and made clear by the addition of dilute sulfuric acid. The ethereal solution was washed with water, dilute sodium bicarbonate and water. After drying over sodium sulfate, the ether was evaporated and the residue was distilled to give 800 mg. of colorless liquid of b.p. 103–117° (130–137 mm.) and 1.45 g. of higher boiling liquid. Both fractions were combined and refluxed with methanol containing a few drops of concd. sulfuric acid for 5 hours. Water was added and the mixture was extracted with ether. After the usual processing, the extract afforded 1.62 g. (32.85%) of colorless liquid, c.p. 118–119° (150 mm.) or 165° (760 mm.), $[\alpha]_D$ −20.02°, d^{52}_4 0.8035, n^{28}_D 1.4258. The reported constants[22] are b.p. 164°, M^{25}_D +3.2, calcd. max. M^{25}_D +31.2, d^{25}_4 0.828 (for the antipode).

Anal. Calcd. for $C_7H_{16}O$: C, 72.35; H, 13.88. Found: C, 72.39; H, 14.00.

BUNKYO-KU, TOKYO, JAPAN

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. CXXXVII.[1] Synthesis of a New Class of Potent Cortical Hormones. 6α,9α-Difluoro-16α-hydroxyprednisolone and its Acetonide

BY J. S. MILLS, A. BOWERS, CARL DJERASSI AND H. J. RINGOLD

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The syntheses of 6α,9α-difluoro-16α-hydroxyprednisolone (XVb) and related corticoids are described. In one sequence, 16α,17α-oxido-Δ⁵-pregnene-3β,21-diol-20-one 21-acetate (I) was converted to 6α-fluoro-16α-hydroxy substance "S" (IXb) which on adrenal incubation gave 6α-fluoro-16α-hydroxyhydrocortisone (Xa) transformed to XV by the Fried sequence followed by selenium dioxide oxidation. Alternately 6α-fluorohydrocortisone and 6α,9α-difluorohydrocortisone were hydroxylated at C-16α by *Streptomyces roseochromogenus*. 6α,9α-Difluoro-16α-hydroxyprednisolone and its corresponding acetonide (XVc) exhibited high anti-inflammatory activity without retaining sodium.

Following the basic finding of Fried and his coworkers[2] that the glycogenic, anti-inflammatory and mineralocorticoid activity of cortical hormones may be potentiated by the substitution of halogen, in particular fluorine, at C-9α, considerable effort has been devoted to the synthesis of cortical hormone analogs bearing substituents at other sites of the molecule in the hopes of obtaining high anti-inflammatory activity without undesirable sodium retention. This search has led to the preparation, among others, of 2α-methyl-,[3] 4-methyl-,[4] 4-halo-,[5] 6α-methyl-,[6] 6α-chloro-,[7] 6α-fluoro-,[8] 6α-nitro-,[9] 7β-methyl-,[10] 11α-methyl-,[11] 12α-chloro-,[12] 14α-hydroxy-,[13] 16α-hydroxy-,[8d,14] 16α-methyl-[8c,e,f,15] and 16β-methyl-[15b,16] cortical hormone analogs with the 6- and 16-substituted compounds appearing to be of particular interest. Thus, the 6-methyl, -chloro and -fluoro groups potentiate anti-inflammatory activity and promote sodium excretion but as single modificants are incapable of completely overcoming the profound sodium retention induced by a 9α-fluoro atom. Introduction of a 16α-hydroxy group into 9α-fluoroprednisolone leads to a compound devoid of sodium retention[14d] but with anti-inflammatory activity considerably lower[14d,e] than the parent 9α-fluoro compound, while 16α-[15c,d] and 16β-methyl-9α-fluoro-prednisolone,[16] also free of sodium retention, are more potent than the parent compound. Peculiarly conversion of 16α-hydroxy-

