The acid-catalyzed cyclization of pseudosapogenins to neosapogenins and to sapogenins in terms of structures I, II, III and V is of interest mechanistically. The axial methyl group is energetically acceptable in the formation of the thermodynamically stable I by *trans*-ring closure of pseudosarsasapogenin. In the smilagenin series, however, ring-closure to the kinetically-favored (and thermodynamically-unfavored) spiroketal apparently proceeds in the *cis* fashion to V (equatorial methyl) because of a higher transition energy to the *trans*-product IV (axial methyl). The steric and mechanistic complexities of this system are clearly in need of further study.

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[Contribution from the Lederle Laboratories Division, American Cyanamid Co.]

16-Hydroxylated Steroids. VI.[1] The Synthesis of the 16α-Hydroxy Derivatives of 9α-Substituted Steroids

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The synthesis of 9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (triamcinolone) (VIIIh) and other 9α-substituted-16α-hydroxy-steroids is described.

In an earlier communication[2] from this Laboratory, there was announced the synthesis of the 16α-hydroxy analogs of the interesting 9α-halo-corticoids. It is the purpose of this paper to expand upon this previous report.

Biological studies on 16α-hydroxyhydrocortisone (IIIa)[3,16] have demonstrated that this type of corticoid derivative still maintains a considerable activity in the usual types of assays (glycogen deposition, thymus involution and anti-inflammatory tests). It was, therefore, of interest to prepare the 16α-hydroxy derivatives of the more potent 9α-halo-steroids.[4]

Treatment of 21-acetoxy-4,9(11),16-pregnatriene-3,20-dione (I)[5] with osmium tetroxide in benzene and pyridine according to the procedure previously described[3] furnished 21-acetoxy-16α,17α-dihydroxy-4,9(11)-pregnadiene-3,20-dione (IIa). The conversion of I to IIa was also accomplished with potassium permanganate in acetone and a small amount of acetic acid according to the method of Petrow and co-workers.[6] Acetylation of IIa under mild conditions afforded the 4,9(11)-diene-16,-21-diacetate IIb. The method of Fried[4a,1] was then adapted for the introduction of the ring C substituents. The diene 16,21-diacetate IIb was treated with N-bromoacetamide and 10% perchloric acid in dioxane to give 16α,21-diacetoxy-9α - bromo - 11β,17α - dihydroxy - 4 - pregnene-3,20-dione (IIId). Reaction of the latter with anhydrous potassium acetate followed by reacetylation afforded 16α,21-diacetoxy-9β,11β-epoxy-17α-hydroxy-4-pregnene-3,20-dione (IVa). Prolonged treatment of the bromohydrin IIId with potassium acetate, or formation of the epoxide with potassium hydroxide in methanol, simultaneously deacetylated the product to furnish the epoxy 16α,-17α,21-triol IVb.

Addition of a saturated solution of hydrogen chloride in chloroform to a solution of the epoxy 16,21-diacetate IVa in chloroform yielded 16α,21-diacetoxy - 9α - chloro - 11β,17α - dihydroxy - 4-pregnene-3,20-dione (IIIe). Treatment of the latter with sodium methoxide in methanol gave 9α-chloro - 11β,16α,17α,21 - tetrahydroxy - 4 - pregnene-3,20-dione (IIIf) with an unexpectedly high melting point. Oxidation of the chlorohydrin 16,21-diacetate IIIe with a chromic anhydride–pyridine mixture[7] gave 16α,21-diacetoxy-9α-chloro-17α-hydroxy-4-pregnene-3,11,20-trione (VIa).

In a similar fashion reaction of the epoxy 16,21-diacetate IIIa with hydrogen fluoride in alcohol-free chloroform[8] afforded 16α,21-diacetoxy-9α-fluoro - 11β,17α - dihydroxy - 4 - pregnene - 3,20-dione (IIIg) in 33% yield. The free steroid, 9α-fluoro - 11β,16α,17α,21 - tetrahydroxy - 4 - pregnene-3,20-dione (9α-fluoro-16α-hydroxy-hydrocortisone (IIIh)), was obtained on hydrolysis with sodium methoxide in methanol. Oxidation of the fluorohydrin 16,21-diacetate IIIg in the manner described above yielded 16α,21-diacetoxy-9α-fluoro-17α-hydroxy-4-pregnene-3,11,20-trione (VIb).

Treatment of the fluorohydrin 16,21-diacetate IIIg with 2,4-dinitrophenylhydrazine in the usual manner furnished the expected mono-3-(2,4-di-

(1) Paper V, S. Bernstein, M. Heller, R. Littell, S. M. Stolar, R. H. Lenhard and W. S. Allen, This Journal, **79**, 4555 (1957).

(2) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, *ibid.*, **78**, 5693 (1956).

(3) W. S. Allen and S. Bernstein, *ibid.*, **78**, 1909 (1956).

(4) (a) J. Fried and E. F. Sabo, *ibid.*, **75**, 2273 (1953); (b) **76**, 1455 (1954); (c) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, *ibid.*, **77**, 4181 (1955); (d) R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett and M. Tishler, *ibid.*, **77**, 3166 (1955); (e) A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, *ibid.*, **77**, 4184 (1955); (f) J. A. Hogg, F. H. Lincoln, A. H. Nathan, A. R. Hanze, W. P. Schneider, P. F. Beal and J. Korman, *ibid.*, **77**, 4438 (1955); (g) E. Vischer, Ch. Meystre and A. Wettstein, *Helv. Chim. Acta*, **38**, 1502 (1955); (h) R. F. Hirschmann, R. Miller, J. Wood and R. E. Jones, This Journal, **78**, 4956 (1956); (i) J. Fried and E. F. Sabo, *ibid.*, **79**, 1130 (1957).

(5) W. S. Allen and S. Bernstein, *ibid.*, **77**, 1028 (1955).

(6) G. Cooley, B. Ellis, F. Hartly and V. Petrow, *J. Chem. Soc.*, 4373 (1955)

(7) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, This Journal, **75**, 422 (1953).

(8) Addition of tetrahydrofuran as an organic base according to the method of R. F. Hirschmann and co-workers[4h] increased the yield to 75–80%. We wish to thank Dr. S. Fox and M. Blicharz of the Lederle Laboratories Division for this information.

nitrophenylhydrazone) derivative IIIi. That the *cis*-glycol system was present was confirmed by the ready formation of an acetonide XI,[6,15] which gave a positive Blue Tetrazolium color test for the ketol side-chain. Acylation of the fluorohydrin IIIh with a more highly substituted acid chloride such as *t*-butylacetyl chloride gave as the only isolated product a mono-*t*-butylacetate V formed at the primary C21-hydroxyl group.

The epoxy 16,21-diacetate IVa was also cleaved with perchloric acid in aqueous dioxane[9] to form the 9α,11β,17α-triol 16,21-diacetate IIIj. Saponification gave 9α,11β,16α,17α,21-pentahydroxy-4-pregnene-3,20-dione (IIIk). Compound IIIj was also oxidized to afford 16α,21-diacetoxy-9α,17α-dihydroxy-4-pregnene-3,11,20-trione (VIc). Fission of the epoxy 16,21-diacetate IVa with methanol or ethanol in the presence of perchloric acid afforded the 9α-methoxy-16,21-diacetate IIIl[10] or the 9α-ethoxy 16,21-diacetate IIIm.

In view of the potentiation of glucocorticoid activity provided by introduction of a Δ^1-double bond in cortisone and hydrocortisone[4e,11] and also in 9α-halo-steroids[4c-g] it was felt desirable to prepare the Δ^1-analogs of the most potent glucocorticoids which contain both the 9α-halo atom and the 16α-hydroxyl group.

Microbiological dehydrogenation of 16α,21-diacetoxy - 9α - fluoro - 11β,17α - dihydroxy - 4 - pregnene-3,20-dione (IIIg) with *Corynebacterium simplex*[4e] or *Nocardia corallina* furnished, after reacetylation, the highly solvated 16α,21-diacetoxy-9α - fluoro - 11β,17α - dihydroxy - 1,4 - pregnadiene-3,20-dione (VIIIg). This reaction could also be effected by selenium dioxide dehydrogenation[12] of IIIg in *t*-butyl alcohol and acetic acid. Saponification of the 1,4-diene 16,21-diacetate VIIIg with potassium hydroxide in methanol afforded 9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (9α-fluoro-16α-hydroxyprednisolone) (triamcinolone, VIIIh).[13,14] Reacetylation of the latter gave the 16,21-diacetate VIIIg (highly solvated as previously mentioned).

The 1,4-diene-16,21-diacetate VIIIg was oxidized to the 11-carbonyl 16,21-diacetate Xc with chromium trioxide–pyridine, and also formed the mono-3-(2,4-dinitrophenylhydrazone)VIIIi. The ultraviolet and infrared absorption spectra of the latter further confirmed the presence of the $\Delta^{1,4}$-3-one moiety in triamcinolone. Formation of the acetonide XII[15] of triamcinolone (VIIIh) which gave a positive Blue Tetrazolium test for C17-ketol grouping further supported the assigned structure.

