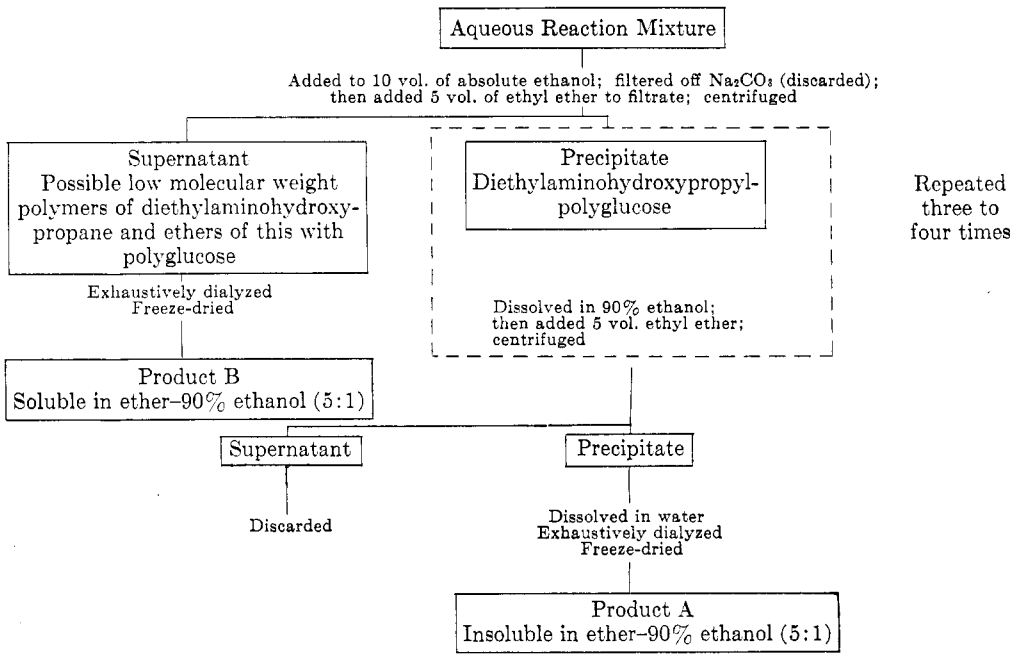
FIGURE 2

PURIFICATION OF THE ALCOHOL-SOLUBLE DIETHYLAMINOHYDROXYPROPYL ETHERS OF POLYGLUCOSE



when freeze-dried gave 0.44 g. of a light tan, fluffy powder.

Anal. Calcd. for $\{C_6H_{8.4}O_5[—CH_2CH(OH)CH_2N^+(C_2H_5)_3Cl^-]_{1.6}\}_n$: N, 4.75; Cl, 12.02. Found: N, 4.49; Cl, 10.38.

Attempted Reductions of Polyglucose Nitrate Ester.—Polyglucose nitrate ester (D.S. = 2.2)[25] was treated with sodamide in liquid ammonia. Only highly degraded low molecular weight products resulted, in line with similar experience on reduction attempts of cellulose dinitrate.[26]

(25) J. W. Wood and P. T. Mora, *J. Org. Chem.*, **27**, 658 (1962).

Acknowledgment.—We wish to thank Mr. H. G. McCann for the analytical determinations, and Dr. Harry A. Saroff, National Institute of Arthritis and Metabolic Diseases, for helpful suggestions and interpretations in connection with the pK' determinations.

(26) P. C. Sherer and J. M. Feild, *Rayon Textile Monthly*, **22**, 607 (1941).

2-Fluoroprednisone

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2-Fluoroprednisone (V) has been synthesized in four steps from cortisone bismethylenedioxy derivative (I) *via* alkoxalylation, treatment with perchloryl fluoride, removal of the blocking group, and 1,2-dehydrogenation by *Nocardia corallina*.

