tration, washed with acetone and dried *in vacuo* at 50°; yield, 21.1 g. (58%), m.p., 208–211° dec.

Anal. Calcd. for $C_{23}H_{30}ClN_3O_3 \cdot 2HCl \cdot 0.25H_2O$: C, 54.23; H, 6.43; N, 8.25; Cl^-, 13.92; H_2O, 0.88. Found: C, 54.39; H, 6.42; N, 8.37; Cl^-, 13.91; H_2O, 0.99.

Mono- and Difluorination of Steroids at C-16 through Enamine Intermediates

ARTHUR H. GOLDKAMP

Chemical Research Division, G. D. Searle & Co., Chicago 80, Ill.

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Reaction of 17-oxosteroids with 4-pipecoline followed by treatment of the crude reaction product with perchloryl fluoride leads to 16,16-difluoro- and 16-monofluoro-17-oxosteroids. The reaction very likely proceeds through an enamine intermediate.

Recent reports[1,2] of the fluorination of steroids at C-16 by use of perchloryl fluoride have shown that this reagent can be used with suitable intermediates such as the 16-hydroxymethylene[1] and 16-ethoxalyl[2] derivatives of 17-oxosteroids. The present report concerns the application of the enamine procedure[3] to the same over-all transformation.

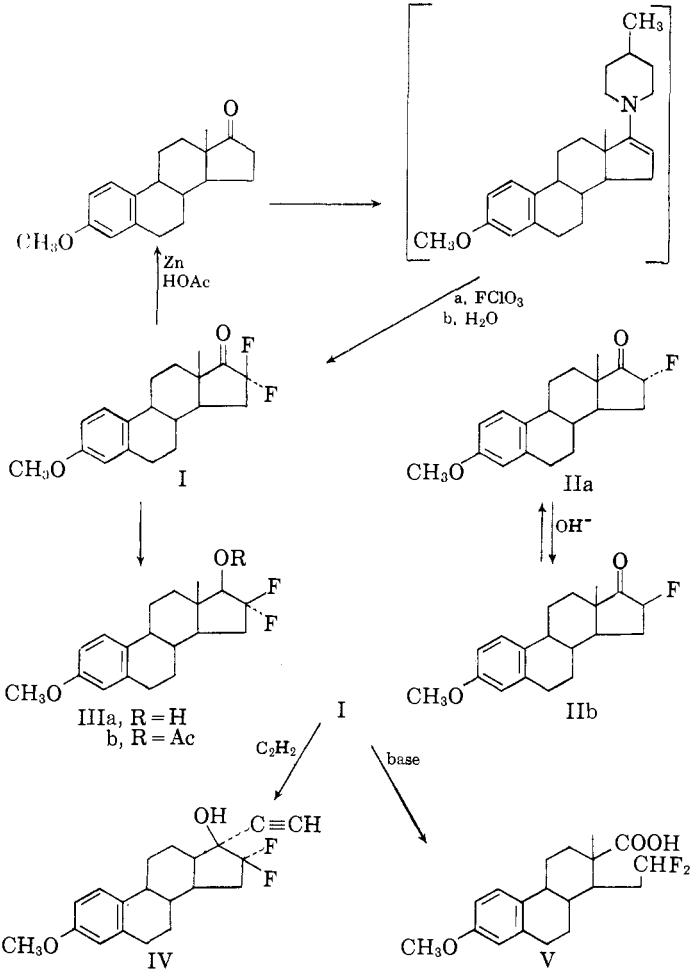
When applied to C-17, formation of enamines proved difficult and required far more vigorous conditions than at other positions.[4] Partial reaction at this position was accomplished by use of a high boiling amine, 4-pipecoline, with 3-methoxyestra-1,3,5(10)-trien-17-one or 3β-hydroxyandrost-5-en-17-one. Though enamine formation in this study proceeded at best to approximately 50% completion, it is hoped that improved procedures can be found. These observations are in essential agreement with those of Herr and Heyl,[5] who noted that enamines from 17-oxosteroids and pyrrolidine could not be prepared except for the unusual case of adrenosterone in which

(1) C. H. Robinson, N. F. Bruce, E. P. Oliveto, S. Tolkdsorf, M. Steinberg and P. L. Perlman, *J. Am. Chem. Soc.*, **82**, 5256 (1960).
(2) H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *J. Org. Chem.*, **26**, 973 (1961).
(3) R. B. Gabbard and E. V. Jensen, *ibid.*, **23**, 1406 (1958).
(4) F. W. Heyl and M. D. Herr, *J. Am. Chem. Soc.*, **75**, 1918 (1953).
(5) M. D. Herr and F. W. Heyl, *ibid.*, **75**, 5927 (1953).

the 11-oxo function influences the reactivity at C-17.

In the estratriene series, 16,16-difluoro-3-methoxyestra-1,3,5(10)-trien-17-one (I) was prepared as shown (I–V). It was identical with material prepared by the synthetic route reported by Robinson, *et al.*,[1] utilizing the 16-formyl derivative for the fluorination reaction with perchloryl fluoride. Removal of the fluorine atoms with zinc dust in glacial acetic acid established its relationship to the starting ketone.

