 17,20-dihydroxypregn-4-en-21-oic acids and it seemed probable that they would not be valid for the 17-deoxy-20-hydroxypregnanoic derivatives in Table III.

TABLE III

MOLECULAR ROTATIONS[a] OF 5β-PREGNANES WITH SUBSTITUENTS AT C-3, C-11, C-20 AND C-21

Pair	Substituents				Epimers		Δ[b]
no.	C-3	C-11	C-20	C-21	20α	20β	α-β
1	α-OH	=O	OH	O_2H	+182	+91	+91
2	α-OH	=O	OH	O_2CH_3	+148	+95	+53
3	α-OH	β-OH	OH	O_2CH_3	+197	+102	+95
4	α-OAc	=O	OAc	O_2H	+350	+188	+162
5	α-OAc	=O	OAc	O_2CH_3	+351	+152	+199
6	α-OAc	β-OH	OAc	O_2CH_3	+366	+162	+204
7	α-OH	=O	OAc	O_2H	+248	+85	+163
8	α-OH	=O	OAc	O_2CH_3	+256	+63	+193
9	=O	=O	OAc	O_2H	+281	+93	+188

[a] Molecular rotations, MD, are $[\alpha]_D \times$ mol. wt./100. [b] Δ = MD 20α − MD 20β.

Because of these considerations, 3α,20β-diacetoxy-11-oxo-5β-pregnan-21-oic acid of known absolute configuration at C-20[22] was synthesized. With this compound available, it was possible to establish the 20β-configuration for the acetoxy group in the 3α,20-diacetoxy-11-oxo-5β-pregnan-21-oic acid which melts at 199–200°, and to assign formula VIIIb to this substance. Since VIIIb is already correlated configurationally at C-20 with the various other 20-asymmetric compounds which are described, it follows that their configurations are as indicated in the figures.

Experimental

General Procedures.—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 a temperature of 24 ± 2° unless otherwise designated. Analyses were by J. F. Alicino, Metuchen, N. J.

Paper chromatography was used extensively for evaluating complex reaction mixtures, developing solvent systems for chromatography on columns, and judging homogeneity of purified samples. Chromatography on columns was employed to separate preparative quantities of pure products. In general, the procedures discussed in detail by Neher[23] were followed.

For detection of Δ⁴-3-ketones on paper chromatograms, ultraviolet absorption and sodium hydroxide-induced fluorescence were employed. Alkaline blue tetrazolium was used for α-ketols and the Porter–Silber reagent was employed[6] for detection of dihydroxyacetone or glyoxal groupings. For several other classes of compounds phosphomolybdic acid, which is nonspecific and relatively insensitive, was used. With this reagent, the methyl esters of the 20-epimeric 3α,20-dihydroxy-11-oxo-5β-pregnan-21-oic acids gave very little color, even at levels of 100 μg., whereas the 11β-hydroxy analogs gave intense spots with as little as 10 μg. Acids were detected by dipping the paper chromatograms into a 0.01% alcoholic solution of chlorophenol red and then holding the paper over a mild current of steam to drive off the residual acetic acid; steroidal acids gave yellow spots on a purple background. The test was sensitive to 2 μg. of carboxyl group in the steroidal acids examined.

Columns were prepared for chromatography with either Bush- or Zaffaroni-type solvent systems by pretreating Celite 545 (100–200 mesh) with an excess of mobile phase mixed with approximately 50% (v./w.) of stationary phase. For the preparation of columns greater than 2 cm. in diameter, the use of a conventional close-fitting Martin packer did not result in uniform packing of the supporting medium. Chromatography of sudan III indicated that the center of the column was packed more loosely than was the periphery. When a close-fitting packer was used in conjunction with a packer of small diameter, which was used to tamp the center of the column, satisfactory uniformity of the supporting medium was achieved. A column was judged suitable for use if a test sample of sudan III was eluted in less than 20% of the effluent collected after applying sudan III to the column. Rates of flow of the solvents through the columns were in the range of 4 to 16 ml./sq. cm. surface area/hr.

