[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Halogenated Corticoids. I. 9α-Halogen Derivatives of Cortisone and Hydrocortisone *

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The synthesis of 9α-halogen derivatives of hydrocortisone and cortisone from 11-epi-hydrocortisone is described. Although originally intended as intermediates in a synthesis of hydrocortisone from its 11α-epimer the main significance of these substances derives from their unusual biological properties. The most potent compound, 9α-fluorohydrocortisone acetate, is obtained in an over-all yield of 26%. The preparation of 9α-halogenated androstane derivatives by degradation of the correspondingly substituted hydrocortisones is described. Acid-catalyzed opening of the 9β,11β-epoxide IV in the presence of water or the lower alcohols furnishes the 9α-hydroxy and alkoxy derivatives, respectively.

The synthesis of a series of modified corticoids possessing a halogen atom in the 9α-position and their remarkable biological properties were first announced from this Laboratory in 1953.[1,2] It is the purpose of this paper to present in detail and to expand on the material summarized in these earlier communications.

The impetus for undertaking these studies arose from the fact that microbiological hydroxylation of Reichstein's Compound S by *Rhizopus nigricans*[3a] and by several Aspergillus species[3b] had rendered 11-epi-hydrocortisone (I) an attractive intermediate not only for the synthesis of cortisone[3] but, it was hoped, also for the preparation of hydrocortisone. To achieve the latter it was our intention to cause Walden inversion of the 11α-hydroxyl group by conversion into the tosylate or mesylate to be followed by a substitution reaction with sodium acetate or formate to yield the corresponding 11β-acetoxy or 11β-formoxy derivative. Contrary to expectations, the reaction of both the 11α-tosylate 21-acetate (Ib) and the corresponding 11α-mesylate Id with sodium acetate in boiling acetic acid furnished in 80% yield $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione 21-acetate (II) with no trace of the desired 11β-acetoxy derivative to be found even after careful chromatography of the mother liquors. When the less bulky formate ion was substituted for acetate essentially the same result was obtained except that a small amount of the 17α-formate IIa also was isolated. The unusual facility with which *cis*-elimination of the equatorial 11α-sulfonyloxy group and the 9α-hydrogen atom occurs in this case must undoubtedly be ascribed to the excessive steric requirements of both acetate and formate ion at C-11β[4] as well as to the great stability of the ring system possessing unsaturation at C-9(11).[5]

Having failed in our original objective of effecting Walden inversion of the 11α-hydroxyl group it appeared attractive to subject the now readily available 9(11)-dehydro derivative II to the hydroxybromination reaction first applied to such steroids by Hicks and Wallis[6] and later by Stavely.[7] These workers added the elements of hypobromous acid to the double bond of methyl 3α-acetoxy-$\Delta^{9(11)}$-cholenate and without isolating the bromohydrin[8] oxidized the crude mixture to the bromoketone and reduced the latter with zinc and acetic acid to methyl 3α-acetoxy-11-ketocholanate, which was isolated in poor yield by chromatography together with much starting material. The formation of the 11-keto derivative proved that the reaction of HOBr with a 9(11)-double bond proceeds with addition of a hydroxyl group in the 11- and consequently of a bromine atom in the 9-position. On mechanistic grounds it was reasonable to predict that the orientation of the hydroxyl group was 11β and that of the bromine atom 9α.[9] Indeed, application of this reaction to $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione 21-acetate (II) using dioxane as the solvent and dilute perchloric acid to liberate bromonium ion furnished in a completely stereospecific reaction (80–90% yield)[10] the de-

* This paper was to have been published simultaneously with the paper "The Reaction of Epoxides with Anhydrous Hydrogen Fluoride in the Presence of Organic Bases. The Preparation of 9α-Fluoro-4-pregnene-11β,11α,21-triol-3,20-dione 21-Acetate and its 1-Dehydro Analog," by R. F. Hirschmann, R. Miller, J. Wood and R. E. Jones, THIS JOURNAL, **78**, 4956 (1956), which reports related work, but was not, as a result of regrettable oversights in the Editorial Offices.—The Editors.

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953).

(2) J. Fried and E. F. Sabo, *ibid.*, **76**, 1455 (1954).

(3) (a) D. H. Peterson, S. H. Epstein, P. D. Meister, B. J. Magerlein, H. C. Murray, H. M. Leigh, A. Weintraub and L. M. Reineke, *ibid.*, **75**, 419 (1953). (b) *Aspergillus niger:* J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *ibid.*, **74**, 3962 (1952). *Aspergillus nidulans:* J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman in "Recent Progress in Hormone Research," XI, Academic Press, Inc., New York, N. Y., 1955, p. 149.

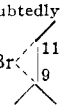
(4) It is of interest in this connection that acetolytic scission of methyl 3α-acetoxy-11α,12α-oxidocholanate (T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, **162**, 495 (1946)) furnishes in about 25% yield methyl 3α,11β-diacetoxy-12α-hydroxycholanate (substitution) in addition to methyl 3α-acetoxy-12α-hydroxy-$\Delta^{9(11)}$-cholenate (elimination), which latter constitutes the major product (50%) of this reaction. The fact that substitution at C_{11} occurs to the extent of 25% in this case while none is observed in our own may in part be ascribed to the presence of the epoxide ring, which so alters the conformation of ring C as to reduce steric hindrance at $C_{11}\beta$.

(5) Since the appearance of our communication (ref. 2) others have reported the elimination of the 11α-tosyloxy group by means of basic reagents; *cf.* S. Bernstein, R. H. Lenhard and J. H. Williams, *J. Org. Chem.*, **19**, 41 (1954); G. Rosenkranz, O. Mancera and F. Sondheimer, THIS JOURNAL, **76**, 2227 (1954).

(6) E. M. Hicks, Jr., and E. S. Wallis, *J. Biol. Chem.*, **162**, 641 (1946).

(7) H. E. Stavely, *Federation Proc.*, **9**, (Part I) 233 (1950).

(8) In the Introductory section of their paper, Hicks and Wallis state that a crystalline bromohydrin was formed. However, in the Experimental part the only product isolated from this reaction and characterized as crystalline was unchanged starting material.

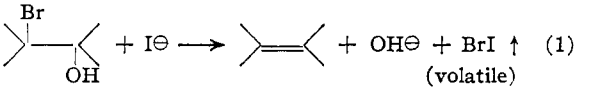
(9) Attack of bromonium ion on the double bond would undoubtedly occur from the less hindered α-side resulting in the cation 

which upon attack of water from the opposing β-face would form the conjugate acid of the bromohydrin III.

(10) When the hydroxybromination reaction was conducted in a medium containing dilute sulfuric acid (*cf.* ref. 6) the yield was as low

sired 9α-bromohydrocortisone acetate (III).[11] The structure of III rests mainly on the fact that in line with the original objective of this work reduction with zinc and alcohol furnished hydrocortisone acetate in about 15% yield. Other supporting evidences are the resistance of the hydroxyl group to acetylation and the oxidation of III to 9α-bromocortisone acetate (VII), in which the bromine atom could be removed either reductively with zinc and acetic acid to form cortisone acetate or by elimination with collidine to form 8(9)-dehydrocortisone acetate (XIV).

The yields in the reductive debromination of 9α-bromohydrocortisone acetate and also of 9α-iodohydrocortisone acetate (*vide infra*) were discouragingly low in spite of the variety of reducing agents and conditions employed. In our hands the only reagent to produce hydrocortisone was the above-mentioned zinc in dilute alcohol. Catalytic reduction in a basic medium or using a basic carrier, conditions which have been shown to transform 3,4-bromohydrins to the corresponding halogen-free alcohols,[12] as well as Raney nickel, which was used successfully in the dehalogenation of 12α-iodo-5α,22a-spirostane-3β,11β-diol,[13] effected mainly elimination to the 9(11)-olefin and epoxide formation accompanied in most cases by reduction of the 4,5-double bond.[14] Chromous chloride and zinc in acetic acid[15] produced the pure 9(11)-dehydro compound in about 80% yield. The reaction of the bromohydrin III with sodium iodide in acetone at reflux temperature afforded a mixture of the 9(11)-ene II and the 9β,11β-epoxide IV. The formation of the former parallels the well-known elimination reaction of vicinal dihalides. The less orthodox formation of the latter in the same reaction may be ascribed to the presence of hydroxyl ion formed in the elimination reaction according to equation 1



When the reaction with sodium iodide was conducted at room temperature for 7 days it took an entirely different course. There was isolated instead a trienone different from the trienone XVII,[10] to which structure XXI is assigned because of its ultraviolet absorption spectrum (bands at 244, 285–300 and 385 mμ) and of its unusually high positive rotation ([α]D +550°).[16] The formation of the trienone XXI may be assumed to proceed by elimination of HBr to form the 8(9)-ene, followed by acid-catalyzed 1,4-elimination of water to form the 7,9(11)-diene XVIIa. The latter would be expected to, and actually does isomerize under the influence of acid to the conjugated trienone XXI.

The preparation of 9α-iodohydrocortisone acetate required for the above-described dehalogenation to hydrocortisone acetate necessitated as an intermediate the 9β,11β-epoxide IV. The latter was prepared readily by treatment of the bromohydrin with excess potassium acetate in absolute alcohol at reflux temperature. Potassium bicarbonate in methanol at room temperature produced the epoxide 21-ol IVa. The epimeric 9α,11α-epoxide IVb was prepared in the conventional manner by treatment of the 9(11)-ene II with perbenzoic acid.

The interesting and unusual fission reactions of the readily available 9α,11α-epoxides, particularly those involving intramolecular interaction with functional groups at C-3, have been studied in great detail by Heyman and Fieser.[17] In contrast, the chemistry of the heretofore more difficultly accessible 9β,11β-epoxides has received scant attention. One of the most characteristic properties of the β-oxides, it has now been found, is the ease with which they undergo fission with hydrogen halides to form the expected diaxial halohydrins. Thus treatment of IV with hydrogen bromide at 0° for 10 minutes furnished the original 9α-bromohydrin III. Hydriodic acid in chloroform at −18° for 20 minutes produced 9α-iodohydrocortisone acetate (Vb). Even under these relatively mild conditions a small amount of the 9(11)-dehydro derivative was formed, which became the major product when the temperature was raised to −5° and the reaction time was extended to three hours.

While up to this point our main purpose for preparing the above halohydrins was to provide intermediates for the synthesis of hydrocortisone the interest in this group of substances increased considerably when it was discovered that both 9α-bromo- and 9α-iodohydrocortisone possessed corticoid activity. The fact that the bromo compound was the more active of the two raised the question of what influence substitution by the lighter halogens, chlorine and fluorine would have upon biological activity. Moreover, the introduction of sub-

as 45%. It was noted that the remaining 55% could not be accounted for in the chloroform-soluble fraction of the reaction mixture, and it was therefore assumed that that portion had been transformed into the water-soluble 11β-sulfate ester IIIb as a result of substitution by sulfate ion rather than water in the 11β-position. On that basis the use of an acid whose anion possesses low nucleophilicity such as perchloric acid should lead exclusively to III, and this was indeed found to be the case. From the aqueous phase containing the sulfate ester IIIb upon standing in the refrigerator for a period of 40 days a crystalline chloroform-soluble substance separated, which is formulated as $\Delta^{4,7,9(11)}$-pregnatriene-17α,21-diol-3,20-dione (XVII) mainly because of its characteristic ultraviolet absorption spectrum (λ_{max}^{alc} 243 mμ, shoulders at 237 and 250 mμ). The formation of the latter from IIIb can be rationalized as a sequence of eliminations first of HBr followed by sulfuric acid (1,4).

(11) The hydroxybromination reaction has been employed independently by L. B. Barkley, M. W. Farrar, W. S. Knowles and H. Raffelson (THIS JOURNAL, **76**, 5017 (1954)) for the introduction of oxygen into C_{11} in the final stages of the Woodward total synthesis of cortisone.

(12) L. F. Fieser and R. Ettore, *ibid.*, **75**, 1700 (1953); L. F. Fieser and X. A. Dominguez, *ibid.*, **75**, 1704 (1953).

(13) J. Schmidlin and A. Wettstein, *Helv. Chim. Acta*, **36**, 1241 (1953).

(14) Among the reagents causing largely elimination were Raney nickel, with or without hydrogen at room temperature, and at 80°, palladium-on-calcium carbonate, palladium-on-barium sulfate in pyridine and zinc–copper in alcohol. Reagents without effect on 9α-bromohydrocortisone acetate were zinc dust in *t*-butyl alcohol, with or without water, cadmium or iron powder in aqueous alcohol, sodium amalgam in 90% acetic acid and lead-poisoned palladium-on-calcium carbonate and hydrogen.

