Michaelis–Menten constant having the value of 1.7×10^{-6} *M*.

The requirement of sulfhydryl groups for citrovorum factor cyclodehydrase activity is indicated by the inhibitory effect of *p*-chloromercuribenzoate and *o*-iodosobenzoate on the rate of formation of the intermediate compound from citrovorum factor and the reversal of inhibition in both cases by reduced glutathione (Fig. 6).

Rabinowitz and Pricer[8] have reported the occurrence in *Clostridium cylindrosporum* of an enzyme, formimino-THF cyclodeaminase, which catalyzes a reaction which is analogous to that proposed for citrovorum factor cyclodehydrase. ATP is not required in the reaction they describe.

Experimental

Apparatus and Materials.—A Beckman model DU spectrophotometer and 1 cm. Corex cuvettes were used for the enzyme assays. 1 cm. quartz cuvettes were used for the determinations of protein concentration from the ultraviolet absorption by the method of Warburg and Christian[9] and a Cary recording spectrophotometer was used for the comparison of the spectra of anhydroleucovorin and the enzymatically-formed intermediate in the region 320–360 mμ. A Beckman model G *p*H meter was used for *p*H determinations.

Calcium leucovorin was generously supplied by the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, N. Y., and Solka-Floc BW 200 used for the preparation of carboxymethyl-cellulose[3] (sodium form), was a gift of the Brown Company, Boston, Mass. ATP was obtained from the Sigma Chemical Company, St. Louis, Mo.

Sheep liver acetone powder was prepared as previously described.[2] Hydroxylapatite was prepared by the method of Tiselius, *et al.*,[10] and calcium phosphate gel was prepared by the method of Keilin and Hartree.[11] Dowex-1 (200–400 mesh) (Microchemical Specialties Company, Berkeley, Calif.) was converted to the chloride form and columns were prepared by the method of Cohn.[6b]

(8) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 5702 (1956).

(9) O. Warburg and W. Christian, *Biochem. Z.*, **310**, 384 (1941–1942).

(10) A. Tiselius, S. Hjertén and Ö. Levin, *Arch. Biochem. Biophys.*, **65**, 132 (1956).

(11) D. Keilin and E. F. Hartree, *Proc. Roy. Soc. London, Series B*, **124**, 397 (1938).

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DEPARTMENT, SCHERING CORP.]

Introduction of Oxygen into Ring B of Corticoids

BY A. L. NUSSBAUM, G. BRABAZON, T. L. POPPER AND E. P. OLIVETO

RECEIVED JANUARY 2, 1958

Epoxidation of 6-dehydroprednisone acetate gave the 6α,7α-epoxide as well as some 17-ketone V. The former was converted to the 7α-hydroxy derivative either directly with chromous acetate or *via* a bromohydrin. Corresponding reactions with 6-dehydrocortisone acetate led to 7α-hydroxycortisone acetate. The latter was converted microbiologically to the new 7α-hydroxyprednisone. The Δ^4-compound dehydrates normally, but the $\Delta^{1,4}$-diene is resistant to dehydration. Arguments in support of the indicated structures are presented.

The introduction of an additional double bond into the prednisone molecule by Gould, *et al.*,[1] and by Agnello and Laubach[2] furnished a means for further modification of the steroidal B ring in structures possessing physiological activity. Preferential attack of the 6,7-double bond by peracids was observed when 17α,21-dihydroxy-$\Delta^{1,4,6}$-pregnatrien-3,11,20-trione 21-acetate[1,2] (I) was exposed to such reagents. Two products were isolated: the 6α,7α-epoxide IIa and its corresponding 17-ketone V.

The assignment of structure of substance IIa follows from the following considerations: Elementary analysis of both 21-acetate and the corresponding 21-ol IIb showed the presence of an additional oxygen atom in the molecule. The ultraviolet spectrum of the cross-conjugated trienone starting material had disappeared, and the new substance had instead a single absorption maximum at 240 mμ. This excluded attack at either of the two ring A double bonds, a consideration that was confirmed by the infrared spectrum. The latter showed absorption peaks at 6.18 and 6.24 μ, characteristic of cross-conjugated dienones,[3] and at 11.88 μ, assigned to the epoxide function.[4] The nature of the newly-introduced oxygen was further indicated by reversion of IIa to starting material upon treatment with hydriodic acid.[5] Stereochemical assignment of the epoxide function to the α-side of the molecule rests on steric considerations (rule of the rear[6]) as well as the nature of certain transformation products now to be described.