An alternate pathway to the synthesis of 16α-hydroxy-9α-substituted-prednisolones was desired. The starting material for this pathway was 11β,16α,17α,21 - tetrahydroxy - 4 - pregnene - 3,20-dione (16α-hydroxyhydrocortisone) (IIIa).[3,16] Microbiological dehydration of the latter with *Nocardia corallina* or *Corynebacterium simplex* afforded 11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (16α-hydroxy-prednisolone) (VIIIa). The 16,21-diacetate VIIIb was obtained on acetylation. The latter was also prepared in low yield by selenium dioxide dehydrogenation of 16α,21 - diacetoxy - 11β,17α - dihydroxy - 4 - pregnene-3,20-dione (IIIb) according to the method of Ringold and co-workers.[17] Oxidation of VIIIb gave 16α,21-diacetoxy-17α-hydroxy-1,4-pregnadiene-3,11,20-trione (16,21-diacetate of 16α-hydroxyprednisone) (Xa).

Formation of the mono-3-(2,4-dinitrophenylhydrazone) VIIIc of 16α,21-diacetoxy-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIIb) and its comparison by ultraviolet and infrared spectral analysis with the mono-3-(2,4-dinitrophenylhydrazone) IIIc of 16α,21-diacetoxy-11β,17α-dihydroxy-4-pregnene-3,20-dione (IIIb) further confirmed the presence of the $\Delta^{1,4}$-3-one moiety.

Treatment of 16α,21-diacetoxy-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIIb) with thionyl chloride in pyridine resulted in the selective dehydration of the 11β-hydroxyl group to afford 16α,21 - diacetoxy - 17α - hydroxy - 1,4,9(11)-pregnatriene-3,20-dione (VII). Addition of the elements of hypobromous acid in the usual manner gave the amorphous bromohydrin VIIId. Treatment of the latter with potassium acetate in ethanol yielded 16α,21-diacetoxy-9β,11β-epoxy-17α-hydroxy-1,4-pregnadiene-3,20-dione (IX). Oxide opening with hydrogen chloride gave 16α,21-diacetoxy - 9α - chloro - 11β,17α - dihydroxy - 1,4-pregnadiene-3,20-dione (VIIIe). Saponification gave the free steroid, 9α-chloro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (VIIIf).[18] Oxidation of the chlorohydrin diacetate VIIIe afforded 16α,21-diacetoxy-9α-chloro-17α-hydroxy-

(9) The preparation of 9α-hydroxy-steroids by essentially this procedure was first described by (a) N. L. Wendler, R. P. Graber, C. S. Snoddy, Jr., and F. W. Bollinger, THIS JOURNAL, **79**, 4476 (1957), and by (b) J. Fried and E. F. Sabo, *ibid.*, **79**, 1130 (1957).

(10) The 9α-methoxy 16,21-diacetate IIIl was solvated and could not be brought to analytical purity. Reference 9b first reported the preparation of 9α-alkoxy steroids by this procedure.

(11) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science*, **121**, 176 (1955).

(12) (a) C. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); (b) S. Szpilfogel, T. Posthumus, M. De Winter and D. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956).

(13) Since our original publication it has been shown by R. W. Thoma, J. Fried, S. Bonnano and P. Grabowich, THIS JOURNAL, **79**, 4818 (1957), that 9α-fluorohydrocortisone may be microbiologically hydroxylated with *Streptomyces roseochromogenus* to form 16α-hydroxy-9α-fluorohydrocortisone (IIIh) which was further converted microbiologically to triamcinolone (VIIIh). The latter was also prepared microbiologically by hydroxylation of 9α-fluoro-prednisolone. The Squibb work has been subsequently confirmed in these laboratories.

(14) This compound has been assigned the generic name triamcinolone. The registered trademark of the American Cyanamid Co. for triamcinolone is Aristocort.

(15) J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, THIS JOURNAL, **80**, 2338 (1958), have recently reported on the preparation and biological activities of triamcinolone acetonide (XII) and related compounds. The potentiation of the glucocorticoid activity of 9α-halo-16α-hydroxycorticoids by acetonide formation has been confirmed in these laboratories.

(16) In the liver glycogen deposition assay in adrenalectomized rats 16α-hydroxyhydrocortisone (IIIa) was active but less than that of hydrocortisone. In the same assay 16α-hydroxyprednisolone (VIIIa) had an activity of two times that of hydrocortisone. Neither IIIa nor VIIIa in the electrolyte assay display any sodium-retaining properties. We wish to thank Drs. P. H. Bell, F. I. Dessau and I. Ringler and their associates (Experimental Therapeutics Research Section of these laboratories) for the biological assays.

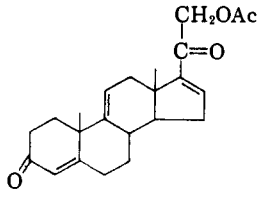
(17) H. Ringold, G. Rosenkranz and F. Sondheimer, *J. Org. Chem.*, **21**, 239 (1956).

(18) It is interesting to note that preliminary attempts to prepare 9α - chloro - 11β,16α,17α,21 - tetrahydroxy - 1,4 - pregnadiene - 3,20-dione (VIIIf) by microbiological dehydrogenation of the 9α-chloro-Δ^4-3-one IIIe were unsuccessful.

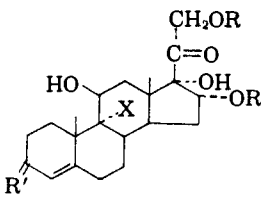
1,4-pregnadiene-3,11,20-trione (Xb). Treatment of the epoxy 16,21-diacetate IX with hydrogen fluoride gave the 16,21-diacetate VIIIg of triamcinolone identical with the material described above.

The biological activities of many of the compounds described above will be published elsewhere by the Experimental Therapeutics Section of these laboratories.

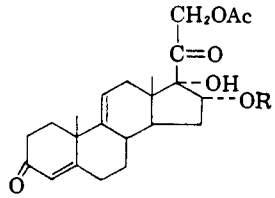
Acknowledgment.—We wish to thank Louis M. Brancone and associates for the analyses, and William Fulmor and associates for the infrared and ultraviolet absorption spectra and optical rotation data. We further wish to express our appreciation to Charles Pidacks and his co-workers for advice and help in developing the partition chromatography systems.



I



IIIa, X = H; R = H; R′ = O
IIIb, X = H; R = Ac; R′ = O
IIIc, X = H; R = Ac; R′ = DNP[a]
IIId, X = Br; R = Ac; R′ = O
IIIe, X = Cl; R = Ac; R′ = O
IIIf, X = Cl; R = H; R′ = O
IIIg, X = F; R = Ac; R′ = O
IIIh, X = F; R = H; R′ = O
IIIi, X = F; R = Ac; R′ = DNP[a]
IIIj, X = OH; R = Ac; R′ = O
IIIk, X = OH; R = H; R′ = O
IIIl, X = OCH_3; R = Ac; R′ = O
IIIm, X = OC_2H_5; R = Ac; R′ = O



IIa, R = H
IIb, R = Ac

Experimental

Melting Points.—All melting points are uncorrected, and were determined with uncalibrated Anschütz thermometers unless otherwise noted.

Optical Rotations.—The rotations are for chloroform solutions unless otherwise noted.

Absorption Spectra.—The ultraviolet absorption spectra were determined in absolute alcohol unless otherwise noted. The infrared absorption spectra were determined in potassium bromide.

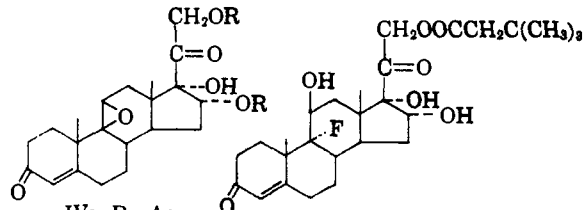
Petroleum Ether.—The fraction used, unless otherwise specified, had a b.p. 60–70° (Skellysolve B).

All evaporations were carried out under reduced pressure.

21-Acetoxy-16α,17α-dihydroxy-4,9(11)-pregnadiene-3,20-dione (IIa). A.—A solution of 0.70 g. of 21-acetoxy-4,9(11),16-pregnatriene-3,20-dione (I) and 0.50 g. of osmium tetroxide in 10 ml. of benzene and 0.5 ml. of pyridine was allowed to stand at room temperature for 18 hours. The osmate ester was decomposed by the addition of 35 ml. of water, 10 ml. of benzene, 23 ml. of methanol and 3.58 g. each of sodium sulfite and potassium bicarbonate. After the mixture was stirred for 5 hours, approximately 150 ml. of chloroform was added and the stirring was continued for 0.5 hour.[19] The mixture was filtered through a diatomaceous silica product, the residue washed with hot chloroform and the organic layer separated. The aqueous phase was extracted several times with chloroform, and the combined extracts were washed with saturated saline and with water. The dried extract was evaporated and the residue was crystallized from acetone–petroleum ether to give 0.62 g of crude product, m.p. 172–174° with previous softening. Three recrystallizations from acetone–petroleum ether gave 0.42 g. of pure IIa, m.p. 195–197.5° with previous softening. One additional recrystallization did not alter the melting point; λ_{max} 238.5 mμ (ε 16,700); ν_{max} 3425, 1751(shoulder), 1732, 1675, 1626(shoulder) and 1239 cm.$^{-1}$; $[\alpha]^{25}D$ + 93° (*c* 0.637).