(15) Zinc in acetic acid has been shown to produce olefins from both *cis*- and *trans*-bromohydrin ; *cf.* ref. 12 and a recent paper by D. R. James, R. W. Rees and C. W. Shoppee, *J. Chem. Soc.*, 1370 (1955).

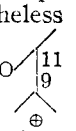
(16) *Cf.* R. Yashin, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 4654 (1951).

(17) H. Heyman and L. F. Fieser, *ibid.*, **73**, 5252 (1951); **74**, 5938 (1952).

stituents other than halogen became worthy of study.

The opening of the epoxide ring with hydrogen chloride in chloroform at 0° proceeded smoothly in 80% yield. However, the analogous reaction with hydrogen fluoride required more intimate study. A survey of the literature revealed that the reaction of epoxides with hydrogen fluoride had received little attention, the most comprehensive account being a recent paper by Knunyants, *et al.*[18] These workers prepared fluoroethanol and other aliphatic fluorohydrins in about 40% yield by treating the requisite epoxides with anhydrous hydrogen fluoride in ether at 100° for six hours. Significantly, as we shall see later, no fluorohydrins were found in the absence of ether. Instead, polymerization of the oxide to a mixture of polyethylene glycols took place. In the steroid field, Hicks, Berg and Wallis,[19] with the objective of isomerizing 9α,11α-epoxides to 11-ketones, studied the reaction of several epoxides with hydrogen fluoride at −40° and obtained rearrangement products of unknown structure. No fluorohydrins were encountered under the conditions used. Unaware, and therefore protected from the discouraging aspects of this latter publication, we attempted the reaction of the epoxide IV with hydrogen fluoride using chloroform as a solvent. When hydrogen fluoride was passed into a solution of the epoxide in redistilled chloroform at 0° there resulted at first a single colorless phase which on continued addition of the acid separated into two layers with the color of the upper layer deepening to an intense red. When the addition of hydrogen fluoride was interrupted before the intense red color had developed fully, only unchanged epoxide could be recovered even after a period of 4 or more hours at 0°. However, when the intensity of the color was permitted to develop to a level corresponding to a 5–10%[20] solution of hydrogen fluoride there was isolated after 1–2 hours reaction time in about 50% yield the desired 9α-fluorohydrocortisone acetate (VI). In addition, there was formed in the reaction a small amount of cortisone acetate and a fluorine-free isomer (XVIII) of the epoxide, the amount of which depended upon the exact conditions employed. This latter compound became the major reaction product when hydrogen fluoride was used as the solvent at −70°.[21] This observation calls to mind the failure of the Russian workers to produce fluoroethanol from ethylene oxide under essentially identical conditions.

An inquiry into the nature of the fluorine-free by-product[22] became of interest primarily as part of a rational approach to avoid its formation and thereby to increase the yield of fluorohydrocortisone. The infrared spectrum of XVIII showed bands at 5.75 and 5.80 μ indicating that the acetylated side chain had not been altered. The preparation of a crystalline propionate (XVIIIa) and mesylate (XVIIIb) under mild conditions demonstrated the presence of a readily acylable hydroxyl group.[23] In a titration with perphthalic acid one mole of the oxidant was consumed indicative of an isolated double bond. Fractionation of the resulting mixture of products furnished two isomeric epoxides (A and B) melting at 208 and 182°, respectively (XXII), in which the α,β-unsaturated ketone system was still present. Both epoxides could be converted into diacetates (XXIIa) with pyridine and acetic anhydride at room temperature. Treatment of the mesylate XVIIIb with sodium acetate in acetic acid or with pyridine produced the homoannular diene XXIV, the absorption spectrum of which showed a shoulder at 275 mμ (3,700) in the region characteristic of such dienes. The absorption maximum associated with this chromophoric system was more clearly defined in XXIVa, which was obtained when XVIII was first hydrogenated and the mesylate XVIIIc of the resulting 4,5-dihydro derivative was demesylated by heating with pyridine. The preparation of XXIVa has also been reported by the Merck workers.[22]

The above data, although not sufficient to support a definite structural assignment, nevertheless strongly suggested that the carbonium ion  was an intermediate in the formation of the fluorine-free by-product from the epoxide IV. This view was supported by the findings that XVIII was likewise formed, albeit in small yield, when the epoxide was treated with glacial acetic acid containing a small amount of perchloric acid or when 9α-bromohydrocortisone acetate (III) was treated with silver nitrate in aqueous dioxane. Normal fission of an epoxide to form the halohydrin requires a concentration of halide ion sufficiently large to sustain SN2 attack of the latter on the protonated epoxide. It is well known that hydrogen fluoride, although one of the strongest acids known, dissociates only to a very small extent into hydrogen and fluoride ion.[24] It was reasonable to assume then that the formation of the isomer XVIII was the result of an insufficient supply of fluoride ion and that its formation could be suppressed if an increase in the concentration of the latter could be achieved. Compounds containing oxygen functions are strongly basic toward hydrogen fluoride and promote

(18) I. L. Knunyants, O. V. Kildisheva and I. P. Petrov, *J. Gen. Chem.* (*U.S.S.R.*), **19**, 95 (1949); *C. A.*, **43**, 6163 (1949).

(19) E. M. Hicks, Jr., C. J. Berg and E. S. Wallis, *J. Biol. Chem.*, **162**, 645 (1946).

(20) It is noteworthy that once the appropriate concentration of hydrogen fluoride has been attained the concentration of the epoxide can be varied between 0.5 and 5%.

(21) The authors are indebted to Dr. H. Cords of these laboratories for permission to report these data.

(22) A substance having essentially the same properties as our fluorine-free by-product has recently been described by R. P. Graber, C. S. Snoddy, Jr., and N. L. Wendler, *Chemistry and Industry*, 57 (1956), who proposed structure XVIII (11β-OH) for that product. Undoubtedly, the two substances are identical and our previous findings *cf.* ref. 2) as well as new findings reported in this paper are in agreement with the main features of the structure advanced by the Merck workers. We do prefer, at present, to leave the orientation of the 11-hydroxyl group unassigned because we feel that a structure possessing an 11α-hydroxyl group and a 9α-hydrogen atom can equally well account for the facile esterification of the 11-hydroxyl group in XVIII and in the epimeric epoxides XXII as the 11β,9β-structure implied by Graber, *et al.* Formula XVIII is therefore used in this paper as the most satisfactory representation to date.

(23) Acetylation furnished an amorphous diacetate which could be utilized advantageously in the separation of mixtures of fluorohydrocortisone acetate and the very difficultly soluble by-product XVIII.

the formation of fluoride ions.[24] The addition of such low molecular weight oxygen bases as alcohol, ethyl ether, acetone, etc., should therefore lead to increased fluoride ion concentrations, and thus prevent or minimize the formation of XVIII with a concomitant rise in the yield of the fluorohydrin.[25] This was indeed found to be the case, and as a result chloroform containing 5% ethanol is now being used as a solvent in our hydrofluorination reactions. In this manner yields of about 60–65%[26] can be obtained routinely from IV.

The hydrolysis of 9α-fluorohydrocortisone acetate to the free ketol VIa was accomplished in 90% yield by means of potassium carbonate in methanol. The free ketol was in turn converted into the lipid-soluble heptanoate VIb and into the water-soluble sodium hemisuccinate VIc. The above alkaline conditions are obviously not applicable for the deacetylation of the chloro and bromo derivatives, since the rate of epoxide formation in such a medium equals or surpasses that of saponification, particularly in the case of the bromo compound. For this reason we have devised a hydrolysis procedure employing 0.25 *N* perchloric acid in methanol at room temperature. The free ketols have been found to be remarkably stable in that medium, 9α-fluorohydrocortisone remaining unchanged for as long as 64 hours. An equal concentration of hydrogen chloride in methanol or chloroform applied for the same length of time is known to cause cortisone to undergo the Mattox rearrangement,[27] and does in fact catalyze the formation of the dimethyl acetals XV and XVI from 9α-chloro- and 9α-fluorohydrocortisone acetate, respectively.

The free ketols IVa and VIa have been degraded to the corresponding 9(11)-substituted Δ^4-androstene-3,17-diones X and XII either by chromic acid or when preservation of the 11β-hydroxyl group was essential by means of sodium bismuthate. Of particular utility is the 9β,11β-epoxide X which has served as an intermediate in the preparation of all the 9α-halo-11β-hydroxyandrostenediones.[28]

Cleavage of the epoxide IV by agents other than the hydrogen halides has been successfully utilized for the introduction of the 9α-hydroxy[29] as well as the corresponding lower alkoxy groups. The former was accomplished by fission with sulfuric acid in aqueous dioxane solution, the latter by employing a medium consisting of the requisite alcohol and a trace of perchloric acid. The resulting hydrocortisone derivatives XIX and XIXa have been oxidized to the corresponding 11-ketones XX and XXa.

Certain regularities in the positions of the ultraviolet maxima and some of the molecular rotation relationships of 9,11-substituted Δ^4-pregnene-17α,-21-diol-3,20-dione 21-acetates are of interest and the pertinent data are presented in Table I. It will be noted that in the case of both the 9α-substituted cortisones and hydrocortisones the position of the maximum associated with the chromophore in ring A is a function of the electronegativity of the 9α-substituent, the largest hypsochromic shifts being caused by the most negative element fluorine. In contrast, steric factors appear to be responsible for the difference of 5 mμ in the positions of the maxima for the 9,11α- and β-epoxides. As to the molecular rotation relationships the 9α-halohydrocortisones show a steady rise in rotation with increasing atomic weight of the halogen. A different pattern is observed in the case of the corresponding halocortisones where an unexpectedly large gap separates the value for the fluoro derivative from those for the chloro and bromo derivatives.

TABLE I

MOLECULAR ROTATIONS AND ULTRAVIOLET MAXIMA FOR 9,11-SUBSTITUTED Δ^4-PREGNENE-17α,21-DIOL-3,10-DIONE 21-ACETATES

Substituent 9α	11	[M]D CHCl3	$\lambda^{alc.}_{max}$, mμ
H	β-OH	+630°	242
I	β-OH	+790	243
Br	β-OH	+661	243
Cl	β-OH	+631	241
F	β-OH	+604	238
OH	β-OH	+643	242
OCH_3	β-OH	+700	243
OC_2H_5	β-OH	+610	..
H	=O	+820	238
Br	=O	+1160	237
Cl	=O	+1140	236
F	=O	+673	234
OH	=O	+885	238
OCH_3	=O	+870	238
$\Delta^{8(9)}$	=O	+1700	235[a]
$\Delta^{9(11)}$		+413	238
9β,11β-oxido		+ 84	243
9α,11α-oxido		+398	238

[a] This curve which slopes gently, especially toward the longer wave length end of the spectrum, represents the summation of the curves for the Δ^4-3-keto and $\Delta^{8(9)}$-11-keto systems. Since the latter would be expected to possess a maximum in the vicinity of 255 mμ (9,000) (*cf.* ref. 17) the maximum for the Δ^4-3-ketone system should be somewhat below 235 mμ in order to accommodate the composite peak at 235 mμ. The vicinal influence of the $\Delta^{8(9)}$-11-keto system is therefore comparable to that of 9α-fluoro-11-ketones.

Acknowledgments.—The authors are indebted to Mr. J. F. Alicino and his collaborators for the microanalyses, to Dr. N. H. Coy, Mr. Charles Fairchild, and Mr. Carl Sabo for the ultraviolet and infrared spectra, and to Mr. P. Grabowich and Mr. E. A. Paredes for assisting in some of the experiments. Special thanks also are due to Dr. A. Borman and Dr. F. M. Singer whose excellent bioassays have served to steer this investigation into productive channels.

(24) For a review on the properties of hydrogen fluoride, *cf.* J. H. Simons in "Fluorine Chemistry," Academic Press, Inc., New York, N. Y., 1950, p. 225.

