SYNTHESIS OF TRIAMCINOLONE ACETONIDE-4-^{14}C

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SUMMARY

The free hydroxyl groups in triamcinolone acetonide (1) were protected as silyl ethers to give the bis-silyloxy derivative 4. Compound 4 was then hydrogenated over 5% palladium on carbon to give the Δ^4-3-oxo steroid 5 which was then converted to the enol-lactone 7 under selective reaction conditions. The enol-lactone 7 was subjected to Grignard reaction with ^{14}C-methylmagnesium iodide to give an adduct 8a which on subsequent treatment with lithium 2,6-di-*t*-butylphenoxide gave 4-^{14}C-Δ^4-3-oxo derivative 9. Compound 9 was heated with selenium dioxide in *t*-butanol containing pyridine to give the $\Delta^{1,4}$-3-oxo derivative 10. Removal of the silyl protecting groups was readily accomplished by heating 10 with hydrochloric acid in methanol-tetrahydrofuran solution to give the title compound 11.

Key Words: 4-^{14}C-triamcinolone acetonide, lithium 2,6-di-*t*-butylphenoxide, high pressure liquid chromatography (HPLC)

INTRODUCTION

Triamcinolone acetonide (TAC) (1, Scheme 1) is a synthetic anti-inflammatory steroid widely used for chronic allergic disorders, dermatologic and inflammatory conditions in both male and female patients. Hendrickx et al. [1,2] recently demonstrated that TAC can cause some teratogenic effects on the skeletal and lymphoid systems in nonhuman primates. They observed that two different malformation syndromes can be produced with this drug. One is seen when the drug is given in early pregnancy, which includes defects of face, cranium, brain and induction of cleft palate. A second syndrome appears if the drug is given during late pregnancy and is usually limited to hypoplasia of the thymus gland and other effects on the lymphoid system. This condition can greatly influence the immune capability of the newborn infant. To investigate the effects of maternal and fetoplacental metabolism on the teratogenicity of TAC, it was necessary to obtain ^{14}C-labeled TAC. Previously, the metabolism of TAC in laboratory animals was studied [3,4] with ^{14}C-labeled material but the label was located at the 2-position of the acetonide group. For present investigations it was desirable to incorporate the ^{14}C-label in a biochemically stable position in the steroid nucleus and we now describe an efficient synthesis of the title compound.

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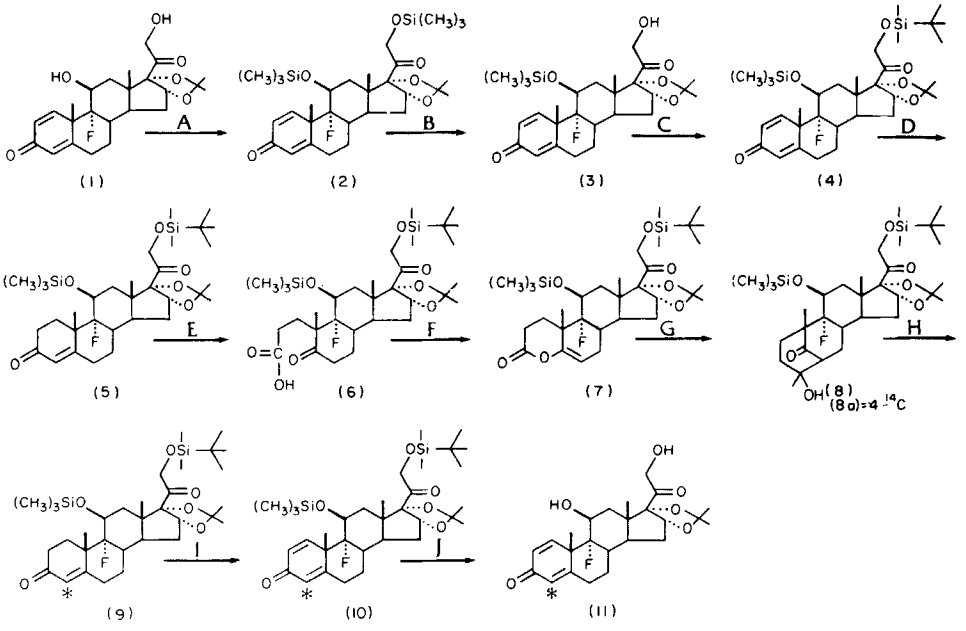
Scheme I



A: DMF, imidazole, $(CH_3)_3SiCl$

B: THF-H_2O-HOAc, room temp.

