16,16-Difluoro Steroid Hormone Analogs[1]

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The reaction of 16-formyl-17-keto steroids with perchloryl fluoride, in the presence of strong base, gives directly the 16,16-difluoro-17-keto system. The spectroscopic properties of, and some reactions shown by, the latter system are described, together with the synthesis of 16,16-difluoro steroid hormone analogs.

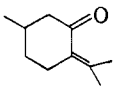
For some time we have been interested, as have others, in modified estrogens[2] (which might show serum cholesterol lowering activity without undesirable estrogenic activity) and in modified androgens[3] (which might show favorable myotrophic–androgenic ratios). At the inception of this work, it was known[2] that, in the estrone series, substitution by chlorine or iodine at C-16α results in improved lipid-shifting-feminizing ratios, while in the androgen area 16-bromo- and 16-chloro-testosterones[4] have been reported, but few or no biological data were available. In the case of fluorine substitution at C-16, 16-monofluoroestrones and -testosterones had been described only in the patent literature,[5,6] using apparently uncongenial syntheses.

The advent of perchloryl fluoride provided an effective method for mono- and polyfluorination of active methylene compounds,[7] and we decided to study the action of perchloryl fluoride on the β-dicarbonyl system in 16-formylestrone 3-methyl ether[8] (VIII) and 16-formyl-5-androsten-3β-ol-17-one[9] (I) as a route to 16-fluorinated 17-ketones.

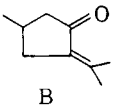
Both of the 16-formyl (16-hydroxymethylene) compounds (I and VIII) were prepared, in good yield from the 17-ketones with minor modification of the published[8,9] procedures. In our experience, the use of sodium methoxide and ethyl formate in tetrahydrofuran gave perfectly satisfactory results, and the crude products were obtained in sufficiently pure condition for subsequent reactions.

The 16-hydroxymethylene 17-ketones VIII and I showed λ_{max}^{MeOH} 268 mμ (9,600) and λ_{max}^{MeOH} 266 mμ (9,100), respectively, shifted to 305 mμ (20,800) and 303 mμ (20,500) in 0.07 *N* methanolic sodium hydroxide solution. These figures differ markedly from those shown[3] by 2-hydroxymethyleneandrostan-3-ones ($\lambda_{max} \sim$ 282 mμ, shifted to 315 mμ in basic solution). However, hydroxymethylenecamphor shows[10] λ_{max}^{EtOH} 264.5 mμ (12,900), in reasonable agreement with the values for steroidal 16-hydroxymethylene 17-ketones which also bear the chromophoric system in a five-membered ring.

The compounds A and B have very similar maxima[11] at 252 mμ and 254 mμ, respectively, ring size having no effect in these cases. The substantial difference between the maxima of the six-membered and five-membered ring hydroxymethylene ketones may be due to differences in ease of chelation of the enolized β-dicarbonyl system in the latter cases.



A



B

The 16-formyl compound (I) was then allowed to react with perchloryl fluoride in a *t*-butyl alcohol-potassium *t*-butoxide system (six moles of potassium *t*-butoxide per mole of steroid). The rate of disappearance of the β-dicarbonyl system was conveniently followed by measurement of the ultraviolet absorption at 303 mμ (and qualitatively by the ferric chloride test). Depending on the flow-rate of perchloryl fluoride, concentration, etc., the reaction was judged to be complete after 45–90 min., and the latter reaction time was routinely used.

Paper chromatographic examination of the crude product showed that a number of products were present, one of which was present in substantial amount.

Chromatography on Florisil gave this crystalline product in 25–30% yield. The analytical data and infrared spectrum (λ_{max}^{Nujol} 2.88, 3.05, 5.63 μ; $\lambda_{max}^{CS_2}$ 5.62 μ (1779 cm.$^{-1}$) were consistent with structure IIa, 16,16-difluoro-5-androsten-3β-ol-17-one. The compound IIa was further characterized as the 3-acetate (IIb) which could be reduced easily by zinc-acetic acid to give 5-androsten-3β-ol-17-one 3β-acetate.

Similarly, 16-hydroxymethylene estrone 3-methyl ether (VIII) gave the difluoro compound (IX) which regenerated estrone 3-methyl ether on reduction with zinc-acetic acid.

The spectroscopic properties of the 16,16-difluoro-17-keto system deserve brief comment. First of all, the infrared absorption of the 16,16-difluoro-17-keto system in IIa, at 1779 cm.$^{-1}$ (CS_2), is in reasonable accord with additive[12] shifts due to the fluorine atoms at C-16. Thus, the shift of the 17-ketone absorption due to the *gem*-difluoro system at C-16 is $\sim$37 cm.,$^{-1}$ while the $\Delta\nu$ values reported[2] for 16α- and 16β-fluoro substituents in the estrone series are, respectively, 18 cm.$^{-1}$ and 24 cm.$^{-1}$.

(1) (a) A preliminary account of part of this work has appeared earlier: *J. Am. Chem. Soc.*, **82**, 5256 (1960); (b) Presented, in part, at the 138th National Meeting of the American Chemical Society, New York, N. Y., September 11–16, 1960.

(2) *Cf.* G. P. Mueller and W. F. Johns, *J. Org. Chem.*, **26**, 2403 (1961), and references therein.

(3) *Cf.* R. O. Clinton, *et al.*, *J. Am. Chem. Soc.*, **83**, 1478 (1961), and references therein.

(4) B. Ellis, D. Patel, and V. Petrow, *J. Chem. Soc.*, **800**, (1958).

(5) G. P. Mueller, U. S. Patent 2,855,411 (1958). See also ref. 2.

(6) J. Fried and G. H. Thomas, U. S. Patent 2,857,403 (1958).

(7) C. E. Inman, T. A. Tyczkowski, R. E. Oesterling, and F. L. Scott, *Experientia*, **14**, 355 (1958); C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, *J. Am. Chem. Soc.*, **80**, 6533 (1958).

(8) J. C. Bardhan, *J. Chem. Soc.*, 1848 (1936).

(9) L. Ruzicka, V. Prelog, and J. Battegay, *Helv. Chim. Acta*, **31**, 1296 (1948).

(10) A. E. Gillam, J. I. Linas-Gray, A. R. Penfold, and J. L. Simonsen, *J. Chem. Soc.*, **60** (1941).

(11) H. S. French and L. Wiley, *J. Am. Chem. Soc.*, **71**, 3702 (1949).

(12) See for example, T. Nambara and J. Fishman, *J. Org. Chem.*, **26**, 4569 (1961). These workers noted such an additive effect for 16,16-dibromo- and 16,16-dichloro-17 keto steroids of the 14β-series.