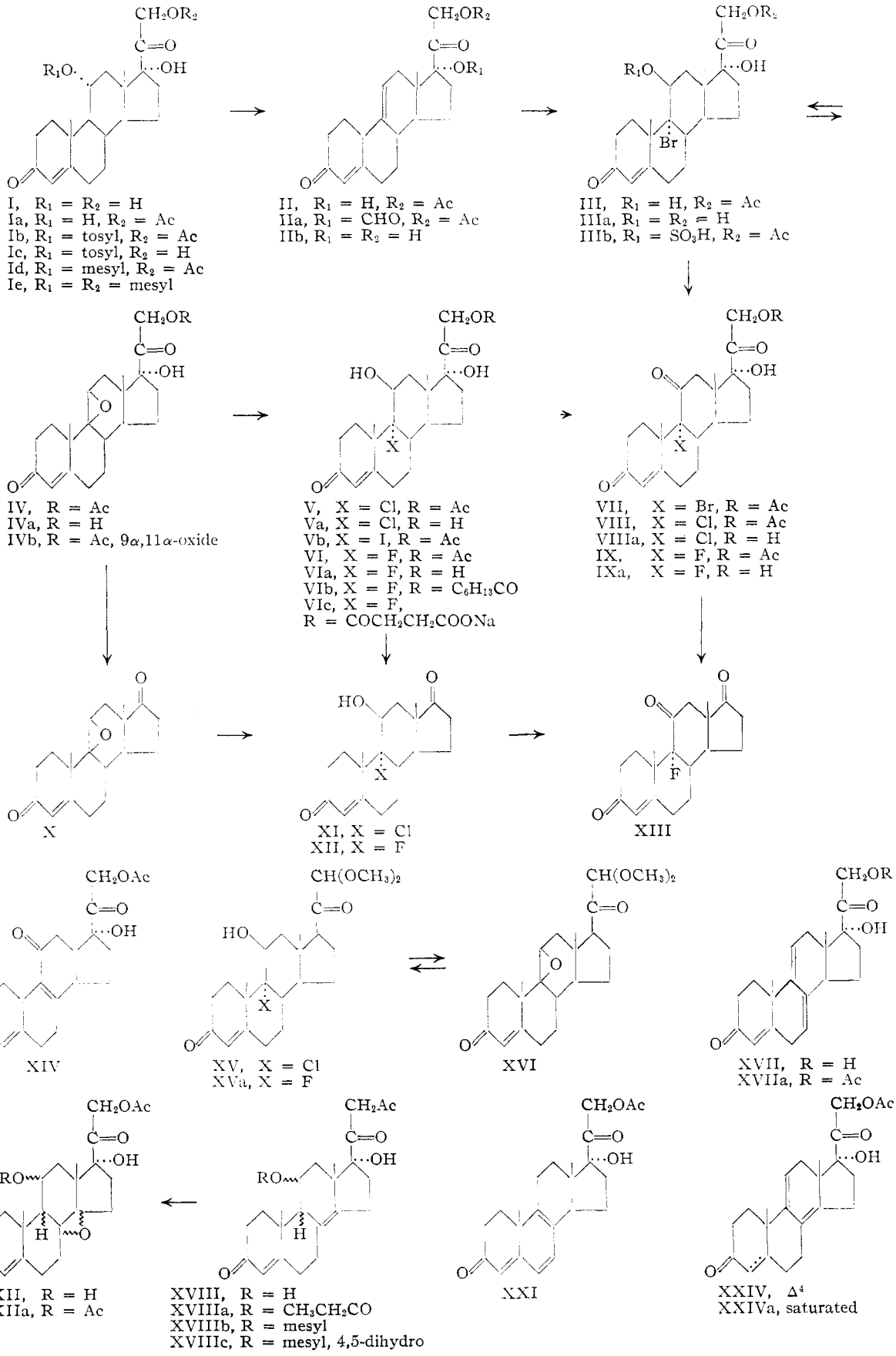
(25) It was observed early during this work that chloroform solutions of the epoxide IV would dissolve considerably more hydrogen fluoride than the solvent alone. This was interpreted as being due to protonation of the 6 oxygen functions of IV resulting in the release of an equivalent number of fluoride ions. The success of our early experiments, which were conducted in alcohol-free chloroform, may be ascribed to this phenomenon.

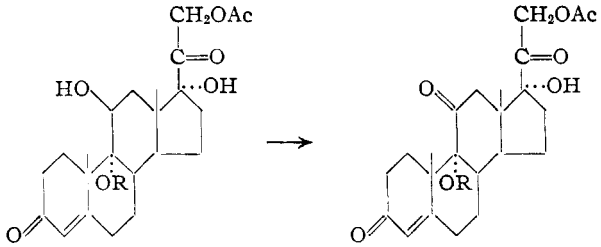
(26) We are indebted to Dr. Florey of these laboratories for carrying out this series of experiments.

(27) V. R. Mattox, THIS JOURNAL, **74**, 4344 (1952).

(28) After completion of this work R. H. Lenhart and S. Bernstein (*ibid.*, **77**, 6665 (1955)) have described an alternate procedure for the preparation of this series of derivatives.

(29) After completion of this work the preparation of 9α-hydroxyhydrocortisone has been reported by (a) Littell and Bernstein, *ibid.*, **78**, 984 (1956) (21-acetate), and by (b) R. P. Graber, *et al.*, ref. 22 (21-alcohol).


I, R1 = R2 = H
Ia, R1 = H, R2 = Ac
Ib, R1 = tosyl, R2 = Ac
Ic, R1 = tosyl, R2 = H
Id, R1 = mesyl, R2 = Ac
Ie, R1 = R2 = mesyl
II, R1 = H, R2 = Ac
IIa, R1 = CHO, R2 = Ac
IIb, R1 = R2 = H
III, R1 = H, R2 = Ac
IIIa, R1 = R2 = H
IIIb, R1 = SO3H, R2 = Ac
IV, R = Ac
IVa, R = H
IVb, R = Ac, 9α,11α-oxide
V, X = Cl, R = Ac
Va, X = Cl, R = H
Vb, X = I, R = Ac
VI, X = F, R = Ac
VIa, X = F, R = H
VIb, X = F, R = C6H13CO
VIc, X = F,
R = COCH2CH2COONa
VII, X = Br, R = Ac
VIII, X = Cl, R = Ac
VIIIa, X = Cl, R = H
IX, X = F, R = Ac
IXa, X = F, R = H
X
XI, X = Cl
XII, X = F
XIII
XIV
XV, X = Cl
XVa, X = F
XVI
XVII, R = H
XVIIa, R = Ac
XXII, R = H
XXIIa, R = Ac
XVIII, R = H
XVIIIa, R = CH3CH2CO
XVIIIb, R = mesyl
XVIIIc, R = mesyl, 4,5-dihydro
XXI
XXIV, Δ4
XXIVa, saturated



XIX, R = H
XIXa, R = CH_3
XIXb, R = C_2H_5

XX, R = H
XXa, R = CH_3

Experimental[30]

11-Epihydrocortisone 21-Acetate (Ia).—To a solution of 11-epihydrocortisone (I) (360 g., m.p. 217°, $[\alpha]^{23}D$ +117°) in anhydrous pyridine (1.8 l.) was added with stirring at 0–4° acetic anhydride (107 ml., 1.05 mole equivalents). After 15 hours at 0° the solution was concentrated *in vacuo* (bath temperature 30°) to a thick sirup. The sirup was dissolved in chloroform (2.5 l.) and extracted with water and with 1 *N* sulfuric acid until free of pyridine. After final washes with dilute sodium bicarbonate and water the chloroform solution was dried and concentrated to a volume of 1.4 l. Seeding with epihydrocortisone and cooling to 0° for 24 hours produced 10 g. of unreacted starting material, which was filtered off. The chloroform filtrate containing the monoacetate was used directly in the next step.

11-Epihydrocortisone 11α-Tosylate 21-Acetate (Ib).—To a chloroform solution of 11-epihydrocortisone 21-acetate (Ia) derived from 50 g. of 11-epihydrocortisone (200 ml.) was added anhydrous pyridine (250 ml.) and the mixture cooled to 0°. Pure *p*-toluenesulfonyl chloride (70 g.) was then added with stirring and the mixture allowed to remain at 0° for four hours and at room temperature for an additional 15 hours. Ice (10 g.) was added, the mixture poured into water and the tosylate Ib isolated with chloroform. The crystalline residue (88.4 g.) remaining after evaporation of the chloroform was triturated with absolute alcohol (200 ml.) and the mixture filtered after short cooling in the refrigerator. The first crop of crystals amounted to 58.6 g. and melted at 165–166° dec. A second crop (5.6 g.) was isolated from the mother liquors (yield 83.5% from I). An analytical sample was recrystallized from ethyl acetate and dried at 56° over P_2O_5; m.p. 165.5–166°, $[\alpha]^{23}D$ +109° (*c* 1.0).

Anal. Calcd. for $C_{30}H_{38}O_8S$ (558.60): C, 64.51; H, 6.81; S, 5.73. Found: C, 64.55; H, 6.84; S, 5.77.

11-Epihydrocortisone 11α-Tosylate (Ic).—To a solution of 11-epihydrocortisone 11α-tosylate 21-acetate (Ib, 115 mg.) in 1 ml. of chloroform and 5.5 ml. of methanol was added at 40° a solution of potassium bicarbonate (86 mg.) in water (1.6 ml.). The yellow solution was allowed to remain at room temperature for 18 hours and after the addition of water (2 ml.) was concentrated *in vacuo*. The hydrolysis product Ic was isolated with chloroform and the resulting crystalline material (110 mg.) recrystallized from acetone; m.p. 130.5–131° dec., $[\alpha]^{23}D$ +71° (*c* 1.0).

Anal. Calcd. for $C_{28}H_{36}O_7S$ (516.57): C, 65.12; H, 6.97. Found: C, 65.29; H, 7.22.

(30) The melting points were taken in open capillaries and are corrected for stem exposure. The rotation measurements were carried out in 1-dm. semi-micro tubes, with chloroform as the solvent unless indicated otherwise. The ultraviolet spectra were measured in a Cary self-recording spectrophotometer, model 11. The infrared spectra were determined to the greater part in a Perkin–Elmer double-beam self-recording instrument, model 21. Some of the earlier work was done with a Perkin–Elmer single-beam instrument, model 12B. The alumina used for chromatography (Harshaw) was washed with dilute sulfuric acid and water to *p*H 4.5 and reactivated by heating at 150° for 48 hours. Isolation of products after completion of the reaction was achieved by extraction into redistilled chloroform, washing of the chloroform solution with water and removal of acidic and basic reagents with dilute sodium bicarbonate and 1 *N* sulfuric acid, respectively. These washes were always followed by an additional water wash. Chloroform extracts were dried over sodium sulfate, filtered and the solvents removed *in vacuo*. This procedure was followed throughout unless indicated otherwise.

Reacetylation of Ic with pyridine and acetic anhydride furnished Ib in quantitative yield.

11-Epihydrocortisone 11α-Mesylate 21-Acetate (Id).—To a solution of 11-epihydrocortisone 21-acetate (Ia) (equivalent to 351 g. of I) in chloroform (1.4 l.) and anhydrous pyridine (350 ml.) was added at 0° with stirring over a 20-minute period methanesulfonyl chloride (111 ml., 1.5 mole equivalents) in redistilled chloroform (250 ml.). After 16 hours in the refrigerator, ice (50 g.) was added and the mesylate Id recovered from the reaction mixture with chloroform. The chloroform solution was concentrated to a sirup at a bath temperature not exceeding 25° and absolute alcohol (1.3 l.) was added with swirling. Crystallization ensued rapidly which was complete after several hours in the refrigerator. The first crop amounted to 185 g. and concentration of the mother liquors furnished an additional 200 g. (83% of theory from I), m.p. 159–160° (dec.), $[\alpha]^{23}D$ +119° (*c* 1.09).

Anal. Calcd. for $C_{24}H_{34}O_8S$ (482.57): C, 59.73; H, 7.10; S, 6.64. Found: C, 59.47; H, 7.13; S, 6.36.

11-Epihydrocortisone 11α,21-Dimesylate (Ie).—To a solution of 11-epihydrocortisone (I) (10 g.) in anhydrous pyridine (110 ml.) was added with swirling a mixture of methanesulfonyl chloride (6.6 ml.) and redistilled chloroform (10 ml.). After 15 hours at 0°, 1 g. of ice was added and the dimesylate Ie recovered with chloroform. The crystalline residue (12.4 g.) after removal of the solvent was recrystallized from 95% alcohol and yielded 10.4 g. melting at 154° dec. Analytically pure material was obtained after two more crystallizations from the same solvent; m.p. 162° dec., $[\alpha]^{23}D$ +97° (*c* 0.98 in dioxane).