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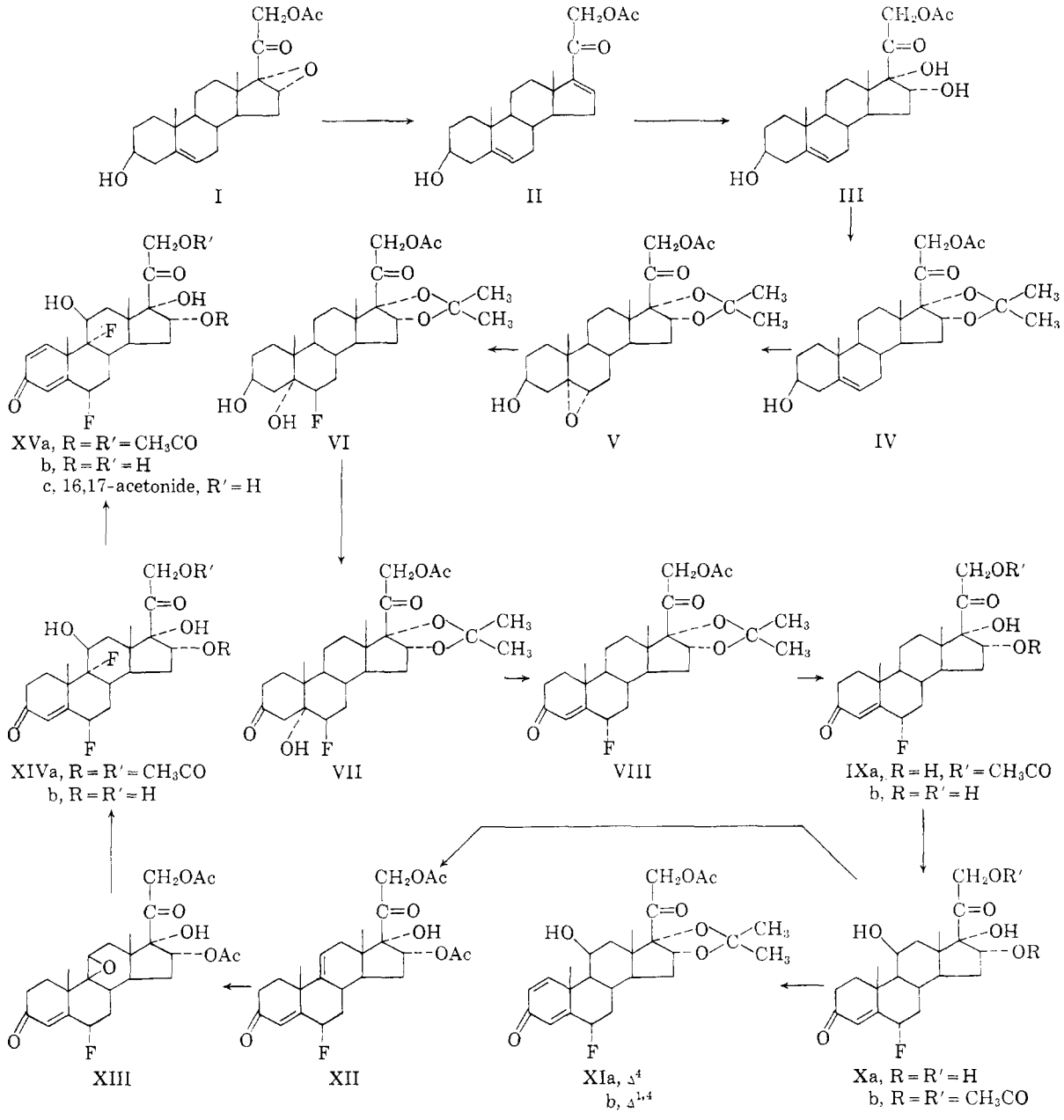

9α-fluoroprednisolone to the 16,17-acetonide[14e] markedly increases topical activity without significantly affecting oral anti-inflammatory activity.

Since the 6α-fluoro substituent appears to be the single most active potentiating group and since the 6α,9α-difluoro-corticoids[8,b,e,g] are still not devoid of sodium retention, an important extension was the preparation of 6α,9α-difluoro-16α-hydroxy and 6α,9α-difluoro-16α-methyl-corticoids.[8e,f] This paper is concerned with the syntheses of 6α-fluoro-16α-hydroxy corticoids[17] by combined chemical–biological routes, one synthesis involving the introduction of the 9α-fluoro-11β-hydroxy system into 6α-fluoro-16α-hydroxy compound "S" while the other involves the microbiological 16α-hydroxylation of 6α-fluoro- and 6α,9α-difluorohydrocortisone.[18]

(17) For a preliminary report see ref. 8d.

Treatment of the readily available[19] 16α,17α-oxido-Δ5-pregnene-3β,21-diol-20-one 21-acetate (I) with chromous chloride in acetic acid gave a mixture of the 16α-hydroxy compound[20] and Δ16-compound[21] II which, without purification, was treated with hydrochloric acid in acetone to complete dehydration, yielding about 65% of II. Petrow[22] had demonstrated that potassium permanganate in acetone solution *cis*-hydroxylates a Δ16-20-keto steroid. Thus when Δ5,16-pregna-

(18) Another synthesis based upon introduction of a 6-fluoro substituent into 9α-fluoro-16α-hydroxyhydrocortisone will be published at a later date.

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diene-3β,21-diol-20-one 21-acetate (II) was treated in acetone containing a small amount of acetic acid with potassium permanganate in aqueous acetone the crude 16α,17α-glycol III was obtained. Conversion of crude III to the 16,17-acetonide IV was accomplished by brief reaction with acetone–perchloric acid, the yield of IV from II averaging 25% in a number of runs.

With the 16- and 17-hydroxyl groups adequately protected by the acetonide function it was now possible to introduce a 6-fluoro substituent into IV by conversion to the 5α,6α-oxide followed by reaction with boron trifluoride. The opening of such an epoxide to the 5α-hydroxy-6β-fluoro compound by boron trifluoride had been previously demonstrated by Henbest and Wrigley[23] and by Bowers and Ringold.[24] Thus IV was converted to the 5α,6α-oxide V in 75% yield by oxidation with ethereal monoperphthalic acid whence cleavage with boron trifluoride etherate in anhydrous ether–benzene gave a mixture of recovered V and 5α-hydroxy-6β-fluoro compound VI, readily separated by chromatography on alumina. Oxidation of VI with chromic acid in acetone–sulfuric acid[25] yielded the 3-keto compound VII which on treatment with anhydrous hydrogen chloride in acetone underwent concomitant elimination of the 5α-hydroxyl group as well as inversion of the axial 6β-fluoro atom to the stable 6α-fluoro-Δ⁴-pregnene-16α,17α,21-triol-3,20-dione 16,17-acetonide 21-acetate (VIII). While anhydrous hydrogen chloride in glacial acetic acid also effected dehydration and inversion, acetone was preferred as a solvent since some hydrolysis of the acetonide occurred with the acetic acid conditions. Heating of VIII in boiling 60% formic acid cleaved the acetonide function yielding 6α-fluoro-16α-hydroxy "S" as a mixture of 21-acetate IXa and 21-hydroxy compound IXb, crystallized to pure IXa, or preferably saponified with methanolic potassium hydroxide to IXb since the free compound was desired for the next step.