Methyl 3α,20α-(and 20β)-Dihydroxy-11-oxo-5β-pregnan-21-oates and Three Unknown Compounds from I and Methanolic Cupric Acetate.—A solution of 3.48 g. (10 mmoles) of 3α,21-dihydroxy-5β-pregnane-11,20-dione[5] (I) in 250 ml. of methanol was mixed with 1.0 g. (5 mmoles) of cupric acetate in an equal volume of methanol at room temperature. After 117 hr., only 7% of the glyoxal remained, as indicated by analysis[6] with the Porter–Silber reagent. After addition of 2.0 g. of disodium ethylenedinitrilotetraacetate (EDTA) in 50 ml. of water, the methanol was evaporated *in vacuo*, and the aqueous residue was extracted with methylene chloride. The extract was washed with 5% sodium bicarbonate and then water, and concentrated to dryness. To remove unchanged glyoxal II, the residue in 20 ml. of methanol was heated on a steam bath with 500 mg. of sodium bisulfite in 50 ml. of water. The cooled mixture was extracted with methylene chloride, and the organic layer, after being washed with water, was concentrated to dryness. The residue gave 1500 mg. (m.p. 190–195°) of fine needles from 5 ml. of benzene. Recrystallization from acetone gave 1170 mg. (31%, m.p. 200–203°) of pure **methyl 3α,20β-dihydroxy-11-oxo-5β-pregnan-21-oate (IIIb)** as prisms.

The residue (2127 mg.) from the benzene and acetone mother liquors was chromatographed in benzene (1800), cyclohexane (1200), formamide (200) on a 6-cm. diameter column packed to a height of 40 cm. with 350 g. of Celite plus 57.5 ml. of stationary phase. Before fraction no. 1 was collected 800 ml. of effluent was discarded. Each fraction contained 20 ml. Weight of the residue from every fifth fraction was obtained and plotted against the corresponding fraction number. Fractions were pooled and the solvent was removed. Three compounds of unknown structure, X_1, X_2, and X_3, emerged before the dihydroxy esters.

Compound X_1. Fractions 17–40.—The residue gave long needles from methyl ethyl ketone (288 mg., m.p. 164–165°). A sample for analysis prepared from the same solvent melted at 167–168.5°; $[\alpha]_D$ +112° ± 2°.

Anal. Calcd. for $C_{22}H_{32}O_5$: C, 70.18; H, 8.57; CH_3O, 8.24. Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05; CH_3O, 8.20. Found: C, 70.35; H, 8.63; CH_3O, 8.19.

Compound X_2. Fractions 48–80.—The residue gave crystals (84 mg., m.p. 189–190°) from acetone. Recrystallization from acetone did not alter the melting point; $[\alpha]_D$ +139° ± 1°.

Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51. Found: C, 74.99; H, 9.38; CH_3O, 0.0.

Compound X_3. Fractions 116–164.—Crystals (121 mg., m.p. 198–198.5°; and 9 mg., 196–198°) were obtained from acetone–ether. The sample for analysis was recrystallized from acetone–ether; m.p. 200–201°; $[\alpha]_D$ +15° ± 2°.

Anal. Calcd. for $C_{24}H_{38}O_6 \cdot CH_3COCH$: C, 67.47; H, 9.23; $3CH_3O$, 19.40. Found: C, 67.46; H, 9.19; CH_3O, 19.17.

Compound X_3 Acetate.—Acetylation at room temperature in acetic anhydride–pyridine and crystallization from ether–petroleum ether gave a product with m.p. 124.5–125°; $[\alpha]_D$ +47° ± 1°.

Anal. Calcd. for $C_{28}H_{42}O_8$: C, 66.38; H, 8.36; 2 CH_3CO, 17.00. Found: C, 66.24; H, 8.34; CH_3CO, 17.88.

Methyl 3α,20α-Dihydroxy-11-oxo-5β-pregnan-21-oate (IIIa). Fractions 210–280.—Rosettes (580 mg.) were obtained from acetone–petroleum ether. Recrystallization from methyl ethyl ketone gave 436 mg. (11.5%, m.p. 175–178°) of pure IIIa. The sample for analysis was recrystallized from acetone–ether; m.p. 177.5–178°; $[\alpha]_D$ +39° ± 1°.

(21) We are indebted to R. M. Dodson who, in view of these findings, emphasized the chance of being in error when using molecular rotations to assign configurations to the 17-deoxy-20-acetoxy-5β-pregnan-21-oic compounds described in this paper.

(22) V. R. Mattox, unpublished data.

(23) R. Neher, "Chromatographie von Sterinen, Steroiden und Verwandten Verbindungen," Elsevier Publishing Co., New York, N. Y., 1958, 100 pp.

Anal. Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.89; H, 9.05.