In addition, a 16-monofluorinated product (IIa) was obtained which, on the basis of equilibration with its 16-epimer[6] (IIb) and its



(6) G. P. Mueller and W. F. Johns, *J. Org. Chem.*, **26**, 2403 (1961).

mode of formation (presumed α-side attack of $FClO_3$), has been assigned the 16α-configuration.

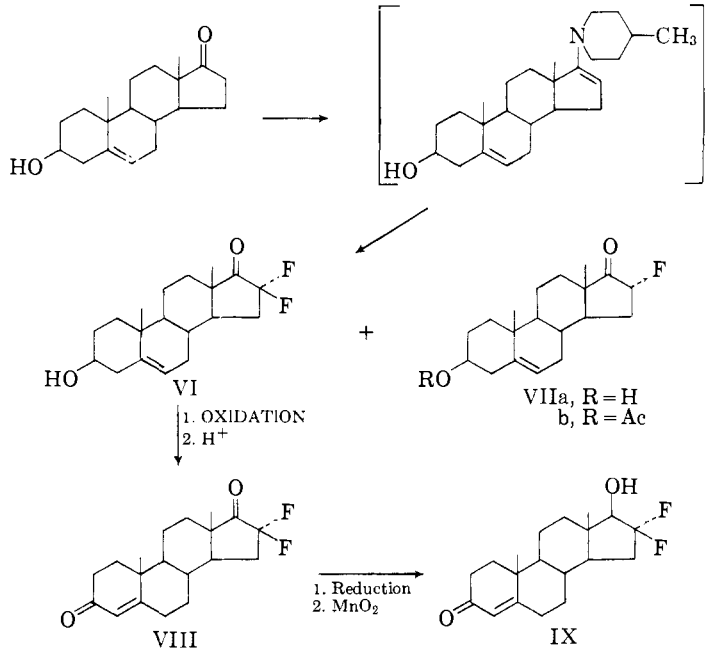
The equilibration experiments with (IIa) and (IIb) were carried out in a manner similar to that reported for base-catalyzed epimerization of 16α-bromo-17-oxosteroids.[7] Thus, the monofluoro epimers, on treatment with base, led to mixtures whose infrared spectra were essentially identical and showed good agreement with what would be expected for a mixture of pure epimers; the total crude mixture from the equilibration of the α-epimer contained approximately 43% of the β-epimer and 48% of the α-epimer based on calculations from the intensities of the bands at 9.94 and 10.42 microns, which are medium-strong bands due exclusively to the α and β epimers, respectively. These experiments establish the epimeric relationship of the two monofluoro ketones, though they do not provide any additional evidence for their configurations. Examination of a number of chloroform spectra of other 16-halo-17-ketones[6] along with the fluoro compounds did not suggest any definitive relationship which could be used to correlate configurations of the different halogen derivatives. Thus, the assignments rest primarily on the optical rotations and their relationship to the rotatory dispersion curves of α-fluoro ketones.[6,8]

Enamine formation with 3β-hydroxyandrost-5-en-17-one occurred less readily than in the estratriene series. Treatment of the crude product mixture with perchloryl fluoride gave the difluoro derivative VI and a monofluoro derivative VIIa; again, the α-configuration seems probable from its mode of formation. Attempts to utilize the latter for preparation of a 16-fluorotestosterone for comparison with the fluorotestosterone produced by the route of Kissman, *et al.*,[2] were unsuccessful.

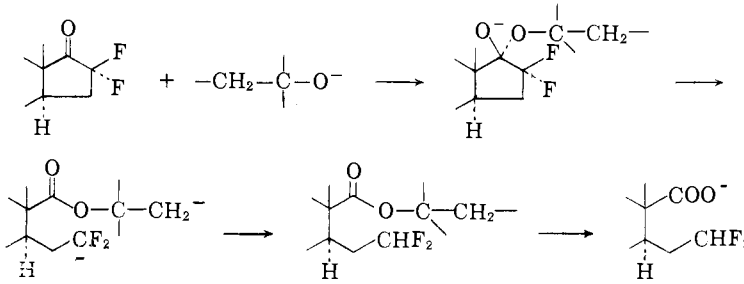
The difluoro compound (VI) was converted to the androstenedione (VIII) by oxidation with chromium trioxide and isomerization of the double bond.[9,10] Reduction and re-oxidation of the allylic hydroxyl group gave 16,16-difluoro-17β-hydroxyandrost-4-en-3-one (IX).

