A Stereospecific Synthesis of C-21-Methylated Corticosteroids[1a,d]

E. J. AGNELLO, S. K. FIGDOR, G. M. K. HUGHES, H. W. ORDWAY, REX PINSON, JR., B. M. BLOOM, AND G. D. LAUBACH

Medical Research Laboratories, Chas. Pfizer and Company, Inc., Groton, Connecticut

Received November 16, 1962

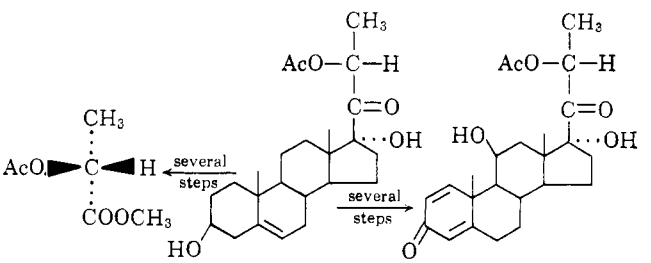
Reduction of 20,21-diketones with fermenting yeast occurs selectively and stereospecifically at C-21 to produce *one* of four possible dihydro derivatives, the 21α_F-hydroxy-21-methyl corticoid. The required 20,21-diketones are best prepared from halohydrins obtainable by hydrogen halide cleavage of 21-methyl-21,21a-epoxides. The latter are prepared by the action of diazomethane on 21-aldehydes.

The rationale for the synthesis of C-21-methylated corticosteroids has been presented in preliminary communications from this laboratory[1] and the glucocorticoid and mineralocorticoid activities of this class of compounds have been reported in recent publications.[2] The present communication provides the details of a synthesis reported earlier in preliminary form.[1a,d] The sequence in its preferred form involves the following transformations: (1) treatment of a C-21 aldehyde with diazomethane to produce a 21-methyl-21,21a-epoxide, (2) opening of the epoxide with hydrogen halide, (3) dehydrohalogenation of the halohydrin with concomitant rearrangement to a 21-methyl-20,21-diketone, and (4) selective reduction of the C-21 carbonyl group to afford the desired C-21-methylated corticoid.

One of the noteworthy advantages of this short synthetic sequence is its applicability to a large variety of complex starting materials, a feature arising from the selective nature of the reagents employed. Another important characteristic of this route is the formation of only *one* (the 21α_F-hydroxy derivative[5]) of each pair of epimeric 21-methyl corticoids by virtue of the stereospecific nature of the reduction of 20,21-diketones by fermenting yeast or sodium borohydride. These tures make available a convenient synthesis of 21-methyl-21α_F-hydroxy corticoids from virtually all of the common steroids containing a dihydroxyacetone side chain, even those bearing several nuclear substituents, without the necessity for employing protective groups.

The usefulness of this synthetic sequence is further enhanced by the availability of a method, recently reported from this laboratory,[1c] for interconverting 21α_F- and 21β_F-hydroxy corticoids *via* their 21-mesylates by displacement with acetate. Thus, the direct conversion of a highly substituted corticoid to either of its two epimeric 21-methyl derivatives can be accomplished readily. Forthcoming publications will describe the details of alternate routes to pairs of epimeric 21-methyl corticoids which involve introduction of the 21-methyl group into simple, readily available starting materials prior to elaboration of the remainder of the molecule.

11β,17 - Dihydroxy - 3,20 - dioxopregna - 1,4 - dien-21-al (prednisolone 21-aldehyde) (I)[6] reacted rapidly[7] with ethereal diazomethane and the major product (40% yield) was 21-methyl-21,21a-epoxy-11β,17-dihydroxypregna-1,4-diene-3,20-dione (II). The epoxide structure was assigned to this product on the basis of its elemental analysis, spectral properties, and reactions with hydrogen halides. Confirmation of the presence of the intact tetracyclic steroid nucleus was obtained by cleavage of the side chain of II with periodic acid (a slow reaction, as expected) and isolation of 11β-hydroxyandrosta-1,4-diene-3,17-dione.[9] Among the five diazomethane reaction by-products detectable by paper chromatography an isomer of II was isolated which



D-(−)-Lactic acid methyl ester acetate

XXIV

11β,17,21β_F-Trihydroxy-21-methylpregna-1,4-diene-3,20-dione 21-acetate (formerly 21A-ol)

establishes its absolute configuration and makes it possible to designate the stereochemistry of XXIV and all other members of the series as 21β_F-ols. Compounds of the opposite configuration (originally designated 21B-ols) are therefore correctly designated 21α_F-ols.

(1) For preliminary reports of this work see: (a) E. J. Agnello, S. K. Figdor, G. M. K. Hughes, H. W. Ordway, R. Pinson, Jr., B. M. Bloom, and G. D. Laubach, Abstracts of Papers presented at the 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April, 1960, p. 20-N; (b) S. K. Figdor, R. Pinson, Jr., H. W. Ordway, E. J. Agnello, B. M. Bloom, and G. D. Laubach, *ibid.*, p. 21-N; (c) H. J. Hess, S. K. Figdor, G. M. K. Hughes, R. Pinson, Jr., and W. T. Moreland, Abstracts of Papers presented at the 138th National Meeting of the American Chemical Society, New York City, N. Y., September, 1960, p. 39-P; (d) E. J. Agnello, R. Pinson, Jr., S. K. Figdor, G. M. K. Hughes, H. W. Ordway, B. M. Bloom, and G. D. Laubach, *Experientia*, **16**, 357 (1960).

(2) The recently reported results of pharmacological[3] and clinical[4] studies with 21-methyl-9-fluoro-11β,17,21α_F-trihydroxypregna-1,4-diene-3,20-dione (P-1742) (XIV) demonstrate the effectiveness of the 21-methyl function in eliminating the undesirable salt-retaining activity of 9-fluoroprednisolone with only partial reduction of its glucocorticoid activity. Thus, introduction of this substituent transforms 9-fluoroprednisolone into a systemically useful anti-inflammatory agent.[4]

(3) (a) J. G. Llaurado and J. A. Schneider, *Fed. Proc.*, **19**, 159 (1960); (b) J. G. Llaurado, *Acta Endocrinol.*, **38**, 137 (1961).

(4) E. W. Boland, *Am. J. Med.*, **31**, 581 (1961).

(5) (a) Prior to the present publication, the 21-methyl corticoids which were obtained by the reduction of 20,21-diketones with fermenting yeast were arbitrarily designated 21B-ols to differentiate them from the epimeric compounds (designated 21A-ols) prepared by another route. Subsequent stereochemical studies[5b] (the details of which will appear in a forthcoming publication) have established the absolute configuration of the epimeric 21-methyl corticoids, thus allowing the designation of the yeast reduction products as 21α_F-hydroxypregnane derivatives (Fischer convention) or as 17β-[(S)-2-acetoxypropionyl]androstane derivatives (Cahn–Ingold–Prelog convention[27b]). (b) one of the intermediates (XXIV)[1c] belonging to the series originally designated 21A-ols was cleaved at the C-17–C-20 bond and the nonsteroidal fragment was converted in several steps to methyl α-acetoxypropionate. Identification of this product as a derivative of D-(−)-lactic acid

(6) B. G. Christensen, N. G. Steinberg, and R. Hirschmann, *Chem. Ind.* (London), 1259 (1958).

(7) The rapidity of this reaction is noteworthy in view of the recently reported[8] sluggish nature of the reaction of C-20 and C-3 ketones with diazomethane. The difference in rates makes possible the use of the present reaction sequence with complex steroids.

(8) A. L. Nussbaum and F. E. Carlon, *J. Am. Chem. Soc.*, **79**, 3831 (1957).

(9) H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro E. P. Oliveto, and E. B. Hershberg, *ibid.*, **77**, 4781 (1955).

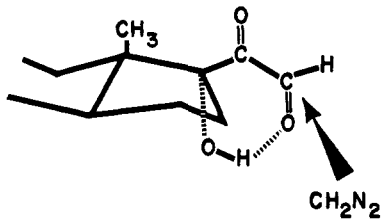
proved to be 21-methyl-11β,17-dihydroxypregna-1,4-diene-3,20,21-trione (III).[10] The α-diketone structure of III was demonstrated by its reaction with *o*-phenylenediamine to form a quinoxaline (VIIIb) possessing ultraviolet absorption spectral characteristics closely resembling those of the quinoxaline (VIIIa) obtained from prednisolone 21-aldehyde (I). The infrared spectrum, elemental analysis, and subsequent transformations of this product also were consistent with the assigned α-diketone structure.

Treatment of the 21,21a-epoxide with hydrogen halides opened the ring predominantly at the terminal carbon atom affording the desired halohydrins [*e.g.*, 21 - chloromethyl - 11β,17,21 - trihydroxypregna - 1,4-diene-3,20-dione (IV)] with hydroxyl at C-21.[11,12] Location of this new hydroxyl function was based on cleavage of IV with periodic acid, which afforded 11β,-17α - dihydroxyandrosta - 1,4 - dien - 3 - one - 17-carboxylic acid,[13] and upon subsequent reactions described.