Anal. Calcd. for $C_{23}H_{34}O_9S_2$ (518.62): C, 53.26; H, 6.60; S, 12.36. Found: C, 53.42; H, 6.29; S, 11.00.

$\Delta^{4,9(11)}$-Pregnadiene-17α,21-diol-3,20-dione 21-Acetate (II).—To a solution of anhydrous sodium acetate (385 g.) in glacial acetic acid (3.5 l.) was added at a temperature of 110° with agitation the mesylate Id (285 g.). The resulting mixture was heated to reflux (7 minutes) and maintained at that temperature for 30 minutes. The reaction mixture was then immersed in an ice-bath and allowed to cool with agitation. Water (5.4 l.) was added and the fine crystals[31] of the 9(11)-dehydro compound II filtered off and washed first with dilute acetic acid and finally with water. The material after drying *in vacuo* at 80° amounted to 243 g. (79% of theory) and had m.p. 233–235°. An analytical sample was prepared by recrystallization from ethyl acetate. Crystallization from acetone gave an adduct with one mole of acetone; m.p. 236–237°, $[\alpha]^{26}D$ +120° (*c* 1.0), λ_{max}^{alc} 238 mμ (16,500); reported[32] m.p. 231.5–234.5, $[\alpha]^{22}D$ +124° (*c* 1.04).

Anal. Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.50; H, 7.77. Found: C, 71.57; H, 7.85.

$\Delta^{4,9(11)}$-Pregnadiene-17α,21-diol-3,20-dione 21-acetate has also been prepared by applying the above procedure to the 11α-tosylate Ib (72% yield) and to the 11α,21-dimesylate Ie (32%).

The free alcohol IIb was prepared from the acetate with potassium carbonate in methanol. After recrystallization from 95% alcohol it melted at 247–250° dec., $[\alpha]^{23}D$ +103° (*c* 0.32 in dioxane), λ_{max}^{alc} 239 mμ (17,600); λ_{max}^{Nujol} 2.89, 5.88, 6.04 and 6.21 μ; reported[33] m.p. 259–261° dec., $[\alpha]^{23}D$ +88° (*c* 0.6 in pyridine).

Anal. Calcd. for $C_{21}H_{28}O_4$ (344.44): C, 73.22; H, 8.19. Found: C, 72.95; H, 7.98.

Treatment of 11-Epihydrocortisone 11α-Tosylate 21-Acetate (Ib) with Sodium Formate in Formic Acid.—A solution of the 11α-tosylate Ib (2.0 g.) and sodium formate (3.50 g.) in 98% formic acid (30 ml.) was heated at reflux for one hour. After removal of the bulk of the formic acid *in vacuo*, the reaction products were isolated by extraction with chloroform. Evaporation of the solvent left a residue (2.0 g.) which upon trituration with acetone yielded 630 mg. of $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione (II) melting at 233–234°. Chromatography of the vacuum-dried mother

(31) The crystal size and form obtained in this manner is essential for satisfactory performance in the hydroxybromination reaction.

(32) R. P. Graber, A. C. Haven, Jr., and N. L. Wendler, THIS JOURNAL, **75**, 4722 (1953).

(33) S. Bernstein, R. Littell and J. H. Williams, *ibid.*, **75**, 4830 (1953).

liquors (873 mg. dissolved in 8 ml. of benzene and 2 ml. of hexane) on alumina (17 g.) furnished in the benzene–hexane eluates (8:2, 400 ml.) 300 mg. of a crystalline fraction consisting of the 17-formate ester IIa, which was recrystallized twice from acetone–hexane and finally from acetone; m.p. 215–216°, $[\alpha]^{23}D$ +41° (*c* 1.07), λ_{max}^{ale} 238 mμ (16,400); λ_{max}^{Nujol} no OH band, 5.76, 5.87, 5.95 and 6.18 μ.

Anal. Calcd. for $C_{24}H_{30}O_6$ (414.48): C, 69.54; H, 7.30. Found: C, 69.71; H, 7.54.

Continued elution of the column with benzene–chloroform (3:1) gave a small amount of II.

9α-Bromohydrocortisone Acetate (III). (a) Perchloric Acid Procedure.—To a suspension of $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione (II) (243 g.) in pure peroxide-free dioxane (2.43 l.) and 0.46 *N* perchloric acid (365 ml.) was added in the dark at room temperature with stirring over a one-hour period solid N-bromoacetamide (114 g., 1.3 mole equivalents). During the addition the suspension began to thin and after a total reaction time of 1.5 hours all the starting material was dissolved. After an additional half-hour 10% sodium sulfite solution (250 ml.) was added with stirring until KI–starch paper was no longer blued. Ice (1 kg.) and chloroform (2.5 l.) was added and the layers separated. During the subsequent washings of the chloroform–dioxane phase the temperature was held at 15–20° by the addition of ice. The extract was concentrated *in vacuo* at a bath temperature of less than 25° to a viscous sirup and acetone (930 ml.) was mixed into the residue with swirling. Crystallization ensued rapidly, which was complete after several hours in the refrigerator. Filtration furnished a first crop (205 g.), which was followed by two additional crops (28 and 15 g.) when the mother liquors were evaporated to dryness and allowed to crystallize from acetone (130 ml.). The total yield was 248 g. or 80% of theory. When the reaction was performed on a small scale yields as high as 90% were obtained. Analytically pure material was obtained by additional crystallization from acetone. It was dried *in vacuo* at room temperature; m.p. 132–133° dec. In one instance a sample was obtained which melted at 183–188° with slight decomposition, $[\alpha]^{23}D$ +137° (*c* 0.75), λ_{max}^{ale} 243 mμ (14,500); λ_{max}^{Nujol} 2.98, 5.76, 5.93, 6.05 and 6.16 μ.

Anal. Calcd. for $C_{23}H_{31}O_6Br$ (483.48): C, 57.17; H, 6.42; Br, 16.54. Found: C, 57.40; H, 6.56; Br, 16.11.

(b) Sulfuric Acid Method. Isolation of $\Delta^{4,7,9(11)}$-Pregnatriene-17α,21-diol-3,20-dione (XVII).—To a suspension of the $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione (II) (3.0 g.) in dioxane (300 ml.), water (30 ml.) and 1 *N* sulfuric acid (30 ml.) there was added in one portion N-bromoacetamide (1.34 g.) and the mixture allowed to react as described above. From the chloroform–dioxane extract was isolated 1.80 g. of 9α-bromohydrocortisone acetate (48% of theory). The aqueous phase when allowed to remain in the refrigerator for 40 days deposited crystals (231 mg.) of the triene XVII which were removed by chloroform extraction and recrystallized from acetone after evaporation of the chloroform *in vacuo;* m.p. 219–221°, $[\alpha]^{23}D$ +240° (*c* 0.99); λ_{max}^{ale} 243 mμ (18,000), shoulders at 237 mμ (16,600) and 250 mμ (14,700)[34]; λ_{max}^{Nujol} 3.05, 5.77, 5.87 and 6.00 μ.

Δ[M]D for the 7,9(11)-diene system in XVII is +402°; Δ[*M*]D for the 7,9(11)-diene system in $\Delta^{4,7,9(11)}$-pregnatriene-3,20-dione is +486°.[34]

Anal. Calcd. for $C_{21}H_{26}O_4$ (342.42): C, 73.66; H, 7.66. Found: C, 73.75; H, 7.61.

The 21-acetate XVIIa was prepared with pyridine and acetic anhydride for 18 hours. After recrystallization from acetone it melted at 200–202°; λ_{max}^{ale} 243 mμ (21,900), shoulders at 237 mμ (20,300) and 250 mμ (18,000); λ_{max}^{Nujol} 3.02, 5.77, 5.81, 5.88 and 6.06 μ.

9α-Bromohydrocortisone (IIIa).—A suspension of 9α-bromohydrocortisone acetate (III) (101 mg.) in 0.27 *N* methanolic perchloric acid (4 ml., prepared by mixing 19.5 ml. of methanol with 0.5 ml. of 72% perchloric acid) was shaken at room temperature for 20 hours. The resulting solution was mixed with sodium acetate solution and chloroform and the crystalline precipitate filtered off after 2 hours in the refrigerator. Recrystallization from alcohol furnished pure 9α-bromohydrocortisone (IIIa), which melted at 152° dec. and had $[\alpha]^{23}D$ +156° (*c* 0.49 in 95% alcohol); λ_{max}^{Nujol} 2.92, 5.85 and 6.15 μ.

Anal. Calcd. for $C_{21}H_{29}O_5Br$ (441.35): C, 57.15; H, 6.62. Found: C, 57.45; H, 6.89.

9α-Bromocortisone Acetate (VII).—To a solution of 9α-bromohydrocortisone acetate (III) (5.0 g.) in glacial acetic acid (80 ml.) was added at room temperature over a period of 10 minutes a solution containing chromic acid (1.5 g.) in acetic acid (75 ml.). After a total reaction period of 30 minutes, alcohol (2 ml.) was added and the mixture was concentrated to a sirup. The 9α-bromocortisone acetate was isolated with chloroform and the chloroform residue (5.19 g.) recrystallized from 95% alcohol. The total yield was obtained in three successive crops and amounted to 2.68 g. (54%). After an additional recrystallization, VII melted at 219° dec., $[\alpha]^{23}D$ +242° (*c* 0.61), λ_{max}^{ale} 237 mμ (16,100); λ_{max}^{Nujol} 2.85, 3.11, 5.72, 5.81, 5.88, 6.04 and 6.19 μ.

Anal. Calcd. for $C_{23}H_{29}O_6Br$ (481.46): C, 57.41; H, 6.03; Br, 16.61. Found: C, 57.30; H, 6.16; Br, 16.15.

An attempt to oxidize 9α-bromohydrocortisone acetate (102 mg.) with N-bromoacetamide (41 mg.) in a medium consisting of dioxane (10 ml.), water (0.13 ml.) and pyridine (0.25 ml.) for 20 hours at room temperature failed; only starting material could be recovered (83 mg., m.p. 119–122°).

Reduction of 9α-Bromocortisone Acetate (VII) to Cortisone Acetate.—A solution of 9α-bromocortisone acetate (VII) (25 mg.) in glacial acetic acid (2 ml.) was treated portionwise with zinc dust (48 mg.) on the steam-bath for a total of 15 minutes. The residual zinc was removed by centrifugation and the acetic acid solution evaporated to dryness *in vacuo*. The cortisone acetate was isolated with chloroform and recrystallized from acetone. A total of 15 mg. was obtained, which melted at 238–241° and had an infrared spectrum identical with that of an authentic sample.

8-Dehydrocortisone Acetate (XIV).—A suspension of 9α-bromocortisone acetate (VII) (102 mg.) in freshly distilled collidine (0.5 ml.) was refluxed for ten minutes. The reaction product was isolated by chloroform extraction and the crystalline residue (77 mg.) recrystallized from 95% alcohol with the aid of Darco G-60; 51 mg. (60%) of material melting at 248–249° dec. was obtained, $[\alpha]^{23}D$ +424° (*c* 0.55), λ_{max}^{ale} 235 mμ (broad) (17,200); λ_{max}^{Nujol} 2.96, 5.72, 5.82, 5.88, 6.06, 6.26 and 6.35 μ; reported[22] m.p. 249–250° dec., $[\alpha]^{CHCl_3}D$ +422°.

Anal. Calcd. for $C_{23}H_{28}O_5$ (400.45): C, 68.98; H, 7.05. Found: C, 68.81; H, 7.28.

Hydrocortisone Acetate from 9α-Bromohydrocortisone Acetate (III).—A solution of 9α-bromohydrocortisone acetate (III) (100 mg.) in alcohol (30 ml.) and water (5 ml.) was shaken with zinc dust at room temperature for 19 hours. At the end of this period the zinc dust (1.0 g.) was removed by centrifugation and washed with alcohol. The alcohol solution was concentrated *in vacuo* and the aqueous suspension extracted with chloroform. The residue from the chloroform extract (84 mg.) was dissolved in chloroform (1 ml.)–benzene (4 ml.) and chromatographed on silica gel (Davison No. 923, 2 g.). Elution of the column with chloroform–benzene (1:1) eluted $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione 21-acetate (II) (39 mg.) which after recrystallization from acetone melted at 233–236°. It was identified by infrared comparison with an authentic sample. This fraction was followed by lower melting mixed fractions. Chloroform (250 ml.) eluted material (25 mg.), which after trituration with chloroform and recrystallization of the chloroform-insoluble portion from acetone furnished pure hydrocortisone acetate, melting at 216–218.5°, $[\alpha]^{23}D$ +156° (*c* 0.36), λ_{max}^{ale} 241 mμ (16,700). Its infrared spectrum was identical with that of an authentic sample.