In an initial attempt to prepare the 17-ethynyl derivative of I using potassium acetylide in *tert*-amyl alcohol,[11] there was obtained a 69% yield of the seco acid V. This structure was suspected on the basis of its acidic properties, analysis, and infrared absorption which did not show any absorption for an acetylenic hydrogen, but did show

(7) J. Fajkős, *J. Chem. Soc.*, 3966 (1959).
(8) C. Djerassi, J. Osiecki, R. Riniker and B. Riniker, *J. Am. Chem. Soc.*, **80**, 1216 (1958).
(9) C. Djerassi, R. R. Engle and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).
(10) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946)
(11) H. Stavely, *J. Am. Chem. Soc.*, **61**, 79 (1939).



carbonyl absorption. Repetition of this experiment under slightly altered conditions without the use of acetylene gave the same seco acid. The reaction probably proceeds by opening of the ring as indicated. The observed isolation of the free acid can be attributed to base-catalyzed β-elimination from the tertiary ester, alkyl-oxygen cleavage, or hydrolysis in the work-up.



The ethynylation itself finally was achieved by the use of lithium acetylide in liquid ammonia to give the 17-ethynyl compound IV.

Infrared Spectra.—The shifts in chloroform for the 17-keto absorption in the 16,16-difluoro and 16-monofluoro steroids were quite noticeable due to the high electronegativity of fluorine.[12] The

(12) E. J. Little and M. M. Jones, *J. Chem. Ed.*, **37**, 231 (1960).

TABLE I

DERIVATIVE AND DISTINGUISHING BANDS, μ[a]

Parent Ketone	16α-Fluoro	16β-Fluoro	16,16-Difluoro
3-Methoxyestra-1,3,5-(10)-trien-17-one	10.6, 10.73	10.42, 10.7, 10.9, 11.9	10.13, 10.53
Estra-1,3,5(10)-trien-17-one	10.52, 10.63, 10.78, 10.95, 11.30, and 11.54 (crude)		10.13, 10.54, 10.74, 11.04, 11.34
3β-Hydroxyandrost-5-en-17-one	10.25, 10.75, 11.52		10.2, 10.63, 11.22, 11.53
3β-Acetoxyandrost-5-en-17-one	10.25, 10.75, 11.52		
Androst-4-ene-3,17-dione			10.12, 10.23, 10.62, 11.08
17β-Hydroxyandrost-4-en-3-one			11.12

[a] Bands noted are in the 10–12 μ portion of the fingerprint region where the most notable differences from the parent compounds occur. The spectra were all obtained in chloroform. Where no bands are listed, the corresponding derivatives were not available.

normal value of 5.75 μ for the unsubstituted ketone is shifted to 5.68 μ in the monofluoro[6] and to 5.62 μ for the difluoro compounds,[1] as previously reported. In addition, the 16-fluoro substitution in 17-oxosteroids results in more complex spectra in the 10–12 μ region.

The bands listed in Table I are those which are observed in chloroform solution which are either not present in, or are extremely weak absorptions in, the parent ketone. The bands noted between 11 and 11.5 μ in the androstene compounds and in the estratriene-17-one not bearing an A-ring substituent (see Experimental) seem, from these few examples, to be the most generally occurring changes observed as a result of α-fluorination. Changes in the shapes of bands in this region for the 3-methoxy case suggest that similar new absorptions have been added, though this is difficult to ascertain.

Both 16,16-difluoroestra-1,3,5(10)-trien-17-one and the parent ketone show a series of bands in the 11–12 μ region which are of lower intensity and at slightly different positions in the unsubstituted ketone. Only the two strongest of these absorptions are listed in Table I for the difluoro ketone; however, the similarity in the total number of bands in this region for the two compounds may indicate that the C-16 methylene and difluoromethylene groups contribute in a similar way to skeletal vibrations observed in this part of the spectrum.

Bio-assays.—Preliminary biological studies with the 16,16-difluoroestratrienes give a range from approximately one-quarter

to equal the activity of estrone in reducing the plasma cholesterol/phospholipid ratio in cholesterol-fed cockerels.[13] The least active was the 3-deoxy compound, 16,16-difluoroestra-1,3,5(10)-trien-17-one (see experimental section); the most active was 16,16-difluoro-17α-ethynyl-3-methoxyestra-1,3,5(10)-trien-17β-ol(IV).

Feminizing activity was measured by a mouse uterine growth test.[14] In this test, 16,16-difluoroestra-1,3,5(10)-trien-17-one has greater than 1% the estrogenicity of estrone. Of the 16,16-difluoro-3-methoxyestratrienes, the 17-ethynyl derivative (IV) is representative and has given estrogenic potencies of approximately 4% that of estrone. Robinson, *et al.*,[1] have also reported lipid shifting and feminizing activities for compounds in this series as well as for the free phenol, 16,16-difluoroestrone. In their tests, estradiol-17β was the reference compound. In view of the ranges of activity observed due to non-parallel dose-response curves for the standard and test compounds and correcting for the difference in standards used, the data obtained in the two laboratories seem to be in essential agreement.