Preliminary exploration of methods for transforming the C-22 steroids prepared in this way to one or both of the epimeric 21-methylprednisolones led to the choice of diketone III as the most useful intermediate and, therefore, to a search for a more efficient synthesis of 20,21-diketones. It was hoped that the desired diketone would be formed readily from epoxide II by one of the methods commonly employed for epoxide-to-ketone conversion.[14a] However, most of the attempts to convert the epoxide directly to III resulted in disappointingly low yields. For example, treatment of II with anhydrous hydrogen chloride in refluxing ethyl acetate afforded 30% of crystalline diketone.[15]

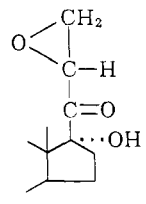
An unexpected and superior method of preparing diketone III was discovered during an attempt to oxidize the 20,21-ketol moiety of IV to the 21-chloromethyl 20,21-diketone VII, from which halogen presumably would be removed more readily by hydrogenolysis. For this purpose a modification of the method of Rigby,[16] which utilizes bismuth trioxide for the oxidation of acyloins to diketones, was employed. However the reaction did not proceed as expected (there was no apparent reduction of the reagent to elemental bis-

(10) With the exception of III the structures of the by-products (each of which assayed less than 5% by paper chromatographic analyses) have not been elucidated. The possibility exists that one of them is the C-21-epimer of epoxide II. However, the absence of significant quantities of the epimer indicates either that it is formed in less than 5% yield due to attack of diazomethane on I from a preferred direction or that the epimer is unstable and decomposes to other products. We favor the former explanation (attack from the less hindered side as shown in XXVI) in view of the likelihood that the aldehyde exists in a *trans* configuration.[10a] This conformation

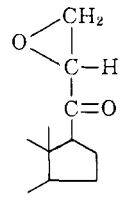


XXVI

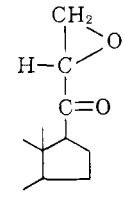
is favored not only by the strong repulsive forces of the dipoles of the carbonyl groups but by the possibility for hydrogen bonding between 17-hydroxyl and 21-carbonyl when the configuration is as shown in XXVI. If the attack of diazomethane on the 21-carbonyl carbon is as postulated above, the absolute stereochemistry of the resulting epoxide can be predicted as belonging to the 21βF-series (XXVII). Supporting data for this configurational assignment will be presented in a subsequent paper from this laboratory. An observation which may be relevant to the degree of



XXVII



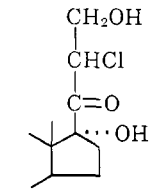
XXVIII



XXIX

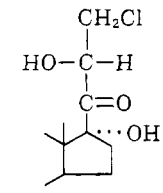
hydrogen bonding in XXVI is the isolation of *two* major products (rather than one) from the reaction of a 17-desoxysteroid 3,20-dioxopregn-4-en-21-al (desoxycorticosterone 21-aldehyde) with diazomethane. The products are isomeric $C_{22}H_{28}O_3$ compounds and are tentatively assigned the epoxide structures XXVIII and XXIX on the basis of their elemental analyses, infrared spectra, and their conversion to halohydrins. (a) J. Hine, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1956, pp. 233–234.

(11) The reaction of epoxide II with hydrogen chloride invariably produced a minor by-product for which the not unlikely isomeric chlorohydrin structure VI cannot be ruled out by the available data. In contrast to chlorohydrin II, the minor product did *not* produce a color in the tetrazolium test for 20,21-ketols.



VI

(12) If the stereochemistry of epoxide II is as shown in structure XXVII,[10] then the halohydrins will of necessity have the same absolute configuration at



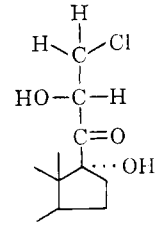
IV

C-21 as the epoxides, *i.e.*, they will belong to the 21βF-hydroxy series (*e.g.*, IV above). Supporting evidence for this assignment was obtained by the use of molecular rotation differences and optical rotatory dispersion and will be presented in a forthcoming publication.

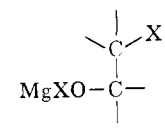
(13) R. Hirschmann, G. Bailey, and J. M. Chemerda, *Chem. Ind.* (London), 682 (1958).

(14) (a) S. Winstein and R. R. Henderson, "Heterocyclic Compounds," Vol. I, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 27–58; (b) S. Winstein and R. R. Henderson, *ibid.*, p. 49.

(15) Examination by paper chromatography of aliquots of the reaction mixture during the course of the reaction showed the presence of a compound identical to chlorohydrin IV in mobility and behavior toward blue tetrazolium reagent. Confirmation of the existence of IV as an actual intermediate in the conversion of II to III by isolation of IV might be useful in the elucidation of the mechanism of oxide openings. It has been pointed out by Winstein and Henderson[14b] that it is often not clear whether the oxide rearranges or whether an intermediate is produced (by the acid catalyst) which actually rearranges, *e.g.*, in the magnesium bromide-catalyzed rearrangement of oxide to ketone the halomagnesium salt (XXX) of the halohydrin is formed and this species rearranges. In the case presently under discussion the intermediate which would be analogous to XXX would be chlorohydrin IV. It is known (see text) that IV is converted to III under these conditions.



IV



XXX

(16) W. Rigby, *J. Chem. Soc.*, 793 (1951).

muth), and the major steroidal product proved to be the diketone III.[17a]

Subsequent to the discovery of the effectiveness of bismuth trioxide it was found that other reagents, *e.g.*, anhydrous hydrogen chloride in refluxing ethyl acetate, also were effective in the dehydrohalogenation and rearrangement of chlorohydrin IV to diketone III. Furthermore, the dehydrohalogenation occurred even more readily when bromohydrin V or iodohydrin XI were employed. In contrast to the more stable chlorohydrin, bromhydrin V lost the elements of hydrogen bromide simply upon being heated in refluxing ethyl acetate (without added acid), and solutions of iodohydrin underwent significant decomposition even at room temperature. The most efficient of the above methods utilized bromohydrin V as an intermediate. Thus, when oxide I was treated with methanolic hydrogen bromide and the resultant crude bromohydrin was heated in refluxing ethyl acetate for a period of one to two hours, the elements of hydrogen bromide were lost and 21-methyl-11β,17-dihydroxypregna-1,4-diene-3,20-21-trione (III) was isolated in approximately 50% yield (based on I).

Although the mechanism(s) of the above dehydrohalogenative rearrangement reactions have not been elucidated, it has been established that the analogous 17-unsubstituted halohydrins are stable under reaction conditions which are effective in the 17-hydroxylated series. It would appear, therefore, that the 17-hydroxyl group participates in the reaction, *e.g.*, by assisting in the ionization of halogen *via* hydroxyl–halogen interaction[17b] in C-20–C-21 enol form (Fig. A) which is a

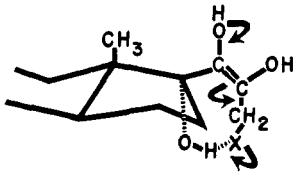


Figure A

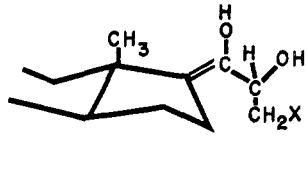


Figure B

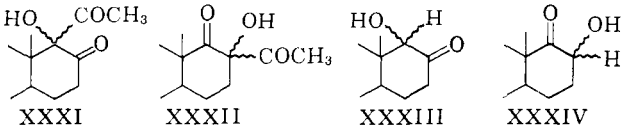
likely intermediate. A less reasonable alternative for the lack of reactivity in the 17-desoxy series involves enolization in the opposite direction to afford the C-17–C-20 enol (Fig. B) which would be unfavorable for further reaction. This alternative is not likely, however, since there is evidence that 17-desoxy-20,21-ketols do indeed enolize to the C-20–C-21 enol form.[17c]

The effectiveness of bismuth trioxide in this transformation probably is related to the complexing ability of bismuth and/or the tendency for the formation of the sparingly soluble bismuthyl chloride (BiOCl)[18] since other oxides (*e.g.*, aluminum oxide, cupric oxide) or acetates (*e.g.*, potassium acetate) are without effect on IV under identical conditions.[19] The role of acid in the observed loss of the elements of HX from the halohydrins in ethyl acetate[20] might consist of promoting their conversion to the proposed enol form (Fig. A) which would be favorable for elimination.

Selective reduction of the 21-carbonyl group of diketone III, required to complete the synthesis of 21-methylprednisolone, could be accomplished by only two of the numerous methods explored. The more satisfactory one was the action of fermenting yeast[21] which gave 21-methyl-11β,17,21α_F-trihydroxypregna-1,4-diene-3,20-dione (XII)[5] in 50–60% yield. No major by-products were detectable.

Reduction of the 20,21-diketone III with sodium borohydride also occurred predominantly at C-21 to form the desired ketol XII, but this was accompanied by a substantial amount of the 20-dihydro derivative 21-methyl-11β,17,20-trihydroxypregna-1,4-diene-3,21-dione (XIII). In view of the well known tendency of sodium borohydride reduction of 20-ketosteroids to produce 20β_F-hydroxyl derivatives[22] the β-configuration would be predicted for the C-20 hydroxyl group of XIII. Additional support for this tentative assignment of configuration was obtained from the observation that microbiological reduction of III with *Streptomyces erythreus*,[23] an organism which is known to reduce other 20-keto steroids to the 20β_F-hydroxy derivatives,[24a,b] afforded the same 20-dihydro product XIII. Assignment of the 20β_F-hydroxy configuration to by-product XIII is also favored by the probable *trans* oriented nature of the diketone[10a] and the expected attack of the borohydride anion from its less hindered side,[22] *i.e.*, the side away from the angular methyl group. Thus, the product which would be predicted from attack on the 21-carbonyl group (XXXV) is the observed 21α_F-hydroxy derivative. Attack from this same side on the 20-carbonyl group

(17) (a) An isomeric by-product (XX) also was isolated from this reaction. An attempt to oxidize IV with cupric acetate also gave XX as a major product (part of a mixture) but no diketone III was detected. Although the structure of XX has not been established definitely, the D-homo structures XXXI and XXXII are likely possibilities for the following reasons: (1) treatment with strong alkali gave a new product resulting from the loss of



XXXI XXXII XXXIII XXXIV

C_2H_2O fragment; (2) elemental analyses and infrared spectra were consistent with structures XXXI or XXXII for the original compound XX and with XXXIII or XXXIV for the alkaline degradation product. (b) Another case of hydrogen bonding to halogen (C-17α hydroxyl and C-12α halogen) was reported recently: P. A. Diassi, J. Fried, R. M. Palmere, and E. F. Sabo, *J. Am. Chem. Soc.*, **83**, 4249 (1961). (c) G. A. Fleisher and E. C. Kendall, *J. Org. Chem.*, **16**, 573 (1951).