Halohydrins III were prepared by standard procedures. Thus, a bromohydrin IIIa, a chlorohydrin IIIc and a fluorohydrin IIIe, as well as the corresponding diacetates IIIb, d and f were obtained. Normal oxide opening would lead to the halohydrins formulated as in III, Chart I (6β-halo, 7α-hydroxy) and a consideration of their ultraviolet absorption spectra lends support to this formulation. There is a progressive drop in the α,β-unsaturated ketone absorption; thus, bromohydrin IIIa has its maximum at 244 mμ, chlorohydrin IIIc at 240 mμ and fluorohydrin IIIe at 236 mμ. A similar picture has been observed by Bird and co-

(1) D. Gould, E. L. Shapiro, H. L. Herzog, M. J. Gentles, E. B. Hershberg, W. Charney, M. Gilmore, S. Tolksdorf, M. Eisler, P. L. Perlman and M. Pechet, THIS JOURNAL, **79**, 502 (1957).

(2) E. J. Agnello and G. D. Laubach, *ibid.*, **79**, 1257 (1957).

(3) J. Fried, R. W. Thoma and A. Klingsberg, *ibid.*, **75**, 5764 (1953).

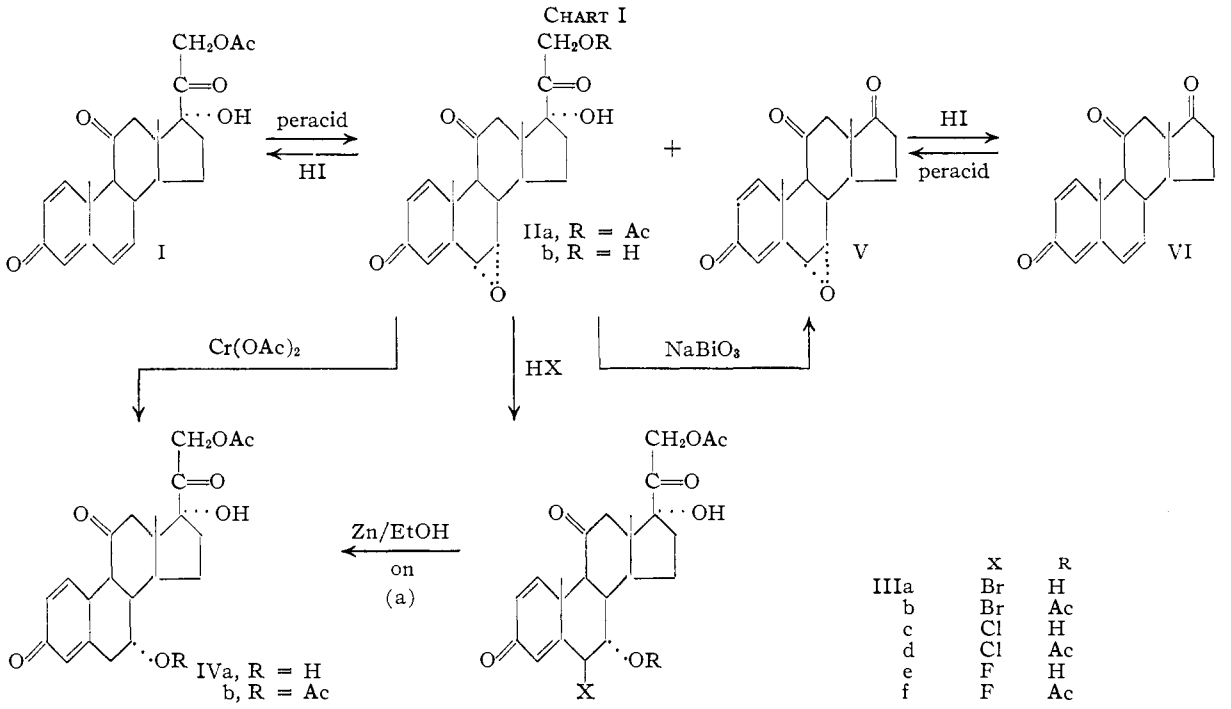
(4) W. A. Patterson, *Anal. Chem.*, **26**, 823 (1954), finds an absorption band at 11.6 μ for a series of simple aliphatic epoxides.