Incubation of IXb with freshly ground beef adrenals[26] in saline buffered medium introduced an 11β-hydroxy group in almost 50% yield producing a key intermediate, 6α-fluoro-16α-hydroxyhydrocortisone (Xa). We have now demonstrated that the adrenals can 11β-hydroxylate derivatives of compound "S" containing one or more of the following substituents: 6α-fluoro,[8a] 6α-chloro,[7] 16α-methyl,[8c] 10-hydrogen[26] (*i.e.*, 19-nor steroids) as well as corresponding Δ¹-dehydro compounds.[7] Hayano and co-workers[27] have shown convincingly that adrenal 11β-hydroxylation proceeds by removal first of the 11β-hydrogen of an 11-methylene compound which implies enzymatic oxidase attack on the β-face of the steroid. The apparent lack of discrimination with which the adrenal oxidase system 11β-hydroxylates derivatives of compound "S" containing equatorial substituents on the α-face of the molecule is in accord with Hayano and Dorfman's findings. It is tempting to speculate that the judicious placement of bulky axial substituents on the β-face of the "S" molecule would prevent adrenal 11β-hydroxylation by steric interference. Should hydroxylation be so inhibited considerable insight would be gained into the steric requirements of this enzyme system and the actual portions of the steroid molecule in contact with the enzyme during hydroxylation.

An alternate synthesis of 6α-fluoro-16α-hydroxyhydrocortisone (X) was realized by microbiological hydroxylation of 6α-fluorohydrocortisone[8a,b] with *Streptomyces roseochromogenus*, Rutgers Collection No. 3689, an organism first demonstrated by a Squibb group[14c] to accomplish 16α-hydroxylation. The products obtained by the two alternate routes were shown to be identical by the usual criteria.

Since the acetonides were also desired for biological evaluation, 6α-fluoro-16α-hydroxyhydrocortisone (Xa) was converted to the 16,17-acetonide 21-acetate XIa by consecutive treatment with acetone–perchloric acid and acetic anhydride–pyridine. Prolonged treatment (88 hours) of XIa with selenium dioxide in boiling *t*-butyl alcohol gave the corresponding Δ¹,⁴-dienone XIb.

For introduction of a 9-fluorine substituent by the now classical Fried sequence,[2] 6α-fluoro-16α-hydroxyhydrocortisone (Xa) was converted to the 16,21-diacetate Xb which was dehydrated by heating with mesyl chloride in dimethylformamide–pyridine[28] solution yielding 6α-fluoro-Δ⁴,⁹-pregnadiene-16α,17α,21-triol-3,20-dione 16,21-diacetate (XII). Addition of hypobromous acid by N-bromoacetamide in dioxane–perchloric acid gave the 9α-bromo-11β-hydroxy compound, converted to the 9β,11β-oxide XIII by treatment with potassium acetate in acetone. Opening of XIII with hydrogen fluoride in tetrahydrofuran–methylene dichloride[29] gave 6α,9α-difluoro-16α-hydroxyhydrocortisone diacetate (XIVa). Alternatively, microbiological oxidation of 6α,9α-difluorohydrocortisone[8b,e,g] by *Streptomyces roseochromogenus* yielded 6α,9α-difluoro-16α-hydroxyhydrocortisone (XIVb) whose diacetate was idential with XIVa obtained above. Selenium dioxide oxidation of XIVa produced 6α,9α-difluoro-16α-hydroxyprednisolone diacetate (XVa), saponified to the free tetrol XVb which in turn was converted to the 16,17-acetonide XVc by acetone–perchloric acid.

Biological Activity (Table I).—Introduction of a 16α-hydroxyl group into 6α,9α-difluoroprednisolone led to a compound (XVb) with the anticipated favorable biological spectrum, namely, high anti-inflammatory activity[30] (35x hydrocortisone; 7x Triamcinolone) and, in contrast to the C-16 unsubstituted compound, no retention of so-

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(30) All assays were carried out in adrenalectomized rats, the salt assays (subcutaneous) without sodium chloride load and the anti-inflammatory assays (oral) by cotton pellet implant. The anti-inflammatory assays were carried out by The Endocrine Laboratories, Madison, Wisc., and by Dr. R. I. Dorfman, The Worcester Foundation, Shrewsbury, Mass. We wish to thank Dr. Dorfman and Dr. E. Rosemberg of The Worcester Foundation for the salt assays.

dium.[30,31] The corresponding 16,17-acetonide XVc exhibited 100x the anti-inflammatory activity of hydrocortisone with no sodium retention. In preliminary clinical trials 6α,9α-difluoro-16α-hydroxyprednisolone was found to be a potent suppressor of inflammatory conditions such as rheumatoid arthritis as well as allergic conditions such as asthma, while its acetonide XVc proved to be highly effective as a topical corticoid. Details of these clinical investigations will be published elsewhere.