(18) (a) The tendency of bismuth salts to be converted to very insoluble bismuthyl halides (BiOX) is well known and can occur at pHs at least as low as 1.2. *Cf.* J. D. Moyer and H. S. Isbell, *Anal. Chem.*, **30**, 957 (1958). (b) K. B. Yatsimirskii and V. P. Vasilev, "Instability Constants of Complex Compounds," Consultants Bureau, New York, N. Y., 1960. (c) Chelates of the dihydroxyacetone moiety with trivalent metals are known. *Cf.* K. Bernauer and S. Fallab, *Helv. Chim. Acta*, **40**, 1690 (1957), and J. W. Fisher, U. S. Patent 3,010,975 (November 28, 1961).

(19) The ineffectiveness of potassium acetate in *acetic acid* eliminates the possibility that the basic properties of bismuth trioxide might be involved in the elimination of the elements of hydrogen chloride. Interestingly, however, when IV was heated with potassium acetate *in acetone* a good yield of diketone III was isolated.

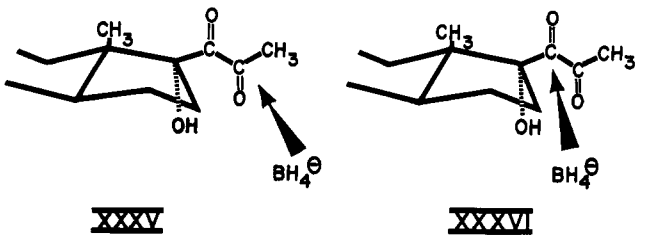
(20) (a) The observed order of stability of the halohydrins is the one which would be predicted on the basis of the ease of heterolysis of the carbon–halogen bond.[20b] Thus chlorohydrin requires an added catalyst while bromohydrin and iodohydrin do not (possibly due to the presence of sufficient amounts of residual HX in these more favorable cases). (b) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 338–339.

(21) C. Neuberg, "Advances in Carbohydrate Chemistry," Vol. 4, Academic Press, Inc., New York, N. Y., p. 86.

(22) L. L. Smith, J. J. Garbarini, J. J. Goodman, M. Marx, and H. Mendelsohn, *J. Am. Chem. Soc.*, **82**, 1437 (1960), and references contained therein.

(23) We are indebted to Dr. J. Sardinas of our Fermentation Research Department for carrying out this microbiological conversion.

(24) (a) G. M. Shull, unpublished results; (b) T. Takahashi and Y. Uchibori, *Agr. Biol. Chem.*, **26**, 89 (1962).



XXXV XXXVI

(XXXVI) instead of on C-21 would, therefore, be expected to produce the 20-dihydro derivative of *opposite* configuration, the 20β_F-hydroxy compound.

Reduction of the diketone polarographically[25a,b] produced a complex mixture of products from which the 20-dihydro derivative XIII was isolated. None of the 21α_F-ol (XII) could be detected.

Application of XIIa of the familiar sequence for introducing a 9α-fluorine substituent[26] proceeded in the expected fashion *via* the intermediate 9(11)-ene (XXXVII), 9α-bromo-11β-hydroxy (XXXVIII), and 9β,11β-oxido (XXXIX) derivatives to 21-methyl-9-fluoro - 11β,17,21α_F - trihydroxypregna - 1,4 - diene-3,20-dione 21-acetate (XIV).[27] This product was identical to that obtained from 9-fluoro-11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-al (XV) *via* the oxide (IX), bromohydrin (XXI), and α-diketone (XVIII). It is noteworthy that when the diazomethane sequence was applied to the preparation of the 9-fluoro and 6α,9-difluoro derivatives XIV and XVII, the reaction proceeded essentially in the same manner as described for the transformation of prednisolone 21-aldehyde to XII except for the slowness of the final yeast reduction step, which was most probably due to the lower solubility of the 9-fluoro and 6α,9-difluoro 20,21-diketones XVIII and XIX.

Experimental[28]

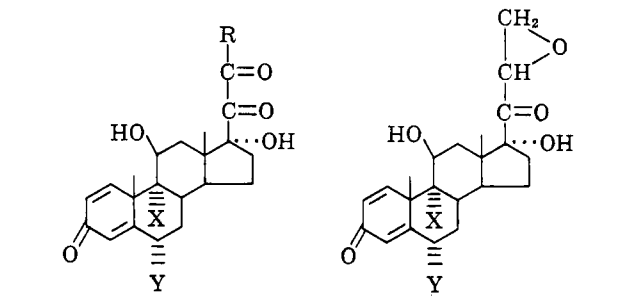
11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-al (Prednisolone-21 Aldehyde) (I).—Prednisolone was oxidized with cupric acetate according to the method of Christensen, *et al.*[29] An analytical sample, from methanol, exhibited m.p. 186–188° dec., λ_{max} 244 mμ (15,700), infrared λ_{max} 1699 cm.

Anal. Calcd. for $C_{21}H_{26}O_5 \cdot CH_3OH$: C, 67.67; H, 7.74; methoxyl, 7.94. Found: C, 67.28; H, 7.64; methoxyl, 7.23.

21-Methyl-21,21a-epoxy-11β,17-dihydroxypregna-1,4-diene-3,-20-dione (II).—An ice-cold solution of diazomethane prepared from 10 g. of N-methyl-N-nitroso-N'-nitroguanidine in 100 ml. ether was added to an ice-cold solution of 3.88 g. of prednisolone 21-aldehyde in 350 ml. methanol and 170 ml. ether. After 15 min. at 5° and 2 hr. at room temperature the excess diazomethane was destroyed by the addition of dilute acetic acid. The reaction mixture was concentrated to 20 ml. and the residue taken up in 500 ml. of chloroform. The chloroform solution was washed with 5% sodium bicarbonate and water and taken to dryness. The amber residue, upon trituration with 1:2 ethyl acetate–ether, afforded 1.45 g. white microcrystals, m.p. 225–227° dec. After two recrystallizations from ethyl acetate the product (300 mg.) exhibited m.p. 239–241° dec., λ_{max} 243 mμ (15,600), infrared λ_{max} 1709 cm.$^{-1}$, $[\alpha]_D$ + 166°.

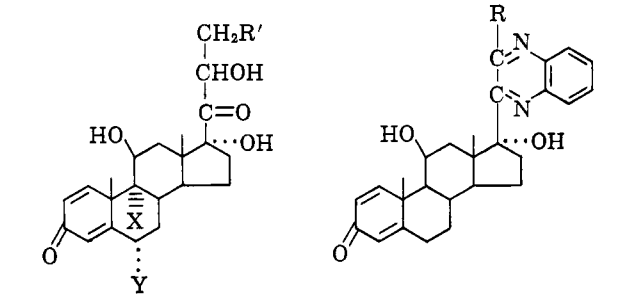
Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 70.71; H, 7.77.

Minor Products of Reaction of Prednisolone 21-Aldehyde with Diazomethane.—After removal of the crude epoxide compound by trituration (see above), the mother liquor residues from several experiments were combined and taken up in methylene chloride and chromatographed on neutral alumina. The components which were isolated (quantitative paper chromatographic assays



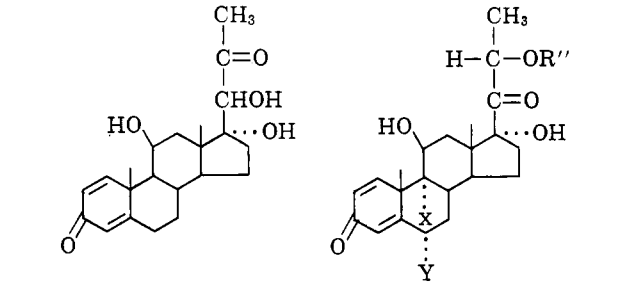
I. R = X = Y = H
III. R = CH_3; X = Y = H
XV. R = Y = H; X = F
XVI. R = H; X = Y = F
XVIII. R = CH_3; X = F; Y = H
XIX. R = CH_3; X = Y = F

II. X = Y = H
IX. X = F; Y = H
X. X = Y = F



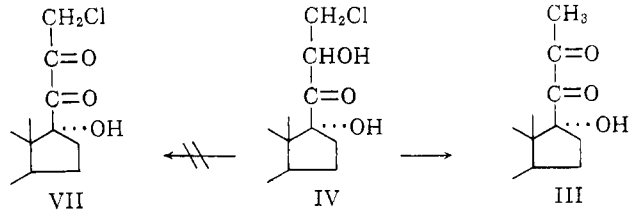
IV. R' = Cl; X = Y = H
V. R' = Br; X = Y = H
XI. R' = I; X = Y = H
XL. R' = Cl; X = F; Y = H
XXI. R' = Br; X = F; Y = H
XXII. R' = Cl; X = Y = F
XXIII. R' = Br; X = Y = F

VIIIa. R = H
VIIIb. R = CH_3



XIII
XIIIa. Acetate of XIII

XII. R'' = X = Y = H
XIIa. Acetate of XII
XIV. R'' = Y = H; X = F
XIVa. Acetate of XIV
XVII. R'' = H; X = Y = F
XVIIa. Acetate of XVII



VII IV III

(25) (a) S. Wawzonek, *Anal. Chem.*, **28**, 638 (1956); (b) we are grateful to Mr. W. McMullen and Mr. L. Ciaccio of our Analytical Department for performing the polarographic reduction experiments.

(26) J. Fried and E. F. Sabo, *J. Am. Chem. Soc.*, **79**, 1130 (1957); R. F. Hirschmann, R. Miller, J. Wood, and R. E. Jones, *ibid.*, **78**, 4956 (1956).

(27) (a) P-1742, previously named 9α-fluoro-21-methyl-1,4-pregnadiene-11β,17α-21B-triol-3,20-dione 21-acetate[1a,d]; according to Cahn–Ingold–Prelog convention for specifying asymmetric configuration[27b] compound XIV would be named 17β-[(S)-2-acetoxypropionyl]-9-fluoro-11β,17-dihydroxyandrosta-1,4-dien-3-one. (b) R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956).