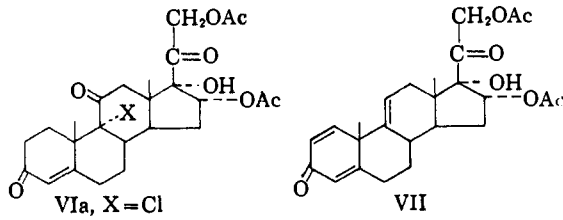
Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.72; H, 7.79.

B.—To a stirred, ice-cold solution of 1 g. of I in 28.5 ml. of acetone and 0.285 ml. of acetic acid was added a solution of 460 mg. of potassium permanganate in 85% aqueous acetone over a 15-minute period. Sulfur dioxide was then bubbled through the mixture until the brown color changed. The resultant light yellow solution was filtered and evaporated at room temperature until most of the acetone was removed. The mixture was extracted with ethyl acetate, dried, treated



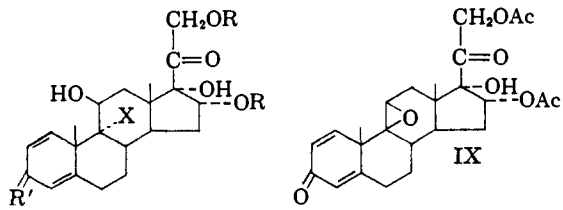
IVa, R = Ac
IVb, R = H

V



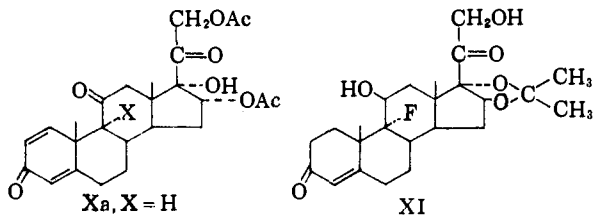
VIa, X = Cl
VIb, X = F
VIc, X = OH

VII



VIIIa, X = H; R = H; R′ = O
VIIIb, X = H; R = Ac; R′ = O
VIIIc, X = H; R = Ac; R′ = DNP[a]
VIIId, X = Br; R = Ac; R′ = O
VIIIe, X = Cl; R = Ac; R′ = O
VIIIf, X = Cl; R = H; R′ = O
VIIIg, X = F; R = Ac; R′ = O
VIIIh, X = F; R = H; R′ = O
VIIIi, X = F; R = Ac; R′ = DNP[a]

IX



Xa, X = H
Xb, X = Cl
Xc, X = F

XI

[a] DNP = N–NH– (2,4-dinitrophenyl)

XII

(19) It was found in subsequent runs that treatment with osmium tetroxide for 0.5 to 1 hour followed by decomposition for 3 hours gave more consistent results and material that was more easily worked up.

with activated carbon and evaporated to dryness. Two crystallizations from acetone–petroleum ether gave 0.321 g. of IIa, m.p. 190–192.5°.[20] The infrared absorption spectrum was identical to that of the sample described in A.

16α,21-Diacetoxy-17α-hydroxy-4,9(11)-pregnadiene-3,20-dione (IIb).—To a solution of 325 mg. of IIa in 20 ml. of pyridine was added 2 ml. of acetic anhydride and the mixture was allowed to stand at room temperature for 3 days. The solution was poured into water, cooled and the product was collected by filtration and washed with water to give 0.33 g. of practically pure IIb, m.p. 193–195° with previous softening. Two crystallizations from acetone–petroleum ether gave 0.28 g. of pure 4,9(11)-diene-16,21-diacetate, m.p. 194–195° with previous softening, λ_{max} 238–239 mμ (ε 17,400); ν_{max} 3571, 1751, 1684, 1634 and 1241 cm.$^{-1}$; $[\alpha]^{25}D$ + 43° (*c* 0.635).

Anal. Calcd. for $C_{25}H_{32}O_7$ (444.51): C, 67.55; H, 7.26. Found: C, 67.31; H, 7.49.

16α,21-Diacetoxy-9α-bromo-11β-17α-dihydroxy-4-pregnene-3,20-dione (IIId). A.—The 4,9(11)-diene 16,21-diacetate (IIb, 0.50 g.) in dioxane (20 ml.) and water (4 ml.) was treated with N-bromoacetamide (0.31 g.) and 10% perchloric acid (2 ml.). The mixture was allowed to stand for 15 minutes at 20° when an excess of saturated sodium sulfite solution and water was added. The resultant soft solid was extracted with chloroform, and the extract was washed with saturated saline, dried, filtered through a diatomaceous silica product and evaporated to afford a glass. Crystallization from acetone–petroleum ether gave 0.45 g. of crude bromohydrin IIId, m.p. 130–133.5° dec. with previous browning. Three crystallizations from acetone–petroleum ether gave 0.31 g. of the analytical sample, m.p. 125–126° dec. with previous browning, λ_{max} 243 mμ (ε 14,100), ν_{max} 3546, 1751, 1715, 1675, 1626 and 1236 cm.$^{-1}$; $[\alpha]^{25}D$ + 76° (*c* 0.818).

Anal. Calcd. for $C_{25}H_{33}O_8Br$ (541.43): C, 55.45; H, 6.14; Br, 14.76. Found: C, 55.73; H, 6.54; Br, 14.51.

B.—In another run with 25.0 g. of IIb, 9.7 g. of N-bromoacetamide, 500 ml. of dioxane, 100 ml. of water and 46 ml. of 10% perchloric acid there was obtained 25.4 g. of IIId, m.p. 122–125° dec. with previous browning.

16α,21-Diacetoxy-9β,11β-epoxy-17α-hydroxy-4-pregnene-3,20-dione (IVa). A.—A solution of 49.6 g. of IIId and 11.3 g. of anhydrous potassium acetate in 3.1 l. of absolute alcohol was refluxed for 19 hours. The reaction mixture was evaporated and the residue was dissolved in ethyl acetate and water. The aqueous phase was extracted with ethyl acetate and the combined extracts were washed with saturated sodium bicarbonate solution and saline. The dried extract was evaporated, and the residue was dissolved in 50 ml. of pyridine, treated with 25 ml. of acetic anhydride and allowed to stand at room temperature for 16 hours. Methanol and benzene were added and the solution was evaporated to dryness. Crystallization of the residue from methanol gave 28.6 g. of crude epoxide IVa, m.p. 177–194° with previous softening. This material was suitable for use in further transformations.

B.—In another run, the product obtained by essentially the above procedure was purified for characterization; m.p. 191.5–193.5° with previous softening (recrystallized from ether), λ_{max} 243–243.5 mμ (ε 15,000); ν_{max} 3571, 3436, 1757, 1745(shoulder), 1673, 1626 and 1239 cm.$^{-1}$; $[\alpha]^{25}D$ −48° (*c* 0.740).

Anal. Calcd. for $C_{25}H_{32}O_8$ (460.51): C, 65.20; H, 7.00. Found: C, 65.00; H, 7.32.

9β,11β-Epoxy-16α,17α,21-trihydroxy-4-pregnene-3,20-dione (IVb). A.—In an initial attempt to prepare the epoxy 16,21-diacetate IVa, a solution of 0.78 g. of IIId and 0.78 g. of anhydrous potassium acetate in 150 ml. of absolute alcohol was refluxed for 53 hours. The reaction mixture was worked up as above except that the residue was not acetylated. Crystallization of the glassy residue from acetone–petroleum ether afforded 0.12 g. of the crude triol, m.p. 187–193.5° with previous softening. Three recrystallizations from acetone–petroleum ether gave 74 mg. of IVb, m.p. 206–209° with previous softening. One additional crystallization from the same solvents did not alter the melting point (compound may be solvated with acetone); λ_{max} 243.5 mμ (ε 15,000 or 17,200)[21]; ν_{max} 3520, 3265, 1728, 1676 and 1628 cm.$^{-1}$; $[\alpha]^{24}D$ − 44° (*c* 0.750, pyridine).

Anal. Calcd. for $C_{21}H_{28}O_6$ (376.44): C, 67.00; H, 7.50. Calcd. for $C_{21}H_{28}O_6 \cdot CH_3COCH_3$ (434.51): C, 66.34; H, 7.89. Found: C, 66.36, 66.48; H, 7.70; 7.91.