Saponification of the mother liquor with aqueous methanolic sodium hydroxide followed by acetylation of the acidic fraction gave an additional 5.8% (260 mg., m.p. 259–261°) of the 20α-epimer as **3α,20α-diacetoxy-11-oxo-5β-pregnan-21-oic acid (VIIIa)** and brought the total yield of 20α-epimer from I to 17.3%.

Methyl 3α,20β-Dihydroxy-11-oxo-5β-pregnan-21-oate (IIIb). Fractions 284–344.—Crystallization from acetone gave 170 mg. (4.5%, m.p. 198–200°) of 20β-epimer IIIb. The total yield of IIIb, obtained by direct crystallization and after chromatography, was 35.5%. Recrystallization from acetone gave the analytical sample; m.p. 202.5–204°; $[\alpha]_D$ +25° ± 2°.

Anal. Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05; CH_3O, 8.20; mol. wt., 378. Found: C, 70.12, 70.21; H, 8.59, 9.10; CH_3O, 8.17; mol. wt., 373.

3α,20α- (and 20β)-Diacetoxy-11-oxo-5β-pregnan-21-oic Acids (VIIIa and VIIIb) from II and Sodium Hydroxide.—To 3.64 g. (10 mmoles) of 3α,21,21-trihydroxy-5β-pregnane-11,20-dione[5] (hydrate of II) suspended in 100 ml. of water was added slowly, while stirring vigorously, 20 ml. of 2 *N* sodium hydroxide. After being stirred for 8 hr. at room temperature, the mixture was acidified with *N* hydrochloric acid and extracted three times with 20-ml. portions of ethyl acetate. The ethyl acetate extract was washed four times with a total volume of 60 ml. of 5% sodium bicarbonate. The combined alkaline washes were extracted with ethyl acetate which, along with the original organic phase, was discarded. Acidification of the aqueous phase and extraction with ethyl acetate gave 3.75 g. of crude acidic material. This product was acetylated with 5 ml. each of pyridine and acetic anhydride for 11 hr. at room temperature. The acetylated product, recovered in the usual fashion except for omission of the bicarbonate wash, was subjected to fractional crystallization and gave 1.91 g. (42.6%, m.p. 260–262°) of pure **3α,20α-diacetoxy-11-oxo-5β-pregnan-21-oic acid (VIIIa)** from ethyl acetate. The analytical sample was prepared by recrystallization from acetone; m.p. 261.5–262°; $[\alpha]_D$ +78° ± 1°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.77; H, 8.04.

The addition of petroleum ether to the mother liquor from VIIIa gave 1.76 g. (39.3%, m.p. 189–192°) of impure **3α,20β-diacetoxy-11-oxo-5β-pregnan-21-oic acid (VIIIb)**. This fraction, after recrystallization from acetone, saponification with aqueous methanolic alkali, and esterification with diazomethane, afforded 822 mg. (21.8% over-all from II, m.p. 202–203.5°) of pure **methyl 3α,20β-dihydroxy-11-oxo-5β-pregnan-21-oate (IIIb)**. This product did not depress the melting point of IIIb which was prepared from I by treatment with cupric acetate.

A sample of pure **3α,20β-diacetoxy-11-oxo-5β-pregnan-21-oic acid (VIIIb)** was obtained by acetylation of the 3α,20β-dihydroxy acid (VIIb) with acetic anhydride and pyridine for 3 hr. at room temperature. From 250 mg. of VIIb were obtained three crops of crystals (185 mg., m.p. 197–199°; 84 mg., m.p. 195.5–197°; and 8 mg., m.p. 194–195.5°) from acetone in a yield of 90%. A sample for analysis was prepared by recrystallization from acetone and drying for 2 hr. at 100° and 5–10 μ; m.p. 199–200°; $[\alpha]_D$ +41° ± 1°.

Anal. Calcd. for $C_{25}H_{36}O_7 \cdot {}^1/_2H_2O$: C, 65.62; H, 8.18. Found: C, 65.66, 65.73; H, 8.11, 8.19.

3α,20β-Dihydroxy-11-oxo-5β-pregnan-21-oic Acid (VIIb) from IIIb.—To a solution of 400 mg. of methyl 3α,20β-dihydroxy-11-oxo-5β-pregnan-21-oate (IIIb) in 3 ml. of methanol was added 12 ml. of water, then 4 ml. of 2 *N* sodium hydroxide. The milky suspension became clear in about 1 min. After 15 min. at room temperature, the solution was acidified with dilute hydrochloric acid. The product was extracted with ethyl acetate and crystallized from acetone–ether in a yield of 87% (300 mg., m.p. 244–245°; and 37 mg., m.p. 243–245°). Recrystallization from acetone–ether did not elevate the melting point; $[\alpha]_D$ +25° ± 2°.