Our studies on 16,16-difluoro-17β-hydroxyandrost-4-en-3-one have shown that this compound is essentially inactive as an anabolic agent (rat *levator ani* test) and has 2.5% the androgenic activity of testosterone propionate as measured by its effect on seminal vesicle weight in rats.[15,16]

Experimental[17]

16,16-Difluoro-3-methoxyestra-1,3,5(10)-trien-17-one (I) and 16α-Fluoro-3-methoxyestra-1,3,5(10)-trien-17-one (IIa).—Estrone methyl ether (10 g.), 15 ml. of 4-pipecoline and 1.6 g. of *p*-toluenesulfonic acid monohydrate were placed in a flask fitted with a Dean and Stark trap (capacity 30 ml.) and a reflux condenser. The trap was filled with xylene and the reaction mixture was heated under reflux for 3$^1/_2$ hr. Then 100 ml. of dry xylene was added to the reaction mixture followed by distillation of 60 ml. of xylene over a period of 1.25 hours. During these operations the pot vapor temperature changed from 126 to 139°. The reaction mixture was cooled and 50 ml. of dry benzene and 0.5 ml. of pyridine were added. Perchloryl fluoride was bubbled through the reaction mixture for 20 min. while

(13) D. L. Cook, R. A. Edgren and F. J. Saunders, *Endocrinology*, **62**, 798 (1958).

(14) R. A. Edgren, *Proc. Soc. Exptl. Biol. Med.*, **92**, 569 (1956).

(15) F. J. Saunders and V. A. Drill, *ibid.*, **94**, 646 (1957).

(16) The author wishes to express his appreciation to Drs. Donald L. Cook and Francis J. Saunders and their staffs for the observations reported in this section.

(17) All melting points were taken on a Fisher–Johns melting point apparatus and are corrected. Unless otherwise stated, the rotations were determined at 24 ± 2° in chloroform and the ultraviolet spectra in methanol. The author wishes to thank Drs. R. T. Dillon and H. W. Sause of the Analytical Division of G. D. Searle & Co. for the analytical and optical data reported and Dr. E. G. Daskalakis of the Chromatography Department for the papergrams run during the course of this work.

cooling in an ice bath. The resulting solution was diluted with methylene chloride, extracted once with dil. potassium carbonate solution and the methylene chloride evaporated. An infrared spectrum in chloroform had a band due to the difluorinated product at 5.61 μ and a band at 5.74 μ indicating approximately 50% of unreacted estrone methyl ether. Also, a shoulder at 5.67 μ suggested the presence of the 16-monofluoro derivative. The solution of the reaction products in benzene was diluted with an equal volume of petroleum ether (b.p. 60–71°) and chromatographed over 750 g. of silica gel. The column was eluted successively with petroleum ether (b.p. 60–71°), 50% benzene–petroleum ether,[18] 75% benzene–petroleum ether, benzene, and finally 5% ethyl acetate in benzene.

The later fractions of 75% benzene and the first 3 l. of benzene gave solid residues with melting points in the range 127–131°. The combined fractions (2.35 g.) were recrystallized from methylene chloride–methanol to give 1.70 g. (15%) of 16,16-difluoro-3-methoxyestra-1,3,5(10)-trien-17-one, m.p. 129–131° (reported[1] 126–128°); $[\alpha]_D$ + 166.0° (reported[1] +167°, dioxane); λ_{max} 277.5 mμ (ϵ 2240) and 286.5 mμ (ϵ 1990); $\lambda_{max}^{CHCl_3}$ 5.62, 6.22, 6.33, 6.63, 10.13, 10.54, and 10.72 μ. A paper chromatogram of this material showed only one component. A mixture melting point with material of m.p. 127.5–129° obtained by the method of Robinson, *et al.*,[1] was 127.5–129°.

Anal. Calcd. for $C_{19}H_{22}F_2O_2$: C, 71.23; H, 6.92. Found: C, 71.44; H, 7.02.

The later fractions of benzene gave crystalline materials with melting points in the range of 137–150°. Attempted crystallization from ether gave a gel which lost solvent on standing, m.p. 152–158°. Crystallization from ethanol then gave 210 mg. (2%) of 16α-fluoro-3-methoxyestra-1,3,5(10)-trien-17-one as transparent needles, m.p. 155.5–164.5°; $[\alpha]_D$ + 176.5°; λ_{max} 278 mμ (ϵ 2110) and 286.5 mμ (ϵ 1960); $\lambda_{max}^{CHCl_3}$ 5.68, 6.22, 6.33, 8.49, 8.69 and 9.94 μ.

Anal. Calcd. for $C_{19}H_{23}FO_2$: C, 75.47; H, 7.67; F, 6.28. Found: C, 75.94; H, 7.62; F, 6.30.

A paper chromatogram of this material showed only one component. Final elution with 5% ethyl acetate in benzene gave 5.47 g. of unreacted estrone methyl ether.

Equilibration of 16α- and 16β-Fluoro-3-methoxyestra-1,3,5(10)-trien-17-ones.—A solution of 24.1 mg. of 16α-fluoro-3-methoxyestra-1,3,5(10)-trien-17-one in 5 ml. of methanol and 0.2 ml. of 8.5 *N* aqueous potassium hydroxide was boiled on the steam plate for 3–5 min. and then stirred at room temperature overnight. The solution was then warmed to dissolve some precipitate which had formed and was transferred with ether. After extracting twice with water, the ether layer was dried and solvent removed to give 21.6 mg. of residue. An infrared spectrum of this material (3% in $CHCl_3$) showed bands at 9.94 and 10.42 μ. The first of these is found in the α epimer and the second in the β epimer. Calculations from these bands indicated that the mixture contained 43% of the β epimer and 48% of the α epimer.