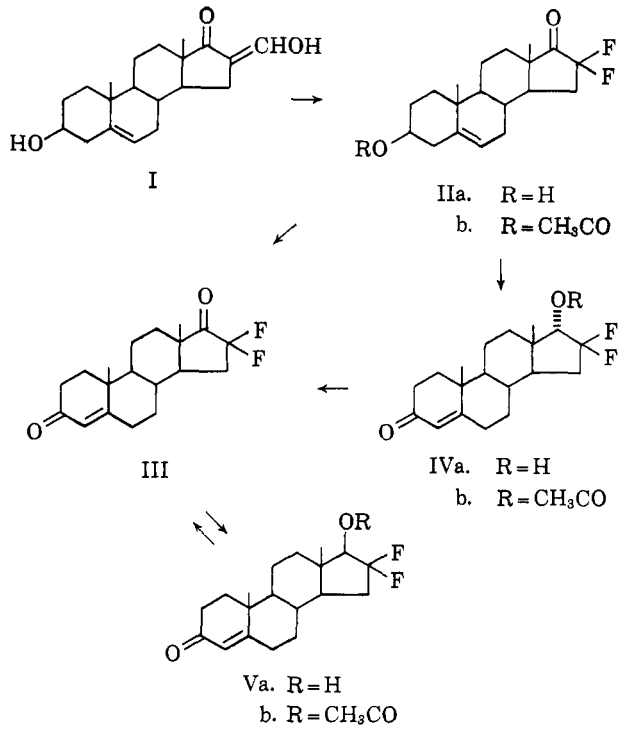


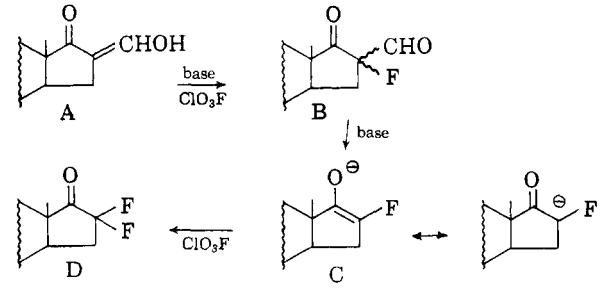
CHART I

The rotatory dispersion curve[13] (in methanol) of IIa showed a strong positive Cotton effect: ϕ peak +5576 (350 mμ); ϕ trough −6731 (298 mμ); amplitude = 12,300.

The 16-monofluoroestrone methyl ethers also show[2] strong positive Cotton effects, the position of the first extremum being 338 mμ for both the 16α- and 16β-isomers, while the unsubstituted 17-ketone has the first extremum at 315 mμ.

The proton magnetic resonance spectrum[14] of IX revealed splitting of the C-18 methyl resonance into a doublet (64 and 65 c.p.s. from tetramethylsilane) presumably due to proton-fluorine coupling.[15] Further work with 16-monofluoro compounds would have been of obvious interest, but due to purification difficulties with a mixture of C-16 monofluoro epimers (*vide infra*), we were not able to pursue this matter further.

The mechanism of formation of the *gem*-difluoro system may be plausibly formulated as shown.



(13) Determined through the courtesy of Professor Carl Djerassi, Stanford University, Palo Alto, Calif.

(14) Measured in deuteriochloroform solution at 60 Mc, using a Varian A60 spectrometer, with tetramethylsilane as internal reference. We thank Dr. Leon Mandell, Emory University, Atlanta, Ga., for this spectrum.

(15) See A. D. Cross and P. W. Landis, *J. Am. Chem. Soc.*, **84**, 1736 (1962), and references cited therein. The Syntex and Lilly workers describe splitting of the steroid C-19 methyl resonance due to long range proton-fluorine coupling.

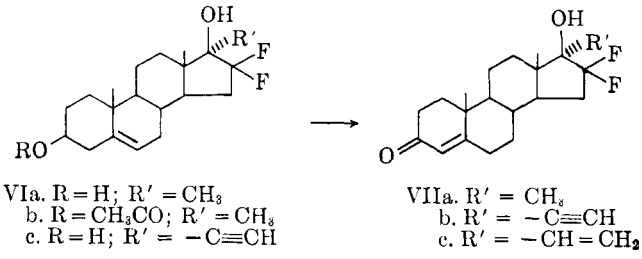


CHART II

Nucleophilic attack by the mesomeric carbanion of A on perchloryl fluoride[16] to give the fluoroformyl intermediate[17] (B) which can then suffer base-induced cleavage to give the monofluoro ketone (shown as the enolate anion C). Further attack by the fluoro ketone carbanion on perchloryl fluoride would then give the difluoro ketone D.[18]

Support for this postulated sequence is derived from the presence of 16-monofluoro-17-ketones in our reaction mixtures. Thus, we were able to isolate, in the estrone series, after chromatography and crystallization, an apparently homogeneous 16-monofluoroestrone 3-methyl ether (showing λ_{max}^{Nujol} 5.68 μ, and the correct analysis). However, this turned out to be a mixture of the 16α- and 16β-fluoro epimers as judged by infrared comparison[19] with spectra of the pure epimers and of equilibrium mixtures of both. No 16,16-difluoro 17-ketone was present in this material, as judged by paper or thin layer chromatography.

This mixture of 16α- and 16β-monofluoroestrone 3-methyl ether was now subjected to exactly the same reaction conditions used for the conversion of the 16-formyl compound (VIII) to the *gem* difluoro compound (IX). As expected, the 16,16-difluoro 17-ketone (IX) was formed, and was isolated, pure, in about 25% yield. This lends further support to the postulated reaction sequence.

The key intermediate II a was then converted, by known reactions, to a variety of hormone analogs (Chart I).

Oppenauer oxidation of IIa gave III, and lithium aluminum hydride reduction of the 3-pyrrolidylenamine[20] of III gave 16,16-difluorotestosterone (Va). The hydride reduction appeared to be stereospecific and no 17α-epimer (which was encountered in another connection, *vide infra*) was observed.

Conversion of IIa to the methyltestosterone analog VIIa (Chart II) was accomplished uneventfully by reaction with methylmagnesium iodide, followed by Oppenauer oxidation.

(16) The propensity of mesomeric carbanions for nucleophilic displacement on fluorine, compared with alkoxides, amines and oxime anions which displace on chlorine, in reactions with perchloryl fluoride, has been discussed recently by J. P. Freeman, *ibid.*, **82**, 3869 (1960), and by A. S. Kende and P. MacGregor, *ibid.*, **83**, 4197 (1961).

(17) *Cf.* J. Edwards and H. J. Ringold, *ibid.*, **81**, 5262 (1959). For other references to fluorination of steroidal β-dicarbonyl systems see ref. 1a.

(18) Analogous conversions of a 21-ethoxalyl-20-keto steroid to the 21,21-difluoro 21-ketone have been noted by Edwards and Ringold (ref. 17) and by S. Nakanishi, K. Morita and E. V. Jensen, *ibid.*, **81**, 5259 (1959). The conversion of a steroidal 21,21-difluoro 20-ketone to the 21,21,21-trifluoro compound by the action of perchloryl fluoride, in basic solution, has also been observed by the latter workers.