TABLE I

ANTI-INFLAMMATORY ACTIVITY OF 6α-FLUORO-16α-HYDROXY-CORTICOIDS

Compound	Anti-inflammatory activity[30]
Hydrocortisone	1
9α-Fluoro-16α-hydroxyprednisolone (triamcinolone)	5
6α-Fluoro-16α-hydroxyhydrocortisone	5
6α-Fluoro-16α-hydroxyhydrocortisone 16,17-acetonide 21-acetate	4
6α-Fluoro-16α-hydroxyprednisolone 16,17-acetonide 21-acetate	20
6α,9α-Difluoro-16α-hydroxyhydrocortisone	15
6α,9α-Difluoro-16α-hydroxyprednisolone	35
6α,9α-Difluoro-16α-hydroxyprednisolone 16,17-acetonide	100

Experimental[32]

$\Delta^{5,16}$-Pregnadiene-3β,21-diol-20-one 21-Acetate (II).—16α,17α-Oxido-Δ^5-pregnene-3β,21-diol-20-one 21-acetate[21] (I) (7.8 g.) in acetic acid (200 ml.) was treated under carbon dioxide with 40 ml. of a solution of chromous chloride (prepared from 13.3 g. of chromic chloride hexahydrate and 4 ml. of concentrated hydrochloric acid[33]). After 5 minutes the product was precipitated with water and filtered. The crude product was heated for one hour in acetone (150 ml.) containing hydrochloric acid (1 ml.) and then concentrated to incipient crystallization to yield 5 g. of II, m.p. 170–173°. The analytical sample, from acetone, had m.p. 177.5–179°, $[\alpha]_D$ −41°, λ_{max} 242 mμ, log ε 3.93 (reported[21] m.p. 174–177°, $[\alpha]_D$ −29° (ethanol)).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66; O, 17.18. Found: C, 73.71; H, 8.75; O, 17.32.

Δ^5-Pregnene-3β,16α,17α,21-tetrol-3,20-dione 21-Acetate (III) and Δ^5-Pregnene-3β,16α,17α,21-tetrol-3,20-dione 16,17-Acetonide 21-Acetate (IV).—To a solution of II (40 g.) in acetone (1.2 l.) and acetic acid (8 ml.) at 0° was added, in one portion, a solution of potassium permanganate (18 g.) in acetone (850 ml.) and water (150 ml.). After 5 minutes the mixture was filtered through Celite and the pale yellow filtrate concentrated *in vacuo*, poured into iced brine and filtered. One crystallization from acetone yielded 13.5 g. of crude III, m.p. 186–190°, which was used for the preparation of the acetonide. An attempt to prepare an analytical sample led to material which was apparently still impure. After eight crystallizations from acetone, III had m.p. 223–227°, $[\alpha]_D$ −136.5° (pyridine).

Anal. Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43; O, 23.62. Found: C, 68.74; H, 7.93; O, 23.18.

(31) We have found that 6α,9α-difluoro-hydrocortisone and -prednisolone exhibit definite retention of sodium in the experimental animal although to a lesser degree than the C-6 unsubstituted compound.

(32) Melting points are uncorrected. Rotations were determined in chloroform unless noted otherwise and ultraviolet absorption spectra in 95% ethanol solution. Infrared spectra were determined with a Perkin–Elmer model 21 spectrophotometer. We are grateful to Dr. L. Throop for determination of rotations and spectral data.

(33) See G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, THIS JOURNAL, **72**, 4077 (1950) for the preparation of chromous chloride.

The once-crystallized material (14.8 g.) was stirred in acetone (370 ml.) containing perchloric acid (72%, 2.5 ml.). After 10 minutes all the material had dissolved and after 1 hour the product was precipitated with water and filtered. Crystallization from acetone gave, in two crops, 12.3 g. of the acetonide IV, m.p. 210–214°. Further crystallization gave the analytical sample, m.p. 215–216.5°, $[\alpha]_D$ +8°.

Anal. Calcd. for $C_{26}H_{38}O_6$: C, 70.24; H, 8.16; O, 21.59. Found: C, 70.02; H, 8.58; O, 21.74.

5α,6α-Oxidoallopregnane-3β,16α,17α,21-tetrol-20-one 16,17-Acetonide 21-Acetate (V).—The acetonide (IV, 13.8 g.) in chloroform (275 ml.) at 0° was treated with 59 ml. of an ethereal solution containing 1.4 molar equivalents of monoperphthalic acid. The solution was left for 1 hour at 0° and overnight at room temperature, washed with aqueous sodium carbonate and water, dried and evaporated. Crystallization from acetone–hexane gave 10.3 g. of the α-epoxide V, m.p. 186–194°. Recrystallization led to the analytical sample, m.p. 195–196°, $[\alpha]_D$ ±0°.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28; O, 24.21. Found: C, 67.42; H, 8.16; O, 23.96.