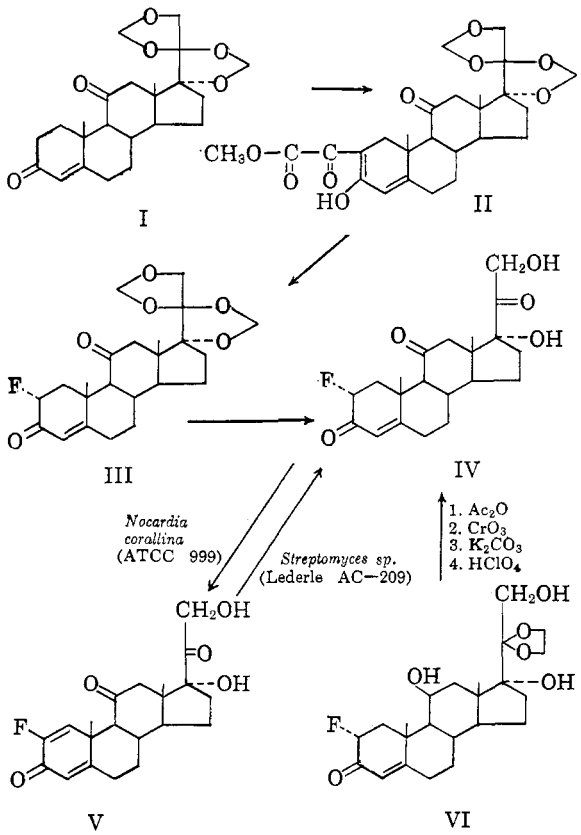
The synthesis of 2α-fluorohydrocortisone has recently been reported from this laboratory.[1] Despite the apparent lowering of glucocorticoid activity caused by the introduction of the 2α-fluorine atom,[1] it was of interest to prepare a Δ1,2-dehydro derivative, since the fluorine atom would then become essentially coplanar with ring A and perhaps confer enhanced biological activity. The electronic interrelationship between the electronegative fluorine atom and the biologically important C_1-C_2 double bond might also be important in this regard. It was decided that our initial effort would be directed to the preparation of 2-fluoroprednisone (V) from 2α-fluorocortisone (IV).

A pathway to 2α-fluorocortisone (IV) which did not involve 2α-fluorohydrocortisone as an intermediate was desirable, since the latter compound is somewhat tedious to prepare in quantity. Introduction of a fluorine atom at the 2α-position of a Δ^4-3-keto steroid is conveniently achieved *via* the reaction of perchloryl fluoride with a 2-alkoxalyl derivative.[1] For this purpose the bismethylene-

(1) H. M. Kissman, A. M. Small, and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 2312 (1960).

dioxy (BMD) derivative (I)[2] of cortisone appeared to be a suitable and conveniently available starting material.

Treatment of cortisone BMD (I) with methyl oxalate and sodium methoxide afforded the crystalline 2-methoxalyl derivative II in 66% yield. The assignment of the methoxalyl group to C-2 was based on the known course of alkoxalylation of Δ^4-3-keto steroids,[3] the apparent inability of 11-keto steroids to undergo alkoxalylation, at least under the above conditions,[4] and an analysis of infrared data. The assignment of the particular tautomeric form II to this product is based on spectroscopic evidence.[5] Perchloryl fluoride treatment of the sodium salt of II followed by acetate-induced demethoxalylation afforded the 2α-fluorocortisone bismethylenedioxy derivative (III) in 78% yield. Removal of the bismethylenedioxy blocking group by hydrolysis with hot 60% formic acid[2] then gave the desired 2α-fluorocortisone in 38% yield. Confirmation of the C-2 position for the fluorine atom in III and IV, and the methoxalyl group in II, was obtained by conversion of 2α-fluorohydrocortisone 20-ketal (VI)[1] to IV *via* 21-acetylation, chromic oxide oxidation to the 11-ketone, deacetylation, and 20-ketal hydrolysis.



(2) R. E. Beyler, R. M. Moriarity, F. Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, **80**, 1517 (1958).

(3) G. R. Allen, Jr., and M. J. Weiss, *ibid.*, **81**, 4968 (1959) and references cited therein.

The synthesis of a 2-fluorocortisone 21-acetate *via* the reaction of perchloryl fluoride with an ethoxalyl derivative has been reported previously by Nathan, Magerlein, and Hogg,[6] who considered the configuration of the 2-fluorine atom to be equivocal on the basis of optical rotary dispersion studies. However, pending a further report from these authors we continue to assign the α-configuration to the fluoro ketones prepared by the alkoxalyl-perchloryl fluoride method, for the following reasons.