(19) We wish to express our best thanks to Dr. A. H. Goldkamp, G. D. Searle and Co., for kindly making this infrared comparison possible.

(20) See, for example, M. E. Herr, J. A Hogg, and R. H. Levin, *J. Am. Chem. Soc.*, **78**, 500 (1956).

TABLE I [a-c]

R_f VALUES FOR PAIRS OF EPIMERIC 17-OLS

Compound	R_f in systems I	II	III	IV
16,16-Difluoro-1,3,5(10)-estratriene-3,17β-diol 3-methyl ether	0.30	0.13		
16,16-Difluoro-1,3,5(10)-estratriene-3,17α-diol 3-methyl ether	.41	.26		
1,3,5(10)-Estratriene-3,17β-diol 3-methyl ether	.40	.16		
1,3,5(10)-Estratriene-3,17α-diol 3-methyl ether	.45	.23		
16,16-Difluoro-4-androsten-17β-ol-3-one			0.37	6.3 cm.
16,16-Difluoro-4-androsten-17α-ol-3-one			.46	12.6 cm.
4-Androsten-17β-ol-3-one			.26	6.8 cm
4-Androsten-17α-ol-3-one			.31	8.8 cm.

[a] Papergrams were all descending, and were run at 24–25°. System I = Heptane/methyl cellosolve; System II = Heptane/phenyl cellosolve; System III = Ligroin–toluene (7:3)/propylene glycol; System IV = Ligroin/propylene glycol. [b] Systems I, II, and IV were used as described by P. Kabasakalian and A. Basch, *Anal Chem.*, **32**, 458 (1960); System III differs from system IV solely in use of a 7:3 ligroin–toluene mixture for the mobile phase. [c] Papergrams in system IV were run off the paper, over 18 hours, and only migration distances from the origin could be measured.

Ethynylation of IIa was conveniently carried out using the technique described some years ago by Campbell, *et al.*,[21] *viz.*, sodium acetylide–dimethyl sulphoxide. We have found this to be a very simple and useful experimental procedure and worthy of wider notice.

The resulting ethynyl compound VIc then gave the ethynyltestosterone analogue VIIb on Oppenauer oxidation.

We then had occasion to subject the Δ^5-3β-ol (IIa) to the action of *Flavobacterium dehydrogenans*[22] as an alternative route to the Δ^4-3,17-dione (III). However, the product ($C_{19}H_{26}O_2F_2$), isolated in fairly good yield, lacked a 17-carbonyl, while the Δ^4-3-ketone and a new hydroxyl group were present. This product (IVa) was shown to be epimeric at C-17 with the previously isolated 16,16-difluorotestosterone (Va) by oxidation with chromium trioxide to the Δ^4-3,17-dione (111).

Our configurational assignments for IVa and Va rest on the following grounds:

First, the Δ[M]D (Va–IVa) value, +94°, is in excellent agreement with the Δ[M]D (17β–17α) values[23] shown by known pairs of steroidal epimeric 17-alcohols. (Average value for three pairs of 3-oxygenated-Δ^4-androsten-17-ols = +99°.)

Secondly, the acetylation behavior of IVa and Va is consistent with the assigned configurations. The quasiequatorial 17β-ol (Va) is acetylated under conditions (pyridine–acetic anhydride, room temperature overnight) from which the quasiaxial 17α-ol was recovered largely unchanged. (Acetylation of IVa in good yield required heating on the steam bath.)

Thirdly, the 17α-epimer (IVa) was found to migrate faster than the 17β-epimer (Va) in two different paper chromatographic systems (see Table I for R_f values of this pair and of control pairs of 17-epimers), in accord with the generalization[24] that steroids with an axial hydroxyl group migrate faster than the corresponding equatorial isomers.

Finally one would expect the hydride reduction to proceed normally to the 17β-ol, since we have symmetrical substitution at C-16.

This peculiar reduction, by an organism *which converted 5-androsten-3β-ol-17-one to 4-androstene-3,17-dione*, is noteworthy as an example of the drastically modified activity of ketone with electronegative alpha substituents *vis à vis* an enzyme system. The similar reduction of 16,16-difluoroestrone 3-methyl ether (IX) to the 17α-ol (*vide infra*) by *Flavobacterium dehydrogenans* is also of obvious interest.

The preference of the 16,16-difluoro-17-keto system for the reduced state under these conditions is consistent with, for example, the behavior of 9α-fluorocortisone *vs.* cortisone, in man. Thus it has been argued[25] that the lack of urinary 11-ketometabolites in the case of 9α-fluorocortisone, compared with the presence of analogous metabolites in the case of cortisone, suggests that the oxidation–reduction equilibrium at C-11, *in vivo*, favors the 11β-hydroxy form in the case of the 9α-fluoro compound.

It is, however, interesting that, in the case at hand, reduction by the enzyme has apparently taken place from the β-face, rather than the α-side attack which is certainly favored in chemical reactions with this D-ring system.[26]

The reactions described for the androstane series were paralleled in the estrogen series (Chart III).

Treatment of the difluoro 3-methyl ether (IX) with hydriodic acid gave 16,16-difluoroestrone (X), which could be converted back to IX by methylation at C-3.

The 17α-methyl, ethynyl and vinyl compounds (XIIIa, b, and c, respectively) were obtained from the 17-ketone (IX) without any difficulty.

Hydride reduction of IX gave the 17β-ol (XIa) while *Flavobacterium dehydrogenans* converted IX to the 17α-ol (XII). These assignments were supported by the Δ[M]D (17β–17α) value (+74°) and by paper chromatographic evidence.

The biological activities of most of the 16,16-difluoroestrogens have already been delineated.[1] The hormone analogues Va and VIIa showed considerably diminished androgenic and anabolic activity compared with the nonfluorinated compounds, and the ethynyl testosterone analogue VIIb showed less progestational activity than 17α-ethynyl testosterone.

(21) J. A. Campbell, J. C. Babcock, and J. A. Hogg, *J. Am. Chem. Soc*, **80**, 4717 (1958).

(22) A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore, and W. Charney, *ibid.*, **79**, 4814 (1957).

(23) See C. H. Robinson, O. Gnoj, and E. P. Oliveto *J. Org. Chem.*, **25**, 2247 (1960), for a compilation of such values.

(24) *Cf.* K. Savard, *J. Biol. Chem.*, **202**, 457 (1953).

(25) I. E. Bush and V. B. Mahesh, *Biochem. J.*, **69**, 9P (1958).