Equilibration starting with the β epimer was carried out using approximately 15 mg. of the pure material.[6,19] It was dissolved in 5 ml. of methanol on the steam

(18) Removal of solvent from the second fraction (*ca.* 800 ml.) of 50% benzene–petroleum ether gave a residue which detonated on further heating, suggesting possible formation of perchlorylated by-products. In a run in which the crude product mixture was washed with sodium bicarbonate and then 1 *N* potassium hydroxide, this difficulty was not encountered. It may also be desirable to wash the product mixture with a solution of some suitable reducing agent.

plate and 0.2 ml. of 8.5 *N* aqueous potassium hydroxide was added while stirring rapidly. Heating was continued for 10 min., the solution poured into ether, and the ether extracted 3 times with water. After drying, the ether was removed to give 16.1 mg. of residue. The infrared spectrum (3% in chloroform) matched closely that of the mixture obtained from the α-epimer.

17β-Acetoxy-16,16-difluoro-3-methoxyestra-1,3,5(10)-trien (IIIb).—To a solution of 440 mg. of 16,16-difluoro-3-methoxyestra-1,3,5(10)-trien-17-one in 2 ml. of 95% ethanol was added a solution of 200 mg. of sodium borohydride in 1 ml. of water and 2 ml. of ethanol. After standing at room temperature for 10 min. the reaction mixture was diluted with water and the solid filtered off. It was dissolved in methylene chloride, the solution dried and the solvent removed to give a glass which resisted attempts at crystallization. The infrared spectrum in chloroform showed absorption at 2.74 μ and absence of carbonyl absorption.

The 17-hydroxy compound (340 mg.) was acetylated in 5 ml. of pyridine and 4 ml. of acetic anhydride overnight at room temperature. Dilution with water gave material of m.p. 120–124° which was recrystallized from ether–petroleum ether (b.p. 60–71°) to give 200 mg. (40% over-all) of 17β-acetoxy-16,16-difluoro-3-methoxyestra-1,3,5(10)-triene, m.p. 130–31°; $[\alpha]_D$ + 21.7°; λ_{max} 278 mμ (ϵ 2110) and 286.5 mμ (ϵ 1870); $\lambda_{max}^{CHCl_3}$ 5.71, 6.22, 6.34, 7.98, 10.28 and 10.50 μ.

Anal. Calcd. for $C_{21}H_{26}F_2O_3$: C, 69.21; H, 7.19. Found: C, 69.80, 69.39; H, 7.04, 7.07.

16,16-Difluoro-3-methoxy-16,17-secoestra-1,3,5(10)-trien-17-oic Acid (V). A.—To a solution of 250 mg. of potassium in 5 ml. of *tert*-amyl alcohol was added 2.5 ml. of ether and the mixture cooled in an ice bath. There was then added 60 mg. of 16,16-difluoro-3-methoxyestra-1,3,5(10)-trien-17-one (I) and stirring was continued with cooling for 6 hr. The reaction mixture was treated with 20 ml. of ice cold saturated ammonium chloride solution and 50 ml. of benzene. The layers were separated, the benzene layer washed once with water, then dried, and solvent removed to give a paste. The paste was taken up in ether and extracted with 1 *N* potassium hydroxide and the extract acidified with concd. hydrochloric acid to give a solid, m.p. 174–176°. Recrystallization from ether–petroleum ether (b.p. 60–71°) gave 8.6 mg. (14%) with m.p. 175–178°. This material was identical with that obtained below in attempted ethynylation, mixture m.p. 174.5–178°.

B. A stirred solution prepared from 2 g. of potassium in 30 ml. of *tert*-amyl alcohol and 7 ml. of ether was cooled in an ice bath, then saturated with acetylene and 700 mg. of 16,16-difluoro-3-methoxyestra-1,3,5(10)-trien-17-one (I) was added. Stirring and passage of acetylene was continued for 8.5 hr. Ice-cold saturated ammonium chloride solution (100 ml.) and 250 ml. of benzene were then added, the layers separated, and the benzene layer was washed twice with water. It was dried and solvents removed to give 820 mg. of a yellow crystalline solid. This was treated in ether with activated charcoal and recrystallized from ether–petroleum ether (b.p. 60–71°) to give 510 mg. (69%) of V, m.p. 175–7.5°; $[\alpha]_D$ + 63.5°; $\lambda_{max}^{CHCl_3}$ 2.84, 5.73, 5.87, 6.22, 6.33, 9.52, 9.63, 10.08, and 10.40 μ. The infrared spectrum was essentially identical, except for the fingerprint region, with that of the unfluorinated acid, doisynolic acid methyl ether.[20]

(19) The author is grateful to Dr. G. P. Mueller for supplies of this substance for the equilibration experiments.