(28) Unless otherwise noted the ultraviolet absorption spectra were determined in methanol solution, the infrared absorption spectra in pressed potassium bromide, and the optical rotations in dioxane solution. All melting points are uncorrected.

(29) This compound has been described as a monohydrate.[6] Our sample (prepared by the same method) was shown by a methoxyl determination to contain an equivalent of methanol.

indicated there was less than 5% of each of the minor products) are described below.

(A) Eluted in methylene chloride containing 15% ether. Recrystallization from acetone–ether afforded an analytical sample, m.p. 233–235°, λ_{max} 243 mμ (14,700).

Anal. Found: C, 72.79; H, 8.18; O, 19.21; methoxyl, 0.68.

(B) Eluted in ether containing 1% acetone. An analytical sample, recrystallized from acetone ether, exhibited m.p. 220–222°, λ_{max} 243 mμ (14,900), infrared λ_{max} 1693 cm.$^{-1}$.

Anal. Found: C, 71.50; H, 7.64; O, 20.91.

(C) Eluted in ether containing 5–25% acetone. The analytical sample, recrystallized from acetone–ether, exhibited m.p. 206–208°, infrared λ_{max} 1715, 1700 cm.$^{-1}$.

Anal. Found: C, 71.18; H, 7.62. Identical with compound III prepared by a different method (below).

(D) Eluted in ether containing 25–50% acetone. Recrystallization from acetone afforded an analytical sample, m.p. 289–295° dec., λ_{max} 256 mμ (20,800), infrared λ_{max} 1680, 1644 cm.$^{-1}$.

Anal. Found: C, 75.03; H, 7.53; O, 17.69; methoxyl, 0.69.

(E) Eluted in 1:1 acetone–methanol. The product consisted of a white glass which gave a positive Tollens test.

Reaction of Epoxide II with Periodic Acid.—A solution of 900 mg. of periodic acid (dihydrate) in 25 ml. of water was added to a solution of 475 mg. of II in 75 ml. of methanol at 40° and allowed to stand 24 hr. The crystalline precipitate which was removed by filtration (60 mg.) was identical to starting material. The filtrate was extracted with 1:1 ethyl acetate–benzene, and the extract was washed with water and concentrated to 3 ml. Another crop of crystalline starting material (62 mg.) was removed by filtration. The filtrate was concentrated to dryness and the residue, upon trituration with 1:1 ethyl acetate–ether, yielded 124 mg. of crystalline product, identical by infrared and paper chromatographic comparison with 11β-hydroxyandrosta-1,4-diene-3,17-dione prepared by the action of sodium bismuthate on prednisolone as described below.

Reaction of Prednisolone with Sodium Bismuthate.—A solution of 10 g. of prednisolone in 500 ml. of 50% acetic acid was stirred with 29 g. of sodium bismuthate at room temperature overnight. The stirring was stopped and after the solid had settled the colorless supernatant was filtered and diluted with 800 ml. of water with cooling. The white precipitate (2.84 g.), recrystallized from ethyl acetate afforded 1.81 g. of 11β-hydroxyandrosta-1,4-diene-3,17-dione,[10] m.p. 185–187°, λ_{max} 242 mμ, (14,850), infrared λ_{max} 1720 cm.$^{-1}$.

21-Methyl-21,21a-epoxy-17-hydroxypregna-1,4-diene-3,11,20-trione.—A suspension of 650 mg. of oxide II (1.75 mequiv.) in 5 ml. of acetic acid was treated with 130 mg. (1.92 equivalents) of chromic acid in 18.4 ml. of acetic acid–water (9:1). The product isolated by extraction, upon trituration with 1:1 ethyl acetate–ether, consisted of 386 mg. of white crystalline solid, m.p. 215–218° dec. Recrystallization once from ethyl acetate and then from methanol afforded the analytical sample (105 mg.) which had m.p. 226–228° dec., λ_{max} 238 mμ (16,900), $[\alpha]_D$ + 226°.

Anal. Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08. Found: C, 71.39; H, 7.11.

21-Chloromethyl-11β,17,21ξ$_F$-trihydroxypregna-1,4-diene-3,20-dione (IV). Method A.—A suspension of 500 mg. of II in 50 ml. of chloroform was treated with 50 ml. of a solution of anhydrous hydrogen chloride in glacial acetic acid (4.1 mg./ml.). The resultant solution was allowed to stand for 1.5 hr. at room temperature. After adding 125 ml. of chloroform and 75 ml. of water, the layers were separated and the chloroform layer was washed and dried. The residue (592 mg. of pale yellow solid), upon trituration with 2:1 ether–ethyl acetate, afforded 195 mg. of crude chlorohydrin IV, m.p. 188–189° dec. Two recrystallizations from ethyl acetate yielded 65 mg. of analytically pure IV, m.p. 199–200° dec., λ_{max} 243 mμ (14,500), infrared λ_{max} 1697 cm.$^{-1}$, $[\alpha]_D$ +66°.

Anal. Calcd. for $C_{22}H_{29}O_5Cl$: C, 64.61; H, 7.15; Cl, 8.67; O, 19.57. Found: C, 64.76; H, 7.24; Cl, 8.07; O, 20.27.

Method B.—A suspension of 5 g. of II in a solution of 27 ml. of 2.5 *N* methanolic hydrogen chloride in 50 ml. of chloroform was stirred at room temperature for 2 hr. Addition of 200 ml. of water precipitated the product. Filtration yielded 4.4 g. of product which, by paper chromatographic analysis, contained approximately 70% of IV and 10% of a more polar product (presumably VI). Pure chlorohydrin, identical to the product obtained by method A, was obtained by recrystallization from ethyl acetate.

Treatment of 230 mg. of IV with 2 ml. of pyridine and 1 ml. of acetic anhydride overnight at room temperature afforded 190 mg. of crude acetate. After two recrystallizations from ethyl acetate, there was obtained 97 mg. of the 21-acetate of IV which exhibited m.p. 185–186° dec., λ_{max} 243 mμ (15,000), $[\alpha]_D$ +46°.

Anal. Calcd. for $C_{24}H_{31}O_6Cl$: C, 63.91; H, 6.93; Cl, 7.86. Found: C, 63.85; H, 7.02; Cl, 6.97.

The mother liquors obtained from several preparations of chlorohydrin IV (by either method A or B) were combined and dissolved in 20:1 ether–ethyl acetate and filtered through a column of Florisil. The filtrate contained VI free of other steroids. After three recrystallizations from 1:1 acetone–ether, the by-product VI exhibited m.p. 186–187° dec., λ_{max} 243 mμ (15,050), infrared λ_{max} 1709 cm.$^{-1}$.

Anal. Found: C, 63.44; H, 7.01; O, 19.86; Cl, 8.49.

21-Bromomethyl-11β,17,21ξ$_F$-trihydroxypregna-1,4-diene-3,20-dione (V).—A suspension of 2 g. of II in 150 ml. of 0.37 *N* methanolic hydrogen bromide was stirred at room temperature for 2 hr. Addition of 100 ml. of water to the final solution and removal of 100 ml. of methanol *in vacuo* precipitated the crystalline product. Filtration afforded crude bromohydrin, m.p. 135–136° dec. A sample prepared in chloroform–glacial acetic acid containing anhydrous hydrogen bromide and isolated as described for IV above was recrystallized twice from ethyl acetate for analysis. The best sample of V, which was somewhat unstable (see text), exhibited m.p. 141–142°, λ_{max} 243 mμ ($E^{1\%}_{1\,cm}$ 350), infrared λ_{max} 1696 cm.$^{-1}$, $[\alpha]_D$ +99°.

Anal. Calcd. for $C_{22}H_{29}O_5Br$: C, 58.28; H, 6.45; Br, 17.63. Found: C, 59.65; H, 6.51; Br, 16.96.

21-Iodomethyl-11β,17,21ξ$_F$-trihydroxypregna-1,4-diene-3,20-dione (XI).—A suspension of 3.7 g. of II in 300 ml. of methylene chloride was protected from light by wrapping the flask with aluminum foil. The suspension was stirred vigorously with 29 ml. of 50% aqueous hydrogen iodide for 10 min. The organic layer diluted with 150 ml. of methylene chloride and 30 ml. of ethyl acetate and 300 ml. of water and 100 ml. of 5% sodium thiosulfate solution were added. After stirring for 3 min., the organic layer was separated and the aqueous layer extracted three times with 100-ml. portions of 9:1 methylene chloride–ethyl acetate. The combined organic extracts were washed with water, dried, and concentrated to 200 ml. The first crop of crystalline product (2.48 g.) was isolated by filtration. A second crop (0.87 g.) was isolated by concentration of the filtrate to 20 ml. Recrystallization of 400 mg. of the first crop material from ethyl acetate afforded 103 mg. of crystals in two crops, m.p. 149–150° and m.p. 146–147°. A final recrystallization from ethyl acetate yielded a still impure sample of the very unstable iodohydrin (XI), m.p. 145–146° dec., λ_{max} 243 mμ ($E^{1\%}_{1\,cm}$ 331).

Anal. Calcd. for $C_{22}H_{29}O_5I$: C, 52.81; H, 5, 84; I, 25.36. Found: C, 58.17; H, 6.77; I, 21.32.

21-Methyl-11β,17-dihydroxypregna-1,4-diene-3,20,21-trione (III). From Bromohydrin.—A suspension of 1.0 g. of bromohydrin (V) in 150 ml. of ethyl acetate was heated under reflux for 2 hr. during which time the steroid dissolved. The solution was taken to dryness and the tacky crystalline residue (888 mg.) triturated with 1:1 ether–ethyl acetate. The resultant crystals (524 mg.) were identical by infrared spectral comparison with III prepared from chlorohydrin IV with bismuth trioxide (see below) and with one of the minor products (C) isolated from the reaction of aldehyde I with diazomethane.

