Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.50; H, 8.16. Found: C, 74.71; H, 8.22.

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16-Hydroxylated Steroids. XIII.[1] 9α-Fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione

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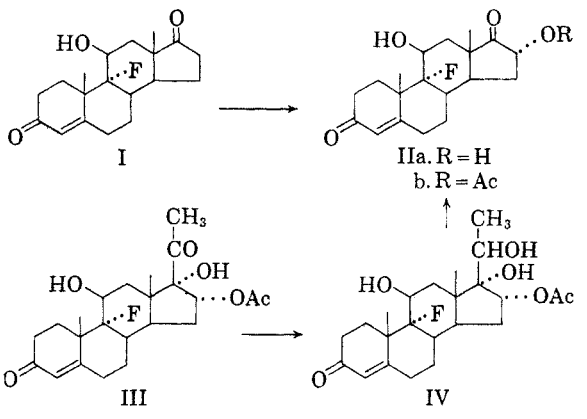
The research program of this laboratory on the preparation of 16-hydroxylated steroids has been extended to include C19-steroids. We wish to report on the synthesis of 9α-fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa).[2]

9α-Fluoro-11β-hydroxy-4-androstene-3,17-dione (I)[3] on microbiological hydroxylation with *Streptomyces roseochromogenus* (Lederle AE 409)[4] afforded 9α-fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa). The structure of the fermentation product was established as follows:

Compound IIa exhibited a positive Blue Tetrazolium test indicative of the 16,17-ketol moiety.[5] Acetylation gave the monoacetate IIb, in turn, synthesized from 16α-acetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (III).[6] Reduction of III in methanol at 0° with sodium borohydride gave 16α-acetoxy-9α-fluoro-11β,17α,20-trihydroxy-4-pregnen-3-one (IV).[7] The latter on the basis of elemental analyses was apparently obtained in a pure state. However, its ultraviolet absorption spectrum, $\lambda_{max}^{methanol}$ 240 mμ (ε 11,000), revealing a low molecular extinction coefficient,[8] indicated that reduction in part of the C3-carbonyl and/or the C4-5-double bond had occurred.[9] The material as such in methanol was treated with an aqueous solution of sodium periodate at room temperature to give after partition chromatography 16α-acetoxy-9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (IIb), identical in all respects with the acetylated fermentation product.

Bioassay. Rosemberg and Dorfman[10] have recently cited 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (I) as the first instance of a highly active sodium retaining substance in the C19-series. These same investigators have now found in a preliminary assay that 9α-fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa) was inactive in the electrolyte assay (saline load, six hours) on adrenalectomized rats at 6, 25, and 100 μg. dose levels.[11]

Flow Sheet



EXPERIMENTAL

All melting points are uncorrected.

9α-Fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa). Forty 500 ml. flasks were charged with 100 ml. each of the following medium: corn steep liquor (30 g.), glucose (30 g.), soybean oil (5 g.), and calcium carbonate (5 g.) in 1 l. of distilled water. Each flask, after the addition of 25 mg. of 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (I) dissolved in 1 ml. of methanol, was inoculated with 4 ml. of a 48 hr. (28°) mycelial growth of *Streptomyces roseochromogenus* (Lederle AE 409). The fermentation was carried out for 78 hr. at 28° with shaking (rotary shaker, 240 RPM).

The pooled fermentation mixture was filtered, and the filtrate was extracted twice with 4 l.-portions of chloroform. The combined extracts were washed with water, treated with animal charcoal, dried and evaporated. The crude residue was subjected to partition chromatography on 300

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(7) The reduction of the C20-carbonyl group presumably provided the 20β-hydroxyl group; see, D. K. Fukushima and E. D. Meyer, *J. Org. Chem.*, **23**, 174 (1958).

(8) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

(9) F. Sondheimer, M. Velasco, E. Batres and G. Rosenkranz, *Chem. & Ind.* (London), 1482 (1954); J. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955).

(10) E. Rosemberg and R. I. Dorfman, *Proc. Exptl. Biol. and Med.*, **99**, 336 (1958).

(11) We wish to thank the Worcester Foundation group for carrying out this assay, the details of which will be reported by them elsewhere.

g. of Celite[12] with a ternary system consisting of 1 part water, 5 parts dioxane, and 4 parts cyclohexane. The fraction collected from 2.3–3.9 hold-back volumes (HBV) (maximum product at 3.3) (1 HBV = 485 ml.) was evaporated to afford 604 mg. of crude IIa. Two crystallizations from acetone gave pure IIa, m.p. 262–270° with previous softening and browning and with decomposition at 271°; $\lambda_{max}^{methanol}$ 237 mμ (ε 17,400); ν_{max}^{KBr} 3510, 3400, 3250, 1754, 1657, 1640–1615 (inflection) cm.$^{-1}$; $[\alpha]_D^{25}$ +157° (methanol); positive Blue Tetrazolium test.

Anal. Calcd. for $C_{19}H_{25}O_4F$ (336.39): C, 67.83; H, 7.49; F, 5.65. Found: C, 67.64; H, 7.65; F, 5.86.