(1) Kende has shown that the equatorial conformer of 2-fluorocyclohexanone is the more stable.[7] The acetate-catalyzed dealkoxalylation step in the alkoxalyl–perchloryl fluoride procedure and also the acid-catalyzed hydrolysis of the side chain blocking groups should allow the formation of the more stable epimer.[8] In fact, 2α-bromo-[9] and 2α-methyl-Δ^4-3-ketones[10] are so obtained from 2-alkoxalyl derivatives.

(2) The differences in molecular rotation resulting from the introduction of the 2-fluorine atom are in the +12 to +228 range. These values are in general agreement with the effect on molecular rotation caused by substitution of chloro,[11] bromo,[11] hydroxy,[12] and methyl[13] groups at the 2α-position of a Δ^4-3-ketone. The change in molecular rotation resulting from 2β-substitution by chloro,[11] bromo,[11] and hydroxy[14] groups is strongly negative. In this connection it may be noted that fluorine at C-6 has an effect on molecular rotation similar to that caused by chlorine and bromine at this position.[15]

(3) The 2-fluorodihydrotestosterone prepared *via* the reaction of 2-hydroxymethylenedihydrotestosterone with perchloryl fluoride, a method quite analogous to the alkoxalyl–perchloryl fluoride procedure, is identical to the product obtained by treatment of dihydrotestosterone pyrrolidine enamine with perchloryl fluoride.[16] Allinger and co-

(4) G. R. Allen, Jr., private communication.

(5) W. Fulmor and G. O. Morton, to be published; *cf.* N. A. Nelson and R. N. Schut, *J. Am. Chem. Soc.*, **80**, 6630 (1958).

(6) A. H. Nathan, B. J. Magerlein, and J. A. Hogg, *J. Org. Chem.*, **24**, 1517 (1959).

(7) A. S. Kende, *Tetrahedron Letters*, No. **14**, 13 (1959).

(8) Nathan, Magerlein, and Hogg[6] recovered unchanged a 2-fluoro-Δ^4-3-ketone, prepared by the perchloryl fluoride–ethoxalyl procedure, after treatment with hydrogen chloride in chloroform at 0° for two hours.

(9) G. R. Allen, Jr., and M. J. Weiss, *J. Am. Chem. Soc.*, **81**, 4968 (1959).

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

(11) B. Ellis and V. Petrow, *J. Chem. Soc.*, 1179 (1956).

(12) G. Rosenkranz, O. Mancera, and F. Sondheimer, *J. Am. Chem. Soc.*, **77**, 145 (1955).

(13) H. J. Ringold and G. Rosenkranz, *J. Org. Chem.*, **21**, 1333 (1956).

(14) H. L. Herzog, M. J. Gentles, E. B. Hershberg, F. Carvajal, D. Sutter, W. Charney, and C. P. Schaffner, *J. Am. Chem. Soc.*, **79**, 3921 (1957).

(15) A. Bowers and H. J. Ringold, *Tetrahedron*, **3**, 14 (1958).

(16) S. Nakanishi, K. Morita, and E. V. Jensen, *J. Am. Chem. Soc.*, **81**, 5259 (1959).

workers[17] have unequivocably demonstrated that the latter procedure, when applied to the preparation of 2-fluorocholestanone, affords the *alpha* epimer.[18,19]

Since attempts to prepare the Δ^1-2-fluoro derivative (V) by selenium dioxide dehydrogenation of 2α-fluorocortisone proved unsuccessful, the possibility of microbiological 1,2-dehydrogenation was investigated. Precedent for microbiological 1,2-dehydrogenation of 2α-substituted steroids is afforded by the formation of 2-hydroxyandrosta-1,4-diene-3,17-dione from 2α-hydroxytestosterone by *Bacillus sphaericus* var. *fusiformis*,[20a] and by *Nocardia corallina* (ATCC 999).[20b] Fermentation of 2α-fluorocortisone with *Norcardia corallina* (ATCC 999) yielded 2-fluoroprednisone (V), which travelled with somewhat greater mobility than 2α-fluorocortisone on papergrams developed with a modified Bush-type system.[22] As shown in Table I, the 1,2-dehydrogenation of IV occurred rapidly, followed by disappearance of total (240-mμ absorbing) steroid. This pattern is similar to that observed with *Nocardia corallina* fermentations of hydrocortisone and other steroids.[23]