III from Chlorohydrin with Bismuth Trioxide in Acetic Acid.—A solution of 7.56 g. of chlorohydrin IV in 380 ml. of glacial acetic acid was immersed in a water bath maintained between 50 and 60°. Bismuth trioxide (29.4 g.) was added and the mixture was stirred for 3 hr. The warm reaction mixture was filtered (Super Cel) and the acetic acid was removed *in vacuo*. The residue was stirred three times with 200-ml. portions of chloroform. The combined chloroform extracts were filtered (Super Cel) and washed with water (twice), 5% sodium bicarbonate (six times), and water (three times), dried, concentrated to a small volume (20 ml.), and filtered to remove 1.71 g. of crystalline material identical to starting material (IV). The filtrate was taken almost to dryness and treated with ethyl acetate. The crystals which were removed by filtration (2.62 g.) were recrystallized once from ethyl acetate and once from acetone–ether to obtain an analytical sample of III, m.p. 206–208°, λ_{max} 243 mμ (15,450), infrared λ_{max} 1715, 1700 cm.$^{-1}$, $[\alpha]_D$ + 91°.

Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 70.91; H, 7.65.

Chromatography of the mother liquor residue of crude III (p. 1535) on Florisil and elution with 2:1 ether–ethyl acetate yielded 310 mg. of a by-product (XX) (see below) identical to the major product isolated from the reaction of chlorohydrin with bismuth trioxide at slightly higher temperatures (60–70°). Compound XX was also obtained as a major product when chlorohydrin IV was treated with cupric acetate (see below).

III from Chlorohydrin IV with Potassium Acetate in Acetone.—A mixture of 101 mg. of IV and 200 mg. potassium acetate in 10 ml. of acetone was heated at reflux for 2 hr. with stirring. Concentration to 3 ml. and slow addition of the residue to 40 ml. of water produced a white crystalline precipitate which was filtered. The dry solid (46 mg., 50%) was identical by paper chromatographic and infrared spectral comparison to α-diketone III prepared by other methods herein described.

III from Chlorohydrin IV in Refluxing Ethyl Acetate Containing Acid.—A suspension of 100 mg. of IV in 15 ml. of ethyl acetate was heated under reflux while bubbling anhydrous hydrogen chloride through the mixture for 2 hr. Evaporation of the solvent and trituration of the residue gave 23 mg. of crystals which were identical to diketone III by paper chromatographic and infrared spectral comparison. Paper chromatographic assay of the reaction solution prior to isolation of the crystalline product indicated the presence of 40% of the theoretical amount of III.

III from Epoxide II.—A suspension of 1 g. of epoxide II in 150 ml. of ethyl acetate was heated with stirring at reflux temperature, and anhydrous hydrogen chloride was bubbled through the reaction mixture for 2 hr. The final solution was concentrated to an oil which was triturated with ether. Filtration afforded 604 mg. of crystals, m.p. 160–168°, which proved to be crude 20,21-diketone III by paper chromatographic and spectral comparison with III prepared as described above. Recrystallization of the crude material from 4:1 methanol–water gave 300 mg. of crystals, m.p. 203–205° identical with a sample of authentic diketone III.

Reaction of III with *o*-Phenylenediamine.—Compound III (200 mg.) in 10 ml. of ethanol was heated with 200 mg. of *o*-phenylenediamine and 1 ml. of 2 *N* hydrochloric acid for 0.5 hr. at 60°. Dropwise addition of 20 ml. of water to the orange-red solution with cooling resulted in precipitation of 195 mg. of yellow crystals, m.p. 178–181° dec., λ_{max} 238 and 321 mμ and infrared absorption spectrum exhibiting no saturated carbonyl absorption. Recrystallization of the product from 1:2 ethanol–water afforded bright yellow crystals (110 mg.) m.p. above 260°, λ_{max} 238 mμ ($E^{1\%}_{1cm}$ 930) and 321 mμ ($E^{1\%}_{1cm}$ 198). The infrared and ultraviolet absorption spectra of this product were very similar to those of the derivative obtained from prednisolone 21-aldehyde under identical conditions.

Reaction of III with Periodic Acid.—A solution of 80 mg. of diketone III in 10 ml. of methanol at 40° was treated with 5 ml. of water containing 180 mg. of periodic acid (dihydrate) and stored at room temperature for 15 hr. The solution was evaporated to dryness and the residue washed with water. The crystalline residue was recrystallized from ethyl acetate. The product was identical to 11β-hydroxyandrosta-1,4-diene-3,17-dione[9] by paper chromatographic and infrared spectral comparison.

Reaction of Chlorohydrin IV with Periodic Acid.—A solution of 300 mg. of IV in 25 ml. of methanol was treated with 15 ml. of periodic acid solution (containing 540 mg. of dihydrate) and after 15 hr. the product was isolated as described above. The product (177 mg.) was almost entirely soluble in 5% sodium hydroxide. It was dissolved in 5 ml. of alkali and extracted three times with 10-ml. portions of methylene chloride. The alkaline solution was made acidic by dropwise addition of 2 *N* hydrochloric acid to reprecipitate the acid (113 mg.), which exhibited m.p. 226–227° and infrared absorption spectrum identical to the product obtained from prednisolone with periodic acid under the same conditions (see below).

Reaction of Prednisolone with Periodic Acid.—Treatment of 468 mg. of prednisolone in 25 ml. of methanol with 900 mg. of periodic acid in 25 ml. of water overnight at room temperature afforded (by the isolation procedure above) 240 mg. of 11β,17α-dihydroxyandrosta-1,4-diene-3-one-17-carboxylic acid,[13] m.p. 224–226° dec.

Reaction of Chlorohydrin (IV) with Bismuth Trioxide at 60–70°.—A suspension of 1 g. of IV in 44 ml. of glacial acetic acid was heated to dissolve the steroid and immersed in a water bath kept at 60–70°. The solution was treated with 3.9 g. of bismuth trioxide for 5 hr. with stirring. The reaction mixture was diluted with 50 ml. of chloroform and filtered (Super Cel). The product, isolated as described before for compound III and triturated with 3:1 ethyl acetate–ether, afforded 295 mg. of XX as microcrystals, m.p. 211–213° dec. The analytical sample of XX, prepared by recrystallization from ethyl acetate, exhibited m.p. 215–216°, λ_{max} 242 mμ ($E^{1\%}_{1cm}$ 407), infrared λ_{max} 3496, 3389, 1693 (broad), 1650, 1620, 1605 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58; O, 21.48. Found: C, 71.15; H, 7.53; O, 20.87.

The infrared spectrum and paper chromatographic behavior of XX differed from that of diketone III.

Treatment of Chlorohydrin IV with Potassium Acetate in Acetic Acid.—A solution of 100 mg. of chlorohydrin IV in 5 ml. of acetic acid containing 200 mg. of potassium acetate was heated at 50–60° for 5 hr. Paper chromatographic analysis of aliquots taken at hourly intervals showed little or no reaction had occurred in this period. Heating was continued for 2 hr. after which 5 ml. of ethanol was added but no reaction was detected paper chromatographically. The solvents were removed *in vacuo* and the residual white solid was triturated with water and filtered. The resulting white solid (60 mg.) was identical to starting material by infrared spectral comparison.

Treatment of Chlorohydrin IV with Bismuth Trioxide in Acetone.—A mixture of 100 mg. of chlorohydrin IV and 200 mg. of bismuth trioxide in 10 ml. of acetone was heated at reflux under an atmosphere of nitrogen for 2 hr. It was cooled to room temperature and 10 ml. of 1:1 chloroform–methanol was added. The bismuth salts were removed by filtration and the filtrate was concentrated to dryness *in vacuo*. Paper chromatographic analysis indicated that the chlorohydrin was unaffected by this treatment.

Chlorohydrin IV in Refluxing Solvents.—A suspension of 100 mg. of IV in 15 ml. of ethyl acetate was heated at reflux for 72 hr. Only partial dissolution occurred. Evaporation of the solvent left a crystalline residue which was starting material (infrared spectral evidence and paper chromatographic analysis).

A suspension of 100 mg. of IV in 15 ml. of *n*-amyl acetate was heated at reflux for 5 hr. Dissolution occurred in 30 min. After 5 hr. the solvent was evaporated and the residual crystals were examined by paper chromatography and spectrally. The starting material was the preponderant steroid present but there was evidence that some degradation had occurred (intensity of absorption at 240 mμ was approximately two-thirds that of the starting material).

Reaction of Chlorohydrin IV with Cupric Acetate.—A suspension of 1.0 g. of chlorohydrin IV in 64 ml. of methanol was treated with a mixture of 1.5 g. of cupric acetate, 6 ml. of water, and 0.5 ml. of glacial acetic acid at 60° for 30 min. The reaction mixture was cooled to room temperature, treated with 1.0 g. of versene and concentrated to about 5 ml. The residue was diluted with water and the resultant blue-green precipitate was filtered and washed with 30% ammonium hydroxide and water. The resultant pale yellow solid (800 mg.) was a mixture (paper chromatographic analysis) from which compound XX could be isolated by chromatography on Florisil as described before for its isolation from the mother liquor of compound III.