6β-Fluoroallopregnane-3β,5α,16α,17α,21-pentol-20-one 16,17-Acetonide 21-Acetate (VI).—The foregoing epoxide (V, 10.17 g.) dissolved in dry ether–benzene (1:1, 1 l.) was treated with freshly distilled boron trifluoride–ether complex (20 ml.) and the solution left overnight at room temperature. After washing with aqueous sodium bicarbonate and water, drying and evaporating, the residue was chromatographed on alumina (300 g.). Elution with benzene and crystallization from acetone–hexane afforded 2.3 g. of starting material, m.p. 193–195°. Elution with ether–benzene (1:9) and crystallization from acetone–hexane gave 3.06 g. of the fluorohydrin VI, m.p. 210–215°. The analytical sample had m.p. 224–226°, $[\alpha]_D$ +29.5°, infrared λ_{max}^{KBr} 5.78(sh) and 5.82 μ.

Anal. Calcd. for $C_{26}H_{39}FO_7$: C, 64.71; H, 8.15; F, 3.94. Found: C, 64.10; H, 8.13; F, 4.11.

6β-Fluoroallopregnane-5α,16α,17α,21-tetrol-3,20-dione 16,17-Acetonide 21-Acetate (VII).—The preceding fluorohydrin VI (1 g.) in acetone (50 ml.) was oxidized at 0° with 8 *N* chromic acid–sulfuric acid solution in the standard manner.[25] After 5 minutes, dilution with water and filtration gave 880 mg. of the ketone VII, m.p. 215–224°. Crystallization from acetone–hexane gave the analytical sample, m.p. 225–227°, $[\alpha]_D$ +48°; infrared λ_{max}^{KBr} 5.76(sh), 5.81 and 5.89 μ.

Anal. Calcd. for $C_{26}H_{37}FO_7$: C, 64.98; H, 7.76. Found: C, 64.68; H, 7.67.

6α-Fluoro-Δ^4-pregnene-16α,17α,21-triol-3,20-dione 16,17-Acetonide 21-Acetate (VIII).—The preceding compound VII (1.5 g.) in dry acetone (150 ml.) was treated with a stream of dry hydrogen chloride at 0° for 30 minutes and the solution maintained at this temperature for a further 3.5 hours. Concentration *in vacuo* to *ca.* 25 ml. and filtration yielded 950 mg. of the 6α-fluoro-Δ^4-3-ketone VIII, m.p. 288–290°, and a further 250 mg. of material of similar melting point was obtained by retreatment of the mother liquors for an additional 2 hours with hydrogen chloride. Recrystallization from acetone–hexane gave the analytical specimen, m.p. 295–296°, $[\alpha]_D$ +104°, λ_{max} 236 mμ, log ε 4.19; infrared λ_{max}^{KBr} 5.74, 5.82, 5.98 and 6.20 μ.

Anal. Calcd. for $C_{26}H_{35}FO_6$: C, 66.34; H, 7.90; F, 4.26. Found: C, 66.74; H, 7.62; F, 4.26.

6α-Fluoro-Δ^4-pregnene-16α,17α,21-triol-3,20-dione 21-Acetate (IXa).—The acetonide VIII (14.9 g.) was added to 1.5 l. of refluxing 60% formic acid. After 10 minutes all the material had dissolved and after a further 10 minutes the solution was cooled and poured into ice-water and filtered to give 11.5 g. of crude product, m.p. 185–190°. Extraction of the filtrate with methylene chloride gave a further 2.5 g. of semi-crystalline material. Recrystallization of a portion of the precipitated material from aqueous acetone afforded the analytical sample of IXa, m.p. 206–208°, $[\alpha]_D$ +86°.

Anal. Calcd. for $C_{23}H_{31}FO_6$: C, 65.38; H, 7.40; F, 4.50. Found: C, 65.92; H, 7.67; F, 4.22.

6α-Fluoro-Δ^4-pregnene-16α,17α,21-triol-3,20-dione (IXb).—The crude product from the cleavage of the acetonide (12 g.) was stirred in methanol (120 ml.) with potassium hydroxide (1.2 g.) under nitrogen at 0° for 1 hour. Excess base was neutralized with acetic acid and the reaction mixture evaporated to dryness *in vacuo*. Addition of water and

filtration afforded 11 g. of crude 6α-fluoro-16α-hydroxy substance "S" (IXb) which was used without further purification for the adrenal incubation. Recrystallization of a small portion from acetone gave the analytical sample, m.p. 228–230°, $[\alpha]D$ +64° (dioxane), λ_{max} 236 mμ, log ε 4.18.

Anal. Calcd. for $C_{21}H_{29}FO_5$: C, 66.30; H, 7.68. Found: C, 66.04; H, 7.81.

6α-Fluoro-16α-hydroxyhydrocortisone (Xa). (a) By Adrenal Incubation of 6α-Fluoro-16α-hydroxy "S."—The following solutions were prepared: 425 ml. of aqueous potassium hydrogen phosphate (1.74%) and 75 ml. of aqueous sodium dihydrogen phosphate (1.38%) were diluted to 5 l. (A); 1 l. of aqueous sodium chloride (4.5%) was mixed with 40 ml. of aqueous potassium chloride (5.75%) and 10 ml. of aqueous magnesium sulfate (19.1%) (B); 20.9 g. of fumaric acid and 14.4 g. of sodium hydroxide were made up to 1.2 l. (C). Crude 6α-fluoro-16α-hydroxy "S" (IXb) (6 g.), in propylene glycol (30 ml.) was added to 9 kg. of minced, defatted fresh beef adrenals suspended in 9 l. of buffer solution prepared by mixing 950 ml. of solution A, 8.64 l. of solution B and 2.4 l. of solution C. The mixture was incubated at 30° for 3 hours with continuous agitation in open erlenmeyer flasks, at the end of which time acetone (30 l.) was added and the mixture stirred well for a further one hour, filtered and washed with acetone. The filtrate was concentrated to about one-third volume *in vacuo* and extracted twice with hexane (4 l.). The aqueous phase was then extracted with methylene chloride (3 × 5 l.) and the extracts dried and evaporated. The residue was swirled with *ca.* 30 ml. of methylene chloride, cooled well and filtered to give 3 g. of colored crystals of 6α-fluoro-16α-hydroxyhydrocortisone (Xa), m.p. 220–225°. Recrystallization from acetone–methanol (charcoal) gave an analytical sample, m.p. 233–236°, $[\alpha]D$ +95° (dioxane), λ_{max} 236–238 mμ, log ε 4.18; infrared λ_{max}^{KBr} 5.73, 6.00 and 6.15 μ. This sample was compared to the product obtained by microbiological hydroxylation of 6α-fluorohydrocortisone (see below) and proved to be identical.