C: DMF, imidazole, t-butyldimethylsilane

D: Dioxane, H_2, Pd/C (5%)

E: K_2CO_3-H_2O, CH_2Cl_2-t-BuOH, $NaIO_4$-H_2O, room temp-40°C; H_2SO_4, 0°C

F: Ac_2O - NaOAc, NEt_3, 145°C

G: Benzene-ether, $CH_3MgI^{14}C$, room temp; H_2O, NH_4Cl, 0°C

H: THF, lithium 2,6-di-t-butylphenoxide, 60°C

I: t-BuOH, pyridine, SeO_2, 85-95°C

J: MeOH-THF, HCl (2N), 60°C

DISCUSSION

In a radiochemical synthesis it is desirable to incorporate the isotope towards the final stage of the synthetic sequence in order to obtain an efficient overall yield of the desired labeled compound. Although many well-documented syntheses of TAC (1) are available, these procedures are unsuitable for the synthesis of 4-^{14}C-TAC. The classical method developed by Turner [5] for the introduction of isotopic carbon at C-4 of the steroid nucleus which was further modified by Fujimoto [6] can be readily followed for the synthesis of the title compound. However, TAC (1) has very sensitive functional groups such as the 20-oxo-21 hydroxy side chain at C-17 and the 9α-fluoro-11β-hydroxy substituents in ring-C. Some of these functional groups, in particular the hydroxyls, must be suitably protected before reactions are carried out on other parts of the steroid skeleton. In addition, TAC (1) has a $\Delta^{1,4}$-3-oxo structure in ring-A and the Δ^1-double bond must be reduced to give a compound with a Δ^4-3-oxo structure required for adapting Turner's procedure [5]. After incorporating the ^{14}C at C-4, the Δ^1-double bond must be re-introduced and the protective groups removed to obtain 4-^{14}C-TAC. In the current synthesis, after ^{14}C is introduced, there are only three steps to obtain the title compound.

To protect the alcoholic hydroxyl groups in TAC at C-11 and C-21, we explored a number of different protecting groups such as tetrahydropyranyl ethers and acetates but found none of them satisfactory. Finally silyl ethers proved to be most useful protecting groups in the present work. We found that the 11β- and 21-hydroxyl groups in TAC can be silylated with trimethylchlorosilane and imidazole in dimethylformamide to give 11β,21-bis(trimethylsilyloxy) (TMS) derivative 2. However, the 21-TMS ether was susceptible to solvolysis in protic media and required a more stable silyl ether derivative. The 21-*t*-butyldimethylsilyl (TBDMS) ether 4 [7] was found to be more stable than the 21-TMS ether 2 and yet readily cleaved under a variety of selective conditions. Catalytic reduction of 4 with 5% palladium on carbon in dioxane gave a mixture of the Δ^1, Δ^4, and saturated 3-oxo derivatives which were readily separated by preparative liquid chromatography and the pure Δ^4-3-oxo compound 5 was isolated. Ring-A of 5 was cleaved by a modified potassium permanganate-sodium metaperiodate oxidation [8] to give the seco-acid 6 in excellent yield. The seco-acid 6 was converted to the enol-lactone 7 by reaction with acetic anhydride in the presence of anhydrous sodium acetate and triethylamine [9]. The reaction conditions were optimized for the Grignard reaction with the enol-lactone 7 employing unlabeled methylmagnesium iodide to obtain the abeo derivative 8. After establishing all the reaction conditions, the actual ^{14}C-labeled compound 8a was prepared by employing methyl-^{14}C iodide. Treatment of 8a with lithium 2,6-di-*t*-butylphenoxide in refluxing tetrahydrofuran led to the desired Δ^4-3-oxo derivative 9. Reaction of the Δ^4-3-oxo compound 9 with selenium dioxide and pyridine in *t*-butanol gave the $\Delta^{1,4}$-3-oxo derivative 10. Finally, the protecting silyl ether groups were removed by reacting 10 with hydrochloric acid in methanol-tetrahydrofuran solution to give the title compound 11.