(26) We have also observed very fast chemical reduction of the 16,16-difluoro-17-keto system, under conditions which leave the unsubstituted 17-keto group essentially unchanged (C. H. Robinson, L. E. Finckenor, and R. Tiberi, to be published). This reduction, like the hydride reductions described in this paper, also leads primarily to the 17 β-alcohol.

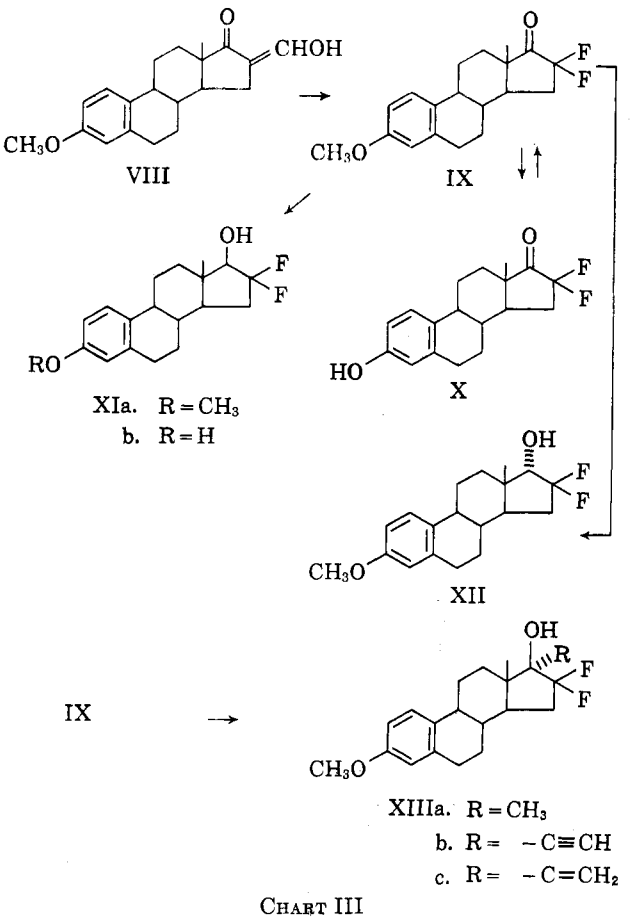


CHART III

Experimental[27]

16-Formyl-5-androsten-3β-ol-17-one (I).—To a stirred solution of 5-androsten-3β-ol-17-one (25 g.) in anhydrous tetrahydrofuran (500 ml.) was added sodium methoxide (25 g.), followed by ethyl formate (200 ml., added over 10 min.). A nitrogen atmosphere was maintained during these operations. The stirred reaction mixture was left at room temperature, under nitrogen, for 15 hr. and was then diluted with water and acidified with concentrated hydrochloric acid. The resulting precipitate was filtered, washed well with water, and dried *in vacuo*, to give the 16-formyl compound (I, 22 g.), λ_{max}^{MeOH} 266 mμ (9,100); λ_{max}^{Nujol} 3.10, 3.25, 5.84, 6.12 μ. [L. Ruzicka, *et al.*, ref. 9, reported λ_{max} 265 mμ (9,540).]

16,16-Difluoro-5-androsten-3β-ol-17-one.—A stirred suspension of 16-formyl-5-androsten-3β-ol-17-one (10 g.) in *t*-butyl alcohol (1.2 l.) was prepared in a reaction vessel fitted with stirrer, two gas inlets and gas outlet. A gentle stream of nitrogen was passed through the liquid, and this nitrogen stream was maintained during the subsequent operations. A solution of potassium *t*-butoxide in *t*-butyl alcohol (1 *M;* 200 ml.) was added to the stirred suspension, and the mixture was stirred until substantially all of the suspended solid had dissolved. A cylinder of perchloryl fluoride gas was then connected, *via* an empty trap and a *t*-butyl alcohol bubbler, to one of the gas inlets of the reaction vessel, and a brisk stream of perchloryl fluoride gas was bubbled through the stirred solution for 2 hr. The reaction vessel was cooled by tap water to maintain the internal temperature at about 30–40°.

At the end of 2 hr., the perchloryl fluoride flow was stopped, and nitrogen was bubbled *vigorously* through the solution for 1 hr. The mixture was then poured cautiously into about 3 l. of water, and the aqueous mixture was extracted with ether. The ethereal extracts were washed with water, dried (Na_2SO_4), and evaporated to give a residue which was chromatographed on Florisil (300 g.).

(27) Melting points were obtained on the Kofler block. Rotations were measured at 25° in dioxan solution at about 1% concentration. We are indebted to the Physical Chemistry Department, Schering Corporation, for measurement of ultraviolet and infrared spectra and rotations. Microanalyses were carried out by Mr. Conner (Microanalytical Laboratory, Schering Corporation), by the Schwarzkopf Microanalytical Laboratory, Woodside, Long Island, N. Y., and by Galbraith Laboratories, Knoxville, Tenn.

Elution with hexane containing up to 25% ether gave a series of fractions from which several crystalline, fluorine containing, substances could be isolated. These were not studied further.

Further elution, with hexane–ether (7:3), afforded a solid, m.p. 185–190° (from acetone–hexane), $[\alpha]_D$ +30°; λ_{max}^{Nujol} 5.63 μ.

The fluorine analyses (Found: F, 9.68, 9.88.) indicated two fluorine atoms per molecule, the molecular weight (Found: mol. wt., 317.) corresponded to a monomeric compound, and methoxyl determinations gave variable results (Found: OCH_3, 5.58, 6.91, 5.0.). Consistent C and H values could not be obtained, and the compound was not studied further.

Elution with hexane–ether (1:1) provided solids (3.0 g.) which migrated as one spot (R_f 0.19) in the ligroin–propylene glycol system. Crystallization from acetone–hexane gave pure 16,16-difluoro-5-androsten-3β-ol-17-one (IIa), m.p. 151–154°, $[\alpha]_D$ +30°. R.D. in methanol (*c*, 0.07), $[\alpha]_{700}$ +11.4°, $[\alpha]_{589}$ +34.2°, $[\alpha]_{350}$ +1719°, $[\alpha]_{298}$ −2075°, $[\alpha]_{265}$ −1477°; λ_{max}^{Nujol} 2.88, 3.05, 5.63 μ.

Anal. Calcd. for $C_{19}H_{26}O_2F_2$: C, 70.34; H, 8.07; F, 11.71. Found: C, 70.08; H, 7.95; F, 11.35.

The derived 3β-acetate (IIb) was obtained by acetylation in pyridine–acetic anhydride at room temperature for 18 hr., and showed m.p. 151–153° (from acetone), $[\alpha]_D$ +18°; λ_{max}^{Nujol} 5.64, 5.75, 5.78, 8.02, 8.08 μ.

Anal. Calcd. for $C_{21}H_{28}O_3F_2$: C, 68.83; H, 7.70; F, 10.37. Found: C, 68.88; H, 7.90; F, 10.54.