(5) E. P. Oliveto, C. Gerold and E. B. Hershberg, THIS JOURNAL, **79**, 3596 (1957).

(6) L. Fieser, *Experientia*, **6**, 312 (1950).

(7) Oxides generally open to the *trans*-diaxial halohydrins; see, for instance, A. Fürst and P. A. Plattner, Abstracts, 12th International Congress of Pure and Applied Chemistry, New York, 1951, p. 409. Abnormal ring openings, however, have been reported: *cf. inter al.*, W. S. Knowles and Q. E. Thompson, THIS JOURNAL, **79**, 3212 (1957).

CHART I



workers[8] in a structurally related Δ^4-cholesten-3-one series substituted in the 6β-position. This effect was not observed in the 6α-position, and certainly could not be expected if the halogens were located at C-7, where intimate interaction of the π-electron system is unlikely. Thus, formula III (Chart I) for the halohydrins is supported and consequent assignment of the oxide II to the α-side follows.

Attempts to oxidize the bromohydrin IIIa, either with chromium trioxide–pyridine[9] or chromic acid in acetone[10] were unsuccessful, as were several dehydration attempts, either on IIIa itself, or *via* a 7α-mesylate.[11]

Treatment with zinc in ethanol furnished a new hydroxyprednisone acetate, formulated as IVa. This compound had the correct elementary analysis, as did a diacetate IVb, and was considerably more polar in a paper chromatographic system[12] than the starting material. Here again several attempts at oxidation and dehydration were unsuccessful, but conversion to a mesylate followed by treatment with refluxing 2,6-lutidine gave a mixture of products, the ultraviolet spectrum of which showed some absorption at 297 mμ, indicating that partial dehydration to the triene I had occurred.

In order to bolster the structure assigned to the presumed 7α-hydroxyprednisone acetate IVa, we subjected substance IIa, the 6α,7α-epoxide, to the action of chromous acetate. This reagent has been used successfully to convert 16α,17α-epoxy-21-acetoxy-Δ^4-pregnene-3,11,20-trione into 16α,21-dihydroxy - Δ^4 - pregnene - 3,11,20 - trione 21-acetate,[13] an analogous case. Prednisone acetate itself proved to be stable toward this reagent. When 6α,7α-epoxy-17α,21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione 21-acetate (IIa) was treated with chromous acetate,[14] there was obtained, in low yield, the same 7α-hydroxyprednisone acetate IVa, that had been previously formed by debromination of bromohydrin IIIa with zinc.

The resistance of IVa toward dehydration must, however, be considered anomalous in view of the fact that 7α-hydroxydesoxycorticosterone acetate can be converted easily into the corresponding dienone.[15] We were able to confirm this observation in the case of a compound possessing the full cortical side chain. 6-Dehydrocortisone acetate[16] was converted into its 6α,7α-oxide VIII, and a corresponding bromohydrin IX[17] was prepared. Debromination with zinc in ethanol gave a highly polar hydroxylated derivative of cortisone acetate, undoubtedly 7α,17α,21-Trihydroxy-Δ^4-pregnene-3,11,20-trione 21-acetate (X). The latter compound, unlike its 1-dehydro analog, was dehydrated with expected ease. When it was subjected to ultraviolet measurement under alkaline conditions,[18] the original maximum at 238 mμ shifted to that of the dienone at 282 mμ. The fact that the two 7α-hydroxycorticoids IV and X differed only in the Δ^1-

(8) C. W. Bird, R. C. Cookson and S. H. Dandegaonker, *J. Chem. Soc.*, 3675 (1956).