TABLE I

1,2-DEHYDROGENATION OF 2α-FLUOROCORTISONE BY *N. Corallina* (ATCC 999)

Time, Hr.	2αF-E (V), Approx. %[a]	Δ^1-2F-E (VI), Approx. %[a]
1/2	70	30
1	50	50
2	40	60
3	30	50
5	0	0

[a] Estimated visually by intensity of ultraviolet-absorbing spots on papergrams.

Subsequent isolation of the product after a two-hour fermentation of IV permitted its characterization as 2-fluoroprednisone (V). The structural assignment is based on several considerations. The elemental analysis fits the proposed structure, thereby rendering unlikely any drastic modification of the substrate. A positive blue tetrazolium reaction and infrared data indicate continued presence of the α-ketol side chain. Data in support of the introduction of a double bond at the C_1-C_2 position stem from ultraviolet, infrared, and polarographic analyses, reaction to isonicotinic acid hydrazide[24] and *p*-phenylenediamine phthalic acid[25] spray reagents on paper chromatograms, and the fact that a known 1,2-dehydrogenating organism was employed. The observed bathochromic shift of 4 mμ in the ultraviolet is in the proper direction for $\Delta^{1,4}$-3-one compounds, but is somewhat lower than that observed when proceeding from 2α-chlorocholest-4-ene-3-one,[11,26] λ_{max} 244 mμ, to 2-chlorocholesta-1,4-diene-3-one,[27] λ_{max} 253 mμ. The infrared bathochromic shift of 0.12 μ for the 3-ketone of V when compared with IV is typical of 2-halogenated steroids (*cf.* Table II).

TABLE II

INFRARED ABSORPTION MAXIMA FOR 2-HALOGENATED-3-KETO STEROIDS

Structure	Reference	Infrared Absorption of 3-Ketone, μ	$\Delta\mu$[e]
2α-Fluoro-Δ^4-3-one[a]	This paper	5.89	....
2-Fluoro-$\Delta^{1,4}$-3-one[b]	This paper	6.01	+0.12
2α-Chloro-Δ^4-3-one[c]	26b	5.95	....
2-Chloro-$\Delta^{1,4}$-3-one[d]	27	6.02	+0.07
2α-Bromo-Δ^4-3-one	28a	5.90	....
2-Bromo-$\Delta^{1,4}$-3-one	28b	6.00	+0.10

[a] Compound IV. [b] Compound V. [c] 2α-Chlorocholest-4-ene-3-one. [d] 2-Chlorocholesta-1,4-diene-3-one. [e] Absorption of $\Delta^{1,4}$-3-keto steroid minus absorption of Δ^4-3-keto steroid.

Further support for the assigned structure is afforded by microbiological 1,2-hydrogenation of V by *Streptomyces* sp. (AC-209) to yield a product with papergram mobility and infrared spectrum typical of authentic 2α-fluorocortisone (IV). This culture is known to effect 1,2-hydrogention of of $\Delta^{1,4}$-3-keto steroids.[29] From the fermentation of V with AC-209, it was theoretically possible

(17) N. L. Allinger, H. M. Blatter, M. A. DeRouge, and L. A. Freiberg, *J. Org. Chem.*, **26**, 2550 (1961).

(18) Our previous utilization[1] of infrared shifts for the assignment of the α-configuration to the 2-fluorine atom is of questionable value since Allinger and co-workers[17] have reported that axial 2-fluorocyclohexanones show a significant bathochromic shift of the carbonyl band.

(19) We wish to thank a referee of this paper for helpful comments on this issue.