Acetylation of the main fraction and the combined mother liquors above gave in both cases the epoxy 16,21-diacetate IVa as shown by melting point, ultraviolet and infrared absorption spectra.

B.—The bromohydrin diacetate (IIId, 1.0 g.) in 50 ml. of methanol was cooled to 0° and treated with a solution of 312 mg. of potassium hydroxide in 0.5 ml. of water and 9.5 ml. of methanol. After standing at room temperature for 1 hour, the solution was acidified with glacial acetic acid and evaporated to a white solid. The solid was triturated with saturated ammonium chloride solution, cooled, filtered and washed with water to afford 0.33 g. of the epoxy 16α,17α,21-triol IVb, m.p. 207–210° with previous softening. Recrystallization from acetone (petroleum ether wash) gave 271 mg. of pure IVb, m.p. 215–217° with previous softening (bubbles in the melt with clearing at 222°). One additional crystallization from acetone (petroleum ether wash) did not alter the melting point; negative Beilstein test for bromine; λ_{max} 244–244.5 mμ (ε 13,400): infrared spectrum identical with that of sample prepared in A above; $[\alpha]^{25}D$ −60° (*c* 0.828, pyridine).

Anal. Found: C, 66.80, 66.56; H, 7.71, 7.74.

16α,21-Diacetoxy-9α-chloro-11β,17α-dihydroxy-4-pregnene-3,20-dione (IIIe).—A solution of the epoxy 16,21-diacetate IVa (100 mg.) in alcohol-free chloroform (2 ml.) was treated with a saturated solution of anhydrous hydrogen chloride in alcohol-free chloroform (10 ml.). After standing at 0° for 4.5 hours, the yellow solution was evaporated to dryness at 0°. The residue was dissolved in acetone, treated with activated carbon and crystallized from acetone–petroleum ether to give 99 mg. of product, m.p. 211.5–213.5°. Three crystallizations from acetone–petroleum ether gave 80 mg. of pure chlorohydrin IIIe, m.p. 214.5–215.5°, λ_{max} 240.5 mμ (ε 15,800); ν_{max} 3604, 3521(shoulder), 1767, 1751(shoulder), 1727(shoulder), 1684, 1637, 1252 and 1242 cm.$^{-1}$; $[\alpha]^{25}D$ +76° (*c* 0.735).

Anal. Calcd. for $C_{25}H_{33}O_8Cl$ (496.97): C, 60.42; H, 6.69; Cl, 7.14. Found: C, 60.65; H, 6.80; Cl, 7.34.

9α-Chloro-11β,16α,17α,21-tetrahydroxy-4-pregnene-3,20-dione (IIIf).—A solution of 144 mg. of the chlorohydrin 16,21-diacetate (IIIe) in 10 ml. of dry methanol was treated with 5 ml. of methanol containing 14 mg. of sodium. Dry nitrogen was bubbled through both solutions for 5 minutes before the addition of the sodium methoxide. The reaction was allowed to proceed at room temperature for 10 minutes, and then was neutralized with 0.05 ml. of glacial acetic acid. By concentration of the reaction mixture to about 10 ml., 46 mg. of crystalline material which did not melt at 300° was obtained. The addition of water to the mother liquor gave a poor-looking solid; both solid and mother liquor were combined, and after addition of saturated saline solution to the mixture, extraction was attempted successively with ethyl acetate, ether, benzene, chloroform and methylene chloride. All of the solvents tried proved unsuitable. The organic extracts were separated and the aqueous phase was filtered to afford 50 mg. of solid which was added to the combined extracts. After evaporation the residue was dissolved in a large amount of boiling acetone (a small amount of material which was also insoluble in water was removed by filtration). Concentration of the acetone gave 37 mg. of the tetrol; darkened but did not melt at 360°. Crystallization from methanol gave 24 mg. of IIIf; began to darken at 190°, but did not melt at 400°; λ_{max} 240–240.5 mμ (ε 15,900); ν_{max} 3425, 1709, 1661 and 1626 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{29}O_6Cl$(412.90): C, 61.08; H, 7.08; Cl, 8.59. Found: C, 61.48; H, 7.22; Cl, 8.29.

16α,21-Diacetoxy-9α-chloro-17α-hydroxy-4-pregnene-3,11,-20-trione (VIa).—A solution of the chlorohydrin 16,21-diacetate (IIIe, 300 mg.) in cold pyridine (6 ml.) was added to a slurry of chromic anhydride (215 mg.) in pyridine (3 ml.). After standing at room temperature (26°) for 17 hours, the

(20) A revised procedure which included addition of permanganate solution in 1.5 minutes, reduction of the resultant mixture with sodium bisulfite and elimination of the final extraction by filtration of the final solid gave yields of 60–65% of material suitable for further transformations. We wish to thank Dr. L. Smith and M. Marx of the Lederle Laboratories Division for this information.

(21) Calculated on basis of one mole of acetone per mole of compound.

solution was poured into ice-water and the precipitate washed well with water. The precipitate was heated in ethyl acetate and the inorganic residue removed by filtration. The filtrate was washed with saturated saline, dried and evaporated to afford a white crystalline solid. Crystallization from acetone–petroleum ether gave 168 mg. of product, m.p. 224–227.5°, brown melt with previous softening. Recrystallization from acetone–petroleum ether afforded 161 mg. of the 3,11,20-trione with unaltered melting point, λ_{max} 235.5 mμ (ε 14,500). Two further recrystallizations from acetone–petroleum ether of a 145-mg. portion of the latter gave 129 mg. of pure VIa, m.p. 226.5–229.5°, brown melt with previous softening, λ_{max} 235.5 mμ (ε 15,400); ν_{max} 3450, 1742, 1725 (shoulder), 1674, 1622 and 1235 cm.$^{-1}$; $[\alpha]^{25}D$ +165° (*c* 0.623).

Anal. Calcd. for $C_{25}H_{31}O_8Cl$(494.96): C, 60.66; H, 6.31; Cl, 7.16. Found: C, 60.63; H, 6.07; Cl, 6.82.

16α,21-Diacetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (IIIg).—A solution of 360 mg. of the epoxy-16,21-diacetate IVa in 25 ml. of chloroform (alcohol-free) was treated with approximately 1 ml. of anhydrous hydrogen fluoride and allowed to stand (with occasional agitation) at about −10° for 2 hours. The reaction mixture was poured into ice-water, neutralized with saturated sodium bicarbonate solution and extracted with chloroform. The washed and dried extract was evaporated and the residue was dissolved in 5 ml. of pyridine, treated with 2.5 ml. of acetic anhydride and allowed to stand at room temperature overnight. Methanol and benzene were added and the solution was evaporated to a glass. Crystallization of the residue from acetone–petroleum ether gave 146 mg. of crude fluorohydrin. Recrystallization from acetone–petroleum ether afforded 123 mg. of IIIg, m.p. 231.5–235.5° (Köfler hot-stage). A 62-mg. portion of the latter was recrystallized from acetone–petroleum ether to give 59 mg. of pure product, m.p. 230.5–234.5° (Köfler hot-stage), 231.5–237.5° (capillary). The long colorless needles as obtained were solvated. The analytical sample when dried for 16 hours over refluxing xylene melted at 237–239° with previous softening (capillary), and there was about a 10% loss in weight; λ_{max} 237.5–238.5 mμ (ε 17,600); ν_{max} 3675, 3495, 1750, 1680, 1640-(shoulder) and 1245 cm.$^{-1}$; $[\alpha]^{24}D$ + 70° (*c* 0.517).

Anal. Calcd. for $C_{25}H_{33}O_8F$ (480.51): C, 62.49; H, 6.92; F, 3.95. Found: C, 62.71; H, 7.06; F, 3.63.

C-3-(2,4-Dinitrophenylhydrazone) IIIi, m.p. 239.5–242°; $\lambda_{max}^{chloroform}$ 261, 292 and 391 mμ (ε 16,200, 11,100 and 29,800, respectively).

Anal. Calcd. for $C_{31}H_{37}N_4O_{11}F$ (660.64): C, 56.36; H, 5.65; N, 8.48. Found: C, 55.88; H, 6.13; N, 7.41.

9α-Fluoro-11β,16α,17α,21-tetrahydroxy-4-pregnene-3,20-dione (IIIh).—Dry nitrogen was bubbled for 15 minutes through a solution of 150 mg. of IIIg in 20 ml. of methanol and through 10 ml. of methanol containing 14 mg. of sodium. The sodium methoxide solution was then introduced to the steroid solution and allowed to remain at room temperature for 10 minutes under nitrogen. After the addition of 0.05 ml. of acetic acid, the reaction mixture was evaporated. The solid residue was triturated with water, collected by filtration and washed with water to afford 85 mg. of the crude tetrol, m.p. 238–244° with previous softening and browning. Two crystallizations from acetone–petroleum ether gave 60 mg. of IIIh, m.p. 257–260°[22] with decomposition and previous browning, λ_{max} 238.5 mμ (ε 16,300); ν_{max} 3440, 1720, 1674 and 1630 cm.$^{-1}$; $[\alpha]^{24}D$ + 91° (*c* 0.570, pyridine).