(20) J. Heer and K. Miescher, *Helv. Chim. Acta*, **29**, 1895 (1946). The author is grateful to Dr. John Baran for a chloroform spectrum of this material.

Anal. Calcd. for $C_{19}H_{24}F_2O_3$: C, 67.44; H, 7.15; F, 11.23. Found: C, 67.64; H, 7.42; F, 10.60.

The material dissolved in dilute sodium bicarbonate solution on warming and remained in solution at room temperature. Acidification of a strongly basic solution gave material of m.p. 171.5–174.5°; recrystallization from methylene chloride–hexane raised the melting point to 174–177.5°; mixture m.p. with material described above, 175–177.5°.

16,16-Difluoro-17α-ethynyl-3-methoxyestra-1,3,5(10)-trien-17β-ol (IV).—Four grams of lithium was dissolved over a period of *ca.* 1 hr. in 1 l. of liquid ammonia. The total volume was then brought to 1.5 l. by further addition of ammonia, acetylene bubbled through for 30 min. and 1.35 g. of the 16,16-difluoro-17-ketone (I) added. Passage of acetylene was continued for 10 min. and the ammonia allowed to evaporate overnight. Ether (500 ml.) and saturated ammonium chloride (100 ml.) were added with stirring, the layers separated, and the ether solution washed three times with water. Drying and evaporation of the solvent gave a residue whose infrared spectrum (chloroform) showed approximately 36% conversion to the ethynyl derivative; the carbonyl absorption indicated that most of the remaining material was unreacted 17-ketone. This material was re-treated as follows. A solution of 15 g. of lithium in 400 ml. of liquid ammonia was treated with acetylene for 15 min. The crude product mixture was then added in 100 ml. of anhydrous ether over approx. 20 min. and passage of acetylene continued for 1 hr. Ether (400 ml.) was added and the reaction mixture gently warmed until all of the ammonia had evaporated. The ether suspension was then heated under reflux with rapid stirring for 2.5 hr. while the bubbling of acetylene was continued. The reaction mixture was cooled, 100 ml. of cold, saturated ammonium chloride was added, the layers separated and the ether solution washed twice with water. After drying, removal of solvent gave 1.05 g. of residue which was chromatographed over 100 g. of silica gel. Elution was carried out with petroleum ether (b.p. 60–71°; 1 l.), 25% benzene (2 l.); 50% benzene (4 l.); 75% benzene (4 l.) and benzene. The 75% benzene fractions gave 450–500 mg. of crude product which was recrystallized from petroleum ether (b.p. 60–71°)-acetone to give 370 mg. (25%) of IV which began to soften on the block at 80° with apparent loss of solvent, finally melting at 96–98°. Air-dried material exhibited partial melting from 97 to *ca.* 105° with gradual resolidification and change of crystal form, final m.p. 121–123°. If held at 110°, the partially melted material completely resolidified on scratching, m.p. 121–123°. When this material was dried under reduced pressure (1 hr. at 80° then 2 hr. at 100°), there was obtained 330 mg. of crystalline material, m.p. 143–146° (reported,[1] 141–143°); $[\alpha]_D$ + 27.2° (reported,[1] + 20°, dioxane); $\lambda_{max}^{CHCl_3}$ 2.76, 3.02, 6.22, 6.34, 6.63, 9.93, 10.3 μ.

Anal. Calcd. for $C_{21}H_{24}F_2O_2$: F, 10.97. Found: F, 10.6.

Material obtained in an earlier experiment which had been crystallized from petroleum ether (b.p. 60–71°) alone and had been dried at 60° under reduced pressure melted at 95–98°; $[\alpha]_D$ + 26°.

Anal. Calcd. for $C_{21}H_{24}F_2O_2$: C, 72.81; H, 6.98. Found: C, 72.44; H, 7.07.

This material had an infrared spectrum essentially identical with that of the higher melting form (m.p. 143–146°), though there was a slight shoulder on the low wave length side of the 6.22 μ band which suggested the presence of an impurity. With both samples, papergrams showed a trace of highly polar material remaining at the origin.

16,16-Difluoro-3β-hydroxyandrost-5-en-17-one (VI) and 16α-Fluoro-3β-

hydroxyandrost-5-en-17-one (VIIa).—A solution of 50 g. of 3β-hydroxyandrost-5-en-17-one in 75 ml. of 4-pipecoline was heated under reflux for 22.5 hr. The refluxing amine was returned to the reaction mixture through a trap packed with calcium carbide[5] and fitted with a "cold finger" condenser. Dry xylene (1 l.) was then added and the reaction mixture freed of solvent until it solidified. A small sample of this material gave an infrared spectrum in chloroform indicating that approximately 50–70% of starting material remained. In addition, there was absorption at 6.28 μ which was not attributable to either starting material or the amine. Therefore, it was assigned to the enamine system. Benzene (800 ml.) was added, the solution cooled to 5–10°, and perchloryl fluoride passed through for 15 min. with rapid stirring. Saturated ammonium chloride solution (200 ml.) was then added and the mixture stirred overnight. The benzene layer was separated, washed twice with 10% sulfuric acid, twice with 1 *N* potassium hydroxide and once with water. The solution was dried, the benzene removed and the residue chromatographed over 1000 g. of silica gel. Elution with benzene (2 l.) and 10% ethyl acetate–benzene (9 l.) gave 36 g. of material without sufficient separation of the 16-fluoro components from unreacted starting material (as evidenced by comparison of the respective heights of the carbonyl bands in the infrared spectra of early, middle and late fractions). Further elution with 50% ethyl acetate–benzene gave pure starting material.