EXPERIMENTAL

Most chemicals and solvents were analytical reagent grade and were used without further purification. Some reagents and solvents, such as acetic anhydride, dihydropyran, dioxane, pyridine, tetrahydrofuran, and triethylamine, were purified according to standard laboratory procedures. Methyl-^{14}C iodide (50 mCi, 55 mCi/mmol) was purchased from New England Nuclear, Boston, Massachusetts, and was used without further purification. All organic extracts were dried over anhydrous sodium sulfate, unless otherwise specified, and evaporated *in vacuo*.

Purity and identity of new compounds were established by normal spectral (IR, UV, NMR, MS) and analytical (TLC, HPLC, chemical analysis) techniques. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. IR spectra were obtained in a potassium bromide disc using a Perkin-Elmer Model 467 grating spectrophotometer. UV spectra were measured in methanol solution using a Varian Cary 210 spectrophotometer. Proton NMR spectra were obtained with a Varian EM-390 spectrometer. Mass spectra were recorded on a Finnigan quadrupole mass spectrometer. "Dry column" chromatography was performed on Woelm silica in a nylon column as described by Loev and Goodman [10]. TLC analyses of unlabeled compounds were done on silica gel GF (Analtech) glass plates (2.5 x 10 cm with 250 μM layer and prescored). TLC analyses of ^{14}C-labeled material were carried out on silica gel GHLF (Analtech) glass plates 5.0 x 20 cm with 250 μM layer) and were monitored by an Atomic Accessories Model RSC-363 radiochromatogram scanner. Preparative HPLC was carried out on a Waters Associates, Inc., Prep LC/System 500 employing PrepPak 500/silica cartridge. HPLC analysis of ^{14}C-labeled material was carried out on Waters Associates, Inc., HPLC equipment (Model 6000A pump) employing a reverse phase C_{18} column (Chromegabond MC-18, 10 μm, 30 cm x 4.6 mm), and monitored by an LDC Spectromonitor III. Radioactivity was determined with a Beckman LS 7500 scintillation counter. Microanalyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana.

11β-21-Bis(Trimethylsilyloxy)-9α-fluoro-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione (2)

Trimethylchlorosilane (10 ml, 79 mmol) was added under nitrogen atmosphere to a solution of triamcinolone acetonide (1, 10 g, 23 mmol) and imidazole (10 g, 147 mmol) in dry dimethylformamide (120 ml). The mixture was stirred at room temperature for 48 hr, poured into phosphate buffer (pH 7) and extracted with chloroform 3x. Evaporation of the solvent followed by recrystallization of the residue from hexanes afforded pure compound 2 (12.0 g); mp 204-206°C; IR: ν_{max} 1720, 1660, 1250, 840, and 750 cm^{-1}; UV: λ_{max} 239 nm (ϵ = 15,282); NMR: 0.17(s, 9H, $Si(CH_3)_3$), 0.24(S, 9H, $Si(CH_3)_3$), 0.87(S, 3H, 18-CH_3) 1.17, (S, 3H, acetonide CH_3), 1.41(S, 3H, acetonide CH_3), 1.48(s, 3H, 19-CH_3), 4.38(m, 1H, 11-H), 4.49(d of d, J = 54 Hz, J = 18.5 Hz, 2H, 21-H_2), 5.05(m, 1H, 15-H), 6.11(broad s, 1H, 4-H), 6.32(d of d, J = 6.3 Hz, 1H, 2-H), 7.03(d, J = 9.9 Hz, 1H, 1-H)ppm; Analysis: Calc'd for $C_{30}H_{47}FO_6Si_2$: C, 62.26; H, 8.18; Found: C, 62.56; H, 8.02.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-hydroxy-1,4-pregnadiene-3,20-dione (3)