Further elution gave solids which, after repeated crystallization from acetone–hexane gave a small quantity of 16ζ-fluoro-5-androsten-3β-ol-17-one, m.p. 167–171°; λ_{max}^{Nujol} 3.04, 5.68 μ.

Anal. Calcd. for $C_{19}H_{27}O_2F$: F, 6.20. Found: F, 6.36.

Lack of material precluded rigorous purification and characterization of this substance.

16,16-Difluoro-4-androstene-3,17-dione (III).—A solution of 16,16-difluoro-5-androstene-3β-ol-17-one (IIa, 400 mg.) in toluene (11 ml.) and cyclohexanone (4 ml.) was distilled until 3 ml. of distillate had been collected, and a solution of aluminum isopropoxide (400 mg.) in toluene (4 ml.) was added. The mixture was then refluxed for 1 hr., 3 ml. of distillate being removed during this period. The reaction mixture was then steam distilled, and the resulting aqueous suspension in the distillation flask was extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated to dryness, and the residue was crystallized twice from acetone–hexane to give III as needles (110 mg.), m.p. 174–177°, $[\alpha]_D$ +185°, λ_{max}^{MeOH} 239 mμ (16,000); λ_{max}^{Nujol} 5.63, 6.00, 6.20 μ.

Anal. Calcd. for $C_{19}H_{24}O_2F_2$: C, 70.78; H, 7.50; F, 11.79. Found: C, 70.49; H, 7.37; F, 12.08.

16,16-Difluoro-4-androsten-17β-ol-3-one (Va) from 16,16-Difluoro 4-androstene-3,17-dione (III) *via* the 3-Pyrrolidyl Enamine.—To a boiling solution of 16,16-difluoro-4-androstene-3,17-dione (III, 430 mg.) in methanol (20 ml.) was added pyrrolidine (0.18 ml.) and the solution was heated for several minutes longer, when yellow needles were deposited. The mixture was chilled and filtered, and the solid was washed with a little ether and dried *in vacuo* to give 289 mg. of the 3-pyrrolidylenamine of III, m.p. 210–213°, λ_{max}^{MeOH} 275 mμ (19,400); λ_{max}^{Nujol} 5.65, 6.12, 6.24 μ.

Anal. Calcd. for $C_{23}H_{31}ONF_2$: C, 73.57; H, 8.32; N, 3.73. Found: C, 73.99; H, 8.17; N, 4.11.

A solution of the above enamine (345 mg.) in tetrahydrofuran (10 ml.) was added to a solution of lithium aluminum hydride (200 mg.) in ether (35 ml.) and the mixture was refluxed for 5 min. The reaction mixture was cooled and water (1.0 ml.) was added, cautiously. A solution of sodium acetate (1.38 g.) in water (1.75 ml.) glacial acetic acid (0.88 ml.) and methanol (17.25 ml.) was then added, and the mixture was refluxed for 5 hr. after the initial removal of 35 ml. of distillate. The reaction mixture was then evaporated to dryness *in vacuo*, and 2 *N* aqueous hydrochloric acid (20 ml.) was added. The resulting suspension was filtered, and the residue on the filter was washed with water, dried and was crystallized twice from acetone–hexane to give Va (190 mg.), m.p. 157–159°, $[\alpha]_D$ +93°, λ_{max}^{MeOH} 240 mμ (15,700); λ_{max}^{Nujol} 2.94, 5.75, 6.02, 6.18 μ.

Anal. Calcd. for $C_{19}H_{26}O_2F_2$: C, 70.34; H, 8.07; F, 11.71. Found: C, 70.32; H, 7.93; F, 11.78.

Regeneration of 16,16-Difluoro-4-androstene-3,17-dione (III) from Va by Chromium Trioxide Oxidation.—To a solution of 16,17-difluorotestosterone (Va, 50 mg.) in glacial acetic acid (2.5 ml.) was added chromium trioxide (22 mg.) in glacial acetic acid (1.0 ml.) and water (0.1 ml.), and the mixture was left at room temperature for 24 hr. The usual work-up and crystallization

from acetone–hexane gave the 3,17-dione (III, 19 mg.), identical with authentic material (melting point, mixture melting point, and infrared comparison).

16,16-Difluoro-4-androsten-17β-ol-3-one 17-Acetate (Vb).—Acetylation of 16,16-difluorotestosterone (Va, 175 mg.) with pyridine–acetic anhydride at room temperature for 18 hr. gave the 17-acetate (Vb, 120 mg.), needles from acetone–hexane, m.p. 218–221°, $[\alpha]_D$ +64°, λ_{max}^{MeOH} 240 mμ (17,000).

Anal. Calcd. for $C_{21}H_{28}O_3F_2$: C, 68.83; H, 7.70. Found: C, 68.79; H, 7.73.

16,16-Difluoro-4-androsten-17α-ol-3-one (IVa).—*Flavobacterium dehydrogenans* was grown in a 1% Difco yeast buffered solution (10 g. of Difco yeast, 4.68 g. of $Na_2HPO_4 \cdot 7H_2O$ and 4.48 g. of KH_2PO_4/l.) with shaking and light for 42 hr. A solution of 16,16-difluoro-5-androsten-3β-ol-17-one (IIa, 1.0 g.) in 95% ethanol (40 ml.) was added, and the transformation was allowed to proceed for 74 hr. with shaking and light. Extraction with chloroform and evaporation of the extract gave an oil which was chromatographed on Florisil. Elution with ether–hexane (1:1) gave solids which were combined and crystallized twice from acetone–hexane to give 16,16-difluoro-17-epitestosterone (IVa, 465 mg.), m.p. 190–193°, $[\alpha]_D$ +64°, λ_{max}^{MeOH} 239 mμ (15,800); λ_{max}^{Nujol} 3.05, 6.08, 6.22 μ.

Anal. Calcd. for $C_{19}H_{26}O_2F_2$: C, 70.34; H, 8.07; F, 11.71. Found: C, 70.36; H, 8.11; F, 11.53.

Regeneration of 16,16-Difluoro-4-androstene-3,17-Dione (III) from the 17α-ol (IVa).—A solution of the 17α-ol (IVa, 100 mg.) in glacial acetic acid (5.0 ml.) was treated with chromium trioxide (44 mg.) dissolved in glacial acetic acid (2.0 ml.) and water (0.2 ml.), and the solution was left at room temperature for 6.5 hr. The usual work-up and crystallization from acetone–hexane gave the 3,17-dione (III, 48 mg.) identical with authentic material (melting point, mixture melting point, and infrared comparison).

16,16-Difluoro-4-androsten-17α-ol-3-one 17-Acetate (IVb).—A solution of 16,16-difluoro-17-epitestosterone (IVa, 150 mg.) in pyridine (2.5 ml.) and acetic anhydride (2.0 ml.) was kept at steam bath temperature for 2 hr., and the acetylated product was crystallized from aqueous methanol, to give the methanol, solvate of IVb (88 mg.), m.p. *ca.* 80° (dried for 18 hr. at room temperature *in vacuo*).