The mixture of carbonyl products (36 g.) was rechromatographed over *ca.* 2.5 kg. of silica gel. The elution scheme was benzene followed by 1, 2, 5, 10 and 20% ethyl acetate in benzene; approximately 800-ml. fractions were taken. By this procedure, essentially pure 16,16-difluoro-3β-hydroxy-androst-5-en-17-one (VI) was obtained in the first 8 fractions of 10% ethyl acetate. These fractions (total of 7.65 g.) were combined and recrystallized from benzene–petroleum ether (b.p. 60–71°) to give 5.60 g. of VI (10%), m.p. 158–159.5°; $[\alpha]_D$ + 27.8°; $\lambda_{max}^{CHCl_3}$ 2.74, 5.61, 6.23, 9.99, 10.2, 10.62, 11.21 μ.

Anal. Calcd. for $C_{19}H_{26}F_2O_2$: C, 70.40; H, 8.08; F, 11.71. Found: C, 70.47; H, 7.96; F, 12.1.

The residue obtained from the filtrate (2.05 g.) had an infrared spectrum identical with that of the recrystallized material.

Fractions 9–14 of 10% ethyl acetate were a mixture of the di- and monofluoro derivatives, as shown by their infrared spectra.

Fractions 15–19 (2.27 g.) were combined and recrystallized from ether–petroleum ether (b.p. 60–71°) to give 1.53 g. (2.9%) of 16α-fluoro-3β-hydroxyandrost-5-en-17-one, m.p. 165.5–168°; $[\alpha]_D$ + 27°; $\lambda_{max}^{CHCl_3}$ 2.74, 5.68, 6.23, 7.25, 9.53, 9.9, 10.74, 11.53 μ.

Anal. Calcd. for $C_{19}H_{27}FO_2$: C, 74.47; H, 8.88; F, 6.20. Found: C, 74.27; H, 8.69; F, 6.00.

16,16-Difluoroandrost-4-ene-3,17-dione (VIII).—To a solution of 2.05 g. of 16,16-difluoro-3β-hydroxyandrost-5-en-17-one in 200 ml. of acetone was added 1.7 ml. of chromium trioxide reagent[9,10] with cooling over *ca.* a 5 min. period. The reaction mixture was diluted with ice-water and the solid filtered; m.p. 148–151°; $\lambda_{max}^{CHCl_3}$ 5.62 and 5.82 μ; no ultraviolet absorption. This material was dissolved in 50 ml. of methanol, 2 ml. of concd. sulfuric acid and 10 ml. of water were added and the mixture heated for 15 min. on the steam bath. The solution was diluted with water, cooled and the product filtered off. Recrystallization from acetone–petroleum ether (b.p. 60–71°) gave 600 mg. of VIII, m.p. 180–182°: λ_{max} 239.2 mμ (ε 16,800); $[\alpha]_D$ +193.5°; $\lambda_{max}^{CHCl_3}$ 5.62 and 6.18 μ. Trace absorption

at 2.75 and 5.75 μ suggested the presence of an impurity.

Anal. Calcd. for $C_{19}H_{24}F_2O_2$: C, 70.78; H, 7.50; F, 11.79. Found: C, 70.79; H, 7.43; F, 11.50.

16,16-Difluoro-17β-hydroxyandrost-4-en-3-one (IX).—A solution of 500 mg. of sodium borohydride in 5 ml. of water and 10 ml. of ethanol was added to a solution of 900 mg. of 16,16-difluoroandrost-4-ene-3,17-dione in 10 ml. of ethanol. After 5 min. the reaction mixture was poured into water and extracted 3 times with methylene chloride. The combined organic extracts were dried and the solvents removed under reduced pressure. An infrared spectrum of the residue showed some 3-keto-Δ^4 system remaining (5.98 μ) and no absorption at 5.61 μ.

The total crude reduction product from above was treated in 100 ml. of chloroform with 10 g. of manganese dioxide for 2 hr. with stirring; the reaction mixture was filtered through Supercel and the chloroform allowed to evaporate overnight. The residue was chromatographed over 100 g. of silica gel, eluting with benzene (500 ml.), 5% ethyl acetate–benzene (1 l.), 10% ethyl acetate–benzene (1.5 l.) and 20% ethyl acetate–benzene (3 l.). A peak was obtained in the 10% and 20% eluates with fractions melting in the range of 130–150°. This material (630 mg.) was recrystallized from ether–petroleum ether (b.p. 60–71°) to give 180 mg., m.p. 156–159° with softening from *ca.* 145°; λ_{max} 240 mμ (ϵ 16,200); $\lambda_{max}^{CHCl_3}$ 2.78, 6.0, and 6.18 μ.