Compound 2 (12.0 g, 20.7 mmol), tetrahydrofuran (100 ml), acetic acid (50 ml), and water (50 ml) were stirred at room temperature for 1 hr. The reaction mixture was then poured into phosphate buffer (pH 7) and extracted with ethyl acetate 3x. Evaporation of the solvent followed by recrystallization of the residue from benzene afforded pure compound 3 (10.3 g); mp 207-209°C; IR: ν_{max} 3440, 1710, 1665, 1630, 1610, 1255, 850, and 755 cm^{-1}; UV: λ_{max} 237 nm (ϵ = 14,643); NMR: 0.22(s, 9H, $Si(CH_3)_3$), 0.89(s, 3H, 18-CH_3), 1.14(s, 3H, acetonide CH_3), 1.42(s, 3H, acetonide CH_3), 1.48(s, 3H, 19-CH_3), 4.35(m, 1H, 11-H), 4.4(d of d, J = 55 Hz, J = 20 Hz, 2H, 21-H_2), 5.05(m, 1H, 15-H), 6.11(broad s, 1H, 4-H), 6.33(d of d, J = 11 Hz, J = 2 Hz, 1H, 2-H), 7.00(d, J = 11 Hz, 1H, 1-H)ppm; Analysis: Calc'd for $C_{27}H_{39}FO_6Si$: C, 64.00; H, 7.76; Found: C, 64.00; H, 7.50.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-1,4-pregnadiene-3,20-dione (4)

The silyl ether 3 (10.2 g, 20.1 mmol) was dissolved in dry dimethylformamide (100 ml) and treated with *t*-butyldimethylchlorosilane (15 g, 99.5 mmol) and imidazole (15 g, 220.3 mmol). The reaction mixture was stirred under nitrogen for 24 hr, at which time TLC (ether:hexanes, 1:1) indicated complete reaction. The reaction mixture was poured into ice water and extracted with ethyl acetate. Evaporation of the solvent followed by recrystallization of the residue from hexanes afforded 4 (10.7 g); mp 202-204°C; IR: ν_{max} 1725, 1670, 1665, 1610, 1255, 850, and 780 cm^{-1}; UV: λ_{max} 238 nm (ϵ = 15,524); NMR: 0.07(s, 6H, $Si(CH_3)_2$), 0.20(s, 9H, $Si(CH_3)_3$), 0.82(s, 3H, 18-CH_3), 0.89(s, 9H, $SiC(CH_3)_3$), 1.14(s, 3H, acetonide CH_3), 1.37(s, 3H, acetonide CH_3), 1.44(s, 3H, 19-CH_3), 4.40(m, 1H, 11-H), 4.48(d of d, J = 50 Hz, J = 18 Hz, 2H, 21-H_2), 5.15(m, 1H, 15-H), 6.06(broad s, 1H, 4-H), 6.29(d of d, J = 10 Hz, J = 2 Hz, 1H, 2-H), 6.98(d, J = 10 Hz, 1H, 1-H)ppm; Analysis: Calc'd for $C_{33}H_{53}FO_6Si_2$: C, 63.83; H, 8.60; Found: C, 64.07; H, 8.74.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-4-pregnene-3,20-dione (5)

Palladium on carbon (5%, 4.0 g) was suspended in dioxane (250 ml) in a 500-ml Erlenmeyer flask equipped with an addition side arm and connected to a hydrogen cylinder by means of a manometer for measurement of hydrogen uptake. Hydrogen was passed through the system for 2 min. The manometer was then charged with hydrogen and the catalyst was reduced with vigorous stirring to a constant manometer reading. Compound 4 (4.0 g) was dissolved in dioxane (100 ml) and added to the catalyst by means of the addition side arm. Hydrogen uptake occurred with vigorous stirring and was monitored by means of the manometer. After 1.25 equivalents of hydrogen was utilized, the reaction mixture was filtered from the catalyst and the dioxane was removed under reduced pressure to give 4.2 g of crude material. NMR analysis of the crude product indicated a product mixture of the Δ^1-, Δ^4- and saturated 3-oxo derivatives of 4. A total amount of 16.0 g of 4