21-Methyl-11β,17,21α_F-trihydroxypregna-1,4-diene-3,20-dione (XII).[5] **A. Reduction of III with Yeast.**—A solution of 24 g. of 11β,17-dihydroxy-21-methylpregna-1,4-diene-3,20-21-trione (III) in 4800 ml. of ethanol was added to a stirred solution of 9600 g. of sucrose in 70 l. of tap water. A suspension of 1680 g. of Fleischmann's "active dry yeast" in 9600 ml. of tap water at 40° was stirred for 30 min. and added to the steroid–sucrose solution. The reaction mixture was stirred gently enough to maintain anaerobic conditions and the pH was kept between 3.8 and 4.7 by periodic addition of ammonium hydroxide. After 24 hr. a suspension of 336 g. of yeast and 1920 g. of sucrose in 1620 ml. of water and 96 ml. of ethanol was added. Paper chromatographic assay of the extract of an aliquot at 24 hr. indicated the presence of 78% of the theoretical amount of XII. At 48 hr. the assay of an aliquot was approximately unchanged (81%). At this time Super Cel (4800 g.) was added and the mixture was filtered. The filtrate was extracted four times with 12-l. portions of chloroform and the combined extract was washed with two 6-l. portions of water. The chloroform solution was clarified by filtration through sodium sulfate and concentrated to dryness. The glassy residue was taken up in pyridine (360 ml.), filtered to clarify and another 120 ml. of pyridine was used to wash the filter cake. Acetic anhydride (240 ml.) was added, and the solution was allowed to stand at room temperature overnight. The acetate of XII was precipitated as an oil by addition of water and was extracted with four 1200-ml. portions of ethyl acetate. The ethyl acetate solu-

tion was washed with dilute hydrochloric acid, saturated sodium bicarbonate, and water, dried, and concentrated *in vacuo*. The white crystalline product which separated during the concentration was collected in two crops (10.0 g. and 2.7 g.) of approximately 90% purity (paper chromatographic assay). The yield of acetate in the two crops was 51%. An additional 3.0 g. of 90% purity was isolated by chromatography on Florisil. An analytical sample of 21-methyl-11β,17,21α$_F$-trihydroxypregna-1,4-diene-3,20-dione 21-acetate (XIIa), obtained from ethyl acetate, exhibited m.p. 221–222°, $[\alpha]_D$ +122°, ultraviolet λ_{max} 244 mμ (14,800).

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 68.76; H, 7.67.

Crystalline alcohol (XII) (1.69 g.) was obtained by saponification of 2.0 g. of XIIa with methanolic potassium carbonate. The analytical sample obtained from 1:1 isopropyl alcohol–water apparently retained solvent of crystallization. It exhibited m.p. 117–120°, ultraviolet λ_{max}^{MeOH} 243 (15,050), $[\alpha]_D$ +111°.

Anal. Calcd. for $C_{22}H_{30}O_5 + {}^1/_2C_3H_7OH$: C, 69.77; H, 8.47. Found: C, 69.68; H, 8.39.

B. Reduction of III with Sodium Borohydride.—A solution of 186 mg. of III in 15 ml. of methanol cooled to 0° was treated with an ice-cold solution of 13.3 mg. of sodium borohydride in 3.6 ml. of methanol and kept at 0° for 1 hr. Two drops of acetic acid were added to the reaction solution and it was taken to dryness. The white glassy residue was taken up in 40 ml. of chloroform and washed with 4 ml. of water. Assay by paper chromatography at this stage indicated the presence of 60% of the theoretical amount of product XII and four minor products, one of which was identified as the 20-dihydro compound XIII (approx. 13%).

The chloroform was removed *in vacuo* and the residue acetylated in 2 ml. of pyridine with 1 ml. of acetic anhydride overnight at room temperature. The product, precipitated by the addition of water and isolated (70% yield) as described before, was very similar to XIIIa by infrared spectral comparison, but paper chromatographic analysis indicated the presence of four other compounds.

In a similar experiment in which 558 mg. of 20,21-diketone was reduced with sodium borohydride and the crude product was acetylated and isolated as described before, a small first crop of crude acetate (45 mg.) was identical to the acetate of the 20-dihydro compound (XIII) obtained by the polarographic reduction of III (see below).

21-Methyl-11β,17,20ξ-trihydroxypregna-1,4-diene-3,21-dione (XIII). A. By Polarographic Reduction of III.[25]—To a solution of 993 mg. of III in 300 ml. of formamide was added 100 ml. of universal buffer.[30] The solution was adjusted to pH 8 by addition of 6 *N* potassium hydroxide. The resulting solution exhibited two half-wave potentials: −0.87 and −1.54 volts. The reduction was performed using a mercury pool electrode with a shielded anode at −1.00 volts *vs.* a saturated calomel electrode until approximately 94% of 2 electrons per mole had been consumed (disappearance of wave at −0.87 volts).

The solution was diluted with 2000 ml. of water and saturated with sodium chloride before extracting with three 2000-ml. portions of chloroform. The chloroform solution was dried (sodium sulfate) and the solvent removed *in vacuo*. The residual pale yellow oil (1.00 g.) was acetylated in the usual way and the crude acetate isolated by chloroform extraction. The residue after removal of the chloroform was a brown glass (1.05 g.) which, upon trituration with ethyl acetate, afforded 293 mg. of tan crystals, m.p. 234–237° dec. Recrystallization of this material from 1:1 ethyl acetate–ethanol and again from ethanol gave 98 mg. of the acetate of XIII, m.p. above 250°, ultraviolet λ_{max} 244 mμ (14,850).

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.50; H, 8.14.

Saponification of 80 mg. of XIII$_2$ with methanolic potassium carbonate in the usual way afforded 37 mg. of alcohol XIII free of other steroids (analysis by paper chromatography).

Treatment of XIII with Periodic Acid.—A solution of 18.5 mg. of XIII, obtained by saponification of the acetate (see above), in 1.25 ml. of methanol was treated with 45 mg. of periodic acid (dihydrate) in 1.25 ml. of water overnight at room temperature. The reaction mixture was concentrated to about 1 ml. by evaporation with a nitrogen stream, and the resultant white precipitate was filtered. The product (8 mg. of white crystalline solid) was identical by infrared spectral comparison with 11β-hydroxyandrosta-1,4-diene-3,17-dione[10] obtained by the reaction of prednisolone with sodium bismuthate.[31]

B. By the Action of *Streptomyces erythreus*.[23]—A sample of 50 mg. of III was subjected to the action of *Streptomyces erythreus* using essentially the procedure described by F. Carvajal, *et al.*[32]

The broth (2 l.) was extracted with four 200-ml. portions of chloroform and the chloroform extract was washed with water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. Paper chromatographic comparison of the crude product with the product of polarographic reduction (see A) indicated that the major product was the 20-dihydro derivative XIII. Acetylation of the residue (237 mg.) in the usual way and isolation of the crude acetate by ethyl acetate extraction afforded 58 mg. of yellow oil which was crystallized by trituration with 1:1 ether–ethyl acetate. The filtered product (13 mg.) was identical by paper chromatographic and spectral comparison with the acetate obtained by acetylation of the polarographic reduction product of III.

21-Methyl-17,21α$_F$-dihydroxypregna-1,4,9(11)-triene 3,20-dione 21-Acetate XXXVII.—To a solution of 70.2 g. of XIIa in 350 ml. of pyridine and 350 ml. of dimethylformamide was added 70.2 g. of anhydrous sodium sulfate and, after stirring at room temperature for 1 hr., 21.1 ml. of methanesulfonyl chloride. Stirring was continued for 20 hr. and the product was precipitated by the addition of 5630 ml. of water to the reaction mixture with cooling. Recrystallization from methanol gave 54.4 g. (80%) of 17,21α$_F$-dihydroxy-21-methylpregna-1,4,9(11)-triene-3,20-dione 21-acetate, m.p. 193–195°. An analytical sample (two additional recrystallizations) exhibited m.p. 199°, λ_{max} 239.5 mμ (14,700), $[\alpha]_D$ +67°.

Anal. Calcd. for $C_{24}H_{30}O_5$: C, 72.33; H, 7.54. Found: C, 72.06; H, 7.47.

21-Methyl-9-bromo-11β,17,21α$_F$-trihydroxypregna-1,4-diene-3,20-dione 21-Acetate (XXXVIII).—N-Bromoacetamide (17.9 g.) and 10% aqueous perchloric (205 ml.) acid were added to a stirred suspension of 17,21α$_F$-dihydroxy-21-methylpregna-1,4,9(11)-triene-3,20-dione 21-acetate (49.5 g.) in a mixture of dioxane (1020 ml.) and water (184 ml.) cooled to 25°. The mixture was stirred 18 min., by which time a solution was obtained. Ice (1550 g.) was added followed by sodium sulfite (51.0 g.) and water (3820 ml., 0°). The product was filtered off after stirring the mixture 0.5 hr. at 0° and an additional 0.5 hr. at room temperature. The majority of this crude product was used in the next step. A portion (2.5 g.) was twice recrystallized from aqueous acetone and had m.p. 173° dec., λ_{max} 243 mμ (13,700), $[\alpha]_D$ +141°.

Anal. Calcd. for $C_{24}H_{31}O_5Br$: C, 58.18; H, 6.31. Found: C, 58.25; H, 6.50.

21-Methyl-9β,11β-oxido-17,21α$_F$-dihydroxypregna-1,4-diene-3,20-dione 21-Acetate (XXXIX).—The preceding moist bromohydrin was heated under reflux in ethanol (1200 ml.) for 5 hr. in the presence of potassium acetate (61.5 g.). The crude oxide (56.6 g., m.p. 200–204°), recrystallized from methanol, afforded two crops of crystals: the first (24.5 g.) had m.p. 210–212°; the second, (8.0 g.) had m.p. 218–219°. An analytical sample prepared from another run had m.p. 221–223°, λ_{max} 251 mμ (14,00), $[\alpha]_D$ +68°.

Anal. Calcd. for $C_{24}H_{30}O_5$: C, 69.54; H, 7.30. Found: C, 69.37; H, 7.64.

21-Methyl-9-fluoro-11β,17,21α$_F$-trihydroxypregna-1,4-diene-3,20-dione 21-Acetate (XIVa).—9β,11β-Oxido-17,21α$_F$-dihydroxy-21-methylpregna-1,4-diene-3,20-dione 21-acetate (15.2 g.) in chloroform (100 ml.) was added at −70° to a solution of hydrogen fluoride (20 ml.) in tetrahydrofuran (34 ml.) chloroform (16 ml.). The reaction was allowed to proceed at 0° for 4 hr., cooled to −70° again and slowly added to a stirred mixture of 10% sodium carbonate solution and chloroform. After separation of the layers, the aqueous was extracted twice with chloroform, and the combined chloroform extracts were dried by percolation through anhydrous sodium sulfate. Concentration *in vacuo* to 30 ml. yielded the crude product which was recrystallized by dissolution in 300 ml. of acetone, concentration to 200 ml., and slow addition of 50 ml. of hexane. The product, 8.29 g., m.p. 251–253°, contained 1 mole of acetone of crystallization, which it lost on heating *in vacuo* at 100° overnight (volatiles, 11.5%, calcd., 11.8%). This material appeared to be superior to analytically

(30) Composition: 0.1 *M* acetic acid, 0.1 *M* phosphoric acid, 0.1 *M* boric acid, and 0.5 *M* potassium chloride.