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.19; H, 8.85. Found: C, 69.28; H, 9.17.

Treatment of acid VIIb with diazomethane gave an ester which melted at 202.5–203.5°; it did not depress the melting point of IIIb that was obtained from II by treatment with cupric acetate.

3α,20α-Dihydroxy-11-oxo-5β-pregnan-21-oic Acid (VIIa) from VIIIa.—To a solution of 1260 mg. (2.8 mmoles) of 3α,20α-diacetoxy-11-oxo-5β-pregnan-21-oic acid in 45 ml. of methanol was added 100 ml. of water and 16.2 ml. of 1.72 *N* sodium hydroxide (28 mmoles). The solution was heated on a steam bath for 1.5 hr., cooled, acidified with dilute hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water, dried, and concentrated to dryness. Crystals were obtained from acetone (813 mg., m.p. 237–238°; and 75 mg., m.p. 231–232°) in 87% yield. A sample for analysis was crystallized from acetone; m.p. 239–241°, $[\alpha]_D$ +50° ± 1°.

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.19; H, 8.85. Found: C, 69.07; H, 8.71.

3α,20α-Dihydroxy-11-oxo-5β-pregnan-21-oic Acid (VIIa) from IIIa.—Treatment of 100 mg. of methyl 3α,20α-dihydroxy-11-oxo-5β-pregnan-21-oate under the conditions described for preparation of VIIb from IIIb gave 82 mg. (m.p. 237–237.5°) of VIIa; it did not depress the melting point of VIIa that was obtained by hydrolysis of VIIIa. The infrared spectra of the two samples of VIIa were identical.

Treatment of acid VIIa with diazomethane gave an ester, m.p. 175–177°; it did not depress the melting point of IIIa that was obtained by the action of cupric acetate on II.

3α-Hydroxy-11-oxo-5β-etianic Acid (V) from VIIa and VIIb.—To separate solutions of 46 mg. each of 3α,20α-dihydroxy-11-oxo-5β-pregnan-21-oic acid and 3α,20β-dihydroxy-11-oxo-5β-pregnan-21-oic acid in 3 ml. of glacial acetic acid was added 40 mg. of potassium permanganate in 3 ml. of water. A precipitate of manganese dioxide formed rapidly. After 1 hr. at room temperature, excess oxidizing agent was decolorized with sodium bisulfite solution and the mixtures were extracted with ethyl acetate. The extracts were washed with water and concentrated to dryness. Crystallization from acetone gave 25 mg. (60%, m.p. 290–292°) of needles from each extract. The infrared spectra of the acids were identical with that of authentic 3α-hydroxy-11-oxo-5β-etianic acid[24] (V) prepared by periodate oxidation of 3α,21-dihydroxy-5β-pregnane-11,20-dione (I).

3α-Hydroxy-20β-acetoxy-11-oxo-5β-pregnan-21-oic Acid (IXb) from VIIIb.—To a solution of 921 mg. (2.05 mmoles) of 3α,20β-diacetoxy-11-oxo-5β-pregnan-21-oic acid in 15 ml. of methanol was added 50 ml. of water and 4.62 ml. of 1.72 *N* sodium hydroxide (8 mmoles). After 20 min. at room temperature, the solution was acidified with hydrochloric acid and extracted with ethyl acetate. The extract was washed with water, dried, and taken to dryness. Crystallization from acetone gave three crops (658 mg., 80%) of 20β-acetoxy acid (IXb) which melted in the range 254.5–256.5°. A purified sample melted at 259.5–261°; $[\alpha]_D$ +21° ± 1°.

Anal. Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.99; H, 8.35.

Methyl 3α-Hydroxy-20β-acetoxy-11-oxo-5β-pregnan-21-oate (Xb) from IXb.—Treatment of 100 mg. of 3α-hydroxy-20β-acetoxy-11-oxo-5β-pregnan-21-oic acid (IXb) with diazomethane gave 99 mg. (96%, m.p. 220–222°) of crystals from acetone. Recrystallization from acetone gave the analytical sample; m.p. 224.5–226°; $[\alpha]_D$ +15° ± 1°.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.28; H, 8.96.