Anal. Calcd. for $C_{21}H_{28}O_3F_2 \cdot CH_3OH$: C, 66.31; H, 8.09; Found: C, 66.78, 66.80; H, 7.99, 8.00.

The solvate was dried *in vacuo*, at 60°, for 48 hr., giving unsolvated IVb, m.p. 114–116° (melted at 98–100° and resolidified), $[\alpha]$ +83°, λ_{max}^{MeOH} 240 mμ (17,000); λ_{max}^{Nujol} 5.73, 6.0, 6.2, 8.14 μ.

Anal. Calcd. for $C_{21}H_{28}O_3F_2$: C, 68.83; H, 7.70; F, 10.37. Found: C, 68.79; H, 7.65; F, 10.32.

16,16-Difluoro-17α-methyl-5-androstene-3β,17β-diol (VIa) and the Derived 3β-Monoacetate (VIb).—To a stirred solution of methylmagnesium iodide [prepared from magnesium (6.0 g.) and methyl iodide (15 ml.)] in ether (400 ml.) was added, dropwise, a solution of 16,16-difluoro-5-androsten-3β-ol-17-one (2.0 g.) in tetrahydrofuran (100 ml.). The mixture was now diluted with more tetrahydrofuran (300 ml.), and was distilled until about 400 ml. of distillate had been collected. The reaction mixture was then refluxed for 1 hr., cooled and treated with cold 10% aqueous ammonium sulfate solution. The resulting suspension was filtered, and the solid on the filter was washed with water and dried *in vacuo* at room temperature. Crystallization of this crude product (1.7 g.) from acetone–hexane gave material (1.0 g.) of m.p. 80–83°. Prolonged drying *in vacuo* raised the m.p. to 137–142°, but satisfactory analyses were not obtained. Chromatography on Florisil and paper chromatographic examination of the fractions indicated that the material was homogeneous. Since the product could not be crystallized satisfactorily from a number of other solvents, a portion (150 mg.) of the once crystallized material (m.p. 137–142°) was acetylated (pyridine–acetic anhydride at room temperature for 18 hr.). The acetylated product crystallized readily from acetone–hexane to give pure 16,16-difluoro-17α-methyl-5-androstene-3β,17β-diol 3-monoacetate (VIb, 80 mg.), m.p. 161–164°, $[\alpha]_D$ −67°; λ_{max}^{Nujol} 2.80, 5.75, 8.1 μ.

Anal. Calcd. for $C_{22}H_{32}O_3F_2$: C, 69.08; H, 8.43; F, 9.44. Found: C, 69.34; H, 8.41; F, 10.10.

16,16-Difluoro-17α-methyl-4-androsten-17β-ol-3-one (VIIa).—A solution of 1.0 g. of 16,16-difluoro-17α-methyl-5-androstene-3β,17β-diol (VIa, m.p. 137–142°) in cyclohexanone (12 ml.) and toluene (36 ml.) was distilled until 12 ml. of distillate had been collected. To the boiling solution was now added aluminum isopropoxide (1.0 g.) n toluene (12 ml.), and the mixture was boiled for 1 hr., 12 ml. of distillate being removed during this period. The reaction mixture was then steam distilled, and the aqueous suspension remaining in the distilling flask was cooled, and extracted with ethyl acetate. The organic extract was washed with water, dried (Na_2SO_4), and evaporated *in vacuo* to give an oil. The oil was chromatographed on Florisil and the hexane–ether (2:3) eluates gave solids (470 mg.). These solids were combined and crystallized from acetone–hexane to give 16,16-difluoro-17α-methyltestosterone (VIIa, 200 mg), m.p. 157–160°, $[\alpha]_D$ +70°, λ_{max}^{MeOH} 239 mμ (15,500); λ_{max}^{Nujol} 2.88, 6.05, 6.24 μ.

Anal. Calcd. for $C_{20}H_{28}O_2F_2$: C, 70.98; H, 8.34; F, 11.23. Found: C, 70.62; H, 8.26; F, 11.42.

16,16-Difluoro-17α-ethynyl-5-androstene-3β,17β-diol (VIc).—To a stirred solution of 16,16-difluoro-5-androsten-3β-ol-17-one (IIa, 200 mg.) in dimethyl sulfoxide (3 ml.), at room temperature, was added a solution of sodium acetylide in dimethyl sulfoxide, prepared by centrifuging 1.5 ml. of a 17% suspension of sodium acetylide in xylene (Air Reduction Chemical Company), decanting the supernatant liquid, and dissolving the residue of sodium acetylide in dimethyl sulfoxide (4 ml.). The mixture was stirred at room temperature for 40 min., and was then cautiously diluted with iced water. The resulting aqueous suspension was filtered, and the solid on the filter was washed with water and dried, to give the crude product (194 mg.), m.p. 175–183° (after melting at *ca.* 40° and resolidifying). Crystallization from acetone–hexane gave the analytical sample, m.p. 184–186°; $[\alpha]_D$ −94°; λ_{max}^{Nujol} 3.05, 4.72 μ.

Anal. Calcd. for $C_{21}H_{28}O_2F_2$: C, 71.97; H, 8.05; F, 10.84. Found: C, 71.87; H, 8.32; F, 11.04.

16,16-Difluoro-17α-ethynyl-4-androsten-17β-ol-3-one (VIIb).—A solution of 16,16-difluoro-17α-ethynyl-5-androstene-3β,17β-diol (400 mg.) in toluene (15 ml.) and cyclohexanone (5 ml.) was distilled until 4 ml. of distillate had been collected. A solution of aluminum isopropoxide (400 mg.) in toluene (4 ml.) was then added, and the mixture was boiled for 1 hr., during which time 5 ml. of distillate was removed. The reaction mixture was then steam-distilled, and the aqueous suspension remaining in the distillation flask was cooled and extracted with ethyl acetate. The organic extract was washed with water, dried (Na_2SO_4), and evaporated *in vacuo* to give an oil, which was chromatographed on Florisil. Elution with hexane–ether (3:2) gave solids which were combined and crystallized from methylene chloride–hexane, yielding (VIIb, 76 mg.), m.p. 209–211°, $[\alpha]_D$ +40°, λ_{max}^{MeOH} 240 mμ (16,300); λ_{max}^{Nujol} 3.0, 3.10, 4.75, 6.05, 6.20 μ.

Anal. Calcd. for $C_{21}H_{26}O_2F_2$: C, 72.39; H, 7.52; F, 10.91. Found: C, 72.07; H, 7.37; F, 11.51.