(31) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(32) F. Carvajal, O. F. Vitale, M. J. Gentles, H. L. Herzog, and E. B. Hershberg, *J. Org. Chem.*, **24**, 695 (1959).

pure material prepared in an earlier run and recrystallized from ethyl acetate. The sample thus obtained exhibited m.p. 251–253°, λ_{max} 239 mμ (15,350), $[\alpha]_D$ +87°.

Anal. Calcd. for $C_{24}H_{31}O_6F$: C, 66.34; H, 7.19. Found: C, 65.84; H, 7.06.

Saponification of 500 mg. of XIVa in the usual way with methanolic potassium carbonate afforded, after recrystallization from acetone (7 ml.)–hexane (10 ml.), 241 mg. of 9-fluoro-11β,17,-21α_F-trihydroxy-21-methylpregna-1,4-diene-3,20-dione, m.p. 223–224°, λ_{max} 239 mμ (14,900), $[\alpha]_D$ +139°.

Anal. Calcd. for $C_{22}H_{29}O_5F$: C, 67.32; H, 7.40; F, 4.85. Found: C, 67.00; H, 7.46; F, 4.96.

9-Fluoro-11β,17-dihydroxy-3,20-dioxopregna-1,4-diene-21-al (9-Fluoroprednisolone 21-Aldehyde) (XV).[33a]—9-Fluoroprednisolone (500 mg.), treated with cupric acetate as described earlier for the preparation of I, gave 428 mg. of crude aldehyde which was identical to a sample prepared *via* the nitrone.[33b] After two recrystallizations from acetone–water the aldehyde exhibited m.p. 226–228°, λ_{max} 239 mμ, $E^{1\%}_{1\,cm}$ 390.

Anal. Calcd. for $C_{21}H_{25}O_5F \cdot CH_3OH$: C, 64.68; H, 7.16. Found: C, 64.37; H, 7.48.

21-Methyl-9-fluoro-21,21a-epoxy-11β,17-dihydroxypregna-1,4-diene-3,20-dione (IX).—A solution of 394 mg. of 9-fluoroprednisolone 21-aldehyde in 35 ml. of methanol and 17 ml. of ether was treated with 10 ml. of ethereal diazomethane (approximately 5 mmoles). Removal of the solvents *in vacuo* gave 380 mg. of amorphous residue which crystallized upon treatment with 1:1 ether–ethyl acetate. The crystalline product (80 mg.) was recrystallized twice from ethanol to obtain the analytical sample which had m.p. 254–255° dec., λ_{max} 239 mμ (15,600), $[\alpha]_D$ +123°.

Anal. Calcd. for $C_{22}H_{27}O_5F$: C, 67.67; H, 6.97. Found: C, 68.00, H, 6.95.

21-Chloromethyl-9-fluoro-11β,17,21ξ_F-trihydroxypregna-1,4-diene-3,20-dione (XXXV).—A suspension of 178 mg. of oxide IX in 2.3 ml. of 4.5 *N* hydrogen chloride in methanol was diluted with 1.9 ml. of methanol and 7.8 ml. of chloroform and stirred for 2.5 hr. Addition of 35 ml. of water to the resultant solution precipitated the product as an oil which crystallized during removal of the chloroform *in vacuo*. The crude product (814 mg.), isolated by filtration, was recrystallized from ethyl acetate and afforded 418 mg. of white microcrystals, m.p. 178° dec., $[\alpha]_D$ +79°. An analytical sample, obtained by a second recrystallization, had m.p. 174–174.5° dec., λ_{max} 239 mμ (15,250), $[\alpha]^{24}_D$ +72°.

Anal. Calcd. for: $C_{22}H_{27}O_5FCl$: C, 61.89; H, 6.61. Found: C, 61.39; H, 6.63.

Acetylation of XXXV in the usual way and recrystallization of the crude acetate from methanol afforded an analytical sample which had m.p. 117–119°, $[\alpha]_D$ + 73°.

Anal. Calcd. for $C_{24}H_{30}O_6FCl$: C, 61.47; H, 6.45. Found: C, 61.28; H, 6.99.

21-Bromomethyl-9-fluoro-11β,17,21ξ_F-trihydroxypregna-1,4-diene-3,20 dione (XXI).—A suspension of 720 mg. of oxide IX was stirred in 36 ml. of methanolic hydrogen bromide (0.37 *N*) for 2 hr. at room temperature. The product, precipitated by addition of 72 ml. of water, consisted of 531 mg. of ivory microcrystals, m.p. 105–110° dec. Found: Br, 9.34. This material was used without purification for the preparation of the 20,21-diketone XVIII.

21-Methyl-9-fluoro-11β,17-dihydroxypregna-1,4-diene-3,20,21-trione (XVIII).—A solution of 500 mg. of crude bromohydrin XXI (as obtained above) was heated at reflux for 3 hr. and the solvent evaporated *in vacuo*. The residue was triturated with ether to crystallize the crude product, m.p. 110–130°. Recrystallization from ethyl acetate–cyclohexane gave 148 mg. of white microcrystals, m.p. 182–185°. A second recrystallization from ethyl acetate gave an analytical sample which had m.p. 219–220° dec., λ_{max} 239 mμ (15,700), $[\alpha]_D$ +88°.

Anal. Calcd. for $C_{22}H_{27}O_5F$: C, 67.67; H, 6.97; F, 4.87. Found: C, 67.64; H, 6.91; F, 4.75.

Reaction of XVIII with *o*-Phenylenediamine.—Treatment of 20 mg. of XVIII with 20 mg. of *o*-phenylenediamine in 3 ml. of ethanol for 0.5 hr. at steam bath temperature gave 14 mg. of light orange crystals; λ_{max} 238 mμ ($E^{1\%}_{1\,cm}$ 1000) and 321 mμ ($E^{1\%}_{1\,cm}$ 190). The infrared spectrum of the product exhibited no saturated carbonyl absorption bands.

21-Methyl-9-fluoro-11β,17,21α$_F$-trihydroxypregna-1,4-diene-3,20-dione (XIV) from XVIII.—A solution of 500 mg. of 20,21-diketone (XVIII) in 100 ml. of ethanol was added to a solution of sucrose (200 g.) in tap water (1500 ml.). Precipitation of some of the steroid occurred. Active dry yeast (37.5 g.) in 200 ml. of water at 40° was added, and the mixture was stirred for 53 hr. at room temperature with additions of yeast (7.5 g.) and sucrose (4.0 g.) at 24, 48, and 72 hr. After 6 days, paper chromatographic analysis indicated that most of the starting material had been reduced, and the product was isolated as described for XII. Trituration of the crude residue with ethyl acetate afforded 155 mg. of crystalline alcohol.

Acetylation of XIV in the usual way gave 108 mg. of acetate XIVa which was identical in every respect with the sample prepared by the introduction of 9-fluorine into XIIa (described previously).

6α,9-Difluoro-11β,17-dihydroxy-3,20-dioxopregna-1,4-diene-21-al (XVI).—A solution of 217.8 g. of 6α,9-difluoroprednisolone,[34] m.p. 254° dec., was oxidized with cupric acetate in methanol according to the procedure described for the preparation of I. The white crystalline product (172 g.), m.p. 181–184° dec., λ_{max} 238 mμ ($E^{1\%}_{1\,cm}$ 387), was free of starting material (paper chromatographic analysis) and was suitable for use in the next reaction. A portion of the crude aldehyde, recrystallized from methanol–water (2:1), afforded the analytical sample which had m.p. 197–204°, λ_{max} 238 mμ (16,350), $[\alpha]_D$ +90°.

Anal. Calcd. for $C_{21}H_{24}O_5F_2 \cdot CH_3OH$: C, 61.96; H, 6.62. Found: C, 62.26; H, 6.89.

21-Methyl-6α,9-difluoro-21,21a-epoxy-11β,17-dihydroxypregna-1,4-diene-3,20-dione (X).—A solution of 14.8 g. of 6α,9-difluoroprednisolone 21-aldehyde (XVI) in 650 ml. of acetone was treated at 0° with 400 ml. of ethereal diazomethane obtained from 14.7 g. of N-nitroso-N-methyl-N′-nitroguanidine. After standing at 0° for 2 hr., the excess diazomethane was destroyed with acetic acid and the solvent removed *in vacuo*. The semicrystalline residue was triturated with 1:1 acetone–ether, and filtration afforded 3.57 g. of ivory crystals which had m.p. 237–238° dec., 95% pure by paper chromatographic analysis. Another 0.83 g. of product of equal quality was isolated by chromatography of the filtrate residue on Florisil and elution of the product in ethyl acetate containing 1% acetone. The analytical sample, obtained by recrystallization of the crude product from another run from methanol and then from ethyl acetate (acetone), had m.p. 227–228° dec., λ_{max} 238 mμ (16,500), $[\alpha]_D$ +109°.

Anal. Calcd. for $C_{22}H_{26}O_5F$: C, 64.69; H, 6.42; F, 9.30. Found: C, 64.83; H, 6.53; F, 9.21.

21-Chloromethyl-6α,9-difluoro 11β,17,21ξ_F-trihydroxypregna-1,4-diene-3,20-dione (XXII).—A soultion of 816 ml. of oxide X in 4.4 ml. of 4.6 *N* methanolic hydrogen chloride, 3.6 ml. of methanol, and 8 ml. of chloroform was allowed to stand at room temperature for 2 hr. Addition of 33 ml. of water and evaporation of the chloroform and methanol gave the product as a white solid (653 mg.). The analytical sample, obtained from ethyl acetate, exhibited m.p. 181° dec., λ_{max} 237 mμ (17,400), $[\alpha]_D$ +70°.

