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Steroids CCCI [1]. Radiochemical Syntheses Part I. Preparation of Tritiated 6α, 9α-Difluoro Cortical Hormones

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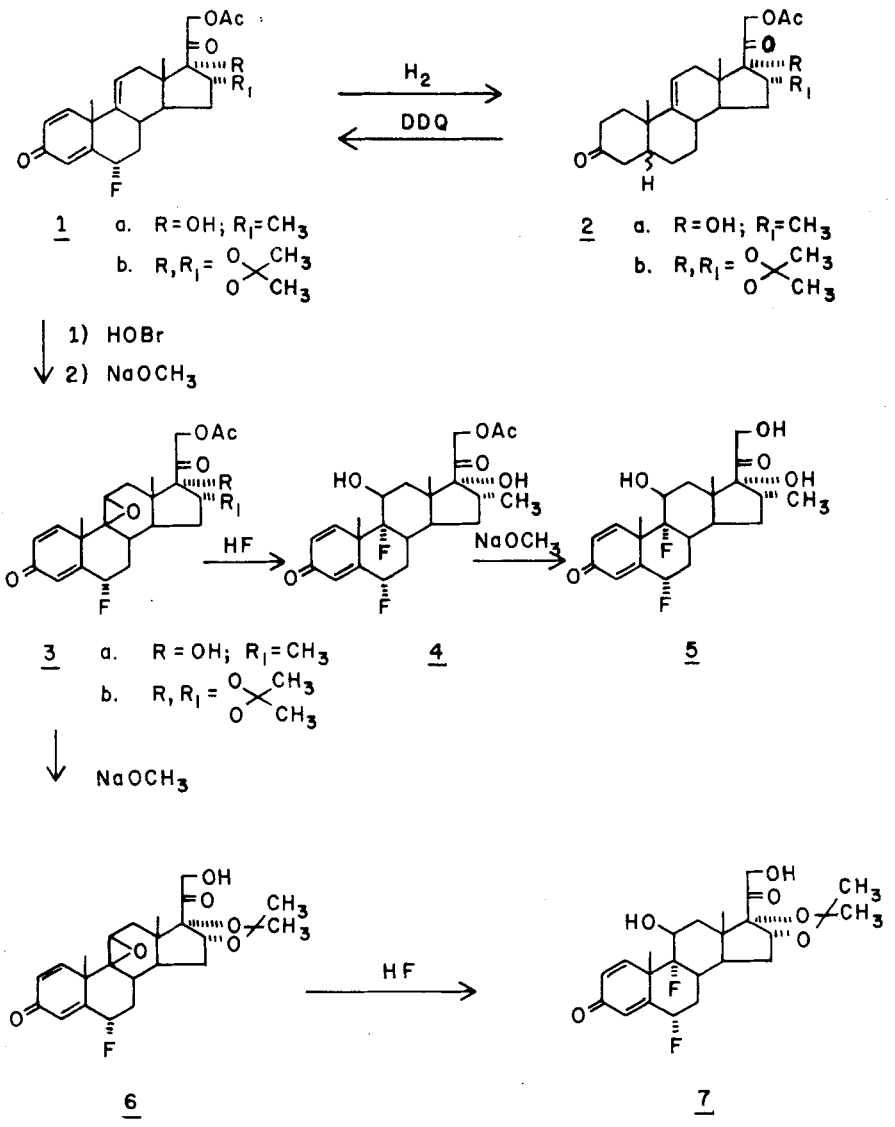
SUMMARY

A six-step synthesis of tritiated 6α, 9α-difluoro-16α-methyl-11β, 17α,21-trihydroxypregna-1,4-diene-3,20-dione (5) *and 6α,9α-difluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide* (7) *from the appropriate* $\Delta^{1,4,9(11)}$*-3-keto intermediates is described. Isotope incorporation was achieved by selectively reducing the 1,2 and 4,5-double bonds with tritium gas followed by regeneration of the* $\Delta^{1,4}$*-3-keto system by dehydrogenation with D.D.Q.*

INTRODUCTION.