16,16-Difluoro-17α-vinylandrost-4-en-17β-ol-3-one (VIIc).—A solution of 16,16-difluoro-17α-ethynyltestosterone (69.6 mg.) in pyridine (5 ml.) was added to pre-reduced 15% palladium–strontium carbonate catalyst (30 mg.) in pyridine (5 ml.) and hydrogenated at 25°. After 10 min., 4.48 ml. (0.98 mole) of hydrogen had been taken up, and the hydrogenation was stopped. The usual work-up and crystallization from acetone–hexane gave the vinyl compound (VIIc, 30 mg.), m.p. 132–134°, $[\alpha]_D$ +71°, λ_{max}^{MeOH} 240 mμ (15,500); λ_{max}^{Nujol} 3.0, 6.05 μ.

Anal. Calcd. for $C_{21}H_{28}O_2F_2$: C, 71.97; H, 8.05; F, 10.84. Found: C, 72.38; H, 8.50; F, 10.92.

16-Formyl-1,3,5(10)-estratrien-3-ol-17-one 3-Methyl Ether (VIII).—To a stirred solution of estrone 3-methyl ether (16 g.) in anhydrous tetrahydrofuran (450 ml.) was added sodium methoxide (16 g.), followed by ethyl formate (150 ml.; added over 10 min.). An atmosphere of nitrogen was maintained during these operations. The reaction mixture was stirred at room temperature for 18 hr., under nitrogen, and was then diluted with water and acidified with concentrated hydrochloric acid. The resulting suspension was filtered and the solid on the filter was washed with water and dried *in vacuo* to give the 16-formyl compound (VIII, 14 g.), m.p. 164–167°,[28] λ_{max}^{MeOH} 268 mμ (9,600); λ_{max}^{Nujol} 3.1, 3.24, 5.85, 6.15, 6.65 μ.

16,16-Difluoro-1,3,5(10)-estratriene-3-ol-17-one 3-Methyl (IX).[29]—A brisk stream of perchloryl fluoride gas was bubbled through a well stirred solution of 16-formyl estrone 3-methyl ether (VIII, 4.2 g.) in *t*-butyl alcohol (450 ml.) and a 1 *M* solution

(28) Bardhan, ref. 8, reported m.p. 170–171°. No spectroscopic data were given.

(29) This experiment was run in exactly the same manner as the preparation of 16,16-difluoro-5-androsten-3β-ol-17-one (IIa) from the 16-formyl compound (I). Since the latter preparation is described in detail above it should be consulted for details such as apparatus, etc.

of potassium *t*-butoxide in *t*-butyl alcohol (85 ml.) at 30–40° for 90 min. Nitrogen was then passed through the reaction mixture for 1 hr. to remove most of the excess perchloryl fluoride, and the reaction mixture was poured into water (1 l.). The aqueous mixture was extracted with ether, and the ethereal extract was washed with water, dried (Na_2SO_4), and evaporated *in vacuo* to a residue which was chromatographed on Florisil (130 g.). Elution with hexane–ether (7:3) mixtures gave solids which were combined and crystallized from acetone–hexane to give 16,16-difluoroestrone 3-methyl ether (IX, 1.34 g.), m.p. 126–128°, $[\alpha]_D$ +167°; λ_{max}^{Nujol} 5.63, 6.22, 6.35, 6.68, 7.98 μ.

Anal. Calcd. for $C_{19}H_{22}O_2F_2$: C, 71.23; H, 6.92; F, 11.86. Found: C, 71.61; H, 7.07; F, 11.60.

Further elution afforded solids, which were crystallized from acetone–hexane to give 16 (α + β)-fluoroestrone 3-methyl ether,[30] m.p. 137–141°, $[\alpha]_D$ +159°; λ_{max}^{Nujol} 5.68 μ.

Anal. Calcd. for $C_{19}H_{23}O_2F$: C, 75.47; H, 7.67; F, 6.28. Found: C, 75.18; H, 7.55; F, 6.01.

Estrone 3-Methyl Ether from 16,16-Difluoroestrone 3-Methyl Ether, by Zinc–Acetic Acid Reduction.—To a refluxing solution of 16,16-difluoroestrone 3-methyl ether (IX, 100 mg.) in 90% aqueous acetic acid (10 ml.) was added zinc dust (1 g.), and reflux was continued for 2 hr. More zinc dust (1 g.) was added, and reflux was continued for 1 hr. more. The mixture was filtered, while hot, into water, and the aqueous mixture which resulted was filtered to give crude estrone 3-methyl ether. Crystallization gave 42 mg. of pure estrone 3-methyl ether, identical in all respects with an authentic specimen.

16,16-Difluoro-1,3,5(10)-estratriene-3,17β-diol 3-Methyl Ether (XIa).—To a refluxing solution of 16,16-difluoroestrone 3-methyl ether (IX, 1.50 g.) in isopropyl alcohol (124 ml.) was added sodium borohydride (2.6 g.), and reflux was continued for 1 hr. Dilution with water gave a suspension which was filtered, and the residue on the filter was washed with water and dried. Attempted crystallization from a variety of solvents and solvent-mixtures gave gels. However, after being left for 3 days in an acetone–ether–hexane mixture, crystallization ensued. The crystalline material was removed by filtration, and was dried at 80° *in vacuo* for 24 hr., giving the 17β-ol (XIa, 750 mg.), m.p. 124–127°, $[\alpha]_D$ +71°; λ_{max}^{Nujol} 2.92, 6.20, 6.36, 6.68, 7.98 μ.

Anal. Calcd. for $C_{19}H_{24}O_2F_2$: C, 70.78; H, 7.50; F, 11.79. Found: C, 70.63; H, 7.55; F, 11.88.

16,16-Difluoro-1,3,5(10)-estratrien-3-ol-17-one (X) from the 3-Methyl Ether (IX).—To a solution of 16,16-difluoroestrone 3-methyl ether (IX, 5 g.) in boiling glacial acetic acid (25 ml.) was added 47% aqueous hydriodic acid (18 ml.). The solution was boiled for 12 min., and then was diluted (after cooling) with 750 ml. of 5% aqueous sodium bisulfite. The resulting suspension was filtered and the residue on the filter was washed with water and was then extracted with boiling 10% aqueous potassium hydroxide solution. The resulting suspension was filtered hot, and the filtrate was cooled, acidified with *N* hydrochloric acid, and extracted with ether. The ethereal extract was washed with water, dried (Na_2SO_4) and evaporated *in vacuo* to give an oily residue which was chromatographed on Florisil (150 g). Elution with hexane–ether (3:1) gave solids which were crystallized from acetone–hexane to give 16,16-difluoroestrone (X, 2.55 g.), m.p. 173–175°, $[\alpha]_D$ +161°; λ_{max}^{Nujol} 2.9, 5.62, 6.17, 6.63 μ.