(9) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(10) R. G. Curtis, I. A. N. Heilbron, E. R. H. Jones and G. F. Woods, *J. Chem. Soc.*, 461 (1953).

(11) N. L. Wendler, D. Taub, S. Dobriner and D. K. Fukushima, THIS JOURNAL, **78**, 5027 (1956).

(12) G. M. Shull, D. A. Kita and J. W. Davisson, U.S. Patent 2,658,023.

(13) P. L. Julian, W. Cole, E. W. Meyer and B. M. Regan, THIS JOURNAL, **77**, 4601 (1955).

(14) W. Cole and P. L. Julian, *J. Org. Chem.*, **19**, 131 (1954).

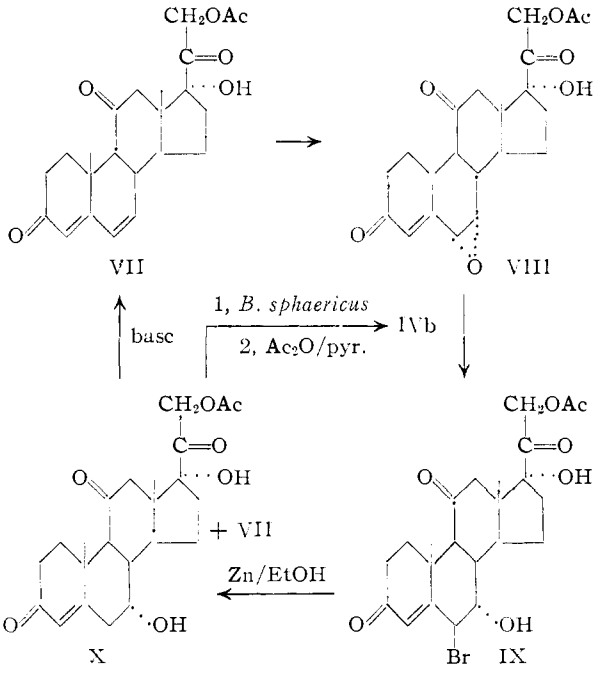
(15) Ch. Meystre, E. Vischer and A. Wettstein, *Helv. Chim. Acta*, **38**, 38 (1955).

(16) V. R. Mattox, E. L. Woroch, S. A. Fleisher and E. C. Kendall, *J. Biol. Chem.*, **197**, 261 (1952).

(17) F. B. Colton, in U.S. Patent 2,738,348 (March 13, 1957), reported an α-oxide and the corresponding bromohydrin from 6-dehydrocortisone acetate. However, preparation of our compounds are given in the Experimental portion, since physical constants of the two series are quite different.

(18) A. S. Meyer, *J. Biol. Chem.*, **203**, 469 (1953).

CHART II



double bond present in IV but absent in X was finally proved by the microbiological introduction of this double bond into X. When the latter compound was subjected to the dehydrogenating action of *B. sphaericus*, the crude extract gave IVb after acetylation and chromatography.

It was mentioned earlier that when 17α,21-dihydroxy-$\Delta^{1,4,6}$-pregnatriene-3,11,20-trione 21-acetate (I) was subjected to peracids, a second product was isolated in addition to the 6α,7α-epoxide IIa. This was not the corresponding β-epoxide since it did not give a purple color with 2,3,5-triphenyltetrazolium chloride,[19] indicating the absence of the dihydroxyacetone side chain. The infrared spectrum showed absence of hydroxyl absorption and a band that could be attributed to a five-membered ring carbonyl. The compound gave a positive Zimmermann[20] test and, accordingly, structure V, 6α,7α-epoxy-$\Delta^{1,4}$-androstadiene-3,11,-17-trione, was assigned to the substance in question. This structure was proved by the following transformations: (1) reaction of V with hydriodic acid gave $\Delta^{1,4,6}$-androstatriene-3,11,17-trione (VI), a known compound[1]; (2) the latter, when treated with perphthalic acid, reverted to V; (3) degradation of 6α,7α-epoxy-17α,21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione (IIb) by sodium bismuthate[21] also furnished V. Degradation of the side chain by peracids had been observed earlier.[22]

Acknowledgment.—We are indebted to Drs. D. H. R. Barton, J. Meinwald and R. K. Hill for helpful advice, to Dr. W. Charney for the microbiological transformation, and especially to Dr. E. B. Hershberg for his kind help with the preparation of the manuscript.