Anal. Calcd. for $C_{21}H_{29}FO_6$: C, 63.62; H, 7.37. Found: C, 63.47; H, 7.42.

(b) By Microbiological Hydroxylation of 6α-Fluorohydrocortisone.—*Streptomyces roseochromogenus*, Rutgers Collection No. 3689,[14c] was grown on the following medium: soybean meal (15 g.), corn sirup (25 g.) and calcium carbonate (25 mg.) diluted with distilled water to 1 l. To 200 ml. of a 24-hour culture medium growth was added 40 mg. of 6α-fluorohydrocortisone in ethanol (2 ml.) and the medium incubated at 25° with vigorous agitation for 72 hours. Fifty such incubations (*i.e.*, 2.0 g. of compound) were combined and the mixture extracted with methylene dichloride to afford a residue which was adsorbed from methylene dichloride on to a column containing a mixture of silica gel (75 g.) and Celite (20 g.). Elution with methylene dichloride–acetone (70:30, 900 ml.) afforded 6α-fluoro-16α-hydroxyhydrocortisone (Xa) (540 mg.), m.p. 215–225°, raised by one crystallization from acetone to 232–234° (140 mg.). The analytical sample exhibited m.p. 234–236°, $[\alpha]D$ +95° (dioxane), λ_{max} 236–238 mμ, log ε 4.18, and was identical with the product obtained as in (a).

Anal. Calcd. for $C_{21}H_{29}FO_6$: C, 63.62; H, 7.37; F, 4.79. Found: C, 64.05; H, 7.19; F, 4.62.

6α-Fluoro-16α-hydroxyhydrocortisone 16,21-Diacetate (Xb).—6α-Fluoro-16α-hydroxyhydrocortisone (Xa) (1.2 g.) in pyridine (8 ml.) and acetic anhydride (4 ml.) was warmed at *ca.* 60° for 2 hours and left a further 2 hours at room temperature. Ice and water were added and the crystalline product collected. Crystallization from methylene chloride–methanol afforded 1.05 g., m.p. 187–192°, and a second crop of 0.13 g., m.p. 184–187°, of the diacetate Xb. Recrystallization from the same solvent pair gave a lower melting form, m.p. 175–177°, $[\alpha]D$ +70° (dioxane), λ_{max} 236–238 mμ, log ε 4.20; infrared λ_{max}^{KBr} 5.75 (broad), 6.00 and 6.13(sh) μ.

Anal. Calcd. for $C_{25}H_{33}FO_8$: C, 62.49; H, 6.92; F, 3.95. Found: C, 62.82; H, 7.33; F, 3.50.

6α-Fluoro-14α-hydroxyhydrocortisone 16,17-Acetonide 21-Acetate (XIa).—6α-Fluoro-16α-hydroxyhydrocortisone (140 mg.) was suspended in acetone (5 ml.) containing 2 drops of perchloric acid (72%). After stirring for 5 minutes all the material dissolved and after a further 15 minutes the solution was diluted with water and the product extracted with methylene chloride. The resulting oil was acetylated overnight with pyridine (2 ml.) and acetic anhydride (1 ml.). Dilution with ice-water and filtration gave 155 mg. of crystals of XIa, m.p. 248–256°. Recrystallization from acetone–hexane raised the melting point to 261–263°, $[\alpha]D$ +135°, λ_{max} 236–238 mμ, log ε 4.18.

Anal. Calcd. for $C_{26}H_{35}FO_7$: C, 65.25; H, 7.37. Found: C, 65.08; H, 7.13.

6α-Fluoro-16α-hydroxyprednisolone 16,17-Acetonide 21-Acetate (XIb).—The preceding compound XIa (155 mg.) in boiling *t*-butyl alcohol (25 ml.) containing pyridine (0.5 ml.) and selenium dioxide (80 mg.) was oxidized under nitrogen for a total of 88 hours. The reaction mixture was diluted with ethyl acetate and filtered through Celite and the filtrate evaporated to dryness. The residue was treated with water and isolated by ethyl acetate extraction. The product was recrystallized successively from ethyl acetate, methylene chloride–methanol and acetone–hexane to yield 48 mg. of the dienone XIb, m.p. 266–268°, $[\alpha]D$ +97°, λ_{max} 240–242 mμ, log ε 4.16; infrared λ_{max}^{KBr} 5.75, 5.84, 6.08, 6.23 and 6.28 μ. The product was homogeneous and slightly more polar than the starting material, on a paper chromatogram.