Anal. Calcd. for $C_{21}H_{29}O_6F$ (396.44): C, 63.62; H, 7.37; F, 4.79. Found: C, 63.47; H, 7.51; F, 4.49.

16α,21-Diacetoxy-9α-fluoro-17α-hydroxy-4-pregnene-3,11-20-trione (VIb).—A solution of the fluorohydrin 16,21-diacetate (IIId, 240 mg.) in cold pyridine (6 ml.) was added to a slurry of chromic anhydride (180 mg.) in pyridine (2.5 ml.). After standing at room temperature (24°) for 20 hours, the solution was worked up as in the preparation of VIa above. The white crystalline solid so obtained was crystallized from acetone–petroleum ether to afford 150 mg. of product, m.p. 221.5–223° with previous softening. Two additional crystallizations from acetone–petroleum ether gave 131 mg. of pure VIb, m.p. 223–224° with previous softening, λ_{max} 234.5 mμ (ε 16,100); ν_{max} 3390, 1735, 1667, 1618, 1240 and 1230 cm.$^{-1}$; $[\alpha]^{25}D$ + 98° (*c* 0.631).

(22) In a larger run, the m.p. was 259–262.5° when the product was crystallized from acetone.

Anal. Calcd. for $C_{25}H_{31}O_8F$ (478.50): C, 62.75; H, 6.53; F, 397. Found: C, 62.81; H, 6.58; F, 4.10.

9α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxy-4-pregnene-3,20-dione (XI).—A solution of IIIh (200 mg.) in 50 ml. of hot acetone was treated with 5 drops of concentrated hydrochloric acid and boiled for 3 minutes. After standing at room temperature for 17 hours, the reaction mixture was poured into dilute sodium bicarbonate and extracted with ethyl acetate. The extract was washed with saturated saline solution, dried and evaporated to a colorless glass. Crystallization of the residue from acetone–petroleum ether gave 152 mg. of product, m.p. 261.5–262.5° dec. with previous softening and browning. Recrystallization from acetone–petroleum ether afforded 145 mg. of pure XI, m.p. 262° dec. with previous softening and browning, λ_{max} 238–239 mμ (ε 16,100); ν_{max} 3448, 1717, 1660 and 1627 cm.$^{-1}$; $[\alpha]^{25}D$ + 144° (*c* 0.848).

Anal. Calcd. for $C_{24}H_{33}O_6F$ (436.50): C, 66.03; H, 7.62; F, 4.35. Found: C, 65.94; H, 7.78; F, 4.49.

21-*t*-Butylacetoxy-9α-fluoro-11β,16α,17α-trihydroxy-4-pregnene-3,20-dione (V).—A solution of 200 mg. of the tetrol IIIh in 2 ml. of pyridine was cooled in an ice-bath and treated dropwise with 1.6 ml. of a 10% solution (v./v.) of *t*-butylacetyl chloride in chloroform. After standing for 22 hours at 0–5°, the reaction mixture was poured into ice-water and extracted with chloroform. The extract was washed with sodium bicarbonate solution and water, dried and evaporated to yield a white solid. Crystallization from acetone-petroleum ether gave 109 mg. of V, m.p. *ca.* 170–190° with previous softening (solvated). Two crystallizations from acetone–petroleum ether afforded 84 mg. of product, m.p. *ca.* 180–197° with previous softening (solvated), λ_{max} 238.5 mμ (ε 16,100); ν_{max} 3375, 1725, 1710, 1660 and 1230 cm.$^{-1}$; $[\alpha]^{25}D$ + 102° (*c* 0.482).

Anal. Calcd. for $C_{27}H_{39}O_7F$(494.58): C, 65.56; H, 7.95. Calcd. for $C_{33}H_{49}O_8F$ (595.72): C, 66.87; H, 8.33. Found: C, 65.93; H, 8.19.

16α,21-Diacetoxy-9α,11β,17α,-trihydroxy-4-pregnene-3,-20-dione (IIIj).—The epoxy 16,21-diacetate (IVa, 0.80 g.) in dioxane (8 ml.) and water (1.6 ml.) was treated with 3 *N* perchloric acid (3 ml.). After standing at 22° for 5 hours, the deep yellow solution was diluted with water and neutralized with sodium bicarbonate. It was then extracted with ethyl acetate and the extract was washed with saturated saline, dried and evaporated to yield a pale yellow glass. The residue was dissolved in 10 ml. of pyridine, treated with 5 ml. of acetic anhydride and allowed to stand at room temperature overnight. Methanol and benzene were added and the solution was evaporated (wt. of residue, 0.82 g.). A solvent system consisting of 3 parts (by vol.) of ethyl acetate, 2 parts of petroleum ether (90–100°), 3 parts of methanol and 2 parts of water was prepared. The residue was shaken with 15 ml. of the lower phase and 30 ml. of the upper phase. The crystalline solid which did not dissolve was removed by filtration and washed with 15 ml. of the upper phase to afford 460 mg. of a single spot product as indicated by paper chromatography, m.p. 174.5–238.5° with previous softening (solvated). Two crystallizations from acetone–petroleum ether gave 438 mg. of pure IIIj, m.p. 233–238.5° with previous softening (solvated, λ_{max} 242 mμ (ε 16,100)); ν_{max} 3480 1742, 1658, 1625 (inflection) and 1240 cm.$^{-1}$; $[\alpha]^{25}D$ +95° (*c* 0.591).

Anal. Calcd. for $C_{25}H_{34}O_9$ (478.52): C, 62.75; H, 7.16. Found: C, 62.92; H, 7.33.

9α,11β,16α,21-Pentahydroxy-4-pregnene-3,20-dione (IIIk).—The pentol diacetate (IIIj, 150 mg.) was treated with sodium methoxide in the same manner as described above in the preparation of IIIh. The residue obtained after evaporation was tested for water solubility and found to be fairly soluble; therefore the organic residue was dissolved in acetone and the insoluble sodium acetate (50 mg.) removed by filtration. Concentration of the filtrate afforded 71 mg. of the crude pentol, m.p. 247.5–251° with decomposition and previous softening. Three recrystallizations from acetone (petroleum ether wash) gave 37 mg. of pure IIIk, m.p. 259–261.5° with decomposition and previous softening, λ_{max} 241–242 mμ (ε 14,300); ν_{max} 3400, 1718, 1660 and 1635 cm.$^{-1}$ (inflection); $[\alpha]^{25}D$ + 113° (*c* 0.784, pyridine).

Anal. Calcd. for $C_{21}H_{30}O_7$ (394.45); C, 63.94; H, 7.66. Found: C, 64.01; H, 7.74.

16α,21-Diacetoxy-11β,17α-dihydroxy-9α-methoxy-4-pregnene-3,20-dione (IIIl).—A solution of the epoxy 16,21-diacetate IVa (250 mg.) in methanol (20 ml.) was treated with 72% perchloric acid (0.15 ml.) and allowed to remain at room temperature (25°) for 4 hours. The solution was then neutralized with sodium bicarbonate and evaporated to dryness. The residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution and saline. The dried extract was evaporated and the residue was acetylated at room temperature with acetic anhydride (1.5 mg.) and pyridine (3 ml.) for 16 hours. Methanol was added and the solution was evaporated. Crystallization of the residue from acetone–petroleum ether gave 107 mg. of solvated product IIIl, m.p. *ca.* 145–180° with effervescence. Recrystallization from acetone–petroleum ether did not alter appreciably the wide melting range, m.p. *ca.* 148–180° with effervescence, λ_{max} 243–244 mμ (ε 15,800); ν_{max} 3450, 1740, 1655 and 1235 cm.$^{-1}$; $[\alpha]^{25}D$ + 90° (*c* 0.522).

Anal. Calcd. for $C_{26}H_{36}O_9$ (492.55): C, 63.40; H, 7.37. Found: C, 61,83, 61.64; H, 7.89, 7.76.

16α,21-Diacetoxy-9α-ethoxy-11β,17α-dihydroxy-4-pregnene-3,20-dione (IIIm).—A solution of the epoxy 16,21-diacetate IVa (240 mg.) in absolute ethanol (20 ml.) was treated with 72% perchloric acid (0.15 ml.) and allowed to remain at room temperature for 4 days. The reaction mixture was worked up as in the preparation of IIIl above. Crystallization of the residue from acetone–petroleum ether afforded 78 mg. of solvated product IIIm, m.p. 166–177° with effervescence. Four crystallizations from acetone–petroleum ether gave 57 mg., m.p. *ca.* 178–197° with effervescence, λ_{max} 243–244 mμ (ε 15,300); ν_{max} 3450, 1740, 1655 and 1235 cm.$^{-1}$; $[\alpha]^{25}D$ + 74° (*c* 0.516).