(19) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

(20) W. Zimmermann, *Z. physiol. Chem.*, **245**, 47 (1936).

(21) C. J. W. Brookes and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(22) N. S. Leeds, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **76**, 2265 (1954).

Experimental[23]

6α,7α-Epoxy-17α,21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione 21-Acetate (IIa).—17α,21-Dihydroxy-$\Delta^{1,4,6}$-pregnatriene-3,11,20-trione 21-acetate (I),[1] 24.66 g., was dissolved in 2.7 l. of methylene chloride, and 1.4 l. of ethereal monoperphthalic acid (47.3 mg. of peracid/ml.) was added. After 65 hours at room temperature, 3.1 l. of methylene chloride was added, and the solution washed twice with 2.5 l. of saturated sodium carbonate solution. The latter was back-extracted with methylene chloride, and the combined organic layers dried and concentrated. By differential crystallization from isopropyl ether, a total of 19.1 g. of **IIa** as well as 266 mg. of the degraded **6α,7α-epoxy-$\Delta^{1,4}$-androstadiene-3,11,17-trione (V)** could be isolated (see later). (In another run, 17.5 g. of I gave 6.89 g. of IIa and 4.70 g. of V.) Substance **IIa** had a melting point of 257–264°, ϵ_{240}^{MeOH} 15,130. Recrystallizations from isopropyl ether gave an analytical sample, m.p. 262–272° dec., $[\alpha]^{25}D$ +156.3, ϵ_{240}^{MeOH} 15,600, R_f 0.35 to 0.43[12]; λ_{max}^{Nujol} at 2.96, 5.72, 5.84, 6.02, 6.18, 6.26, 7.87, 8.11 and 11.88 μ.

Anal. Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 66.95; H, 6.42.

6α,7α-Epoxy-17α,21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione (IIb).—Acetate IIa, 900 mg., was dissolved in 200 ml. of methanol and 2.33 ml. of a 4% aqueous sodium hydroxide solution was added. After 25 minutes at room temperature, the solution was neutralized with acetic acid and concentrated to a small volume. Water was added, and the resulting crystals filtered; yield 402 mg., m.p. 235–240°. Recrystallizations from acetone gave an analytical sample, m.p. 250–265°, $[\alpha]^{25}D$ +86.5° (pyridine), ϵ_{240}^{MeOH} 14,800; λ_{max}^{Nujol} at 3.00, 5.80(sh), 5.85, 6.01 and 6.15 μ.

Anal. Calcd. for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 68.02; H, 6.42.

Reaction of IIa with Hydriodic Acid.—6α,7α-Epoxy-17α,-21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione 21-acetate (IIa) (200 mg.) was dissolved in 25 ml. of methylene chloride. Aqueous 48% hydriodic acid (2 ml.) was added, and the solution was shaken in the dark for 15 minutes at room temperature. After dilution with 20 ml. of water, the organic layer was separated and the aqueous phase was further extracted twice with methylene chloride. Combined extracts were washed with 20-ml. portions each of 5% sodium bicarbonate and 5% sodium carbonate solutions, and water. After drying and vacuum concentration, the residue was crystallized from methylene chloride–isopropyl ether to give 175 mg. of 17α,21-dihydroxy-$\Delta^{1,4,6}$-pregnatriene-3,11,20-trione 21-acetate (I), identical with genuine material in spectral and melting point comparison.