Anal. Calcd. for $C_{18}H_{20}O_2F_2$: C, 70.57; H, 6.58; F, 12.40. Found: C, 70.44; H, 6.80; F, 12.04.

Methylation of 16,16-Difluoroestrone to Give 16,16-Difluoroestrone 3-Methyl Ether.—To a stirred solution of 16,16-difluoroestrone (200 mg.) in methanol (10 ml.) was added a solution of potassium hydroxide (1.2 g.) in water (2 ml.). Dimethyl sulfate was aded in 1-ml. portions every 0.5 hr., for 2 hr. (total volume of 4 ml.). The mixture was stirred for 1 hr. after the last addition of dimethyl sulfate, and then was diluted with water and filtered. The residue on the filter was washed with water, dried and crystallized from acetone–hexane to give 16,16-difluoroestrone 3-methyl ether (100 mg.), identical in all respects with authentic material.

16,16-Difluoro-1,3,5(10)-estratriene-3,17β-diol (XIb).—To a solution of 16,16-difluoroestrone (440 mg.) in isopropyl alcohol (40 ml.) was added sodium borohydride (300 mg.), and the mixture was refluxed for 1.5 hr. The reaction mixture was diluted with water, and filtered, and the dried residue was crystallized twice from aqueous methanol to give 16,16-difluoroestradiol (XIb, 240 mg.), m.p. 195–198, $[\alpha]_D$ +69°.

Anal. Calcd. for $C_{18}H_{22}O_2F_2 \cdot 0.5\ H_2O$: C, 68.12; H, 7.31; F, 11.97. Found: C, 67.86; H, 7.75; F, 12.06.

16,16-Difluoro-1,3,5(10)-estratriene-3,17α-diol-3 Methyl Ether (XII).—A solution of 16,16-difluoroestrone 3-methyl ether (1.0 g.) in 80% ethanol (40 ml.) was subjected to the action of *flavobacterium dehydrogenans* for 55 hr., as described above for 16,16-difluorotestosterone. The product was isolated by extraction with chloroform, and was chromatographed on Florisil (30 g.). Elution with hexane–ether (9:1) gave, after crystallization from ethyl acetate–hexane, 350 mg. of slightly impure 16,16-difluoro-17-isoestradiol 3-methyl ether (350 mg.), m.p. 99–101°. This material was contaminated by 16,16-difluoroestrone 3-methyl ether as evidenced by infrared and papergram data. Rechromatography on Florisil and elution with hexane–ether (9:1) gave pure XII, m.p. 111–113°, $[\alpha]_D$ +48°.

Anal. Calcd. for $C_{19}H_{24}O_2F_2$: C, 70.78; H, 7.50; F, 11.79. Found: C, 70.85; H, 7.42; F, 11.56.

16,16-Difluoro-17α-methyl-1,3,5(10)-estratriene-3,17β-diol 3-Methyl Ether (XIIIa).—To a stirred suspension of methyl magnesium iodide [prepared from magnesium (15 g.) and methyl iodide (40 mg.)] in ether (1 l.) was added, dropwise, 16,16-difluoroestrone 3-methyl ether (5.0 g.) in tetrahydrofuran (250 ml.). The reaction mixture was then diluted by the addition of 750 ml. of tetrahydrofuran, and the mixture was distilled until about 1 l. of distillate had been collected. The resulting suspension was refluxed for 1 hr. further, and was then cooled and diluted with 10% aqueous ammonium sulfate solution (200 ml.), followed by water (800 ml.). Extraction of the aqueous mixture with ethyl acetate, and evaporation of the dried (Na_2SO_4) extract, gave an oily residue. This residue was dissolved in ether, and the ethereal solution was passed through a short column of Florisil. The eluate was concentrated to small volume, when crystallization ensued, giving 1.6 g. of material m.p. 141–143°. Recrystallization from acetone–hexane gave the analytical sample, m.p. 143–145°, $[\alpha]_D$ +38°; λ_{max}^{Nujol} 2.81, 6.17, 6.30, 6.63 μ.

Anal. Calcd. for $C_{20}H_{26}O_2F_2$: C, 71.40; H, 7.79; F, 11.30 Found: C, 71.50; H, 7.94; F, 11.28.

16,16-Difluoro-17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol 3-Methyl Ether (XIIIb).—To a stirred solution of 16,16-difluoroestrone 3-methyl ether (500 mg.) in dimethyl sulfoxide (7 ml.) at room temperature was added a solution of sodium acetylide (obtained by centrifuging 4 ml. of a 17% suspension of sodium acetylide in xylene, *vide supra*) in dimethyl sulfoxide (5 ml.). The mixture was stirred at room temperature for 45 min. and was then cautiously diluted with iced water and extracted with methylene chloride. The methylene chloride extract was washed with water, and was then evaporated to dryness. The residue was crystallized twice from acetone–hexane, to give the 17-ethynyl compound (XIIIb, 160 mg.), m.p. 140–142°, $[\alpha]_D$ +20°; λ_{max}^{Nujol} 2.96, 3.08, 4.76, 6.22, 6.34, 6.56, 8.10 μ.

Anal. Calcd. for $C_{21}H_{24}O_2F_2$: C, 72.81; H, 6.98; F, 10.97 Found: C, 72.80; H, 7.27; F, 10.88.

16,16-Difluoro-17α-vinyl-1,3,5(10)-estratriene-3,17β-diol 3-Methyl Ether. (XIIIc).—A solution of 16,16-difluoro-17α-ethynylestradiol 3-methyl ether (XIIIb, 2.08 g.) in pyridine (100 ml.) was added to pre-reduced 10% palladium–strontium carbonate catalyst (1.17 g.) in pyridine (100 ml.), and hydrogenated at 25°. The hydrogenation was stopped after 145 ml. of hydrogen had been taken up, and the catalyst was removed by filtration. The crude product, obtained by water precipitation of the filtrate, was crystallized twice from acetone–hexane to give the vinyl compound (XIIIc, 700 mg.), m.p. 132–137°, $[\alpha]_D$ +37°; λ_{max}^{Nujol} 2.86, 6.22, 6.34, 6.68, 11.24 μ.

Anal. Calcd. for $C_{21}H_{26}O_2F_2$: C, 72.39; H, 7.52; F, 10.91. Found: C, 72.47; H, 7.50; F, 11.19.

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(30) After this work was completed, a publication by Johns and Mueller (ref. 2) described both epimers of 16-fluoroestrone 3-methyl ether, *viz.*, 16β-fluoroestrone 3-methyl ether, m.p. 166–169° $[\alpha]_D^{CHCl_3}$ +157°; 16α-fluoroestrone 3-methyl ether, m.p. 155–164°, $[\alpha]_D^{CHCl_3}$ +177°. Infrared comparisons, carried out through the courtesy of Dr. A. H. Goldkamp, indicated that our material was a mixture of 16α- and 16β-isomers.