Anal. Calcd. for $C_{27}H_{38}O_9$ (506.57): C, 64.01; H, 7.56. Found: C, 64.12; H, 7.76.

16α,21-Diacetoxy-9α,17α-dihydroxy-4-pregnene-3,11,20-trione (VIc).—A cold solution of the pentol diacetate (IIIj, 150 mg.) in pyridine (5 ml.) was added to a slurry of chromic anhydride (113 mg.) and cold pyridine (2 ml.). After standing at 18–24° for 17 hours, the reaction mixture was shaken for 1 hour with a solution of sodium sulfite (0.34 g.) in water (4 ml.) and then poured into ice-water. The solution was extracted several times with ethyl acetate and the extract was washed with saturated saline solution, dried and evaporated to a white crystalline solid. Crystallization of the residue from acetone–petroleum ether gave 136 mg. of the crude trione, m.p. 227–251.5° with previous softening. Two recrystallizations from acetone–petroleum ether afforded 128 mg. of pure VIc, m.p. 255.5–257.5° with previous softening, λ_{max} 237 mμ (ε 14,200); ν_{max} 3430, 1750, 1732, 1656 and 1632 (inflection) cm.$^{-1}$; $[\alpha]^{25}_D$ + 122° (*c* 0.335).

Anal. Calcd. for $C_{25}H_{32}O_9$ (476.51): C, 63.01; H, 6.77. Found: C, 63.35; H, 7.11.

16α,21-Diacetoxy-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIIg). **A.**—A three-day old slant of *Nocardia corallina* (ATCC 999) on yeast extract agar (yeast extract [Difco] 0.4%; malt extract [Difco] 1.0%; glucose 0.4%; agar 1.5%) was washed with 7 ml. of sterile 0.9% sodium chloride. The entire suspension was added to 100 ml. of medium #13 (cerelose 1.0%; yeast extract 0.1%; beef extract [Armour] 0.4%; peptone [Bacto] 0.4%; sodium chloride 0.25%; *p*H adjusted to 7.0) in a 500-ml. erlenmeyer flask. The flask was shaken (reciprocating shaker, 120 oscillations/min.) for 8 hours at 37°, at which time 1 ml. of the resulting growth was used to inoculate each of 32 flasks containing 100 ml. of medium #13. Incubation was at 32° on a reciprocating shaker. After 40 hours, 25 mg. of 16α,21-diacetoxy-9α-fluoro-11β,-17α-dihydroxy-4-pregnene-3,20-dione (IIIg) dissolved in 2.5 ml. of ethanol was added to each flask. The flasks were harvested and pooled 53 hours after the addition of steroid when paper chromatography showed a very strong spot of more polar material compared to a weak spot of starting material. The pooled fermentation beers were diluted with a large amount of acetone and filtered through a diatomaceous silica product. After removal of most of the acetone by evaporation, the aqueous phase was saturated with salt and extracted five times with butanol. The combined butanol extracts were evaporated, the residue was dissolved in hot acetone and the mixture was filtered to remove inorganic solids. The filtrate was evaporated and the residue dissolved in 10 ml. of pyridine, treated with 5 ml. of acetic anhydride and allowed to remain at room temperature overnight. Methanol was added and the solvents removed to afford an oil (1.17 g.) which was submitted to partition chromatography on a diatomaceous silica product[23] using a solvent system consisting of 3 parts of ethyl acetate, 2 parts of petroleum ether (b.p. 90–100°), 3 parts of methanol and 2 parts of water. The cut containing the desired product was evaporated and the residue crystallized from acetone-petroleum ether to afford 341 mg. of VIIIg, m.p. 158–200° with previous softening (solvated). An additional 38 mg., m.p. 157–205° with previous softening, was recovered from the mother liquor. A 100-mg. portion of the former was recrystallized from acetone–petroleum ether to give 81 mg. of the solvated product, m.p. 158–235°[24] with previous softening, λ_{max} 239 mμ (ε 15,200); ν_{max} 3390, 1740 (shoulder), 1730, 1660, 1610, 1608 (inflection) and 1235 cm.$^{-1}$; $[\alpha]^{25}D$ + 22° (*c* 0.788).

Anal. Calcd. for $C_{25}H_{31}O_8F$ (478.49): C, 62.75; H, 6.53; F, 3.97. Found: C, 63.45; H, 7.44; F, 4.39.

B.—In another run, 3.0 g. of IIIg was fermented and worked up as in A above to afford, after partition chromatography, 427 mg. of unreacted substrate, m.p. 234.5–239°, and 1.04 g. of product, VIIIg, m.p. 185.5–226° with previous softening. An additional 189 mg. of product, m.p. 181–226° with previous softening, was obtained from the mother liquor.

C.—To a solution of IIIg (1.0 g.) in *t*-butyl alcohol (160 ml.) and glacial acetic acid (1.6 ml.) was added 600 mg. of selenium dioxide. The mixture was placed under a nitrogen atmosphere and heated to 70° for 24 hours. An additional 350 mg. of selenium dioxide was then added and heating at 70° was continued for another 24 hours. The reaction mixture was filtered and the filtrate evaporated. The residue was dissolved in ethyl acetate and washed successively with saturated sodium bicarbonate, water, cold freshly prepared ammonium sulfide solution, cold dilute ammonium hydroxide, cold dilute hydrochloric acid, and finally with water. After being dried and treated with activated carbon, the final solution was evaporated to give 850 mg. of a tan glass. Paper chromatographic analysis showed predominately the desired product plus a small amount of starting material. Partition chromatography of the glass on 320 g. of a diatomaceous silica product[23] using a solvent system consisting of 3 parts of ethyl acetate, 4 parts of petroleum ether (b.p. 90–100°), 3 parts of methanol and 2 parts of water, gave a main fraction in the fifth and sixth hold-back volumes which, upon evaporation and crystallization from acetone–petroleum ether, yielded 173 mg. of VIIIg, m.p. 150–190°. Further crystallization from the same solvent pair gave 134 mg., m.p. 185–189°. Infrared spectroscopic analysis showed identity with an authentic sample.

D.—A solution of 16α,21-diacetoxy-9β,11β-epoxy-17α-hydroxy-1,4-pregnadiene-3,20-dione (IX, 70 mg.) in 25 ml. of alcohol-free chloroform was chilled to 5° and treated with 5 ml. of anhydrous hydrogen fluoride. The mixture was shaken for 20 hours at 5°, poured into ice-water, neutralized with sodium bicarbonate and extracted with chloroform. The washed and dried extract was evaporated and the residue, dissolved in 20 ml. of pyridine, was treated with 4 ml. of acetic anhydride and allowed to stand for 18 hours at room temperature. Methanol and benzene were added and the solution evaporated. The residue was subjected to partition chromatography on a diatomaceous silica product as in example A to give, after crystallization from acetone–petroleum ether, 18.2 mg., m.p. 160–210°. The infrared spectrum was identical to that of an authentic sample.

C-3-(2,4-Dinitrophenylhydrazone) VIIIi, m.p. 258–263° dec. with partial melt at *ca.* 183°; $\lambda_{max}^{chloroform}$ 259, 305 and 398–400 mμ (ε 16,100, 6,600 and 32,400, respectively).

Anal. Calcd. for $C_{31}H_{35}N_4O_{11}F$ (658.62): C, 56.53; H, 5.36; N, 8.51; F, 2.88. Found: C, 57.07; H, 5.68; N, 8.77; F, 3.28.

9α-Fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (VIIIh).—A solution of 100 mg. of VIIIg in 10 ml. of methanol was cooled to 0° and treated with a solution of 35 mg. of potassium hydroxide in 2 ml. of methanol. After standing at room temperature for 1 hour the

(23) For a complete description of this technique see R. Littell and S. Bernstein, THIS JOURNAL, **78**, 984 (1956).

(24) On many occasions the m.p. was about 186–188° with effervescence disappearing at 226°. Crystallization from aqueous acetone or ethyl acetate–petroleum ether (90–100°) did not alter the solvated melt.

solution was acidified with glacial acetic acid and evaporated to afford a white solid. The solid was triturated with water, cooled, filtered and washed with water to afford 52 mg. of the crude tetrol, m.p. 246–249° dec. with previous softening and browning. Three recrystallizations from acetone–petroleum ether gave 29 mg. of VIIIh, m.p. 260–262.5° dec. with previous softening and browning,[25] λ_{max} 238 mμ (ε 15,800); ν_{max} 3388, 1705, 1660, 1620 and 1604 cm.$^{-1}$; $[\alpha]^{25}D$ + 75° (*c* 0.200, acetone).

Anal. Calcd. for $C_{21}H_{27}O_6F$ (394.43): C, 63.94; H, 6.90; F, 4.82. Found: C, 64.19; H, 7.17; F, 4.90.