6β-Bromo-7α,17α,21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,-20-trione 21-Acetate III(a).—The epoxide IIa (2.5 g.), dissolved in 150 ml. of chloroform, was shaken with 63 ml. of 48% aqueous hydrobromic acid. The mixture was extracted with ethyl acetate, and the extract washed with water, 5% aqueous sodium sulfate and again with water. Drying and concentration followed by crystallization from acetone–isopropyl ether gave a product which was seen to be heterogeneous by paper chromatography[12] and could not be purified by recrystallization. The difficulty appeared to be partial hydrolysis of the acetate at C-21, so the entire crystalline residue was dissolved in 10 ml. of pyridine and an equal volume of acetic anhydride was added. After four minutes, the solution was diluted with 75 ml. of water and the resulting solid filtered, washed with water and recrystallized from acetone–isopropyl ether, yielding 1.34 g., m.p. 190–194°. An analytical sample was prepared by several recrystallizations from the same solvent system: m.p. 195–198° dec., $[\alpha]^{25}D$ +153.1°, R_f 0.15, ϵ_{244}^{MeOH} 16,770, λ_{max}^{Nujol} at 2.99, 3.10, 5.70, 5.76, 5.82, 6.01, 6.17 and 8.09 μ.

Anal. Calcd. for $C_{23}H_{27}O_7Br$: Br, 16.11. Found: Br, 15.47.

A diacetate IIIb was prepared with acetic anhydride–pyridine in the usual manner, and crystallized from methyl-

(23) All melting points were taken on a Kofler block. Rotations were taken in a 1-dm. tube at a concentration of *ca.* 1% in dioxane, unless otherwise specified. Analyses and spectral data were obtained by the Microanalytical and Physical Chemistry Departments of these laboratories.

ene chloride–ether. The analytical sample had m.p. 200–204° dec., $[\alpha]^{23}D$ +155.9°, ϵ_{243}^{MeOH} 15,900, R_f 0.63; λ_{max} at 3.01, 5.71, 5.79, 6.01, 6.17, 6.21, 8.08 and 8.19 μ.

Anal. Calcd. for $C_{25}H_{29}O_8Br$: C, 55.87; H, 5.40; Br, 14.90. Found: C, 55.63; H, 5.09; Br, 15.26.

6β-Fluoro-7α,21-trihydroxy-Δ^{1,4}-pregnadiene-3,11,20-trione 21-Acetate (IIIe).—Epoxide IIa (500 mg.) was dissolved in 13 ml. of chloroform and added to 5 ml. of a standard stock solution of hydrogen fluoride in chloroform–tetrahydrofuran[24] at −70°. The solution was allowed to come to room temperature and left to stand for 19 hours. It was then added, with stirring, to 425 ml. of a 5% potassium carbonate–ice slurry and extracted with ethyl acetate. Concentration after drying furnished a residue which was seen to be heterogeneous by paper chromatography.[12] Chromatography over Florisil gave 54 mg. of IIIe, m.p. 252–258°. The analytical sample was obtained by recrystallization from acetone–hexane; m.p. 269–272°, ϵ_{236}^{MeOH} 14,700, R_f 0.15; λ_{max} at 2.29, 5.68, 5.76, 5.84, 6.01, 6.17 and 9.60 μ.

Anal. Calcd. for $C_{23}H_{27}O_7F$: C, 63.57; H, 6.27. Found: C, 63.56; H, 6.53.

The corresponding diacetate IIIf was prepared and recrystallized from methylene chloride–ether; m.p. 254–259°, ϵ_{236}^{MeOH} 15,100, R_f 0.60; λ_{max}^{Nujol} at 3.00, 5.71, 5.79, 6.01, 6.16, 6.21, 8.07, 9.46, 9.56 and 9.80 μ.

Anal. Calcd. for $C_{25}H_{29}O_2F$: C, 63.03; H, 6.09; F, 3.99. Found: C, 63.30; H, 6.36; F, 3.86.

7α,17α,21-Trihydroxy-Δ^{1,4}-pregnadiene-3,11,20-trione 21-Acetate (IV). A. From 6β-Bromo-7α,17α,21-trihydroxy-Δ^{1,4}-pregnadiene-3,11,20-trione 21-Acetate (IIIa).—Bromohydrin IIIa (500 mg.) was dissolved in 150 ml. of absolute alcohol; 25 ml. of water and 5 g. of zinc powder were added. The suspension was stirred at room temperature for 48 hours, the zinc was filtered off, and the filtrate concentrated under vacuum. Recrystallization of the residue from methanol gave 116 mg. of IV, m.p. 300–305° dec. After several recrystallizations from methanol, an analytical sample had a lower melting point, 290–293°, $[\alpha]^{22}D$ +102° (pyridine), ϵ_{237}^{MeOH} 14,500, R_f at origin; λ_{max}^{Nujol} at 3.01, 5.69, 5.76, 5.86, 6.01, 6.17, 6.20 and 8.08 μ.