(20(a) C. Gual, S. R. Stitch, M. Gut, and R. I. Dorfman, *J. Org. Chem.*, **24**, 418 (1959). (b) C. E. Holmlund, L. I. Feldman, R. H. Blank, N. Barbacci, and B. Nielsen, International Fermentation Symposium, May 9–12, 1960, Rome, Italy. Intact cells of *N. corallina* were able to 1,2-dehydrogenate 2α-hydroxytestosterone but not 2β-hydroxytestosterone. It was concluded that a 2β-hydrogen was required for this reaction to occur. Hayano *et al.*,[21] in further studies with *Bacillus sphaericus*, concluded that bacterial dehydrogenation involved diaxial loss of the 1α and 2β-hydrogen atoms. The formation of 2-fluoroprednisone (V), from the fermentation of 2α-fluorocortisone (IV), further substantiates therefore the assignment of α-configuration to the fluorine atom of IV.

(21) M. Hayano, H. J. Ringold, V. Stefanovic, M. Gut, and R. I. Dorfman, *Biochem. and Biophys. Res. Comm.*, **4**, 454 (1961).

(22) Benzene–methanol–petroleum ether (90–100° fraction)–water in the volume ratio of 13:16:7:4. This system was devised by R. H. Blank.

(23) Loss of 240-mμ absorbing steroid is a common observation of Δ^1 fermentations [Sih and Weisenborn, *J. Am. Chem. Soc.*, **82**, 2653 (1960); Feldman, Holmlund, and Barbacci, Abstracts, 140th Meeting, A.C.S., 1P–2P (1961)], and is the result of 1,2-dehydrogenation and 9α-hydroxylation followed by a spontaneous retroaldol-type reaction yielding a 9,10-seco-3 hydroxy-1,3,5(10)-triene-9 keto steroid [Dodson and Muir, *J. Am. Chem. Soc.*, **83**, 4627 (1961); Sih, Abstracts, 140th Meeting, A.C.S., 61C–62C (1961)]. 9,10-Seco-A-aromatic steroids are rapidly metabolized by *N. corallina* (unpublished data of this laboratory).

(24) L. L. Smith and T. Foell, *Anal. Chem.*, **31**, 102 (1959).

(25) The *p*-phenylenediamine phthalic acid spray reagent of A. Bodánszky and J. Kollonitsch, *Nature*, **175**, 729 (1955), yields a yellow to yellow-orange color with Δ^4-3-keto steroids, and only a weakly yellow or negative reaction with $\Delta^{1,4}$-3-keto steroids, according to a private communication from R. H. Blank.

(26)(a) B. Ellis and V. Petrow, *J. Chem. Soc.*, 3869 (1953). (b) J. J. Beereboom and C. Djerassi, *J. Org. Chem.*, **19**, 1196 (1954).

(27) D. N. Kirk and V. Petrow, *J. Chem. Soc.*, 1334 (1958).

(28 (a) J. E. Page, *Chem. Ind.* (London), 58 (1957). (b) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(29) Unpublished data of these laboratories.

to obtain either C-2 epimer or a mixture of both. Since only the α-fluoro compound was observed, it would appear that the 1,2-hydrogenase of this microbial system catalyzed the introduction of hydrogen in the axial position of C-2.

In the thymolytic assay,[30] V was about 1.5 times as active as hydrocortisone, whereas IV was about one third as active as hydrocortisone. The introduction of the C_1-C_2 double bond appears to cause a slight enhancement of activity.

Experimental

Melting Points.—All melting points are uncorrected.

Absorption Spectra.—The ultraviolet absorption spectra were determined in methanol. The infrared spectra were carried out with pressed potassium bromide.