16α,21-Diacetoxy-9α-fluoro-17α-hydroxy-1,4-pregnadiene-3,11,20-trione (Xc).—A cold solution of the 1,4-diene 16,21-diacetate VIIIg (200 mg.) in pyridine (5 ml.) was added to a slurry of chromic anhydride (150 mg.) and pyridine (2 ml.). After standing at 22–25° for 20 hours, the solution was worked up as in the preparation of VIa above. The white solid so obtained was crystallized from acetone–petroleum ether to give 126 mg. of the trione, m.p. 228.5–233.5° with previous softening. Two additional crystallizations from acetone–petroleum ether afforded 103 mg. of pure Xc, m.p. 232–234° with previous softening, λ_{max} 235 mμ (ε 17,000); ν_{max} 3410, 1734(shoulder), 1724, 1666, 1627 and 1230 cm.$^{-1}$; $[\alpha]^{25}D$ + 82° (*c* 0.608).

Anal. Calcd. for $C_{25}H_{29}O_8F$ (476.48): C, 63.01; H, 6.13; F, 3.99. Found: C, 63.22; H, 6.25; F, 4.01.

9α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione (XII).—A solution of 250 mg. of VIIIh in 70 ml. of hot acetone and 7 drops of concentrated hydrochloric acid was treated and worked up in the same manner as in the preparation of XI above. The residue was crystallized from acetone–petroleum ether to afford 166 mg. of the acetonide, m.p. 270–274°, dec. with previous softening and browning. Three crystallizations from acetone–petroleum ether gave 113 mg. of XII, m.p. 274–278° dec. with previous softening and browning, λ_{max} 238–239 mμ (ε 14,600); ν_{max} 3470, 1724, 1677, 1630 and 1623 (inflection) cm.$^{-1}$; $[\alpha]^{25}D$ + 112° (*c* 0.537).

Anal. Calcd. for $C_{24}H_{31}O_6F$ (434.49): C, 66.34; H, 7.19; F, 4.37. Found: C, 66.85; H, 7.23; F, 4.71.

16α,21-Diacetoxy-11β,17α-dihydroxy-20-oxo-4-pregnene-3-(2,4-dinitrophenylhydrazone) (IIIc).—A solution of 16α,21-diacetoxy-11β,17α-dihydroxy-4-pregnene-3,20-dione (IIIb, 30 mg.) was treated with 2,4-dinitrophenylhydrazine (13 mg.) in acetic acid (5 ml.) on the steam-bath for 40 minutes. The solution was then chilled and 5 ml. of methanol and 40 ml. of water were added. This gave crystals which were collected by filtration, washed with water and dried. The yield of IIIc was 27 mg., m.p. 170° dec. Crystallization from chloroform–petroleum ether gave 22 mg., m.p. 172° dec.; $\lambda_{max}^{chloroform}$ 258, 292 and 386 mμ (ε 17,600, 10,300 and 28,600, respectively).

Anal. Calcd. for $C_{31}H_{38}N_4O_{11}$ (642.65): N, 8.78. Found: N, 8.55.

11β,16α,17α,21-Tetrahydroxy-1,4-pregnadiene-3,20-dione (VIIIa). A.—An agar slant of *Corynebacterium simplex*, ATCC 6946, was washed with 5 ml. of sterile 0.9% sodium chloride solution, and the resulting cell suspension was used to inoculate 100 ml. of a sterile medium (Trypticase Soy Broth [Baltimore Biological Laboratories], 3.0%; beef extract [Armour], 0.3%) in a 500-ml. erlenmeyer flask. The inoculum flask was incubated on a reciprocating shaker at 37° for 8 hours, at which time 1 ml. was used to inoculate each of twenty-five flasks containing the same medium. These fermentation flasks were shaken at 32° for 40 hours before addition to each flask of 40 mg. of 11β,16α,17α,21-tetrahydroxy-4-pregnene-3,20-dione (IIIa) dissolved in 4 ml. of ethanol. The fermentation was continued for 8 hours at which time the flasks were harvested and their contents pooled.

The beer was extracted with seven 2-liter portions of methylene chloride and the combined extracts evaporated. This gave 1.59 g. of a crude solid which was chromatographed on a diatomaceous silica product with the partition system; ethyl acetate (3 parts), petroleum ether (b.p. 90–100°, 2 parts), methanol (3 parts), water (2 parts). Elution gave 601 mg. of the desired product which when crystallized from acetone–petroleum ether yielded 278 mg. of VIIIa, m.p. 229–231°. Recrystallization from the same solvent pair raised the melting point to 231–232°, λ_{max} 241–242 mμ (ε 14,800); ν_{max} 3436, 1715, 1664, 1621, 1603, 1129, 1063 cm.$^{-1}$; $[\alpha]^{24}D$ + 77° (*c* 0.421, methanol).

Anal. Calcd. for $C_{21}H_{28}O_6$ (376.44): C, 67.00; H, 7.50. Found: C, 66.82; H, 7.27.

B.—Fermentation of IIIa with *Nocardia corallina*, ATCC 999, was carried out in a manner similar to that described in the above sample A. A cerelose–yeast extract–peptone–beef extract medium was employed with a 1% 8-hour preformed inoculum. The flasks were placed in a reciprocating shaker at 32° for 40 hours prior to the addition to each flask of 20 mg. of IIIa in 2 ml. of ethanol. The flasks were harvested 8.5 hours after the addition of the steroid and the contents of all flasks pooled and extracted in the same manner as in example A to give 352 mg. of crude solid. Two crystallizations from acetone–petroleum ether gave 95 mg. of VIIIa, m.p. 229–231°. The infrared spectrum was identical to that of the material isolated in A, above.

16α,21-Diacetoxy-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIIb). A.—A mixture of 11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (VIIIa, 100 mg.) in pyridine (10 ml.) was treated with acetic anhydride (2 ml.) and allowed to stand overnight at room temperature. The solution was evaporated and the residue crystallized from ethyl acetate–petroleum ether (90–100°) to give 105 mg. (86%), m.p. 141–150°. Recrystallization from the same solvent pair raised the melting point to 161–163°, λ_{max} 242 mμ (ε 14,200); ν_{max} 3458, 1748, 1668, 1632, 1612-(shoulder), 1234, 1060 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{32}O_8 \cdot {}^1/_2H_2O$ (469.52): C, 63.96; H, 7.52. Found: C, 64.00, 63.73; H, 7.23, 7.21.

B.—In a larger run, treatment of VIIIa (2.5 g.) in the manner described above gave 2.23 g., m.p. 215–219°. Recrystallization from ethyl acetate–petroleum ether (90–100°) raised the melting point to 217–219°; ν_{max} 3422, 1742, 1710, 1662, 1625, 1602, 1232 and 1046 cm.$^{-1}$; $[\alpha]^{25}D$ + 70° (*c* 1.154, methanol).

Anal. Calcd. for $C_{25}H_{32}O_8$ (460.51): C, 65.20; H, 7.00. Found: C, 64.88; H, 7.31.

C.—To a solution of 16α,21-diacetoxy-11β,17α-dihydroxy-4-pregnene-3,20-dione (IIIb, 300 mg.) in benzene (30 ml.) and water (0.1 ml.) was added selenium dioxide (180 mg.) and the mixture was refluxed 65 hours. The reaction mixture was cooled, diluted with benzene, washed with water, saturated sodium bicarbonate and with water again. After drying and treatment with Norit, the filtrate was evaporated to dryness to give 280 mg. of a dark glass. A paper strip chromatogram showed about 20% conversion. The above glass was partitioned on 70 g. of a diatomaceous silica product[23] using the solvent system ethyl acetate (2 parts), petroleum ether (b.p. 90–100°, 4 parts), methanol (3 parts) and water (2 parts). Evaporation of hold-back volume 1.5–2.0 to dryness gave 40 mg. of a solvated solid. Crystallization from ethyl acetate–petroleum ether (b.p. 90–100°) yielded 3 mg. of VIIIb. Infrared analysis showed identity with the sample prepared above.

C-3-(2,4-Dinitrophenylhydrazone) VIIIc, m.p. 172° dec.; $\lambda_{max}^{chloroform}$ 258 mμ (ε 18,000), 291 mμ (ε 8,920), 393 mμ (ε 32,400).

Anal. Calcd. for $C_{31}H_{36}N_4O_{11}$ (640.63): N, 8.81. Found: N, 8.60.

16α,21-Diacetoxy-17α-hydroxy-1,4-pregnadiene-3,11-20-trione (Xa).—A solution of 16α,21-diacetoxy-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIIb, 200 mg.) in pyridine (10 ml.) was treated with a solution of chromium trioxide (150 mg.) in pyridine (8 ml.) and the mixture allowed to stand overnight at room temperature. Methanol (10 ml.) was added and the solvents evaporated. Water was added to the residue and the mixture was extracted with ethyl acetate. The extract was washed with water, dried and evaporated to a solid residue which on crystallization from ethyl acetate–petroleum ether (90–100°) gave 128 mg. (64%), m.p. 216–217°. Repeated crystallization from the same solvent pair changed the melting point to 208–209°, $\lambda_{max}^{methanol}$ 238 mμ (14,700); ν_{max} 3413, 1727, 1706, 1664, 1626, 1608, 1232 and 1068 cm.$^{-1}$; $[\alpha]^{26}D$ + 133° (*c* 1.040, methanol).