The synthesis of ^{3}H-labeled steroids may be accomplished efficiently by reduction of the 1,2-double bond of Δ^1 and $\Delta^{1,4}$-3-keto systems with tritium gas [2]. This method furnishes a 1,2-ditritiated species from which the labile isotope at C-2 may be exchanged by equilibration with acid or alkali [3]. The successful extension of this route to C-1 tritiated Δ^1- and $\Delta^{1,4}$-3-ketones depends upon the stereochemical course of the Δ^1-reduction process. Thus, Gut *et al.*, have shown that the selective reduction of the 1,2-double bond of 1-dehydrotestosterone with 1 molar equivalent of tritium gas over palladium catalyst proceeds to a major extent from the β-face to yield the 1β, 2β-ditritiated analog [3]. Since chemical [e.g., 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (D.D.Q.)] and enzymatic dehydrogenations of the latter take place predominantly by the stereo-specific removal of the 1α-proton [4], this two-stage sequence results in the overall incorporation of a nonexchangeable tritium atom at C-1 of 1-dehydrotestosterone [3]. In contrast, saturation of the 1,2-double bond of 5α-androst-1-ene-3,17-dione with tritium gas resulted in



the entry of over 90% of the isotope from the α-face. Subsequent dehydrogenation resulted in loss of the greater part of the label [3]. This shows that the stereochemical requirements for the A ring reduction are remarkably sensitive to minor alterations in molecular shape : consequently, tritiation experiments based on this hydrogenation-dehydrogenation approach often fail to provide labeled material of high specific activity. This problem was encountered when

the preparation of tritiated samples of the potent corticosteroids 6α,9α-difluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione (flumethasone) (*5*) [5] and 6α, 9α-difluoro-11β, 16α, 17α, 21-tetrahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide (fluocinolone acetonide) (*7*) [6] was attempted from "Compound S" type precursors. Specifically, the selective tritiation of the 1,2-double bond of 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4-diene-3,20-dione 21-acetate proceeded smoothly but dehydrogenation of the corresponding dihydro product resulted in the loss of virtually all the label from C-1 [7]. A search for alternate substrates capable of undergoing β-face hydrogenation and chemical elaboration to (*5*) and (*7*) led to the studies described in the sequel.

Discussion.

As a result of earlier chemical investigations aimed at the synthesis of the corticosteroids (*5*) and (*7*) a number of potentially useful intermediates were at hand for isotope incorporation studies. However, the chemical and stereochemical requirements seemed to be fulfilled only by derivatives of $\Delta^{1,4,9(11)}$-3-keto steroids, e.g. 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)-triene-3,20-dione 21-acetate (*1a*) [8] whose trigonal configurations at positions 9 and 11 could facilitate β-face hydrogenation in ring A by overall flattening of the molecule. Entry to the desired fluorohydrin system was also provided by the 9(11)-double bond and since this olefinic linkage is relatively inert to catalytic hydrogenation [9], radiochemical impurities resulting from over-reduction were expected to be minimal.

Therefore, a number of attempts were made to hydrogenate selectively the 1,2-double bond of 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)-triene-3,20-dione 21-acetate (*1a*) but these proved abortive. In all cases, mixtures of starting material and the corresponding dihydro and tetrahydro products were obtained. However, hydrogenation of *1a* with palladium-on-calcium carbonate catalyst in benzene solution proceeded smoothly to the tetrahydro stage to afford a crystalline substance which was shown to be approximately a 1 : 1 mixture of the $\Delta^{9(11)}$-5α and 5β-pregnenes *2a* by t.l.c. and n.m.r. spectroscopy. The 19-H resonance of this product appeared as a broad singlet at 68 c.p.s. (half-band width 3 c.p.s.) thereby indicating the virtual identity of the chemical shifts of the 10-methyl protons of the 5α and 5β-tetrahydro isomers. This observed value compares favorably with the calculated 19-H resonances for 6α-fluoro-5α-androst-9(11)-en-3-one and the corresponding 5β epimer which amount to 71.0 and 71.5 c.p.s. respectively [10].

Dehydrogenation of the tetrahydro mixture with 2.5 molar equivalents of D.D.Q. [11] in boiling dioxane followed by preparative t.l.c. provided the starting triene *1a* (51% yield) identified by mixed melting point and spectroscopic comparison with an authentic sample [8]. The efficiency on this two stage

hydrogenation-dehydrogenation sequence precluded the usual requirement namely that of selectively reducing the 1,2-double bond to achieve isotope incorporation at C-1.