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

3-Hydroxy-2-methoxalyl-17α,20:20,21-bismethylene-dioxypregna—2,4-dien-11-one (II).—A solution of sodium (9.66 g., 0.42 mole) in anhydrous methanol (150 ml.) in a 2-l. flask was evaporated to dryness, and a solution of methyl oxalate (90.0 g., 0.756 mole) in benzene (1,800 ml.) was azeotropically distilled to a final volume of 1,300 ml. 17α,20:20,21-Bismethylenedioxypregn-4-ene-3,11-dione (I)[2] (151.2 g., 0.378 mole) was partially dissolved in the methyl oxalate–benzene solution, and the resulting suspension was allowed to cool to room temperature. The suspension was then added to the sodium methoxide residue; an additional 200 ml. of anhydrous benzene was used to aid in the transfer. The resulting mixture was stirred at room temperature for 21 hr. and then drowned in petroleum ether (*ca.* 6 l.). The solids were filtered, pressed dry, ground in a mortar and dissolved in about 22 l. of water. A small quantity of insoluble material (A) was filtered off. As soon as practicable the filtrate was acidified with dilute, aqueous hydrochloric acid. The resulting white solids were collected by filtration, washed with water, pressed dry, and dissolved in methylene chloride. The methylene chloride solution was extracted with water until the aqueous extracts were no longer acidic and was then dried over magnesium sulfate. The solution was concentrated to about 400 ml. Evaporation (boiling) was continued with the addition of benzene periodically to maintain the volume at 400 ml. When the boiling point reached 69°, the mixture was cooled and the precipitated yellow crystals were filtered to give 96.3 g. of product, melting at 203–207°. Further concentration of the mother liquor afforded an additional 5.5 g. of product (m.p. 202–204°). A third crop (8.5 g., m.p. 202–205°) was obtained by concentration of the second mother liquor to near dryness and the addition of acetone.

The above-mentioned water-insoluble material (A) was washed several times with water and then extracted with benzene. After evaporation of the benzene solvent, the residual material was dissolved in methylene dichloride. The resulting solution was filtered and the solvent was evaporated. The residual solids were dissolved in hot acetone (600 ml.). The acetone solution was concentrated (by boiling) to about 200 ml., and petroleum ether (b.p. 20–40°) was added. The mixture was cooled and the precipitated crystals were collected; yield 16.5 g. (m.p. 202–207°). Three additional crops amounting to 7.7 g., m.p. 202–210°, were obtained on further work-up of the mother liquor.

The total yield of II was 134.5 g. (66% yield). Recrystallization from methanol-benzene of material (m.p. 202–205°) obtained from a pilot preparation gave yellow crystals melting at 208–212°; λ_{max} 5.78, 5.86, 6.12, 6.31 μ; λ_{max} 240 mμ (ϵ 11,500), 320–352 mμ (plateau) (ϵ 4,890); $\lambda_{max}^{0.1\ N\ NaOH}$ 247 mμ (ϵ 16,600), 352 mμ (ϵ 12,000).

Anal. Calcd. for $C_{26}H_{32}O_9$ (488.5). C, 63.92; H, 6.60. Found. C, 63.61; H, 6.79.

2α-Fluorocortisone BMD (**2α-Fluoro-17α,20:20,21-bismethylenedioxypregn-4-ene-3,11-dione, III**).—A suspension of 0.977 g. (2 mmoles) of the methoxalyl derivative II in 35 ml. of methanol was cooled to −10° and 5 ml. of 1N methanolic sodium methoxide solution was added. After all of the solid had gone into solution on stirring, perchloryl fluoride gas was passed in for a few minutes. The solution became neutral but still gave a positive enol test. The reaction mixture was freed from solvent under reduced pressure, and the residue was dissolved in a mixture of chloroform and water. The organic phase was washed once with water and then dried and evaporated. The residue was dissolved in 30 ml. of methanol, and 1.6 g. of potassium acetate was then added. The mixture was refluxed for 75 min. and was evaporated. The residue was again dissolved in a chloroform-water mixture and the organic phase was washed with a little water, dried, and evaporated. The residue was crystallized from ether to afford 0.63 g. (78%) with m.p. 210–213°. Recrystallization from ether gave material with m.p. 215–218°, $[\alpha]^{25}D$ +82.5° (1.17%, chloroform), M_D +347, $\Delta M_0(M_0III - M_0I^2)$ = +17; λ_{max} 239 mμ (ϵ 13,900); λ_{max} 5.86 μ(λ_{max} of I = 5.87, 5.99μ).

Anal. Calcd. for $C_{23}H_{29}FO_6$. C, 65.69; H, 6.95; F, 4.52. Found. C, 65.75; H, 7.35; F, 4.70.