16α-Acetoxy-9α-fluoro-11β,17α,20-trihydroxy-4-pregnen-3-one (IV). A solution of 350 mg. of 16α-acetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (III) in 50 ml. of methanol was cooled to 0° and treated with 47 mg. of sodium borohydride. After remaining at 0° for 1 hr., the solution was acidified with 0.2 ml. of glacial acetic acid and evaporated. The residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution and water. The dried extract was evaporated and the residue crystallized from acetone-petroleum ether to afford 254 mg. of crude IV, m.p. 215–219.5° with previous softening. Two additional crystallizations from the same solvent pair gave 228 mg., m.p. 214.5–217.5° with previous softening; $\lambda_{max}^{methanol}$ 240 mμ (ε 11,000); ν_{max}^{KBr} 3430, 1725, 1666, 1625, 1277, and 1253 cm.$^{-1}$; $[\alpha]_D^{25}$ −19° (acetone).

Anal. Calcd. for $C_{23}H_{33}O_6F$ (424.49): C, 65.07; H, 7.84; F, 4.48. Found: C, 64.99; H, 8.17; F, 4.19.

This material was used as such in the subsequent side chain degradation.

16α-Acetoxy-9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (IIb). A. Forty milligrams of IIa in 2 ml. of pyridine was treated with 1 ml. of acetic anhydride, and the mixture was allowed to stand at room temperature overnight. The crude acetate was subjected to partition chromatography on 31 g. of Celite[12] with the system four parts petroleum ether (b.p. 90–100°), three parts ethyl acetate, four parts methanol, and two parts water. The fraction collected from 3.5–5.5 hold-back volumes (maximum product at 4.7) (1 HBV = 38 ml.) was evaporated, and the residue was crystallized from acetone-petroleum ether (b.p. 35–60°) to afford pure IIb, m.p. 248–250°. Its infrared spectrum was identical with that obtained in preparation B.

B. A solution of 380 mg. of impure 16α-acetoxy-9α-fluoro-11β,17α,20-trihydroxy-4-pregnen-3-one (IV) in 20 ml. of methanol was treated with 7.6 ml. of an aqueous solution of sodium periodate (0.1*M*). After standing at room temperature for 19 hr., the solution was poured into ice water, and the resultant precipitate was filtered and washed with water to afford 178 mg. of crude product, m.p. 227.5–236° with previous softening. Three crystallizations from acetone-petroleum ether (b.p. 60–70°) gave 129 mg. of material having a constant melting point (233–239°). Paper strip chromatography indicated approximately 75% of pure IIb together with 25% of a more polar contaminant. A 110 mg. portion of the above 129 mg. was subjected to partition chromatography on Celite[12] using a solvent system consisting of three parts of petroleum ether (b.p. 90–100°), two parts of ethyl acetate, three parts of methanol and two parts of water. The eluate from the second hold-back volume (1 HBV = 320 ml.) was evaporated and the residue crystallized from acetone-petroleum ether to afford 78 mg. of pure IIb, m.p. 246.5–249° with previous softening. One additional crystallization did not alter the melting point; $\lambda_{max}^{methanol}$ 238 mμ (ε 16,300); ν_{max}^{KBr} 3510, 1770, 1755, 1662, 1630, 1245, and 1220 cm.$^{-1}$; $[\alpha]_D^{25}$ +121° (chloroform). Paper strip chromatography indicated an homogeneous compound.

Anal. Calcd. for $C_{21}H_{27}O_5F$ (378.43): C, 66.73; H, 7.19; F, 5.02. Found: C, 67.16; H, 7.54; F, 4.75.

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(12) The adsorbent was specially treated Celite 545 which was slurried in 6*N* hydrochloric acid and allowed to stand overnight. It was then filtered and was washed with water, followed by a mixture of methanol and ethyl acetate. Finally, it was dried at room temperature. Celite is the trademark of Johns-Manville Company for diatomaceous silica products.

Steroidal Hormone Relatives. VIII. A Synthetic Approach to 6-Aza-equilenin[1,2]

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Estrogens are carcinogenic to experimental animals which have an inherited sensitivity to the development of mammary carcinoma, and many clinicians will not employ them in the treatment of women who have a familial history of malignancy.[3] Yet, estrogens,[3] as well as androgens,[4] may be used in the treatment of inoperable breast cancer, and estrogenic materials are effective in the palliation of prostatic carcinoma and its metastases and may also be useful against lung and skin metastases.[3] Such facts have led us to propose that the aza analogs of the steroids might be of considerable interest as possible carcinolytic agents.[5] Perhaps an azasteroid would fit the enzyme site of the parent hormone in such a manner that only a carcinolytic effect would result.

The favorable effect of estrogens upon the blood levels of cholesterol and presumably upon the course of atherosclerosis[6] raises the question of whether or not an azaestrogen would retain the antiatherogenic effect of the parent hormone without exhibiting the undesirable estrogenic effect. It is possible that a nonestrogenic azasteroid would

(1) Abstracted from a portion of the Ph.D. thesis of John A. Durden, Jr., University of Kansas, 1957.

(2) This investigation was supported in part by Grant CY-3573, from the National Cancer Institute, U. S. Public Health Service.

(3) *New and Nonofficial Drugs*, J. P. Lippincott Co., Philadelphia, Pa., 1959, p. 504.

(4) *New and Nonofficial Drugs*, J. P. Lippincott Co., Philadelphia, Pa., 1959, p. 542.

(5) Application for Research Grant to the National Institutes of Health, February 26, 1957.

(6) H. W. Eder in *Hormones and Atherosclerosis*, G. Pincus, Ed., Academic Press, Inc., New York, N. Y., 1959, Chapter 24.