was reduced in 4.0 g batches giving rise to 16.5 g of crude material. The product mixture was separated by means of preparative liquid chromatography (one PrepPak column, hexanes:ethyl acetate, 8:2, 200 ml/min). The combined yield of 5 was 6.5 g; mp 181-183°C; IR: ν_{max} 1729, 1676, 1630, 1258, 850, and 780 cm^{-1}; UV: λ_{max} 237 nm (ϵ = 16,080); NMR: 0.09(s, 6H, $Si(CH_3)_2$), 0.16(s, 9H, $Si(CH_3)_3$), 0.82(s, 3H, 18-CH_3), 0.90(s, 9H, $SiC(CH_3)_3$), 1.12(s, 3H, acetonide CH_3), 1.40(s, 3H, acetonide CH_3), 1.42(s, 3H, 19-CH_3), 4.29(m, 1H, 11-H), 4.53(d of d, J = 51 Hz, J = 18 Hz, 2H, 21-H_2), 5.71(s, 1H, 4-H)ppm; Analysis: Calc'd for $C_{33}H_{55}FO_6Si_2$: C, 63.63; H, 8.90; Found: C, 63.85; H, 8.96.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-3,5-seco-4-nor-pregna-5,20-dione-3-carboxylic acid (6)

Solutions of anhydrous potassium carbonate (2.5 g) in water (25 ml), sodium metaperiodate (12.3 g) in water (100 ml), and potassium permanganate in water (0.8%) were prepared prior to the reaction. A solution of the enone 5 (5.5 g, 8.8 mmol) in dichloromethane (50 ml) was diluted with *t*-butanol (100 ml). The potassium carbonate solution (25 ml) and a portion of the sodium metaperiodate solution (30 ml) were added to the reaction mixture with vigorous stirring, followed by the addition of a sufficient quantity of potassium permanganate solution to obtain a steady purple color. The remaining periodate solution was added and vigorously stirred for 5 hr with permanganate solution added as needed. At the end of this time the reaction mixture was diluted to twice its volume with water, cooled in an ice bath, and adjusted to approximately pH 4 with sulfuric acid (50%). The mixture was then quickly extracted 3x with ethyl acetate and the organic extract washed with brine. The ethyl acetate was removed under reduced pressure and the residue taken up in benzene and concentrated twice to remove water and *t*-butanol. Crystallization of the residue from hexanes afforded 6 (4.7 g); mp 122-124°C; IR: ν_{max} 3320, 1720, 1258, 845, and 782 cm^{-1}; NMR: 0.09(s, 6H, $Si(CH_3)_2$), 0.17(s, 9H, $Si(CH_3)_3$), 0.83(s, 3H, 18-CH_3), 0.9(s, 9H, $SiC(CH_3)_3$), 1.14(s, 3H, acetonide CH_3), 1.30(s, 3H, acetonide CH_3), 1.39(s, 3H, 19-CH_3), 4.35(m, 1H, 11-H), 4.49(d of d, J = 51 Hz, J = 18 Hz, 2H, 21-H_2), 5.01(m, 1H, 15-H)ppm; Analysis: Calc'd for $C_{32}H_{55}Si_2FO_8$: C, 59.78; H, 8.62; Found: C, 59.45; H, 8.62.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-3,5-seco-4-nor-5-pregnen-3-oic acid 3,5-lactone (7)

The keto acid 6 (0.5 g, 0.8 mmol) and anhydrous sodium acetate (1.0 g) were dissolved in freshly distilled acetic anhydride (20 ml). The reaction mixture was then heated to reflux under nitrogen. Ten minutes after initiation of reflux, dry triethylamine (2 ml) was added. The reaction mixture was stirred at reflux and monitored by TLC (ether), which indicated complete reaction within 45 min. The reaction mixture was cooled to room temperature, diluted to twice its volume with ethyl acetate, and filtered. The solvents were removed under reduced pressure and the residue taken up in ethyl acetate. The organic phase was washed 3x with saturated sodium bicarbonate