2α-Fluorocortisone (**2α-Fluoro-17α,21-dihydroxypregn-4-ene-3,11,20-trione, IV**). (A) **From 2α-Fluoro-17α,20:20,-21-bismethylenedioxypregn-4-ene-3,11-dione** (III).—A solution of 0.53 g. (1.26 mmoles) of 2α-fluoro-17α,20:20,21-bismethylenedioxypregn-4-ene-3,11-dione (III) in 20 ml. of 60% aqueous formic acid[2] was heated on the steam bath for 30 min. and was then evaporated. The residue was dissolved in 30 ml. of methylene chloride, and the solution was washed with a little water, dried, and evaporated. The residue was crystallized from ethyl acetate to afford 0.18 g. (38%), m.p. 192–197°. Recrystallization from the same solvent gave m.p. 215–217°; $[\alpha]^{25}D$ +223° (0.48%, chloroform), M_D + 845, ΔM_D (M_D IV–M_D cortisone) = +10; λ_{max} 238 mμ (ϵ 13,900); λ_{max} 5.86 μ, 5.92 μ (shoulder Δ^4-3-one, probably hydrogen-bonded).

Anal. Calcd. for $C_{21}H_{27}FO_5$. C, 66.65; H, 7.19; F, 5.02. Found. C, 66.65; H, 7.56; F, 5.08.

(B) **From 2α-Fluorohydrocortisone 20-Ethylene Ketal** (**VI**).—Acetic anhydride (1 ml.) was added to a cold solution of 0.35 g. (0.83 mmole) of 2α-fluorohydrocortisone 20-ethylene ketal (VI) in 5 ml. of dry pyridine, and the mixture was kept in an ice bath for 1 hr. and at room temperature overnight. Methylene chloride (30 ml.) was then added and the solution was washed successively with water, saturated sodium bicarbonate solution, and water. The organic phase was dried and evaporated. The residue was dissolved in 5 ml. of cold pyridine, and to the solution was added the complex formed from 400 mg. of chromic oxide and 1 ml. of cold pyridine. The mixture was stirred in an ice bath for 1 hr. and then at room temperature for 16 hr. Methanol (25 ml.) was added and the mixture was evaporated at room temperature. Ethyl acetate (60 ml.) was added to the residue, and the mixture was filtered through diatomaceous earth. The yellow filtrate was washed successively with 1 *N* sulfuric acid, water, sodium bicarbonate solution, and water. The organic phase was dried, evaporated, and the residue was redissolved in 30 ml. of methanol containing 1 ml. of 10% aqueous potassium carbonate solution. The solution was stirred at room temperature under a blanket of nitrogen for 2 hr. and was then acidified with 0.5 ml. of perchloric acid. The mixture was stirred in the presence of nitrogen under refluxing conditions for 1 hr., cooled, and 0.8 ml. of pyridine was added. After evaporation at room temperature, the residue was dissolved in a mixture of methylene chloride

(30) We wish to thank S. Mauer, E. Heyder, R. Partridge, and I. Ringler of the Experimental Therapeutics Research Section for carrying out the thymolytic assays which were performed as described elsewhere.[31]

(31) I. Ringler and R. Brownfield, *Endocrinol.*, **66**, 900 (1960).

and water, and the organic phase was separated, washed with a little water, dried, and evaporated. The residue was dissolved in ethyl acetate, treated with activated charcoal, and evaporated to a small volume. After seeding the solution 135 mg. of crystalline material was obtained [43% over-all from 2α-fluorohydrocortisone-20-ethylene ketal (VI)], with m.p. 195–200°. Material recrystallized from ethyl acetate melted at 210–215° and was shown to be identical with 2α-fluorocortisone (IV) by mixed melting point and infrared spectrum.

2-Fluoroprednisone (2-Fluoro-17α,21-dihydroxypregna-1,4-diene-3,11,20-trione, V).—One percent of a 7-hr. inoculum of *Nocardia corallina* (ATCC 999) was introduced into fifty-one 500-ml. Erlenmeyer flasks, each containing 100 ml. of medium A.[32] The flasks were incubated on a reciprocating shaker at 28°. Seventeen hours after inoculation, 10 mg. of 2α-fluorocortisone (IV), dissolved in 1 ml. of methanol, was added to each flask. 2-hr. after steroid addition, the contents of the flasks were pooled and extracted three times with 1-volume portions of ethyl acetate.