Anal. Calcd. for $C_{23}H_{28}O_7$: C, 66.33; H, 6.78. Found: C, 66.31; H, 6.93.

B. **From 6α,7α-Epoxy-17α,21-dihydroxy-Δ^{1,4}-pregnadiene-3,11,20-trione 21-Acetate (IIa).**—A slurry was prepared of 2.9 g. of chromous acetate in 72 ml. of acetone–water (4:1), and 960 mg. of sodium acetate dissolved in 7.2 ml. of glacial acetic acid and 30 ml. of water were added. The resulting mixture was stirred under carbon dioxide, and 2 g. of epoxide IIa dissolved in 250 ml. of acetone was added through a dropping funnel. The mixture was stirred for six hours and then diluted with 800 ml. of ethyl acetate. The organic layer was extracted with water saturated with sodium chloride, aqueous bicarbonate solution and again water, and the washings back-extracted with ethyl acetate. The combined ethyl acetate extracts were dried and concentrated until crystals appeared. The latter were centrifuged off and washed with ethyl acetate. The 250 mg. of IVa thus obtained was identical in every respect with the product described above.

7α,17α,21-Trihydroxy-Δ^{1,4}-pregnadiene-3,11,20-trione 7α,21-Diacetate (IVb) A. From the Parent Triol IVa.—The usual pyridine–acetic anhydride procedure was employed. Recrystallization from ethyl acetate–benzene gave an analytical sample, m.p. 221–224°, ϵ_{237}^{MeOH} 15,200, R_f 0.45; λ_{max}^{Nujol} at 2.84, 3.01, 5.69, 5.76, 5.84, 6.00, 6.20 and 8.05 μ.

Anal. Calcd. for $C_{25}H_{30}O_8$: C, 65.50; H, 6.55. Found: C, 65.78; H, 6.87.

B. From 7α,17α,21-Trihydroxy-Δ^4-pregnene-3,11,20-trione 21-Acetate (X).—The 21-acetate X (103 mg.) was submitted to the action of *B. sphaericus* in the manner of Stoudt, *et al.*[25]

Extraction with ethyl acetate, acetylation of the crude extract with pyridine and acetic anhydride and chromatography gave a small amount of X, as shown by paper chromatography and comparison of the infrared spectra.

6α,7α-Epoxy-17α,21-dihydroxy-Δ^4-pregnene-3,11,20-trione 21-Acetate (VIII).—17α,21-Dihydroxy-Δ^{4,6}-pregnadiene-3,11,20-trione 21-acetate (VIII), 6.32 g., was dissolved in 700 ml. of methylene chloride. An ethereal perphthalic acid solution, 370 ml. (0.5 meq./ml.), was added and the solution was allowed to stand overnight at room temperature. It was then diluted with 830 ml. of methylene chloride and washed with sodium carbonate solution, dried and vacuum concentrated. The residue was crystallized from methylene chloride–isopropyl ether to give 3.8 g. of the desired material VIII, m.p. 235–240°. An analytical sample had m.p. 238–243°, ϵ_{238}^{MeOH} 13,000.

Anal. Calcd. for $C_{23}H_{28}O_7$: C, 66.35; H, 6.73. Found: C, 66.55; H, 6.99.