The foregoing process was repeated next with deuterium gas in order to determine the extent of isotope incorporation. Mass spectral analysis showed that the resulting deuterated triene *1a* consisted of 32% mono and 35% di-deuterated species together with lesser amounts of tri- and tetradeuterated species and unlabeled material. Thus, a considerable portion of the initially introduced deuterium at the C-2 and C-4 positions was apparently retained during the dehydrogenation step. Since the attempted removal of acid labile deuterium by exchange with hydrochloric acid in acetic acid solution resulted in extensive decomposition of the substrate, the amount of isotope remaining at C-1 after dehydrogenation was not estimated. On the other hand, the extensive incorporation of deuterium by *1a* demonstrated the suitability of this substance for tritium labeling studies.

Accordingly, 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)--triene-3,20-dione 21-acetate (*1a*) was exposed to carrier-free tritium gas in the presence of palladium-on-calcium carbonate catalyst and the resulting product treated as before with D.D.Q. Purification by t.l.c. afforded the tritiated triene (700 mC) which was diluted with carrier and converted to 17α,21-dihydroxy--6α-fluoro-16α-methyl-9β,11β-oxidopregna-1,4-diene-3,20-dione-^{3}H 21-acetate (*3a*) (sp. act. 750 mC/mM) by successive treatment with hypobromous acid and potassium acetate [12]. Exposure of the diluted epoxide to 90% hydrofluoric acid [13] provided flumethasone-^{3}H 21-acetate (*4*) (sp. act. 250 mC/mM), homogeneous by t.l.c. and paper chromatography with a radiochemical purity >95% by dilution analysis. Mild alkaline hydrolysis of the latter acetate completed the radiochemical synthesis of tritiated flumethasone (*5*).

The latter sequence of reactions (with minor variations) was next carried out with 6α-fluoro-16α,17α,21-trihydroxypregna-1,4,9(11)-triene-3,20-dione 21--acetate 16,17-acetonide (*1b*) [8] to afford tritiated fluocinolone acetonide [7] of satisfactory radiochemical purity [14].

EXPERIMENTAL [16].

Hydrogenation of 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)-triene-3,20-dione 21-acetate (1a). — A solution of 210 mg of the triene *1a* in 15 ml of dry benzene was hydrogenated for 18 hr in the presence of 20 mg of prereduced 5% palladium-on-calcium carbonate catalyst. The catalyst was removed by filtration and the resulting solid crystallized from methanol to afford 144 mg of tetrahydro product *2a*, m.p. 188-190° C which was approximately a 1 : 1 mixture of 5α and 5β isomers by t.l.c. : n.m.r. 41 (18-H), 56, J = 7 (16α-methyl-H), 68 (19-H broad singlet), 129 (21-acetoxy-H), 278, 294, 298 and 315 (quartet of 21-H) and 300-350 c.p.s. (2 proton multiplet of 6β-H

and 11-H) : ν_{max}^{KBr} 3400, 1750, 1725 and 1260 cm^{-1}. Mol. Wt. Calcd. for $C_{24}H_{33}FO_5$: 420. Found : M^+ = m/e 420.

Dehydrogenation of the tetrahydro product 2a. — A solution of 105 mg of the foregoing reduction product in 4.5 ml of dioxane was heated under reflux with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (158 mg) for 42 hr and after cooling diluted with methylene chloride. The resulting solution was washed with several portions of 2% sodium hydroxide solution and water, dried (Na_2SO_4) and evaporated to yield a yellow crystalline product. Purification by preparative t.l.c. [17] (hexane-ethyl acetate 2 : 1) followed by crystallization from methanol afforded 54 mg of 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)-triene-3,20-dione 21-acetate (*1a*) : m.p. 193-196° C identical in all respects with an authentic sample [8].

Deuteration of 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)--triene-3,20-dione 21-acetate (1a). — A solution of 2.5 g of the triene *1a* in 105 ml of dry benzene was added to a suspension of 375 mg of predeuterated 5% palladium-on-calcium carbonate in 10 ml of dry benzene. The resulting mixture was stirred in an atmosphere of deuterium gas for 18 hr after which time the catalyst was removed by filtration through celite and the solvent evaporated. A solution of the residue in 95 ml of dioxane was heated under reflux with 7.2 g of D.D.Q. for 42 hr and after cooling, diluted with methylene chloride. This solution was washed with several portions of 2% sodium hydroxide solution and water, dried (Na_2SO_4) and evaporated to yield 960 mg of crude product. Purification of 300 mg of this material by preparative t.l.c. followed by successive crystallizations from methanol and ethyl acetate afforded 55 mg of deuterated triene *1a* m.p. 193-195° C. The isotopic purity as determined by mass spectrometry was as follows : d_0 8.3% (mol. wt. 416), d_1 32%, d_2 34.8%, d_3 18.8% and d_4 6.1%.