20β-Acetoxy-3,11-dioxo-5β-pregnan-21-oic Acid (XIb) from IXb.—To 406 mg. (1.0 mmole) of 3α-hydroxy-20β-acetoxy-11-oxo-5β-pregnan-21-oic acid in 20 ml. of acetic acid was added 1.2 ml. of 1.0 *M* aqueous chromic acid. The solution was heated on a steam bath for 20 min., cooled, and added to 100 ml. of water. The suspension was extracted with ethyl acetate, the organic phase was washed with water, and taken to dryness. Crystallization from acetone gave 337 mg. (83.4%, m.p. 255.5–257°) of 20β-acetoxy-3,11-dioxo acid (XIb). The product gave a positive test for a carbonyl group with Brady's reagent and depressed the melting point of starting material by more than 100°. A sample of XIb, recrystallized from acetone and dried in air, lost 13.0% after further drying at 100° and 1–2 mm.; calcd. for loss of one mole of acetone, 12.6%; m.p. 256–257°; $[\alpha]_D$ +23° ± 1°.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.42; H, 8.27.

3α-Hydroxy-20α-acetoxy-11-oxo-5β-pregnan-21-oic Acid (IXa) from VIIIa.—To a solution of 1.34 g. (3.0 mmoles) of 3α, 20α diacetoxy-11-oxo-5β-pregnan-21-oic acid in 45 ml. of methanol was added 100 ml. of water and 3.64 ml. of 1.72 *N* sodium hydroxide (6.25 mmoles). After 1.5 hr., the solution was acidified with hydrochloric acid and extracted with ethyl acetate. The

(24) J. Von Euw, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, **27**, 1287 (1944).

extract was washed with water, dried, and taken to dryness. Crystallization from acetone gave 963 mg. (79%, m.p. 261.5–263°) of IXa. A sample, purified from the same solvent, melted at 263.5–265°; $[\alpha]_D$ +61° ± 1°.

Anal. Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.73; H, 8.37.

Methyl 3α-Hydroxy-20α-acetoxy-11-oxo-5β-pregnan-21-oate (Xa) from IXa.—Treatment of 150 mg. of 3α-hydroxy-20α-acetoxy-11-oxo-5β-pregnan-21-oic acid with diazomethane gave 133 mg. (86%, m.p. 178–180°) of methyl ester Xa, which, after recrystallization from acetone–ether, had m.p. 179.5–181.5°; $[\alpha]_D$ +61° ± 1°.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.35; H, 8.58.

20α-Acetoxy-3,11-dioxo-5β-pregnan-21-oic Acid (XIa) from Xa.—To a solution of 406 mg. (1.0 mmole) of 3α-hydroxy-20α-acetoxy-11-oxo-5β-pregnan-21-oic acid in 20 ml. of glacial acetic acid was added 1.2 ml. of 1.0 *M* aqueous chromic acid. After being heated on a steam bath for 20 min., the mixture was added to 125 ml. of water and extracted with methylene chloride. The extract was washed repeatedly with 5% sodium bicarbonate, then discarded. The combined alkaline washes were acidified with hydrochloric acid and extracted with methylene chloride. The methylene chloride extract was washed with water and evaporated. The residue gave 267 mg. (66%, m.p. 122–124°) of the dioxo acid (XIa) from aqueous methanol. It gave a positive test with Brady's reagent. A sample was recrystallized from 95% ethanol and dried for 1 hr. at 100° and 1–2 mm. It exhibited dual melting points at 120–122° and 198–200°; $[\alpha]_D$ +62° ± 1° (not corrected for solvent of crystallization).

Anal. Calcd. for $C_{23}H_{32}O_6 \cdot C_2H_5OH$: C, 66.64; H, 8.53. Found: C, 66.16, 67.04; H, 8.18, 8.71.

When a portion of the sample was dried for 2 hr. at 121° and 10–20 μ, it lost 9.60%; calcd. for loss of 1 mole of ethanol, 10.22%. This sample melted at 198–200° without previous softening.

Methyl 3,11,20-Trioxo-5β-pregnan-21-oate (VI) from IIIa.—To 113 mg. (0.25 mmole) of methyl 3α, 20α-dihydroxy-11-oxo-5β-pregnan-21-oate in 2.85 ml. of glacial acetic acid was added 0.16 ml. of aqueous 5 *M* chromic acid. The solution was heated on the steam bath for 20 min., cooled, diluted with water, and extracted with methylene chloride. The extract was washed with 5% sodium bicarbonate and then water, dried, and taken to dryness. Crystallization from methanol gave the trioxo ester (VI) in two crops (52 mg., m.p. 164.5–166.5°; and 5 mg., m.p. 162–164°). A sample, recrystallized from methanol, melted at 167.5–170.5°; $[\alpha]_D$ +119° ± 2°.