solution, once with brine, and concentrated. The crude product was purified using a silica gel "dry" column (2.5 cm x 100 cm) developed with diethyl ether:hexanes (1:1). The enol lactone 7 was isolated 52-60 cm from the top of the column. The material obtained from the column was crystallized from hexanes to afford 7 (0.3 g); mp 174-176°C; IR: ν_{max} 1780, 1765, 1730, 1692, 1255, 845, and 782 cm^{-1}; NMR: 0.04(s, 6H, $Si(CH_3)_2$), 0.10(s, 9H, $Si(CH_3)_3$), 0.75(s, 3H, 18-CH_3), 0.86(s, 9H, $SiC(CH_3)_3$), 1.12(s, 3H, acetonide CH_3), 1.32(s, 3H, acetonide CH_3), 1.39(s, 3H, 19-CH_3), 4.4(m, 1H, 11-H), 4.47(d of d, J = 51 Hz, J = 18 Hz, 2H, 21-H_2), 5.02(m, 1H, 15-H), 5.20(t, J = 3 Hz, 1H, 6-H)ppm; MS: m/e = 567 (M^+-57, *t*-butyl); Analysis: Calc'd for $C_{32}H_{53}FO_7Si_2$: C, 61.50; H, 8.55; Found: C, 61.48; H, 8.64.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-3-hydroxy-3-methyl-3(5→6βH)abeo-A-4-norpregna-5,20-dione (8)

To a magnetically stirred solution of methyl magnesium iodide (1 mmol) in diethyl ether (3 ml) was added dropwise the enol-lactone 7 (0.687 g, 1.1 mmol) dissolved in a mixture of anhydrous ether (2 ml) and benzene (5 ml). After one hour of stirring at room temperature, the reaction mixture was cooled to 0°C in an ice bath and quenched with a saturated ammonium chloride solution. After warming to room temperature the reaction mixture was taken up in ethyl acetate, washed once with water and once with brine. Removal of the solvent *in vacuo* gave 0.62 g of residue. A small quantity (0.2 g) of this material was recrystallized from hexanes for characterization; mp 172-174°C; IR: ν_{max} 3460, 1725, 1385, and 846 cm^{-1}; NMR: 0.07(s, 6H, $Si(CH_3)_2$), 0.14(s, 9H, $Si(CH_3)_3$), 0.73(s, 3H, 18-CH_3), 0.92(s, 9H, $SiC(CH_3)_3$), 1.17(s, 3H, acetonide CH_3), 1.27(s, 3H, 19-CH_3), 1.47(s, 3H, 3-CH_3), 4.47(d of d, J = 52 Hz, J = 18 Hz, 2H, 21-H_2), 5.1(m, 1H, 11-H)ppm; MS: m/e = 582 (M^+-57 *t*-butyl); Analysis: Calc'd for $C_{33}H_{57}FO_7Si_2$: C, 61.84; H, 8.96; Found: C, 61.67; H, 9.06.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-3-hydroxy-3-methyl-3(5→6βH)abeo-A-norpregna-5,20-dione-4-^{14}C (8a)

The above procedure was repeated with compound 7 (625 mg, 1 mmol) and methyl-^{14}C iodide (0.13 g, 0.91 mmol, 50 mCi) and magnesium (30 mg, 1.25 mmol) to give the C-4 labeled compound 8a, indicated by a TLC radiochromatographic scan to consist of a single radioactive product identical in R_f to that of the unlabeled material.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-4-pregnene-3,20-dione (5)

A solution of 2,6-di-*t*-butylphenol (6 g) in dry tetrahydrofuran (100 ml) was de-gassed with dry nitrogen for 30 min followed by the addition of n-butyllithium in hexanes (10 ml, 4.5 mmol). The mixture was then stirred at room temperature for 30 min prior to use.

To a solution of the crude Grignard product, 8, in dry tetrahydrofuran (3 ml) a portion (15 ml) of the lithium 2,6-di-*t*-butylphenoxide was added under nitrogen. The reaction mixture was then stirred at reflux for 2.5 hr, poured into an ice cold phosphate buffer (pH 7), and taken up in ethyl acetate. The organic phase was washed once with water, once with brine, and concentrated *in vacuo*. The residue was purified by "dry column" chromatography (ether:hexanes, 1:1) to afford 5 (0.37 g).