Tritiation of 17α,21-dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)-triene--3,20-dione 21-acetate (1a). — A solution of 100 mg of the triene *1a* in 5 ml of benzene and 50 mg of 5% palladium-on-calcium carbonate catalyst was stirred for 18 hr in an atmosphere of carrier-free tritium gas. The catalyst was removed by filtration and the solvent evaporated to afford a solid which was dissolved in 5 ml of dioxane and boiled for 18 hr with 350 mg of D.D.Q. The reaction mixture was diluted with benzene and filtered through a column of 20 g of acid-washed alumina. Evaporation of the benzene eluates afforded a crystalline product which was purified by preparative t.l.c. on silica gel with benzene-ethyl acetate (3 : 1). This yielded 19 mg (700 mC) of 17α,21--dihydroxy-6α-fluoro-16α-methylpregna-1,4,9(11)-triene-3,20-dione-^{3}H 21-acetate (*1a*) which was diluted with 400 mg of carrier.

17α-21-dihydroxy-6α-fluoro-16α-methyl-9β,11β-oxidopregna-1,4-diene-3,20--dione-^{3}H 21-acetate (3a). — A solution of the foregoing triene (419 mg) in

8 ml of dioxane was treated with 0.5 g of 1,3-dibromo-5,5-dimethylhydantoin and 0.26 ml of 1 N perchloric acid. After 5 hr the excess of hypobromous acid was destroyed with dilute aqueous sodium sulfite solution and the product precipitated by the addition of water and saturated brine. The solid was filtered off and dried to yield 490 mg of crude 9α-bromo-6α-fluoro-16α-methyl--11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione-^{3}H 21-acetate which was dissolved in 57.3 ml of dry methylene chloride containing 2.3 ml of methanol and treated with 1 ml of 1 N methanolic sodium methoxide solution. The reaction mixture was kept for 25 min in a nitrogen atmosphere and then neutralized with acetic acid. Water was added and the crude epoxide was isolated by extraction with ethyl acetate and then purified by filtration of a benzene solution through a short column of Florisil. The resulting solid was twice crystallized from methylene chloride-hexane to yield 172 mg (360 mC) of epoxide (*3a*), m.p. 149-152° C, sp. act. 900 mC/mM, homogenous by t.l.c. and paper chromatography (Bush 3 system).

6α,9α-difluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione-^{3}H- 21-acetate (Flumethasone-^{3}H acetate) (4). — The foregoing epoxide (86 mg; 180 mC) was diluted with 224 mg of carrier and added to 2 ml of 90% hydrofluoric acid maintained at −20° C in a polyethylene bottle. After being allowed to stand at −20 °C for 3 hr the reaction mixture was diluted with water and the precipitate was collected in a sintered glass funnel, washed and dried. The crude product was purified by preparative t.l.c. using benzene-ethyl acetate (1 : 1) followed by crystallization from acetone-hexane to give 63.4 mg (35 mC) of flumethasone-^{3}H 21-acetate (*4*), m.p. 268-271° C, sp. act., 250 mC/mM, homogenous by t.l.c. and paper chromatography (Bush 3 system). The radiochemical purity of this material was >99% by dilution analysis.

6α,9α-difluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20--dione-^{3}H (Flumethasone-^{3}H) (5). — Flumethasone-^{3}H 21-acetate (*4*) (5 mC) was diluted with 36.2 mg of carrier, then dissolved in 2 ml of methanol and treated with 0.1 ml of 1 N methanolic sodium methoxide. The reaction mixture was left standing for 0.5 hr in a nitrogen atmosphere at room temperature and then neutralized with glacial acetic acid. Addition of saturated brine precipitated 24.6 mg of flumethasone-^{3}H (*6*) (3 mC) (sp. act. 51 mC/mM) homogenous by t.l.c. and paper chromatography (Bush 5 and E_2B systems).