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.07; CH_3O, 8.29. Found: C, 70.04; H, 8.24; CH_3O, 8.53.

Methyl 3,11-20-Trioxo-5β-pregnan-21-oate (VI) from IIIb.—A solution of 378 mg. (1.0 mmole) of methyl 3α,20β-dihydroxy-11-oxo-5β-pregnan-21-oate in 9.5 ml. of glacial acetic acid and 0.50 ml. of aqueous 5 *M* chromic acid was heated on a steam bath for 90 min. and worked up as described in the previous paragraph. The product (170 mg., m.p. 163–165°; and 31 mg., m.p. 159–161°) did not depress the melting point of VI obtained from IIIa. Their infrared spectra in Nujol were identical and contained no band for an OH group.

Methyl 3α,20α-Diacetoxy-11-oxo-5β-pregnan-21-oate (IVa) from IIIa.—Treatment of methyl 3α,20α-dihydroxy-11-oxo-5β-pregnan-21-oate with acetic anhydride and pyridine gave the diacetoxy ester (IVa) as needles from ether; m.p. 194–194.5°; $[\alpha]_D$ +76° ±2°.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.50; H, 8.28. Found: C, 67.35; H, 8.19.

Methyl 3α,20β-Diacetoxy-11-oxo-5β-pregnan-21-oate (IVb) from IIIb.—Acetylation of methyl 3α,20β-dihydroxy-11-oxo-5β-pregnan-21-oate with acetic anhydride–pyridine and crystallization from ether gave the diacetoxy ester (IVb) as long needles; m.p. 204–205°; $[\alpha]_D$ +33° ± 1°.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.50; H, 8.28; CH_3CO, 18.59. Found: C, 67.97; H, 8.09;. CH_3CO, 18.59.

Methyl 3α,20α-Diacetoxy-11-oxo-5β-pregnan-21-oate (IVa) from VIIIa.—Treatment of 3α,20α-diacetoxy-11-oxo-5β-pregnan-21-oic acid (derived from glyoxal II through treatment with alkali and acetylation) with diazomethane gave a product with an infrared spectrum identical with that from IVa (derived from glyoxal II through treatment with copper acetate followed by acetylation).

Methyl 3α,11β,20α(and 20β)-Trihydroxy-5β-pregnan-21-oate (XIVa and XIVb) and Two Unknown Compounds from XII and Methanolic Cupric Acetate.—To a solution of 1.75 g. (5 mmoles) of 3α, 11β,21-trihydroxy-5β-pregnan-20-one in 125 ml. of methanol was added 500 mg. (2.5 mmoles) of cupric acetate in an equal volume of methanol (glyoxal XIII is formed *in situ* in less than an hour). After 113 hr. at room temperature, 9.7% of the original glyoxal was present. One gram of EDTA in 25 ml. of water was added and the methanol was evaporated *in vacuo*. The aqueous residue was extracted with methylene chloride and the organic phase, after being washed with water, was concentrated to dryness. After removal of the residual glyoxal in the manner described under the preparation of III from II, there was obtained 590 mg. (m.p. 170–180°) of needles from acetone. Recrystallization from acetone gave 528 mg. (27.8%, m.p. 185–188°) of **methyl 3α,11β,20β-trihydroxy-5β-pregnan-21-oate (XIVb).**

The residue from the acetone mother liquors was chromatographed on a 4.6-cm. diameter column packed to a height of 38 cm. with 200 g. of Celite plus 80 ml. of formamide. The solvent system was benzene (2000), formamide (100). Prior to collection of fraction no. 1, 450 ml. of effluent was discarded. Each fraction contained 15 ml. Appropriate fractions were combined after the weight of the residues had been determined and an elution diagram had been made.

Two compounds of unknown structure, X_4 and X_5, emerged before the trihydroxy esters.

Compound X_4. Fractions 8–20.—Crystallized from acetone–petroleum ether as needles (165 mg., m.p. 147–148.5°).

Compound X_5. Fractions 32–45.—Crystallized from acetone (48 mg., m.p. 233–235°).