Anal. Calcd. for $C_{19}H_{26}F_2O_2$: C, 70.34; H, 8.08. Found: C, 70.38; H, 7.76.

Reductive Removal of Fluorine from 16,16-Difluoro-3-methoxyestra-1,3,5(10)-trien-17-one.—A solution of 200 mg. of 16,16-difluoro-3-methoxyestra-1,3,5(10)-trien-17-one in 5 ml. of acetic acid was heated under reflux and 1.5 g. of zinc dust added over 1.5 hr. with stirring. Refluxing was continued overnight. The reaction mixture was filtered, poured into ether and the ether solution extracted three times with water. Removal of solvent and recrystallization of the residue from methanol gave 110 mg. of estrone 3-methyl ether, m.p. 171–173°; mixture m.p. with authentic material of m.p. 171–174.5° was 171.5–174°. The infrared spectra were identical.

16,16-Difluoroestra-1,3,5(10)-trien-17-one.—In early attempts to form an enamine at C-17, use was made of 1,3,5(10)-estratrien-17-one[21] and pyrrolidine. Two separate runs, one in benzene and one in toluene (total starting ketone *ca.* 6 g.) were carried out using toluenesulfonic acid as catalyst. In these cases, the reaction mixtures were worked up as in the preparation of I after treatment with perchloryl fluoride. Direct crystallization of the crude mixture from benzene–methanol or methanol gave essentially pure starting material.

The residue from the combined filtrates was chromatographed over 100 g. of silica gel and eluted with petroleum ether (b.p. 60–71°), 50% benzene, 60% benzene and 75% benzene–petroleum ether mixtures. The difluoro derivative was obtained in the first three fractions of 50% benzene and recrystallization from ether–petroleum ether (b.p. 28–38°) gave 197 mg. (*ca.* 3%) of 16,16-difluoroestra-1,3,5(10)-trien-17-one, m.p. 132–134.5°. Sublimed at 125° (3 mm.), this gave 121 mg., m.p. 132–135°; $\lambda_{max}^{CHCl_3}$ 5.62, 6.68, 7.23, 7.58, 7.93, 10.13, 10.54, and 10.75 μ.

Anal. Calcd. for $C_{18}H_{24}F_2O$: C, 74.50; H, 6.94; F, 13.09. Found: C, 74.16; H, 6.90; F, 13.7.

Fractions obtained in 60% benzene (130 mg.) had infrared spectra which indi-

(21) A. H. Goldkamp, U. S. Patent 2,917,963, Aug. 2, 1960.

cated the presence of a monofluoro derivative (5.68 μ). The material melted at 143–170°. It was sublimed to give 74.7 mg., m.p. 140–167°; $\lambda_{max}^{CHCl_3}$ 5.68, 9.96, 10.52, 10.63, 10.78, 10.95, 11.30, 11.54. The only absorption in the 9.9–11.6 μ region of comparable intensity in the parent ketone is at 9.94 μ, though approximately the same number of extremely weak bands, slightly shifted, appear.

3β-Acetoxy-16α-fluoroandrost-5-en-17-one (VIIb).—A solution of 5.0 mg. of 16α-fluoro-3β-hydroxyandrost-5-en-17-one in 2 drops of pyridine and 2 drops of acetic anhydride was allowed to stand at room temperature overnight, then diluted with water and the precipitate collected and recrystallized from methanol to give 1.7 mg. of VIIb, m.p. 199.5–201.5°. Material obtained by chromatography following acetylation of a crude product mixture from the fluorination of 3β-hydroxyandrost-5-en-17-one melted at 201–203°; mixture m.p. 200–202°. The infrared spectra (KBr pellet) were identical; $[\alpha]_D$ +18.8°.[22]

Anal. Calcd. for $C_{21}H_{29}FO_3$: C, 72.38; H, 8.39. Found: C, 72.72; H, 8.20.

(22) S. Nakanishi and E. V. Jensen [*J. Org. Chem.*, **27**, 702 (1962)] recently have described the preparation of this compound using perchloryl fluoride and a 16,17-en-17-amide. They report m.p. 205–206°, $[\alpha]_D$ (CHCl)$_3$ + 14°. Their free alcohol had m.p. 182–183°, $[\alpha]_D$ (CHCl$_3$) + 18°.

The Synthesis of Some Acidic Amino Acids Possessing Neuropharmacological Activity

J. C. WATKINS

Department of Physiology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia

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Several new acidic amino acids have been synthesized and tested for neuropharmacological action. *N*-Methyl- and *N*-ethyl-D-aspartic acid and D-homocysteic acid have potent excitatory actions upon mammalian and amphibian neurones.

The observation that L-aspartic and L-glutamic acids excite mammalian nerve cells and cause contraction of crustacean muscle[1–3] led to an extensive survey of the actions of related substances on mammalian and toad spinal neurones.[4–6] It was found that N-methyl-DL-aspartic acid was considerably more potent, both on

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