Tritiation of 6α-fluoro-16α,17α,21-trihydroxypregna-1,4,9(11)-triene-3,20--dione 21-acetate 16, 17-acetonide (1b). — The triene-acetonide *1b* (100 mg) in benzene (5 ml) was reduced with carrier-free tritium gas in the presence of 50 mg of 5% palladium-on-calcium carbonate catalyst and the crude product was dehydrogenated exactly as described for the 16α-methyl compound. Purification by preparative t.l.c. with benzene-ethyl acetate (3 : 1) afforded

9 mg of 6α-fluoro-16α,17α,21-trihydroxypregna-1,4,9(11)-triene-3,20-dione-^{3}H 21-acetate 16,17-acetonide (*1b*) (500 mC) which was diluted with 352 mg of carrier.

6α-fluoro-9β,11β-oxido-16α,17α,21-trihydroxypregna-1,4-diene-3,20-dione-^{3}H 21-acetate 16,17-acetonide (3b). — A solution of the foregoing triene (361 mg) in 8 ml of dioxane was treated with 0.45 g of 1,3-dibromo-5,5-dimethylhydantoin and 0.24 ml of 1 N perchloric acid exactly as described for the 16α-methyl compound. This yielded 400 mg of 9α-bromo-6α-fluoro-11β,16α, 17α, 21-tetrahydroxypregna-1,4-diene-3,20-dione-^{3}H 21-acetate 16,17-acetonide which was dissolved in 57.3 ml of methylene chloride containing 2.3 ml of methanol and treated with 0.8 ml of 1 N methanolic sodium methoxide. The reaction mixture was stirred for 3/4 hr in a nitrogen atmosphere, neutralized with acetic acid and the product precipitated with water. Qualitative t.l.c. indicated that hydrolysis of the 21-acetate grouping had occurred to an appreciable extent by the sodium methoxide treatment and consequently the total crude product was acetylated with 6 ml of acetic anhydride-pyridine (1 : 2) for 8 hr. Crystallization of the resulting precipitate from methylene chloride-hexane afforded 223 mg (142 mC) of 6α-fluoro-9β,11β-oxido-16α,17α,21-trihydroxypregna-1,4-diene-3,20-dione-^{3}H 21-acetate 16,17-acetonide (*3b*), m.p. 250-252° C (sp. act. 300 mC/mM), radiochemical purity *ca.* 95% by dilution analysis.

6α-fluoro-9β,11β-oxido-16α,17α,21-trihydroxypregna-1,4-diene-3,20-dione-^{3}H 16,17-acetonide (6). — The foregoing epoxide (55 mg) was diluted with 295 mg of carrier, suspended in 10 ml of dry methanol and treated with 0.5 ml of 1 N methanolic sodium methoxide. The reaction mixture was kept for 1.5 hr in a nitrogen atmosphere, neutralized with acetic acid and diluted with brine. The precipitate was collected and dried to afford 248 mg of the 21-alcohol *6*.

6α,9α-difluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione-^{3}H 16,17-acetonide (Fluocinolone-^{3}H acetonide) (7). — A solution of the foregoing epoxide (248 mg) in 3 ml of 90% hydrofluoric acid was allowed to stand for 5 hr at —20° C and then poured into water. The dried precipitate was treated with 10 ml of acetone containing two drops of 70% perchloric acid for 15 min and the resulting solution was partitioned between ethyl acetate and 8% aqueous sodium bicarbonate. The organic phase was separated, washed with water, dried (Na_2SO_4) and evaporated to yield 240 mg of oil consisting of a 1 : 1 mixture of the starting epoxide and the desired fluocinolone-^{3}H acetonide (*7*). Chromatography of this mixture on a column of 40 g of silica gel followed by crystallization of the appropriate fractions from acetone-hexane yielded 48 mg (5.7 mC) of fluocinolone-^{3}H acetonide (*7*), sp. act. 53 mC/mM, homogenous by t.l.c. and paper chromatography (Bush 5 system), radiochemical purity 97.5% by dilution analysis.

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15. The success of this approach suggests that the stereochemical requirements for Δ^1 introduction are less specific for selenium dioxide than for D.D.Q.
16. Melting points are uncorrected. N.m.r. spectra were recorded for deuteriochloroform solutions using a Varian A-60 spectrometer and tetramethylsilane as internal reference. Mass spectra were obtained with an Atlaswerke CH-4 spectrometer equipped with a direct inlet system. Spectra were measured at an ionizing potential of 70 eV and an acceleration voltage of 3 kV.
17. Preparative t.l.c. was conducted using silica gels GF and HF (from Brinkmann Instruments, Inc., N.Y.) at thicknesses of 1.3 mm and steroid loadings of 2 mg per cm.