Anal. Calcd. for $C_{23}H_{32}O_6$ (404.49): C, 68.29; H, 7.97. Found: C, 68.47; H, 8.14.

The use of 9α-iodohydrocortisone acetate (Vb) in the above procedure gave hydrocortisone acetate in approximately the same yield. 9α-Chlorohydrocortisone acetate (V) remained unchanged under these conditions.

$\Delta^{4,9(11)}$-Pregnadiene-17α,21-diol-3,20-dione 21-Acetate (II) from 9α-Bromohydrocortisone Acetate (III). (a) With Chromous Chloride.—To a solution of 9α-bromohydrocortisone acetate (III) (200 mg.) in dioxane (10 ml.) was added at room temperature, under a blanket of carbon dioxide, a solution of chromous chloride[35] (4 ml.). After 10 minutes

(34) *Cf.* R. Antonucci, S. Bernstein, D. Giancola and K. J. Sax, *J. Org. Chem.*, **16**, 1453 (1951).

(35) G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, THIS JOURNAL, **72**, 4077 (1950).

the mixture was extracted with chloroform and the chloroform residue (168 mg.) recrystallized from acetone. Pure II melting at 235–237° was obtained in 80% yield (130 mg.).

(b) **With Zinc and Acetic Acid.**—A solution of 9α-bromohydrocortisone acetate (III) (104 mg.) in glacial acetic acid (5 ml.) was heated on the steam-bath with zinc dust (103 mg.) for 20 minutes. The steroids were isolated with chloroform and the crude residue (83 mg.) recrystallized from acetone. A total of 45 mg. (57%) of $\Delta^{,9(11)}$-pregnadiene-17α,21-diol-3,20-dione 21-acetate (II) melting at 230–232° was obtained.

$\Delta^{4,6,8}$-Pregnatriene-17α,21-diol-3,20-dione 21-Acetate (XXI) from 9α-Bromohydrocortisone Acetate (III).—A solution of 9α-bromohydrocortisone acetate (III) (200 mg.) and sodium iodide (400 mg.) in acetone (10 ml.) was allowed to remain at room temperature in the dark for 7 days. After removal of the acetone *in vacuo* the steroids were extracted with chloroform and residual iodine removed with sodium sulfite solution. The chloroform residue (169 mg.) after recrystallization from acetone furnished the triene XXI (106 mg., 67% of theory) melting at 188–190°, $[\alpha]^{23}D$ +547° (*c* 1.02); λ_{max}^{alc} 244 mμ (14,300), 285–300 mμ (3,100) and 385 mμ (6,700); λ_{max}^{Nujol} 3.08, 5.77, 5.88, 6.06 and 6.36 μ.

Anal. Calcd. for $C_{23}H_{28}O_5$ (384.47): C, 71.85; H, 7.34. Found: C, 72.28; H, 7.47.

When 9α-bromohydrocortisone acetate (200 mg.) was refluxed with sodium iodide (242 mg.) in acetone (10 ml.) for 2 hours the triene XXI could not be isolated from the reaction mixture even after chromatography on alumina (2.25 g.). Instead elution with chloroform–benzene (1:4) yielded at first $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione 21-acetate, m.p. 227–229°, $[\alpha]^{23}D$ +115° (*c* 0.3), followed by Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (IV), m.p. 206–210°. The infrared spectra of these compounds were identical with those of authentic samples.

Reaction of 9α-Bromohydrocortisone Acetate (III) with Silver Nitrate in Dioxane. Formation of XVIIa and XVIII.—To a solution of 9α-bromohydrocortisone acetate (500 mg.) in dioxane (10 ml.) was added a solution of silver nitrate (200 mg.) in water (6 ml.). The mixture was allowed to remain at room temperature in the dark for one hour after which time the precipitated silver bromide was removed by filtration. The steroids were isolated by extraction with chloroform, the crystalline residue (400 mg.), after evaporation of the solvents, dissolved in chloroform (2 ml.) and benzene (8 ml.) and chromatographed on silica gel (8 g.). After washing the column with chloroform–benzene (1:1, 100 ml.), elution with chloroform (10 × 50 ml.) afforded a fraction (50 mg.), which was purified by crystallization from acetone. The purest material obtained had the following properties, which indicated that it consisted essentially of $\Delta^{4,7,9(11)}$-pregnatriene-17α,21-diol-3,20-dione 21-acetate (XVIIa) but contained some difficultly removable impurities; m.p. 207–209°, $[\alpha]^{23}D$ +206° (*c* 0.8); λ_{max}^{alc} 243 mμ (25,500), shoulders at 235 mμ (23,600) and 250 mμ (21,000).

Anal. Calcd. for $C_{23}H_{28}O_5$ (384.47): C, 71.85; H, 7.34. Found: C, 70.73; H, 7.35.

Continued elution of the column with the same solvent mixture (700 ml.) and with chloroform containing 5% acetone (250 ml.) produced mixtures which could not be resolved by crystallization. Chloroform containing 10% acetone (150 ml.) furnished XVIII, which after crystallization from acetone melted at 258–261° dec., $[\alpha]^{23}D$ +298° (*c* 0.26). Its infrared spectrum was identical with that of the by-product from the reaction of the 9β,11β-epoxide IV with hydrogen fluoride.

Conversion of $\Delta^{4,7,9(11)}$-Pregnatriene-17α,21-diol-3,20-dione 21-Acetate XVIIa into the $\Delta^{4,6,8}$-Isomer XXI.—To a solution of impure $\Delta^{4,7,9(11)}$-pregnatriene-17α,21-diol-3,20-dione 21-acetate (XVIIa) (10.8 mg.) described in the preceding experiment in acetone (1 ml.) ($[\alpha]^{23}D$ +180°) was added one drop of concentrated hydrochloric acid. There was an immediate darkening of the solution and a change in rotation to +388°. There was no further change in rotation during the next half-hour. The solution was diluted with water and the product isolated with chloroform. After recrystallization from acetone–hexane it melted at 202–203°, $[\alpha]^{23}D$ +390° (*c* 0.29); λ_{max}^{alc} 243 mμ (15,600), 290 mμ (1,950) and 384 (4,500). The high specific rotation and the ultraviolet data clearly indicate the presence of the $\Delta^{4,6,8}$-triene XXI in the above product. Rotation and molecular extinction data indicate a content of 67% of XXI.

Δ^4-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-Acetate (IV).—To a solution of anhydrous potassium acetate (158 g.) in absolute alcohol (1.6 l.) which had been heated just below reflux temperature was added a solution of 9α-bromohydrocortisone acetate (III) (232 g.) in dioxane (745 ml.). The mixture was brought to reflux within 3 minutes and the reaction allowed to proceed for a total of 40 minutes. After cooling in an ice-bath, ice-water (4 l.) was added with stirring, upon which crystallization ensued rapidly. The resulting first crop of crystals amounted to 118 g. and an additional 30 g. was obtained by two successive concentrations of the mother liquors; total yield 77%. Analytically pure material was obtained by recrystallization from acetone, m.p. 209–211°, $[\alpha]^{23}D$ +21° (*c* 0.82), λ_{max}^{alc} 243 mμ (15,500); λ_{max}^{Nujol} 2.98, 5.87, 6.00 and 6.18 μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.88; H, 7.57.

Δ^4-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione (IVa) by Hydrolysis of the 21-Acetate IV.—To a suspension of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (IV) (20 g.) in methanol (200 ml.) was added under a blanket of nitrogen oxygen-free 10% aqueous potassium carbonate (42 ml.). The mixture was agitated for one hour, during which period the acetate dissolved completely and the free alcohol began to crystallize. The reaction was terminated by the addition of glacial acetic acid (3 ml.) and ice-water (1300 ml.). After cooling, the crystalline precipitate was collected and dried *in vacuo*. The crude material (13.8 g., 86%) melted at 206–208°. One crystallization from acetone produced pure IVa melting at 214–216°, $[\alpha]^{23}D$ −1° (*c* 0.77 in 95% alcohol), λ_{max}^{alc} 243 mμ (14,500); λ_{max}^{Nujol} 2.98, 5.85 and 6.06 μ.

Anal. Calcd. for $C_{21}H_{28}O_5$ (360.44): C, 70.02; H, 7.77. Found: C, 70.31; H, 7.95.

Δ^4-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione (IVa) from 9α-Bromohydrocortisone Acetate (III).—To a solution of 9α-bromohydrocortisone acetate (III) (115 mg.) in methanol (10 ml.) was added a solution of potassium bicarbonate (103 mg.) in water (1 ml.) and the mixture allowed to remain at room temperature for 18 hours. Water was added, the methanol removed *in vacuo* and the epoxide IVa isolated by extraction with chloroform. Crystalline material (50 mg., 59%) was obtained from acetone, which melted at 206–208°. Its infrared spectrum was identical with that of a sample obtained by hydrolysis of IV. Reacetylation with pyridine–acetic anhydride gave the 21-acetate which melted at 206–208° (depression when mixed with the 21-ol), $[\alpha]^{23}D$ +27°.

Δ^4-Pregnene-9α,11α-oxido-17α,21-diol-3,20-dione 21-Acetate IVb.—$\Delta^{4,9(11)}$-Pregnadiene-17α,21-diol-3,20-dione 21-acetate (200 mg.) was dissolved in an ice-cold 0.024 *M* solution of perbenzoic acid in chloroform (50 ml.) and the mixture allowed to remain in the refrigerator. Iodometric titration after 24 hours indicated the consumption of 1.06 mole equivalents of perbenzoic acid. The chloroform solution was extracted in the order given with sodium iodide in dilute sulfuric acid, dilute sodium sulfite, sodium bicarbonate and water. The crystalline residue from the chloroform solution (186 mg.) upon recrystallization from acetone furnished the pure α-epoxide IVb, which melted at 248–249°, $[\alpha]^{23}D$ +99° (*c* 1.09), λ_{max}^{alc} 238 mμ (16,000); λ_{max}^{Nujol} 2.96, 5.80, 5.96, 6.04 and 6.18 μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.74; H, 7.38.

Δ^4-Androstene-9β,11β-oxido-3,17-dione (X) from Δ^4-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione (IVa). (a) With Chromic Acid.—To a solution of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione (IVa) (101 mg.) in glacial acetic acid (6 ml.) was added chromic acid (65 mg.) in acetic acid (13 ml.). After 35 minutes the reaction mixture was worked up as described above and the resulting crude steroids (85 mg.) dissolved in benzene (2 ml.) and hexane (1 ml.) and chromatographed on alumina (2 g.). Benzene–hexane (2:1, 300 ml.) eluted 43 mg. of crystals which after recrystallization from acetone–hexane melted at 180–181°, $[\alpha]^{23}D$ +48° (*c* 0.77), λ_{max}^{alc} 243 mμ (15,200); λ_{max}^{Nujol} 5.79, 6.04 and 6.19 μ; reported[28] m.p. 181–182°, $[\alpha]^{24}D$ +38° (*c* 0.525 in $CHCl_3$).

Anal. Calcd. for $C_{19}H_{24}O_3$ (300.38): C, 75.97; H, 8.05. Found: C, 75.71; H, 8.31.

(b) With Sodium Bismuthate.—To a solution of the epoxide (IVa, 1.5 g.) in glacial acetic acid (150 ml.) and water (150 ml.) was added sodium bismuthate (12 g.) and the resulting suspension placed on a shaker at room temperature in the dark and agitated vigorously for 40 minutes. The solution was then filtered, the precipitate washed with chloroform and the total mixture extracted with chloroform. From the chloroform solution was isolated Δ^4-androstene-9β,11β-oxido-3,17-dione (X) (852 mg., 70%) by crystallization from acetone–hexane; m.p. 180–181°. The infrared spectrum of this material was identical with that of the material described above.

9α-Bromohydrocortisone Acetate (III) from Δ^4-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-Acetate (IV).—To a solution of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (25 mg.) in glacial acetic acid (0.5 ml.) and carbon tetrachloride (0.5 ml.) was added 32% hydrobromic acid in acetic acid (0.04 ml.). After 10 minutes at room temperature chloroform (10 ml.) was added and the bromohydrin III isolated and purified by crystallization of the crude product (34 mg.) from acetone. A total of 23 mg. was obtained melting at 126–128° dec., $[\alpha]^{22}D$ +144°, (*c* 0.90) identical in all respects with an authentic sample.