Anal. Calcd. for $C_{22}H_{27}O_5F_2Cl$: C, 59.40; H, 6.12. Found: C, 59.11; H, 6.28.

21-Bromomethyl-6α,9-difluoro-11β,17,21ξ_F-trihydroxypregna-1,4-diene-3,20-dione (XXIII).—A suspension of 10 g. of crude oxide X in 850 ml. of 0.37 *N* methanolic hydrogen bromide was stirred at room temperature for 3 hr. The product (11.1g.), isolated as described for V, was a mixture containing approximately 60% of the desired bromohydrin, approximately 10% starting material, and 15% of a third compound presumably an isomeric bromohydrin. The crude product was suitable for use in the next reaction.

21-Methyl-6α,9-difluoro-11β,17-dihydroxypregna-1,4-diene-3,20,21-trione (XIX).—The crude bromohydrin XXIII, prepared as described earlier, was dissolved in ethyl acetate (1 l.) and heated at reflux for 2 hr. At the end of this time most of the bromohydrin had been consumed, and a new compound was detected as the major component in the reaction solution by paper chromatography. The ethyl acetate solution was concentrated to 260 ml. and 780 ml. of methylene chloride was added prior to

(33) (a) The preparation of this compound has been reported by L. H. Sarett in U. S. Patent 2,846,456 (August 5, 1958), but its constants were not included. The sample reported in the present paper appears to be a methanolate. (b) W. J. Leanza, J. P. Conbere, E. F. Rogers, and K. Pfister, *J. Am. Chem. Soc.*, **76**, 1691 (1954).

(34) J. A. Hogg, G. B. Spero, J. L. Thompson, B. J. Magerlein, W. P. Schneider, D. H. Peterson, O. K. Sebek, H. C. Murray, J. C. Babcock, R. L. Pedersen, and J. A. Campbell, *Chem. Ind.* (London), 1002 (1958).

subjecting the solution to chromatography on Florisil (350 g.). The first seven fractions contained the most product and were combined and were rechromatographed starting with methylene chloride as the eluting solvent and adding ethyl acetate. Two fractions, obtained in 25 and 50% ethyl acetate in methylene chloride, contained 4.12 g. of approximately 60% purity. Trituration of the two fractions rich in XIX with ethyl acetate gave yellow crystals. Two recrystallizations from ethyl acetate afforded an analytical sample; m.p. 161–167°, λ_{max} 237 mμ (16,400), $[\alpha]_D$ +75°.

Anal. Calcd. for $C_{22}H_{26}O_5F_2$: C, 64.69; H, 6.42. Found: C, 65.05; H, 6.54.

A solution of 8 mg. of XIX and 2.37 mg. of *o*-phenylenediamine in 1.5 ml. of ethanol was heated at reflux for 0.5 hr. Addition of water caused the precipitation of a solid, weighing 5 mg., λ_{max}^{alc} 238 mμ ($E_{1\,cm}^{1\%}$ 708) and 320 mμ ($E_{1\,cm}^{1\%}$ 108).

21-Methyl-6α,9-difluoro-11β,17,21α$_F$-trihydroxypregna-1,4-diene-3,20-dione (XVII).—A solution of 10 g. of crude α-diketone XIX in 1200 ml. of ethanol was added to a mixture of 2850 g. of sucrose and 77 g. of active dry yeast in 21.2 l. of tap water. The mixture was stirred slowly for 15 days with daily additions of 60 g. of yeast and 320 g. of sucrose. The reaction mixture was filtered through Super Cel and the product isolated from the filtrate by extraction with ethyl acetate as described above for XIV. Concentration of the washed ethyl acetate extract to 150 ml. gave 6.3 g. of crude crystalline product (66% pure by paper chromatographic assay), which was purified by acetylation with acetic anhydride (12.5 ml.) in pyridine (25 ml.). The crystalline acetate XVIIa was precipitated by the addition of water and recrystallized twice from ethyl acetate. This treatment afforded 3.0 g. of 6α,9-difluoro-11β,17,21α$_F$-trihydroxy-21-methylpregna-1,4-diene-3,20-dione 21-acetate (XVIIa), which had m.p. 256–257° dec., λ_{max} 237.5 mμ (16,200), $[\alpha]_D$ +95°.

Anal. Calcd. for $C_{24}H_{30}O_6F_2$: C, 63.70; H, 6.68. Found: C, 63.51; H, 6.91.

Saponification of a 778-mg. sample of XVIIa with methanolic potassium carbonate in the usual way and recrystallization twice from ethyl acetate afforded an analytical sample which had m.p. 211–211.8°, λ_{max} 237 mμ (15,500), $[\alpha]_D$ +102°. The infrared spectrum of XVII showed that it retained ethyl acetate of crystallization despite drying at 135° for 16 hr. The following analysis also indicates the presence of solvent of crystallization.

Anal. Calcd. for $C_{22}H_{25}O_5F \cdot {}^1/_2\ CH_3COOC_2H_5$: C, 63.44; H, 7.05. Found: C, 63.47; H, 7.13.

Treatment of 21-Methyl-6α,9-difluoro-11β,17,21α$_F$-trihydroxypregna-1,4-diene-3,20-dione with Periodic Acid.—Treatment of 64 mg. of XVII with periodic acid (55 mg.) in 2 ml. of dioxane and 1.5 ml. of water overnight at room temperature afforded 32 mg. of an acid, m.p. 257–260° dec., identical to the etio acid obtained from 6α,9-difluoroprednisolone[34] under the same conditions.

Acknowledgment.—The authors gratefully acknowledge the helpful suggestions of Prof. E. J. Corey and the technical assistance of Sandor Barcza, Bohdan Rakoczy, Gerald P. Ceasar, and Yvette LeBlanc Boyle in the performance of this investigation and the helpful comments on this manuscript by Dr. W. T. Moreland.

Proton Magnetic Resonance and Stereochemistry of 1-Ethynyl-2-tolylcyclohexanols[1]

ALAIN C. HUITRIC, WILLIAM S. STAVROPOULOS, AND BERNARD J. NIST

College of Pharmacy and Department of Chemistry, University of Washington, Seattle 5, Washington

Received November 5, 1962

The diastereoisomers 1-ethynyl-*cis*-2-tolylcyclohexanol and 1-ethynyl-*trans*-2-tolylcyclohexanol, for the *o*-, *m*-, and *p*-tolyl compounds, were separated by gas chromatography and characterized by n.m.r. The n.m.r. spectra of all six isomers are consistent with structures in which the cyclohexane ring is in a chair conformation with the aromatic ring in an equatorial orientation. The long-range shielding effect of the aromatic ring causes different chemical shifts of the acetylenic hydrogen in *cis* and *trans* isomers. The aromatic *o*-hydrogen of each *o*-tolyl isomer exhibits a downfield chemical shift. Upon reduction of the ethynyl group to an ethyl group this downfield shift persists in the *cis* isomer (OH axial) and disappears in the *trans* isomer (OH equatorial).

The synthesis of 1-ethynyl-2-tolylcyclohexanols was reported in an earlier publication.[2] The separation of the resulting mixtures of *cis* and *trans* diastereoisomers has now been accomplished by gas chromatography for each of the *o*, *m*- and *p*-tolyl compounds. The components have been characterized and their stereochemistry established by nuclear magnetic resonance.

The n.m.r. spectra of the six isomers are consistent with structures in which the cyclohexane ring has the chair conformation with the aromatic ring in an equatorial orientation when measured in carbon tetrachloride. This conformation is indicated for each isomer by the quartet given by the signal of the hydrogen on C-2, which, from first-order approximation, becomes the X component of an ABX system; the two hydrogens on C-3 making up the A and B components. Figure 1 shows this signal at τ = 7.06 for 1-ethynyl-*trans*-2-*o*-tolylcyclohexanol and at τ = 6.92 for 1-ethynyl-*cis*-2-*o*-tolylcyclohexanol. The other four isomers give analogous quartets. First-order treatment of the quartets give axial–axial (*a*,*a*) splitting of 11.5 c.p.s. and axial–equatorial (*a*,*e*) splitting of 3.5 c.p.s. for every isomer with the ethynyl group in equatorial orientation. For the compounds with the ethynyl group in axial orientation the splittings are as follows: *a*,*a* = 10 c.p.s. and *a*,*e* = 4 c.p.s. for the *p*-tolyl isomer; *a*,*a* = 10.5 c.p.s. and *a*,*e* = 3.9 c.p.s. for the *m*-tolyl isomer; *a*,*a* = 10.7 c.p.s. and *a*,*e* = 3.5 c.p.s. for the *o*-tolyl isomer. In every isomer it is necessary that the hydrogen at C-2 be in an axial orientation to account for the observed splitting pattern. This interpretation has been described earlier for related compounds.[3–5] Selectively deuterated compounds are being prepared to determine if the observed splittings are true measures of the coupling constants because of the inherent danger of assigning coupling constants from first-order treatment.[6–8]

Configurations were established from the chemical shifts of the acetylenic hydrogens and the chemical

(1) This investigation was supported in part by PHS research grants no. H-3843 (C2) and no. HE-03843-04, from the National Heart Institute, Public Health Service.

(2) A. C. Huitric, C. W. Roscoe, and R. A. Domenici, *J. Org. Chem.*, **24**, 1353 (1959).

(3) A. C. Huitric and J. B. Carr, *ibid.*, **26**, 2648 (1961).

(4) A. C. Huitric, Wm. G. Clarke, Jr., K. Leigh, and D. C. Staiff, *ibid.*, **27**, 715 (1962).

(5) Wm. F. Trager and A. C. Huitric, *ibid.*, **27**, 3006 (1962).

(6) J. I. Musher and E. J. Corey, *Tetrahedron*, **18**, 791 (1962).

(7) F. A. L. Anet, *Can. J. Chem.*, **39**, 2262 (1961).

(8) R. J. Abraham and H. J. Bernstein, *ibid.*, **39**, 216 (1961).