An aliquot of the ethyl acetate extract representing 15 mg. of starting substrate was concentrated to a dry residue *in vacuo*. The residue was dissolved in 1 ml. each of the top and bottom phases of a solvent system (water:dioxane:cyclohexane in the ratio 1:5:5), mixed with 2 g. of acid-washed Celite 545,[33] and added to a 20-g. pack of Celite 545 previously moistened with 10 ml. of the bottom phase. The column was developed with top phase, and 240 mμ absorbing fractions were collected. Two incompletely resolved peaks were observed as follows:

Peak	Hold-back Volume
α-(2-fluoroprednisone)	2.3
S-(2α-fluorocortisone)	3.0

To achieve satisfactory separation of these peaks, the total remaining ethyl acetate extract of the fermentation mash, after concentration *in vacuo* to a residue, was chromatographed on a 200-g. Celite column employing the solvent system water–dioxane–cyclohexane in the ratio 1:5:7. The less polar fraction, which emerged as a peak at 4.7 hold-back volumes, was concentrated *in vacuo* to a dry residue, which was crystallized from acetone–petroleum ether to afford 147.7 mg. (30%) of the desired product. Decolorization with Darco[34] and recrystallization from acetone–petroleum ether gave 112 mg. (23.2%) of 2-fluoroprednisone (V), m.p. 232–236° (with discoloration); $[\alpha]^{25}D$ +145° (methanol); λ_{max} 244 mμ (ε 17,600); λ_{max} 5.86, 6.01, 6.16 μ.

Anal. Calcd. for $C_{21}H_{25}O_5F$: C, 66.99; H, 6.70; F, 5.05. Found: C, 67.01; H, 7.10; F, 5.16.

2-Fluoroprednisone (V) proved difficult to separate from 2α-fluorocortisone (IV) by paper chromatography. A development time of 6 hr. in a modified Bush type system[22] resolved the two compounds, V being slightly more mobile than IV. The reaction of V on paper strips to spraying with isonicotinic acid hydrazide[24] was somewhat weaker than customary for $\Delta^{1,4}$-3-ketosteroids, probably because of the substituent at C-2.

Polarographic assay distinguished IV ($E_{1/2}$ = −1.19 V) from V ($E_{1/2}$ = −1.09 V) and showed a characteristic shift to a lower half wave potential for the latter compound.

Microbiological Hydrogenation of 2-Fluoroprednisone.—One percent of a 72-hour inoculum of *Streptomyces* sp. AC-209 was introduced into a 500-ml. Erlenmeyer flask containing 100 ml. of Medium B[35] and 20 mg. of V. The flask was incubated on a reciprocating shaker at 28° for 47 hr. The mash was extracted three times with 1 volume portions of ethyl acetate. The ethyl acetate mash extract was concentrated *in vacuo* to dryness and the residue was chromatographed on a 20-g. Celite 545 column, employing the system water–dioxane–cyclohexane in the ratio 1:5:7. One major peak at 6.7 hold-back volumes and three less polar minor peaks were noted. The fraction representing the major peak A was concentrated to dryness *in vacuo* and the residue was dissolved in 1 ml. methanol. After decolorization with Darco, the filtrate and Darco washings were concentrated to 0.1 ml.; chloroform was added to effect solution, and cyclohexane to initiate crystallization. After chilling, 1.5 mg. (8.1%) of a crystalline product was collected which displayed an infrared spectrum and papergram mobility typical of an authentic sample of 2α-fluorocortisone (IV).

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(32) The composition of medium A was 0.25% NaCl, 0.4% peptone, 1% glucose, 0.4% beef extract, and 0.1% yeast extract.

(33) Celite is the trade mark of Johns-Manville and Co. for diatomaceous earth.

(34) Darco is the trade mark of the Atlas Powder Co. for activated charcoal.

(35) The composition of medium B was 3% corn steep liquor, 3% glucose, 0.5% (by volume) soybean oil, and 0.5% $CaCO_3$.