6β-Bromo-7α,17α,21-trihydroxy-Δ^4-pregnene-3,11,20-trione 21-Acetate (IX).—Epoxide VIII (2.6 g.) was dissolved in 160 ml. of chloroform, 64 ml. of 48% aqueous hydrobromic acid was added, and the mixture shaken for ten minutes. It was then diluted with 800 ml. of ethyl acetate and washed with water, 5% sodium sulfite solution and again water. The ethyl acetate extract was dried and concentrated to a solid residue. The latter was dissolved in 10 ml. of pyridine, an equal amount of acetic anhydride was added, and the solution was allowed to react for three minutes. It was then poured into 300 ml. of ice-water, and the resulting precipitate was filtered and recrystallized from acetone–isopropyl ether, yield 0.81 g. An analytical sample had m.p. 197–198° dec., ϵ_{240}^{MeOH} 12,000, R_f 0.16.

Anal. Calcd. for $C_{23}H_{29}O_7Br$: Br, 16.08. Found: Br, 15.90, 16.18.

7α,17α,21-Trihydroxy-Δ^4-pregnene-3,11,20-trione 21-Acetate (X).—Bromohydrin IX (600 mg.) was dissolved in 180 ml. of ethanol, 30 ml. of water and 6 g. of zinc powder were added, and the mixture was stirred for 48 hours. The metal was filtered off and the filtrate concentrated *in vacuo* to a solid residue. Paper chromatography showed it to be a complex mixture of at least three components, and preparative chromatography on 18 g. of Florisil was used. Elution with benzene–ether gave a material which appeared to be a mixture of 6α,7α-epoxide (VIII) (λ_{240}^{MeOH}) and Δ^{4,6}-diene VII (λ^{MeOH} 280).

Further elution with 5% MeOH in methylene chloride gave the desired product. Recrystallization from methanol gave 87.2 mg., m.p. 278–283°, ϵ_{238}^{MeOH} 15,000; after 2 hours at 60° in methanolic potassium hydroxide, the maximum shifted to 282 mμ, $[\alpha]^{23}D$ +151.8° (pyridine).

Anal. Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 66.42; H, 7.47.

6α,7α-Epoxy-Δ^{1,4}-androstadiene-3,11,17-trione (V). A. From the Peracid Oxidation of 17α,21-Dihydroxy-Δ^{1,4,6}-pregnatriene-3,11,20-trione 21-Acetate (I).—The isolation, in variable yield, of V has been described earlier in this section. Recrystallization from aqueous methanol gave a monohydrate of variable melting point, $[\alpha]^{23}D$ +230.4° (pyridine), ϵ_{240}^{MeOH} 14,300; λ_{max}^{Nujol} at 5.74, 5.84, 6.02, 6.14, 6.26 and 11.82 μ; TPTZ negative, Zimmermann positive.

Anal. Calcd. for $C_{19}H_{20}O_4 \cdot H_2O$: C, 69.07; H, 6.71. Found: C, 68.75; H, 6.52.

B. From 6α,7α-Epoxy-17α,21-dihydroxy-Δ^{1,4}-pregnadiene-3,11,20-trione (IIb).—The free epoxy-alcohol (400 mg.) was dissolved in 64 ml. of 50% aqueous acetic acid, 9.5 g. of sodium bismuthate was added, and the mixture was stirred for 16 hours, poured into ice-water and thoroughly extracted with methylene chloride. The extracts were washed until neutral with water, dried and concentrated. Crystallization from aqueous methanol gave 178 mg. of V, identical with the product described earlier.

C. From Δ^{1,4,6}-Androstatriene-3,11,17-trione (VI).—The trione[1] (100 mg.) was dissolved in 12 ml. of methylene chloride and 9 ml. of ethereal perphthalic acid (40 mg./ml.) was added, and the resulting solution allowed to stand at room temperature for 70 hours. Methylene chloride (20 ml.) was added, and the solution was extracted with sodium carbonate solution. After drying, concentration gave a residue which was crystallized from aqueous methanol. Thus, 46.4 mg. of V was obtained, and this material proved to be identical with the material described above.

BLOOMFIELD, N. J.

(24) R. F. Hirschmann, R. Miller, J. Wood and R. E. Jones, THIS JOURNAL, **78**, 4956 (1956).

(25) P. H. Stoudt, W. G. McAleer, J. M. Chemerda, M. A. Kozlowski, R. F. Hirschmann, V. Marlatt and R. Miller, *Arch. Biochem. and Biophys.*, **59**, 304 (1955).