Methyl 3α,11β,20β-Trihydroxy-5β-pregnan-21-oate (XIVb). Fractions 85–110.—Crystallization from acetone gave 142 mg. (m.p. 186–188°) of XIVb which, when added to that crystallized before chromatography, brought the yield to 670 mg. (35.5%). The analytical sample melted at 189–189.5°; $[\alpha]_D$ +27° ± 2°.

Anal. Calcd. for $C_{22}H_{36}O_5$: C, 69.43; H, 9.53. Found: C, 69.24, 69.19; H, 9.19, 9.02.

Methyl 3α,11β,20α-Trihydroxy-5β-pregnan-21-oate (XIVa). Fractions 121–165.—The residue from these fractions weighed 489 mg. (25.7%) and was homogeneous by chromatography. Initial attempts to crystallize the trihydroxy ester were unsuccessful. After approximately 4 months the compound, which was stored as a glass, crystallized spontaneously. In the meantime one-half of the residue had been removed for further treatment. Crystallization of the remaining half from ether gave prisms (170 mg., m.p. 163–164°; and 14 mg., 160.5–162°) which, after recrystallization from ether, had m.p. 161–163°; $[\alpha]_D$ +52° ± 2°.

Anal. Calcd. for $C_{22}H_{36}O_5$: C, 69.43; H, 9.53. Found: C, 69.29, 69.35; H, 9.28, 9.17.

Methyl 3α,20β-Diacetoxy-11β-hydroxy-5β-pregnan-20-oate (XVb) from XIVb.—To 380 mg. (1.0 mmole) of methyl 3α,11β, 20β-trihydroxy-5β-pregnan-21-oate was added 1.0 ml. each of pyridine and acetic anhydride. After 11 hr. at room temperature, the product was recovered and crystallized from ether [390 mg. (84%), m.p. 174–176°]. Several recrystallizations from ether–petroleum ether raised the m.p. to 176.5–177.5°; $[\alpha]_D$ +35° ± 1°.

Anal. Calcd. for $C_{26}H_{40}O_7$: C, 67.21; H, 8.68. Found: C, 66.81; H, 8.63.

Methyl 3α,20α-Diacetoxy-11β-hydroxy-5β-pregnan-21-oate (XVa) from XIVa.—Acetylation of 245 mg. of amorphous methyl 3α,11β,20α-triacetoxy-5β-pregnan-21-oate (from fractions 121–165) under the conditions used for acetylation of the corresponding 20β-epimer (XIVb) gave 252 mg. (84%, m.p. 142–145°) of XVa. A sample recrystallized from ether–petroleum ether melted at 143.5–144°; $[\alpha]_D$ +79° ± 2°.

Anal. Calcd. for $C_{26}H_{40}O_7$: C, 67.21; H, 8.68. Found: C, 66.83; H, 8.59.

Methyl 3α,20β-Diacetoxy-11-oxo-5β-pregnan-21-oate (IVb) from XVb.—To 92.8 mg. (0.20 mmole) of methyl 3α, 20β-diacetoxy-11β-hydroxy-5β-pregnan-21-oate in 4.76 ml. of glacial acetic acid was added 0.24 ml. of aqueous 1.0 *M* chromic acid. The solution was heated on a steam bath for 15 min. and the product was recovered and crystallized from ether (83 mg., m.p. 203–204°). This product did not depress the melting point of

IVb which had been prepared from IIIb. The infrared spectra of the two samples of IVb were identical.

Methyl 3α,20α-Diacetoxy-11-oxo-5β-pregnan-21-oate (IVa) from XVa.—Treatment of 92.8 mg. (0.20 mmole) of methyl 3α,20α-diacetoxy-11β-hydroxy-5β-pregnan-21-oate with chromic acid as described in the previous paragraph (except that the reaction period was 45 min.) gave 64 mg. (m.p. 194–195°) of product. Its infrared spectrum was identical with that of IVa which had been prepared from IIIa.

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The Muconomycins. I. Studies on the Structure of Muconomycin A, a New Biologically Active Compound[1]

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The chemistry of Muconomycin A has been investigated. This compound can be reduced catalytically to what appears to be a hexahydro derivative and may be acetylated to a diacetate. Basic hydrolysis yields three products. The structure of two of these is discussed.