Anal. Calcd. for $C_{26}H_{33}FO_7$: C, 65.53; H, 6.98. Found: C, 65.78; H, 7.12.

6α-Fluoro-$\Delta^{4,9(11)}$-pregnadiene-16α,17α,21-triol-3,20-dione 16,21-Diacetate (XII).—6α-Fluoro-16α-hydroxyhydrocortisone 16,21-diacetate (2.94 g., dried by azeotropic distillation with benzene) was heated for 2 hours on a steam-bath with freshly distilled dimethylformamide (60 ml.), pyridine (3.6 ml.) and methanesulfonyl chloride (2.4 ml.). The cooled reaction mixture was poured into aqueous sodium bicarbonate solution and the product, isolated with methylene chloride, was chromatographed on 90 g. of silica gel. Elution with methylene chloride–acetone (9:1) and crystallization from methylene chloride–methanol gave 1.6 g. of the $\Delta^{9(11)}$-compound XII, m.p. 110–114°. The analytical sample had m.p. 115–117°, $[\alpha]D$ +24°, λ_{max} 234–236 mμ, log ε 4.18.

Anal. Calcd. for $C_{25}H_{31}FO_7$: C, 64.92; H, 6.76; F, 4.11. Found: C, 64.96; H, 6.90; F, 3.54.

Further elution with methylene chloride–acetone (4:1) and crystallization from methylene chloride–methanol gave 170 mg. of starting material in the higher melting form, m.p. 188–192°.

6α-Fluoro-9β,11β-oxido-Δ^4-pregnene-16α,17α,21-triol-3,-20-dione 16,21-Diacetate (XIII).—The $\Delta^{9(11)}$-compound XII (1.38 g.) in dioxane (15 ml.) and aqueous perchloric acid (0.5 *N*, 1.9 ml.) was treated with N-bromoacetamide (600 mg.) in the dark in three portions during 0.5 hour. Stirring was continued for a further 1.75 hours whereupon excess reagent was destroyed with aqueous sodium bisulfite. Ice-water was added and the product isolated with methylene chloride as a pale yellow oil. Without further purification the crude bromohydrin was heated in boiling acetone (60 ml.) with potassium acetate (2.5 g.) for 6 hours. The acetone was removed by distillation, water was added to the residue and the product isolated with methylene chloride. Crystallization from methanol afforded 800 mg. of the epoxide XIII, m.p. 120–124°, and a further 180 mg., m.p. 117–119°, was obtained by chromatography of the mother liquors over silica gel. Recrystallization from methanol gave an analytical sample, m.p. 125–127°, $[\alpha]D$ −13°, λ_{max} 238 mμ, log ε 4.14.

Anal. Calcd. for $C_{25}H_{31}FO_8$: C, 62.75; H, 6.53; F, 3.97. Found: C, 62.60; H, 6.57; F, 3.83.

6α,9α-Difluoro-16α-hydroxyhydrocortisone 16,21-Diacetate (XIVa).—A solution of the foregoing epoxide XIII (650 mg.) in dry methylene chloride (20 ml.) was added to a solution of anhydrous hydrofluoric acid (1.6 g.) in tetrahydrofuran (2.85 g.) and methylene chloride (10 ml.) at −60°. The solution was then left at −10° for 72 hours. After pouring into aqueous sodium bicarbonate solution, the organic layer was separated, washed, dried and evaporated and the residue re-acetylated by heating for one hour on the steam-bath with pyridine (6 ml.) and acetic anhydride (3 ml.). The reagents were removed *in vacuo* and the residue chromatographed on 20 g. of silica gel. Elution with methylene chloride–acetone (9:1) and crystallization from methylene chloride–methanol afforded 290 mg. of the difluoro compound XIVa, m.p. 140–150° with solvent loss. Recrystallization from acetone–hexane gave the analytical sample, m.p. 182–

185° (after drying at 130°), $[\alpha]_D$ +77° (dioxane), λ_{max} 234 mμ, log ε 4.20; infrared λ_{max}^{KBr} 5.77 (broad), 6.00, 6.12 and 6.21 μ.

Anal. Calcd. for $C_{25}H_{32}F_2O_8$: C, 60.23; H, 6.47; F, 7.62. Found: C, 60.71; H, 6.78; F, 7.24.

6α,9α-Difluoro-16α-hydroxyhydrocortisone (XIVb).—The incubation of 300 mg. of 6α,9α-difluorohydrocortisone with *Streptomyces roseochromogenus*, Rutgers Collection No. 3689, was carried out exactly as described for the preparation of Xa, part (b). The methylene dichloride extract was adsorbed onto a column containing a mixture of silica gel (10 g.) and Celite (2.5 g.) whence elution with methylene dichloride–acetone (70:30; 300 ml.) afforded a crystalline product which after one recrystallization from aqueous methanol gave 56 mg. of 6α,9α-difluoro-16α-hydroxyhydrocortisone (XIVb), m.p. 247–255°. The analytical specimen from acetone–hexane exhibited m.p. 242–248°, $[\alpha]_D$ +58° (dioxane), λ_{max} 234 mμ, log ε 4.18.