Anal. Calcd. for $C_{25}H_{30}O_8$ (458.49): C, 65.49; H, 6.60. Found: C, 65.21; H, 6.68.

(25) The m.p. was dependent upon the rate of heating and the temperature of the bath at which the capillary was inserted. In a later run, the m.p. was 269–271°.

16α,21-Diacetoxy-17α-hydroxy-1,4,9(11)-pregnatriene-3,-20-dione (VII). A.—To a solution of 200 mg. of the 11β-ol VIIIb in 4 ml. of pyridine at −5° was added 3 ml. of thionyl chloride and the solution was kept at this temperature 2 hours. The resultant mixture was poured into ice-water and extracted with chloroform. After the chloroform was washed with saturated saline, it was dried and evaporated to give a semi-solid. This was allowed to stand 60 hours in a small amount of ethyl acetate to furnish crystals, m.p. 196–197°. A further quantity of VIIIb (200 mg.) in 10 ml. of pyridine was treated with 1 ml. of thionyl chloride as above for 5 hours. Extraction as above gave a glass which was combined with the above-mentioned solid and chromatographed on silica gel. Elution with chloroform yielded 75 mg. of VII, m.p. 195–198°. After recrystallization from ethyl acetate–petroleum ether (b.p. 90–100°) 59 mg., m.p. 200–201°, was obtained, $\lambda_{max}^{methanol}$ 238 mμ (ε 15,800); ν_{max} 3497, 1748, 1727, 1661, 1626, 1605 and 1227 cm.$^{-1}$; $[\alpha]^{25}D$ + 6° (*c* 1.122, methanol).

Anal. Calcd. for $C_{25}H_{30}O_7$ (442.49): C, 67.85; H, 6.83. Found: C, 67.54; H, 7.09.

B.—In a larger run 970 mg. of VIIIb dissolved in 20 ml. of pyridine and treated with 1.6 ml. of thionyl chloride for 2 hours at −5° as above gave without recourse to chromatography 532 mg. of VII, m.p. 199–201°.

16α,21-Diacetoxy-9α-bromo-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIId).—A solution of the triene VII (200 mg.) in dioxane (10 ml.) and water was treated with N-bromoacetamide (80 mg.) and 10% perchloric acid (0.42 ml.). After standing for 20 minutes at 20°, 1 ml. of saturated sodium sulfite and excess water were added. The resultant amorphous solid was filtered off to give 60 mg. of VIIId, m.p. 147° dec., which could not be further purified; ν_{max} 3401, 1739, 1658, 1616 and 1232 cm.$^{-1}$.

16α,21-Diacetoxy-9β,11β-epoxy-17α-hydroxy-1,4-pregnadiene-3,20-dione (IX).—A solution of 620 mg. of the bromohydrin 16,21-diacetate VIIId and 200 mg. of potassium acetate in 150 ml. of absolute alcohol was refluxed for 18 hours. The reaction mixture was evaporated to dryness and the residue extracted with hot ethyl acetate. The extract was washed with saline, dried and evaporated. The semi-solid residue was treated with 5 ml. of pyridine and 2 ml. of acetic anhydride for 18 hours and the resultant solution was evaporated. The residue was chromatographed on a silica gel column and the desired product was eluted with chloroform to yield 303 mg. of a hard glass. Crystallization from acetone–petroleum ether furnished 223 mg., m.p. 211–215°. Recrystallization from the same solvent pair raised the melting point to 239.5–241°, $\lambda_{max}^{methanol}$ 247 mμ (ε 16,200); ν_{max} 3390, 1736, 1661, 1626, 1608 and 1239 cm.$^{-1}$ $[\alpha]^{25}D$ ± 0° (*c* 1.018, methanol).

Anal. Calcd. for $C_{25}H_{30}O_8$ (458.49): C, 65.49; H, 6.60. Found: C, 65.25; H, 6.73.

16α,21-Diacetoxy-9α-chloro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VIIIe).—A solution of the 9β,11β-epoxy 16,21-diacetate IX (200 mg.) in chloroform (20 ml.) was treated with 10 ml. of chloroform saturated with hydrogen chloride at 0° for 4.5 hours. The chloroform was then evaporated at 0° and the resultant solid was crystallized from ethyl acetate–petroleum ether (b.p. 90–100°) to furnish 98 mg. of VIIIe, m.p. 229–231° dec. Recrystallization from the same solvent pair raised the melting point to 234–235° dec., $\lambda_{max}^{methanol}$ 239 mμ (ε 14,600); ν_{max} 3378, 1733, 1664, 1626, 1608(shoulder) and 1242 cm.$^{-1}$; $[\alpha]^{25}D$ + 82° (*c* 0.939, methanol).

Anal. Calcd. for $C_{25}H_{31}O_8Cl$ (494.96): C, 60.66; H, 6.31; Cl, 7.16. Found: C, 60.77; H, 6.60; Cl, 6.76.

9α-Chloro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (VIIIf).—To a solution of the chlorohydrin 16,21-diacetate VIIIe (150 mg.) in methanol was added sodium methoxide (35 mg.) and the solution was allowed to stand at room temperature under nitrogen for 10 minutes. Acetic acid (0.1 ml.) was added and the solution evaporated to yield a solid. After the solid was slurried with water, it was crystallized from methanol–ethyl acetate to give 32 mg., m.p. 224° dec., λ_{max} 239 mμ (ε 15,800); ν_{max} 3340, 1714, 1662, 1618 and 1592(shoulder) cm.$^{-1}$; $[\alpha]^{25}D$ + 101° (*c* 0.561, methanol).

Anal. Calcd. for $C_{21}H_{27}O_6Cl$ (410.89): C, 61.38; H, 6.63; Cl, 8.63. Found: C, 61.40; H, 6.73; Cl, 8.36.

16α-21-Diacetoxy-9α-chloro-17α-hydroxy-1,4-pregnadiene-3,11,20-trione (Xb).—A solution of VIIIe (110 mg.) in 4 ml. of pyridine was treated with a solution of 130 mg. of chromium trioxide in 4 ml. of pyridine and allowed to stand at room temperature for 16 hours. The mixture was poured into ice-water, extracted with ethyl acetate, and the extract was washed with sodium bicarbonate, saline solution and dried. Evaporation gave a semi-solid. Chromatography on silica gel yielded the desired product in the chloroform elutions. Crystallization from ethyl acetate–petroleum ether yielded 31 mg. of Xb, m.p. 231–232°, $\lambda_{max}^{methanol}$ 237 mμ (ε 15,800), ν_{max} 3520, 1738, 1668, 1616, 1612(shoulder) and 1232 cm.$^{-1}$; $[\alpha]^{25}D$ + 172° (*c* 0.448, methanol).

Anal. Calcd. for $C_{25}H_{29}O_6Cl$ (492.94): C, 60.78; H, 5.92; Cl, 7.18. Found: C, 60.57; H, 6.29; Cl, 6.78.

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[CONTRIBUTION FROM THE ORGANIC CHEMICAL AND EXPERIMENTAL THERAPEUTICS RESEARCH SECTIONS, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

16-Hydroxylated Steroids. VII.[1] The Synthesis of the 16α-Hydroxy Derivatives of 2-Methyl Steroids[2]

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The 2α-methyl and Δ^1-2-methyl analogs of 16α-hydroxyhydrocortisone and 9α-fluoro-16α-hydroxyhydrocortisone have been synthesized.

In 1955, Hogg and co-workers[3] described a synthesis of 2α-methyl corticoids among which were 2α-methylhydrocortisone and 9α-fluoro-2α-methylhydrocortisone. Biological assays[3,4] revealed that these two compounds possessed greatly increased gluco- and mineralocorticoid activities when compared to the parent steroids, hydrocortisone and 9α-fluorohydrocortisone, respectively.

Previous work[1,5] from these laboratories has demonstrated that the introduction of a 16α-hydroxy group into a corticoid negated sodium retention activities without concomitant destruction of the glucocorticoid activity. In view of this important finding, it was decided to synthesize various corti-

(1) Paper VI, S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **81**, 1689 (1959).

(2) For a preliminary announcement of part of this work see S. Bernstein, M. Heller, R. Littell, S. M. Stolar, R. H. Lenhard and W. S. Allen, *ibid.*, **79**, 4555 (1957).

(3) J. A. Hogg, F. H. Lincoln, R. W. Jackson and W. P. Schneider, *ibid.*, **77**, 6401 (1955).

(4) W. E. Dulin, B. J. Bowman and R. O. Stafford, *Proc. Soc. Exp. Biol. Med.*, **94**, 303 (1957).

(5) W. S. Allen and S. Bernstein, THIS JOURNAL, **78**, 1909 (1956).