9α-Iodohydrocortisone Acetate (Vb).—To a solution of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (IV) (204 mg.) in chloroform (20 ml.), cooled to −20° in an ice–salt-bath, freshly distilled 55% aqueous hydriodic acid (0.4 ml.) was added. The mixture was agitated thoroughly and, after 20 minutes, water was added and the layers separated. Iodine was removed by a sodium sulfite wash. Careful evaporation of the solvent *in vacuo* left a crystalline residue (279 mg.), which was purified by fractional crystallization from ethyl acetate taking care to keep the temperature below 40°. Pure 9α-iodohydrocortisone acetate decomposed vigorously at 110° with initial browning at 70–80° and sintering at 100°, $[\alpha]^{23}D$ +149° (*c* 0.63), λ_{max}^{alc} 243 mμ (11,000).

Anal. (dried at room temperature for 1.5 hours) Calcd. for $C_{23}H_{31}O_6I$ (530.40): C, 52.08; H, 5.89; I, 23.93. Found: C, 52.54; H, 6.44; I, 22.60.

The more soluble fractions always contained some $\Delta^{4,9(11)}$-pregnadiene-17α,21-diol-3,20-dione 21-acetate. The latter became the main reaction product when the reaction time was extended to two hours, and the temperature allowed to rise to −3°.

9α-Chlorohydrocortisone Acetate (V).—To a solution of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (IV) (40 g.) *in redistilled chloroform* (400 ml.) was added with agitation at 0° over a 35-minute period ice-cold 0.45 *N* hydrogen chloride in chloroform (330 ml., 1.5 mole equivalents). The reaction mixture turned red and was allowed to remain at 0° for an additional hour. Water was added and the chloroform solution washed and evaporated to dryness *in vacuo*. The crystalline residue on recrystallization from acetone (160 ml.) furnished 33 g. (76%) of pure 9α-chlorohydrocortisone acetate V which melted at 201–202° dec., $[\alpha]^{23}D$ +144° (*c* 0.86), λ_{max}^{alc} 241 mμ (15,800); λ_{max}^{Nujol} 2.90, 2.96, 5.74, 5.80, 5.92, 6.03 and 6.17 μ; $\lambda_{max}^{CHCl_3}$ 2.96, 5.80, 6.03 and 6.18 μ.

Anal. Calcd. for $C_{23}H_{31}O_6Cl$ (438.93): C, 62.93; H, 7.12; Cl, 8.07. Found: C, 63.23; H, 7.41; Cl, 7.70.

9α-Chlorohydrocortisone (Va).—A suspension of 9α-chlorohydrocortisone acetate (V) (100 mg.) in 0.27 *N* perchloric acid in methanol (2 ml.) was shaken at room temperature for 17 hours. Water was added and the resulting crystals filtered and washed carefully with sodium acetate solution and with water to remove perchloric acid. Recrystallization from 95% alcohol furnished pure material (78 mg.) which melted at 228° dec., $[\alpha]^{23}D$ +160° (*c* 0.58 in 95% alcohol), λ_{max}^{alc} 241 mμ (17,000); λ_{max}^{Nujol} 2.94, 3.03, 5.87 and 6.15 μ.

Anal. Calcd. for $C_{21}H_{29}O_5Cl$ (396.90): C, 63.56; H, 7.36. Found: C, 63.42; H, 7.22.

9α-Chlorocortisone Acetate (VIII).—9α-Chlorohydrocortisone acetate (V) (100 mg.) was oxidized with chromic acid (24 mg.) in acetic acid (total of 10 ml.) as described above for VII. The pure substance after crystallization from acetone melted at 257–258° dec., $[\alpha]^{23}D$ +260° (*c* 1.10), λ_{max}^{alc} 236 mμ (16,600); λ_{max}^{Nujol} 2.98, 5.71, 5.81, 6.06 and 6.20 μ.

Anal. Calcd. for $C_{23}H_{29}O_6Cl$ (436.91): C, 63.22; H, 6.69; Cl, 8.11. Found: C, 62.97; H, 6.61; Cl, 8.13.

9α-Chlorocortisone (VIIIa).—9α-Chlorocortisone acetate (147 mg.) was hydrolyzed with 0.27 *N* methanolic perchloric acid (4.5 ml.) as described above. Trituration with acetone left practically pure VIIIa (126 mg.) of m.p. 230–231° dec. It was recrystallized once from 95% alcohol, m.p. 230–231°; λ_{max}^{Nujol} 2.86, 2.91, 5.86, 6.05 and 6.16 μ.

Anal. Calcd. for $C_{21}H_{27}O_5Cl$ (394.87): C, 63.88; H, 6.87. Found: C, 63.84; H, 6.98.

Δ^4-Pregnene-9α-chloro-11β-ol-3,20-dione-21-al Dimethyl Acetal (XV) from 9α-Chlorohydrocortisone Acetate (V).—A solution of 9α-chlorohydrocortisone acetate (V) (1 g.) in chloroform (26 ml.), methanol (64 ml.) and 1.5 *N* methanolic hydrogen chloride (14 ml.) was allowed to stand at room temperature for 64 hours. After the addition of anhydrous sodium acetate (2 g.) in water (20 ml.), chloroform and sodium bicarbonate solution was added to neutralize the acid and the steroid was isolated from the chloroform solution. The residue (1.06 g.) after recrystallization from ethyl acetate–hexane furnished pure XV (700 mg., 72%) which melted at 137–138°, $[\alpha]^{23}D$ +197° (*c* 0.93), λ_{max}^{alc} 240 mμ (17,800); λ_{max}^{Nujol} 2.90, 5.80, 5.85 and 6.02 μ.

Anal. Calcd. for $C_{23}H_{33}O_5Cl$ (424.94): C, 65.00; H, 7.82; Cl, 8.34; OCH_3, 14.59. Found: C, 65.25; H, 7.73; Cl, 8.46; OCH_3, 14.79.

Δ^4-Pregnene-9β,11β-oxido-3,20-dione-21-al Dimethyl Acetal (XVI) from XV and Re-opening of the Epoxide Ring to the Chlorohydrin XV.—To a solution of Δ^4-pregnene-9α-chloro-11β-ol-3,20-dione-21-al dimethyl acetal (XV) (103 mg.) in methanol (10 ml.) was added a solution of potassium carbonate (95 mg.) in water (0.5 ml.). A precipitate appeared, which redissolved within 45 minutes. At that time the specific rotation of the solution was +63°. After additional one- and two-hour intervals the rotation had dropped to +48 and +40°, respectively. The epoxide was recovered from the solution with chloroform and the amorphous residue (89 mg.) dissolved in benzene (2 ml.) and hexane (10 ml.) for chromatography on Brockman alumina (basic). Benzene–hexane (1:4) (600 ml.) eluted a band (60 mg.) of material which could not be induced to crystallization. To verify its 9β,11β-oxide structure (XVI) the material (50 mg.) was reconverted to the chlorohydrin XV in chloroform (5 ml.) with 0.45 *N* hydrogen chloride in chloroform (0.8 ml.) at 0° for one hour. The resulting product on recrystallization from ethyl acetate–hexane (30 mg.) melted at 138–140° and there was no depression on admixture of an authentic sample of XV.

9α-Fluorohydrocortisone Acetate (VI).—Into a solution of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (IV) (50 g.) in redistilled chloroform (1 l.) contained in a polyethylene bottle provided with a magnetic stirrer and a polyethylene inlet tube was passed at 0° with thorough agitation anhydrous hydrogen fluoride (approximately 60 g.). During the addition, which lasted about 20 minutes, the color of the solution gradually deepened and at the end of the addition became an intense cherry-red. At the same time the solution separated into two layers, with most of the colored material concentrated in the upper, smaller layer. The appropriate color development can be ascertained easily with some experience (light behind polyethylene vessel) and represents an excellent criterion for the attainment of the proper concentration of hydrogen fluoride. When too little hydrogen fluoride (color pink to bright red) was used only the epoxide could be isolated. After 1.5 hours at 0° a suspension of sodium bicarbonate in water was added carefully with stirring until the mixture became weakly basic. It was then transferred to glass equipment and the steroids isolated from the chloroform extract. The crude crystalline material (55 g.) was slurried up in ethyl acetate (100 ml.) and allowed to crystallize for 45 minutes at 5°. Additional ethyl acetate was then added (375 ml.) and the mixture heated to boiling. The bulk of the material went into solution leaving a sandy residue (4.3 g., m.p. 245–250°) which was filtered off. It consists essentially of the by-product XVIII to be described below. The filtrate was allowed to crystallize at room temperature for 15 minutes and the resulting 9α-fluorohydrocortisone acetate (VI) filtered off (22 g., m.p. 228–229°). Concentration of the mother liquors gave in alternate crystallizates an additional 3 g. of VI and 1.5 g. of impure XVIII. Recrystallization

of the combined high melting fractions (5.8 g.) from acetone (*vide infra*) gave an additional 1.4 g. of 9α-fluorohydrocortisone acetate. The total yield of the latter is therefore 26.4 g. or 50.5% of theory. Analytically pure material was obtained after two recrystallizations from ethyl acetate. The material obtained in this fashion contained varying amounts of ethyl acetate (5–9%) and was therefore dried at 100° for two hours; m.p. 232–233°; $[\alpha]^{23}D$ +143° (*c* 0.50 in $CHCl_3$),[36] +127° (*c* 0.54 in acetone); λ_{max}^{ale} 238 mμ (16,800); λ_{max}^{Nujol} 2.94, 3.03, 5.75, 5.82, 6.07 and 6.11 μ.

Anal. Calcd. for $C_{23}H_{31}O_6F$ (422.48): C, 65.39; H, 7.39; F, 4.52. Found: C, 65.32; H, 7.26; F, 4.50.

When the above reaction was performed in chloroform containing 5% alcohol there was only a single phase and the yield of VI rose to 60–65%.

Purification of the High-melting By-product XVIII.—Initial attempts to purify the high melting by-product from the above reaction with hydrogen fluoride by crystallization from alcohol gave constant melting products (256–260°), which still contained 9α-fluorohydrocortisone. Crystallization from acetone, however, gave pure XVIII, in which the absence of the fluoro compound was most convincingly demonstrated by the lack of activity in the liver glycogen assay in the rat. The pure material melted at 259–263° dec., $[\alpha]^{23}D$ +280° (*c* 0.53 in 95% alcohol), λ_{max}^{ale} 239 mμ (20,200); λ_{max}^{Nujol} 2.93, 3.03, 5.75, 5.82, 6.12 and 6.16 μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.60; H, 7.40.

Acetylation of XVIII produced an amorphous acetate.

Reaction of XVIII (50 mg.) with propionic anhydride (0.5 ml.) in pyridine (1 ml.) at room temperature for 17 hours furnished a crystalline propionate XVIIIa (62 mg.) which after recrystallization from 95% alcohol melted at 261–264°; $[\alpha]^{23}D$ +268° (*c* 0.4), +250° (*c* 0.52 in 95% alcohol); λ_{max}^{ale} 238 mμ (18,200); λ_{max}^{Nujol} 3.05, 5.72, 5.80, 6.07 and 6.11 μ.

Anal. Calcd. for $C_{26}H_{34}O_7$ (458.53): C, 68.10; H, 7.41. Found: C, 67.93; H, 7.36.

The mesylate of XVIII (51 mg.) was prepared with methanesulfonyl chloride (0.02 ml.) in pyridine (3 ml.) and chloroform (0.5 ml.) at 0° for 17 hours. Recrystallization from 95% alcohol gave pure XVIIIb melting at 151–152° dec.; $[\alpha]^{23}D$ +268° (*c* 0.54), +245° (*c* 0.35 in 95% alcohol); λ_{max}^{ale} 237 mμ (17,800); λ_{max}^{Nujol} 3.06, 5.72, 5.81 and 6.11 μ.

Anal. Calcd. for $C_{24}H_{32}O_8S$ (480.50): C, 59.98; H, 6.71; S, 6.67. Found: C, 60.01; H, 6.73; S, 6.38.