Anal. Calcd. for $C_{21}H_{28}F_2O_6 \cdot 1/2\ H_2O$: C, 61.90; H, 7.17. Found: C, 61.61; H, 6.92.

Acetylation as described for the preparation of Xb gave a diacetate identical with XIVa in all respects.

6α,9α-Difluoro-16α-hydroxyprednisolone 16,21-Diacetate (XVa).—The diacetate XIVa (290 mg.), *t*-butyl alcohol (30 ml.), pyridine (0.05 ml.) and selenium dioxide (150 mg.) were heated under reflux in a nitrogen atmosphere for 53 hours. Ethyl acetate was added, the mixture filtered through Celite and the filtrate evaporated to dryness. The residue was stirred well with water, filtered and the product chromatographed on 10 g. of silica. Elution with acetone–methylene chloride (1:19) and crystallization from methylene chloride gave 68 mg. of the dienone XVa, m.p. 212–215°. The analytical sample had m.p. 222–224°, $[\alpha]_D$ +51° (dioxane), λ_{max} 238 mμ, log ε 4.23; infrared λ_{max}^{KBr} 5.75 (broad), 6.00, 6.14 and 6.21(sh) μ. The compound was tenaciously solvated.

Anal. Calcd. for $C_{25}H_{30}F_2O_8$: C, 60.48; H, 6.09; F, 7.65. Found (dried at 130°): C, 60.35; H, 6.29. Calcd. for $C_{25}H_{30}F_2O_8 \cdot H_2O$: C, 58.36; H, 6.27; F, 7.34. Found (dried at 93°): C, 58.83; H, 6.06; F, 7.91.

6α,9α-Difluoro-16α-hydroxyprednisolone (XVb).—The foregoing diacetate XVa (430 mg.) was stirred in methanol (15 ml.) at 0° under nitrogen, and methanolic potassium hydroxide (4%, 2.2 ml.) was added. The material rapidly dissolved and reprecipitated after *ca.* 30 minutes at 0°. After 1 hour excess alkali was neutralized with acetic acid and the methanol removed *in vacuo*. Water was added and the product filtered. Crystallization from ethyl acetate–methanol afforded 285 mg. of XVb, m.p. 258–260°. Recrystallization gave an analytical sample, m.p. 266–268°, $[\alpha]_D$ +43° (dioxane), λ_{max} 238 mμ, log ε 4.23; infrared λ_{max}^{KBr} 5.83, 6.00, 6.15 and 6.20 μ.

Anal. Calcd. for $C_{21}H_{26}F_2O_6 \cdot CH_3OH$: C, 59.45; H, 6.80. Found: C, 59.64; H, 6.40.

6α,9α-Difluoro-16α-hydroxyprednisolone 16,17-Acetonide (XVc).—6α,9α-Difluoro-16α-hydroxyprednisolone (XVb) (250 mg.) was stirred in acetone (15 ml.) containing perchloric acid (70%, 5 drops). After 20 minutes all the material had dissolved and after a further 10 minutes water (50 ml.) containing a little sodium bicarbonate was added and the acetone removed *in vacuo*. The resulting needles (257 mg.) were isolated by filtration; m.p. 261–263°. Recrystallization from acetone–hexane gave the analytical sample, m.p. 265–266°, $[\alpha]_D$ +95°, λ_{max} 238 mμ, log ε 4.21.

Anal. Calcd. for $C_{24}H_{30}F_2O_6$: C, 63.70; H, 6.68; F, 8.40. Found (dried at 130°): C, 63.42; H, 6.78; F, 8.07.

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Steroids. CXL.[1] 11-Methyl Steroids

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The addition of methyllithium to 11-ketones of certain androstane and androstene derivatives is shown to provide the corresponding 11α-methyl-11β-ol's. Further transformations of these products to hormone analogs possessing either the 11α-methyl-11β-ol functions or the 11-methyl-$\Delta^{9(11)}$-system are described.

Advances in knowledge concerning the chemistry of steroidal 11-ketones have dispelled older beliefs concerning their inertness toward transformations other than those involving catalytic or chemical reduction. Thus it has been shown that under forcing conditions 11-ketones may be removed by Wolff–Kishner reduction[2] or converted to their 11-ethylene ketal[3] or oxime[4] derivatives.

In connection with a broad program directed toward the preparation of new anabolic agents, the action of methylmagnesium bromide on 5α-androstan-3β-ol-11,17-dione (I) in benzene–ether solution was investigated. In addition to the expected 17α-methyl-5α-androstan-3β,17β-diol-11-one (II) there was observed a second more polar product whose infrared spectrum exhibited no carbonyl absorption. On the basis of this spectroscopic evidence as well as the analytical data, it was obvious that the 11-ketone had undergone attack by the Grignard reagent and the substance was therefore assigned the structure of 11α,17α-dimethyl-5α-androstan-3β,11β,17β-triol (IIIa). Further characterization of this compound was accomplished by pyridine–chromium trioxide oxidation[5] to the 3-keto derivative IIIb. The assignment of configuration as the 11α-methyl-11β-ol in this series of compounds rests on the assumption that the reaction involves attack of the 11-ketone by a methyl carbanion and that the stereochemical course would be the same as for hydride reductions.[6]

Upon observing that replacement of the methyl Grignard reagent by methyllithium led to very high yields of IIIa, the action of this latter reagent was investigated on both 5β-pregnan-11-one (IVa)[7]

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