During a search for new fungicides from natural sources, a crystalline compound was isolated from the cultures of the mold *Myrothecium verrucaria*.[2] This substance, which has been designated as Muconomycin A, proved to be quite interesting because of its high antifungal activity, its extreme toxicity (0.5–0.75 mg./kg.) to albino mice,[3] and the fact that it possesses allergic properties which result in skin irritation on contact. Recent studies by Guarino showed that some of the toxic properties are manifested by severe creatinurea in vitamin E deficient albino rats, a fact which is indicative of a possible interference with oxidative phosphorylation.[3]

This paper describes some studies on the chemistry of this biologically active compound.

Muconomycin A (I), when purified either by repeated recrystallization from acetone–water or by chromatography on alumina, yielded clear, colorless plates which turned yellow at 240° and slowly decomposed over a wide temperature range. An infrared spectrum of the antibiotic shows a main carbonyl peak at 1725 cm.$^{-1}$ with shoulders at 1710 and 1740 cm.$^{-1}$, a hydroxyl peak at 3557 cm.$^{-1}$, and double bond bands at 1637 and 1591 cm.$^{-1}$. The antibiotic is characterized by a single peak in the ultraviolet spectrum at 258.5 mμ (ε 21,200). A molecular weight determination by boiling point elevation in acetone showed the molecular weight to be 496 ± 15. A formula of $C_{27}H_{34}O_9$ (mol. wt., 502.5) was assigned based on this molecular weight and its elemental analysis. A methyl determination was found to be 8.34% methyl, which corresponds to at least three methyl groups for a compound with a molecular weight of 502.5. The molecule contains one active hydrogen and no methoxyl or ethoxyl groups. No derivative could be obtained with carbonyl reagents; thus it was concluded that the main carbonyl peak of I is that of an ester group, a conclusion which was supported by titration with base. The saponification equivalent of Muconomycin A was found to be 168 as compared with an expected value of 167 based on three ester groups.

When I was hydrogenated over Adam's catalyst, a compound was isolated which recrystallized from ether as colorless crystalline clusters, m.p. 145–146.5°. Elemental analysis suggested that the reduced material is most likely a hexahydro derivative, though the possibility that it is a tetrahydro derivative could not be eliminated on the basis of this analysis alone. In one experiment in which I was reduced with hydrogen at atmospheric pressure over palladium on charcoal, 2.80 moles of hydrogen were absorbed per mole of I.

An infrared spectrum of the reduced material shows a single carbonyl peak at 1742 cm.$^{-1}$ and no evidence of unsaturation. It was concluded from these observations that I contains at least one double bond in conjugation with an ester carbonyl.

Muconomycin A formed an acetate readily when treated with acetic anhydride and pyridine at steam bath temperatures. It is interesting to note that though I contains only one active hydrogen, it forms a diacetate.

When the antibiotic was subjected to hydrolysis with dilute base, three principal fragments were isolated from the reaction mixture. One of these was a dicarboxylic acid which was identified as one of the geometrical isomers of muconic acid by analysis, the infrared and ultraviolet spectra, and the fact that it consumed two moles of hydrogen on catalytic reduction with the formation of adipic acid. The preparation of the benzhydryl ester showed it to be the *cis–trans* isomer (II) (m.p. 142.5–143° as reported[4]), a conclusion which was confirmed by synthesis by conventional methods.[4,5]

In addition to *cis,trans*-muconic acid, two alcohols were isolated from the hydrolysis reaction of I. These were designated as alcohol A (III) and alcohol C (IV).

Alcohol A crystallized from ether in the form of flat needles, m.p. 156–157°. The infrared spectrum shows

(1) The research on elucidation of the structure of Muconomycin A, herein reported, was carried out for the most part at the laboratories of the Rohm and Haas Company, Bristol, Pa. Further characterization of the physical properties of degradation products and derivatives of Muconomycin A was carried out at the University of Rhode Island, supported in part by P.H.S. research grant E-4352 from the National Institutes of Health, Public Health Service.

(2) Patent application allowed, Smythe-Kraskin, assigned to the Rohm and Haas Co. The organism has been deposited with the American Type Culture Collection, Washington, D. C., and has been assigned the number ATCC 13667.

(3) A. Guarino, Chemistry Department, University of Rhode Island, unpublished data. This value is in confirmation of the LD_{50} previously determined under Rohn and Haas sponsorship.

(4) J. A. Elvidge, R. P. Linstead, P. Sims, and B. A. Orkin, *J. Chem. Soc.*, 2235 (1950).

(5) J. Pospishil and V. Ettel, *Chem. Prumysl*, **7**, 244 (1957).