Formation of the High-melting By-product XVIII from IV with Perchloric Acid in Acetic Acid.—A solution of the epoxide IV (300 mg.) in gl. HAc (30 ml.) and 72% perchloric acid (0.225 ml.) was allowed to stand at room temperature for 5 minutes. The reaction was stopped by the addition of dilute sodium bicarbonate and the bulk of the solvent removed *in vacuo*. The steroids were isolated with chloroform (340 mg.) and recrystallized from 95% alcohol. After removal of a crop of mixed crystals (110 mg.) consisting in part of cortisone acetate (infrared) a second crop (41 mg.) was obtained which upon recrystallization from the same solvent melted at 261–263° dec., $[\alpha]^{23}D$ +285° (*c* 0.48 in 95% alcohol). Its infrared spectrum was identical with that of the product from the reaction with hydrogen fluoride.

Preparation of the High-melting By-product XVIII from Δ⁴-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-Acetate (IV) in Anhydrous Hydrogen Fluoride.—To hydrogen fluoride (10 ml.) was added portionwise at −70° the epoxide IV (4 g.) over a period of 5 minutes. The resulting deep-red solution was kept at that temperature for an additional 5 minutes and was then poured into chloroform and ice-cold sodium acetate solution. The chloroform layer was washed and evaporated to dryness *in vacuo*. The residue on recrystallization from acetone furnished 1.6 g. of XVIII, m.p. 246–250°, $[\alpha]^{23}D$ +269°. One additional crystallization furnished analytically pure material identical with an authentic sample.

9α-Fluorohydrocortisone (VIa).—9α-Fluorohydrocortisone acetate (VI) (75 g.) suspended in methanol (750 ml.) was hydrolyzed at room temperature under nitrogen with oxygen-free 10% potassium carbonate solution (158 ml.) for 30 minutes as described above for IV. A yield of 58.2 g. (86%), melting at 254–256° was obtained. Recrystallization from 95% alcohol afforded pure material melting at 260–262° dec.; $[\alpha]^{23}D$ +143° (*c* 0.55), +132° (*c* 0.2 in acetone), + 138° (*c* 0.5 in methanol); λ_{max}^{ale} 239 mμ (17,600); λ_{max}^{Nujol} 2.90–3.00, 5.85, 6.00 and 6.10 μ.

Anal. Calcd. for $C_{21}H_{29}O_5F$ (380.44): C, 66.30; H, 7.68; F, 5.00. Found: C, 66.43; H, 7.66; F, 5.04.

9α-Fluorohydrocortisone 21-Heptanoate (VIb).—A solution of 9α-fluorohydrocortisone (VIa) (200 mg.) and heptanoic anhydride (0.17 ml., 1.3 mole equivalents) in pyridine (2 ml.) was allowed to remain at room temperature for 24 hours. The ester was isolated with chloroform (313 mg.) and recrystallized from acetone–hexane. The pure material (213 mg.) melted at 173–174°, $[\alpha]^{21}D$ + 130° (*c* 0.92), λ_{max}^{ale} 239 mμ (16,100); λ_{max}^{Nujol} 2.99, 5.73, 5.79, 5.87 and 6.06 μ.

Anal. Calcd. for $C_{28}H_{41}O_6F$ (492.60): C, 68.25; H, 8.40. Found: C, 68.10; H, 8.17.

9α-Fluorohydrocortisone 21-Sodium Hemisuccinate (VIc).—A solution of 9α-fluorohydrocortisone (VIa) (40 g.) and succinic anhydride (80 g.) in anhydrous pyridine (400 ml.) was heated at 60–70° for two hours. After cooling to 15° ice (200 g.) was added and the mixture poured slowly with stirring onto crushed ice (1.5 l.) and sulfuric acid (166 ml.). The resulting precipitate was filtered and washed well with water until free of sulfuric acid. The dried material (50.66 g.) was recrystallized from 95% alcohol yielding a total of 45.5 g. of the hemisuccinic acid (90% of theory) melting at 212–215°. Analytically pure material melted at 215–217°, $[\alpha]^{23}D$ + 126° (*c* 0.35 in 95% alcohol), λ_{max}^{ale} 238 mμ (17,700); λ_{max}^{Nujol} 2.90, 3.01, 5.72, 5.85 and 6.17 μ.

Anal. Calcd. for $C_{25}H_{33}O_8F$ (480.50): C, 62.48; H, 6.92. Found: C, 62.16; H, 7.01.

The sodium salt VIc was prepared by careful neutralization of a solution of the above acid (514 mg.) in a minimum of 95% alcohol with 0.107 *N* sodium hydroxide (8.9 ml.). The neutralized solution was freed from alcohol *in vacuo*, extracted with ethyl acetate to remove some remaining acid and then lyophilized; λ_{max}^{Nujol} 2.94–2.96, 5.80, 6.02–6.06 and 6.36–6.39 μ.

Anal. (Dried at 110° for 1.5 hours) Calcd. for $C_{25}H_{32}O_8FNa$ (503.50): Na, 4.57. Found: Na, 4.49.

9α-Fluorocortisone Acetate (IX) and Δ⁴-Androstene-9α-fluoro-3,11,17-trione (XIII).—9α-Fluorohydrocortisone acetate (VI) (3.2 g.) was oxidized in glacial acetic acid (130 ml.) with CrO_3 (825 mg.) for 45 minutes. The residue from the chloroform solution (2.89 g.) on recrystallization from 95% alcohol gave IX (2.31 g., 72%) melting at 254–255°, $[\alpha]^{23}D$ +160° (*c* 0.45), λ_{max}^{ale} 234 mμ (17,000); λ_{max}^{Nujol} 2.86, 5.72, 5.78, 5.83 and 6.05 μ.

Anal. Calcd. for $C_{23}H_{29}O_6F$ (420.46): C, 65.70; H, 6.95. Found: C, 65.62; H, 7.19.

From the mother liquors was isolated 70 mg. of Δ⁴-androstene-9α-fluoro-3,11,17-trione (XIII), m.p. 183–184°, $[\alpha]^{23}D$ +232° (*c* 0.81), λ_{max}^{ale} 234 mμ (17,000); λ_{max}^{Nujol} 5.74, 5.80, 5.97 and 6.19 μ.

Anal. Calcd. for $C_{19}H_{23}O_3F$ (318.38): C, 71.44; H, 7.57. Found: C, 71.45; H, 7.40.

9α-Fluorocortisone (IXa).—9α-Fluorocortisone acetate (IX) was hydrolyzed with potassium carbonate in methanol as described above. The resulting material was recrystallized from 95% alcohol and melted at 261–262° dec., $[\alpha]^{23}D$ +149° (*c* 0.41), λ_{max}^{ale} 234 mμ (16,000); λ_{max}^{Nujol} 2.88, 5.87 and 6.08 μ.

Anal. Calcd. for $C_{21}H_{27}O_5F$ (378.43): C, 66.65; H, 7.19. Found: C, 66.50; H, 6.98.

Δ⁴-Pregnene-9α-fluoro-11β,17α-diol-3,20-dione-21-al Dimethyl Acetal (XVa).—A solution of 9α-fluorohydrocortisone acetate (VI) (1 g.) in chloroform (26 ml.), methanol (64 ml.) and 1.5 *N* methanolic hydrogen chloride (14 ml.) was allowed to remain at room temperature for 64 hours. Sodium acetate (2 g.) in water (40 ml.) was added and the solution neutralized with sodium bicarbonate. The dimethyl acetal was isolated with chloroform and recrystallized from ethyl acetate. A total yield of 572 mg. (60%) was obtained, m.p. 171–172°, $[\alpha]^{23}D$ +189° (*c* 0.71), λ_{max}^{ale} 237 mμ (16,800); λ_{max}^{Nujol} 2.91, 5.80, 5.86, 5.97 and 6.17 μ.

Anal. Calcd. for $C_{23}H_{33}O_5F$ (408.49): C, 67.62; H, 8.14; OCH_3, 15.20. Found: C, 67.56; H, 8.00; OCH_3, 16.43.

(36) In our earlier communication $[\alpha]^{CHCl_3}D$ +123° was reported. That sample was later found to contain 9.5% ethyl acetate.

Δ^4-Androstene-9α-fluoro-11β-ol-3,17-dione (XII) from 9α-Fluorohydrocortisone (VIa).—A solution of 9α-fluorohydrocortisone (VIa) (300 mg.) in acetic acid (25 ml.) and water (25 ml.) was shaken with sodium bismuthate (2.21 g.) at room temperature for 40 min. After filtration of the reaction mixture the steroids were extracted with chloroform. The residue (197 mg.) after recrystallization from 95% alcohol melted at 269–270°, $[\alpha]^{23}D$ +187° (*c* 0.50 in 95% alcohol), yield 170 mg. or 68%, λ_{max}^{alc} 238 mμ (16,600); λ_{max}^{Nujol} 3.03, 5.75 and 6.08–6.12 μ; reported[28] m.p. 249.5–250°, $[\alpha]^{23}D$ +184° (*c* 0.59 in $CHCl_3$).

Anal. Calcd. for $C_{19}H_{25}O_3F$ (320.39): C, 71.24; H, 7.87. Found: C, 71.34; H, 7.72.

Oxidation of XII with chromic acid in acetic acid furnished XIII identical in all respects with the sample described above.

Δ^4-Androstene-9α-chloro-11β-ol-3,17-dione (XI) from Δ^4-Androstene-9β,11β-oxido-3,17-dione (X).—To an ice-cold solution of Δ^4-androstene-9β,11β-oxido-3,17-dione (X) (25.4 mg.) in chloroform (2.5 ml.) was added 0.5 *N* hydrochloric acid in chloroform (0.5 ml.). After one hour at 0° the mixture was diluted with water and the chlorohydrin isolated from the chloroform solution (29 mg.). Recrystallization from 95% alcohol yielded pure XI (17 mg.) melting at 242° dec., $[\alpha]^{23}D$ +196° (*c* 0.73); reported[28] m.p. 243.5–245.5° dec., $[\alpha]^{25}D$ +194° (*c* 0.73 in $CHCl_3$).

Anal. Calcd. for $C_{19}H_{25}O_3Cl$ (336.84): C, 67.75; H, 7.48; Cl, 10.50. Found: C, 67.59; H, 7.35; Cl, 10.65.

9α-Hydroxyhydrocortisone Acetate (XIX) from Δ^4-Pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-Acetate (IV).—A solution of the 9β,11β-oxide IV (390 mg.) in a mixture of dioxane (24 ml.), water (60 ml.) and 1.1 *N* sulfuric acid (4 ml.) was refluxed for 45 minutes. During this period of time the specific rotation of the solution changed from an initial value of +41° to a value of +149°. The addition of chloroform (40 ml.) caused separation of phases and the steroids were isolated from the chloroform–dioxane extract. The dried residue was reacetylated with pyridine (2 ml.) and acetic anhydride (2 ml.) and the resulting mixture (360 mg.), after evaporation of the reagents, was dissolved in chloroform (1 ml.), benzene (3 ml.) and chromatographed on silica gel (7 g.). Elution of the column with chloroform (800 ml.) afforded a fraction (110 mg.) which after several recrystallizations from acetone–hexane melted at 218–219°, $[\alpha]^{23}D$ +107° (*c* 0.49), λ_{max}^{alc} 241 mμ (15,400); λ_{max}^{Nujol} 3.01, 5.80, 5.91, 5.96 and 6.00 μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.72; H, 7.26.

This substance may represent an x-dehydrohydrocortisone acetate.

Elution with 10% acetone in chloroform yielded in the first 125 ml. a mixture which could not be separated readily by crystallization. In the next 1000 ml. this same eluent afforded a homogeneous crystalline fraction (110 mg.), which after recrystallization from acetone–hexane melted at 216–217°, $[\alpha]^{23}D$ + 153° (*c* 0.89 in acetone), λ_{max}^{alc} 242 mμ (16,400); λ_{max}^{Nujol} 2.99, 5.84 and 6.18 μ; reported[29a] m.p. 216.5–217.5°, $[\alpha]D$ +171° (*c* 0.66 in pyridine).

Anal. Calcd. for $C_{23}H_{32}O_7$ (420.49): C, 65.69; H, 7.67. Found: C, 65.85; H, 7.57.

9α-Hydroxycortisone Acetate (XX).—9α-Hydroxyhydrocortisone acetate (XIX) (30 mg.) was oxidized in acetic acid (3.25 ml.) with CrO_3 (5.8 mg.). The resulting product after recrystallization from 95% alcohol melted at 237–239°, $[\alpha]^{23}D$ 211° (*c* 0.51), λ_{max}^{alc} 238 mμ (16,500); λ_{max}^{Nujol} 3.03, 5.76, 5.79, 5.87, 6.03 and 6.08 μ.

Anal. Calcd. for $C_{23}H_{30}O_7$ (418.47): C, 66.01; H, 7.23. Found: C, 66.28; H, 7.37.

9α-Methoxyhydrocortisone Acetate (XIXa).—A solution of Δ^4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (IV) (5 g.) in methanol (250 ml.) and 72% perchloric acid (1.87 ml.) was allowed to remain at room temperature for 3.5 hours. During that period of time the specific rotation rose from +25 to +140°. The solution was neutralized with sodium bicarbonate solution and the bulk of the methanol removed *in vacuo*. The steroids were isolated with chloroform and the residue acetylated with acetic anhydride (5 ml.) in pyridine (5 ml.) for 18 hours. After removal of the reagents the crystalline residue (4.57 g.) was recrystallized from 95% alcohol. The yield was 2.05 g. (38%), m.p. 204–207°. Analytically pure material melted at 208–209°, $[\alpha]^{23}D$ +161° (*c* 0.80), λ_{max}^{alc} 243 mμ (14,800); λ_{max}^{Nujol} 2.84, 3.04, 5.70, 5.80, 6.10 and 6.20 μ.

Anal. Calcd. for $C_{24}H_{32}O_7$ (434.51): C, 66.34; H. 7.89; OCH_3, 7.14. Found: C, 65.91; H, 8.08; OCH_3, 7.28.

9α-Methoxycortisone Acetate (XXa).—9α-Methoxyhydrocortisone acetate (XIXa) (1 g.) was oxidized in acetic acid (30 ml.) with chromic acid (372 mg.). Pure XXa was obtained by crystallization from acetone; m.p. 250–251°, $[\alpha]^{22}D$ +201° (*c* 1.07), λ_{max}^{alc} 238 mμ (16,500); λ_{max}^{Nujol} 3.04, 5.74, 5.80, 5.88, 6.05 and 6.21 μ.

Anal. Calcd. for $C_{24}H_{32}O_7$ (432.50): C, 66.65; H, 7.36. Found: C, 66.42; H, 7.36.

9α-Ethoxyhydrocortisone Acetate (XIXb).—A solution of the epoxide IV (200 mg.) in absolute alcohol (20 ml.) containing 72% perchloric acid (0.15 ml.) was allowed to react at room temperature for 3 days. During this period the specific rotation rose from +19 to +139°. The mixture was worked up and acetylated as described for the 9-methoxy compound and the crystalline residue (208 mg.) recrystallized from 95% alcohol; yield 100 mg. of XIXb (45%) melting at 144–145°, $[\alpha]^{23}D$ +136° (*c* 0.62).

Anal. Calcd. for $C_{25}H_{36}O_7$ (448.54): C, 66.94; H, 8.09; OC_2H_5, 10.03. Found: C, 66.98; H, 7.89; OC_2H_5, 8.43.

Oxidation of the "High-melting By-product" XVIII with Perphthalic Acid.—Titration of XVIII (40.4 mg.) in chloroform (30 ml.) with 0.1436 *M* perphthalic acid in ether (3 ml.) at 0° showed an uptake of 1.06 mole equivalents after 17 hours. In a preparative experiment 340 mg. in chloroform (100 ml.) and ethereal perphthalic acid (25 ml.) was stored at 0° for 16 hours. The resulting reaction product (408 mg.) was crystallized from acetone and furnished 146 mg. of the epoxide A (XXII) melting at 207–208° dec., $[\alpha]^{23}D$ +243° (*c* 0.59), λ_{max}^{alc} 240 mμ (16,400); λ_{max}^{Nujol} 2.92, 3.05, 5.73, 5.80 and 6.11–6.15 μ.

Anal. Calcd. for $C_{23}H_{30}O_7$ (418.47): C, 66.01; H, 7.23. Found: C, 65.89; H, 6.90.

The mother liquors (238 mg.) were dissolved in 4 ml. of benzene and chromatographed on alumina. Elution with chloroform–benzene (1:3, 700 ml.), (1:1, 400 ml.) and chloroform (200 ml.) furnished the isomeric epoxide B (XXII) (145 mg.) contaminated by a small amount of starting material. Recrystallization from acetone–hexane gave pure material melting at 180–182°, $[\alpha]^{23}D$ +206° (*c* 0.58), λ_{max}^{alc} 243 mμ (14,400); λ_{max}^{Nujol} 2.97, 3.18, 5.70, 5.77 and 6.11 μ.

Anal. Found: C, 66.12; H, 7.01.

The free 11-hydroxyl group in both epoxides can be acetylated with acetic anhydride in pyridine at room temperature. Epoxide A acetate XXIIa melted at 138–140°, $[\alpha]^{23}D$ +252° (*c* 0.46); λ_{max}^{Nujol} 2.89, 3.00, 3.16, 5.73, 5.81, 5.87, 6.07 and 6.16 μ.

Anal. Calcd. for $C_{25}H_{32}O_8 \cdot H_2O$ (478.52): C, 62.75; H, 7.16. Found: C, 62.97; H, 6.95.

Epoxide A acetate was also obtained when XVIII was first converted into the amorphous diacetate and the latter epoxidized with perphthalic acid.

Epoxide B acetate melted at 203–205°, $[\alpha]^{23}D$ +207° (*c* 0.52), λ_{max}^{alc} 242 mμ (16,600); λ_{max}^{Nujol} 3.06, 5.68, 5.73, 5.80, 5.92, 6.01 and 6.18 μ.

Anal. Calcd. for $C_{25}H_{32}O_8$ (460.51): C, 65.20; H, 7.00. Found: C, 65.71; H, 7.26.

Conversion of the Mesylate XVIIIb into the Triene XXIV.—A solution of XVIIIb (200 mg.) in pyridine (16 ml.) was refluxed for two hours. The steroids were isolated by extraction with chloroform (178 mg.) and recrystallized from acetone. The resulting material (82 mg.) melted at 183–185° dec., $[\alpha]^{23}D$ + 129° (*c* 0.42); λ_{max}^{alc} 240 mμ (17,400) shoulder at 275 mμ (4,150); λ_{max}^{Nujol} 2.86, 2.96, 5.76, 5.82, 6.05, 6.11 and 6.16 μ. The analytical sample (dried at 100°) contained acetone (iodoform test).

Anal. Calcd. for $C_{28}H_{28}O_5 \cdot C_3H_6O$ (442.53): C, 70.56; H, 7.74. Found: C, 70.78; H, 7.62.

Hydrogenation of the High-melting By-product (XVIII).—To a suspension of 5% Pd-on-charcoal (20 mg.) in glacial acetic acid (5 ml.), which had been prereduced with hydrogen (0.7 ml.), was added a solution of XVIII (78 mg.) in glacial acetic acid (5 ml.) and the mixture agitated in an atmosphere of hydrogen. The hydrogenation came to a

standstill after 20 minutes, when 5.1 ml. (1.05 mole equivalents) had been absorbed. The hydrogenation product was isolated with chloroform and the resulting crystalline product recrystallized from acetone–hexane. A total of 61 mg. of the 4,5-dihydro derivative was obtained which melted at 199–200°, $[\alpha]^{23}D$ +160° (*c* 0.65), $\lambda_{max}^{alc.}$ 288 mμ (95); reported[22] m.p. 195–197°, $[\alpha]D$ +165° ($CHCl_3$).

Anal. Calcd. for $C_{23}H_{32}O_6$ (404.49): C, 68.29; H, 7.97. Found: C, 68.08; H, 7.84.

Mesylation of the 4,5-Dihydro Derivative of XVIII and Preparation of the Triene XXIVa.—To a solution of the 4,5-dihydro derivative of XVIII (300 mg.) in pyridine (5 ml.) was added at 0° methanesulfonyl chloride (0.12 ml.) and the mixture allowed to remain at 0° for 16 hours. The resulting amorphous mesylate XVIIIc (390 mg.) was refluxed in pyridine (13 ml.) for one hour and the bulk of the pyridine removed *in vacuo*. The triene XXIVa was isolated with chloroform and the residual solid (345 mg.) crystallized from 95% alcohol. The crude triene (150 mg.) after additional crystallization from the same solvent melted at 183–184°, $[\alpha]^{23}D$ 126° (*c* 0.94), $\lambda_{max}^{alc.}$ 271 mμ (3,800); λ_{max}^{Nujol} 2.89, 5.71, 5.78, 5.84 and 5.89 μ; reported[22] m.p. 180–185°, $[\alpha]^{CHCl_3}D$ +79°.

Anal. Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82. Found: C, 71.43; H, 7.92.

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[CONTRIBUTION FROM THE BIOCHEMICAL RESEARCH DEPARTMENT, THE ARMOUR LABORATORIES]

Studies on Adrenocorticotropin. XIV. Action of Bovine Fibrinolysin and of Liver Cathepsin on Corticotropin-A: Effect on Biological Activity

BY W. F. WHITE AND A. M. GROSS

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Bovine fibrinolysin splits corticotropin-A after arginine in position 8 and after lysine in position 15 with complete loss of physiological activity. It appears reasonable to conclude that this is the mechanism of the destruction of ACTH by blood *in vitro*. Bovine liver cathepsin splits corticotropin-A after leucine in position 31, phenylalanine in position 35 without loss of physiological activity. Thus bovine liver cathepsin appears not to be responsible for the destruction of ACTH by extracts of liver. In its action on corticotropin-A, fibrinolysin resembles trypsin and liver cathepsin resembles pepsin, although in both cases the tissue enzyme has fewer points of attack than the gastro-intestinal enzyme.

Introduction

It has been shown that both blood[1] and extracts of liver[2] are capable of inactivating ACTH *in vitro*. In the case of both tissues, there is accessory evidence supporting the conjecture that inactivation might be due to proteolytic action.[3,4] The availability of purified fibrinolysin from bovine blood and of purified cathepsin from bovine liver has made it possible to study two new proteolytic agents for their effect on corticotropin-A. In addition to clarifying the relationship of the purified enzymes to the crude ACTH-destroying systems, this study has provided data concerning the specificity of the two enzymes.

Preparations. Corticotropin-A.—This material was prepared from porcine oxycellulose ACTH by chromatography on finely divided carboxylic-type ion-exchange resin using a slight modification of the process previously described.[5] By the use of a slightly lower *p*H (8.35 *vs.* 8.50), chromatograms resembling those of Dixon and Stack-Dunne[6] were obtained in which the major active peak was well separated from the other two active peaks and the unretarded inert peak. On countercurrent distribution of the major peak in the 2-butanol:0.2% aqueous trichloroacetic acid system,[7] little or no impurity was revealed and therefore the chromatographic fraction was used without further purification. The activity of such materials is 100–125 units per mg. when administered intravenously and 200–250 units per mg. when administered subcutaneously. All assays are done by the U. S. P. method.[8]

Fibrinolysin.—The preparation used was No. R_x 0345, obtained from Dr. Eugene C. Loomis of Parke Davis & Company, Detroit, Michigan. The proteolytic activity was approximately one casein unit per mg. nitrogen.[9]

Liver Cathepsin.—This enzyme was obtained from Dr. Kenneth C. Robbins of The Armour Laboratories and was prepared from bovine liver by means of a five-step procedure using the conventional alcohol precipitation technique.[10] The purification was followed by means of the proteolytic assay of Anson[11] and the final product has an activity of 0.39 mg. of liberated tyrosine per mg. dry weight. The activity against hemoglobin was not influenced by cysteine and the enzyme has no appreciable activity against the synthetic substrates for trypsin, chymotrypsin or pepsin.

Methods.—Small scale chromatography was done on Whatman #1 paper and large scale chromatographic separations were made on washed[12] Whatman #3 paper. Solvent systems used were the *n*-butanol:acetic acid:water (4:5:1) system of Partridge and the 2-butanol:aqueous ammonia (3:1) system.[13] Amino acid assays were done chromatographically by the technique of Roland and Gross.[14] Paper electrophoresis was done with the apparatus of Durrum.[15] N-Terminal determinations with dinitrofluorobenzene (DNFB) were carried out according to Sanger and Thompson[16] and the dinitrophenylated amino acids (DNP-amino acids) were identified by the two-dimensional chromatographic method of Levy.[17] The carboxypeptidase used for C-terminal work was a commercial 6 times crystallized product (Armour Laboratories) and the amino-peptidase used for N-terminal work was a purified intestinal prepara-

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