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-4-pregnene-3,20-dione-4-^{14}C (9)

The above procedure was repeated with compound 8a to afford 9 (0.22 g), indicated by a TLC radiochromatographic scan to consist of a single radioactive product identical in R_f to that of the unlabeled material.

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-1,4-pregnadiene-3,20-dione (4)

The enone 5 (0.3 g, 0.35 mmol), selenium dioxide (0.1 g, 0.9 mmol), pyridine (0.04 ml), and *t*-butanol (15 ml) were combined and heated at reflux under nitrogen for 20 hr. At the end of this time the reaction mixture was cooled to room temperature, diluted with ethyl acetate, filtered through Celite, and concentrated. The residue was purified by "dry column" chromatography (ether: hexanes, 1:1) followed by crystallization from hexanes to give the pure $\Delta^{1,4}$-compound 4 (0.16 g).

9α-Fluoro-11β-trimethylsilyloxy-16α,17α-isopropylidenedioxy-21-*t*-butyldimethylsilyloxy-1,4-pregnadiene-3,20-dione-4-^{14}C (10)

The above procedure was repeated with compound 9 (0.22 g, 0.35 mmol), selenium dioxide (0.1 g, 0.9 mmol), pyridine (2 ml) and *t*-butanol (8 ml), heated to reflux for 48 hr to afford the pure $\Delta^{1,4}$-3-oxo-compound 10 (0.13 g) along with recovered Δ^4-3-oxo-compound 9 (0.05 g). The recovered material was recycled to afford an additional 0.013 g of 10. The identification and purity of compound 10 was established by a radiochromatographic scan.

Triamcinolone acetonide (1)

All glassware was initially rinsed with EDTA solution (1%) followed by glass distilled water. The dienone 4 (0.16 g, 0.26 mmol) and de-gassed methanol (5 ml) were combined in a 50-ml, two-necked flask equipped with a reflux condenser and a rubber septum. The system was flushed with nitrogen and then heated at 60°C. A sufficient amount of freshly distilled tetrahydrofuran (2 ml) was then added to complete dissolution of the solid material, followed by 2N hydrochloric acid (1 ml, prepared from Baker analyzed reagent grade). The reaction mixture was then stirred at 60°C for 2.5 hr under nitrogen. At the end of this time the reaction mixture was cooled to room temperature, poured into ice cold phosphate buffer (pH 7), and taken up in chloroform. The organic phase was

washed once with a saturated sodium bicarbonate solution, once with brine, and concentrated *in vacuo*. The residue was crystallized from methanol to afford 1 (0.09 g).

Triamcinolone acetonide-4-^{14}C (11)

The above procedure was repeated with compound 10 (0.143 g, 0.23 mmol) with the exception that the residue obtained upon concentration was triturated with ether to give 0.081 g of crystalline material. Removal of the ether *in vacuo* afforded 0.05 g of a residue. Both the crystalline material and the residue obtained from evaporation of ether, when examined by TLC and HPLC, were indicated to be homogeneous. However, both samples retained undetermined amounts of solvent of crystallization. The samples were dried under vacuum at 60°C for a short period of time. Prolonged heating at elevated temperatures to remove the last traces of solvent of crystallization was not attempted because such heating is known to induce radiolysis of compounds having a corticoid side chain.

HPLC Analysis of Triamcinolone acetonide-4-^{14}C (11)

The radiochemical purity of the title compound was determined by co-injection of the ^{14}C-labeled material with unlabeled triamcinolone acetonide on a reverse phase C_{18} column (Chromegabond MC-18, 10 μm, 30 cm x 4.6 mm) using a methanol:water solvent system (55:45) at a flow rate of 1 ml/min. The unlabeled material was monitored by UV absorption (240 nm) and the labeled material by scintillation counting of 0.5 ml fractions collected every 30 sec.

The method of sample preparation for injection is highly critical and was carried out as follows. All glassware was initially rinsed with 1% EDTA solution followed by glass distilled water. A small amount ($\ll$ 1 mg, estimated) of the ^{14}C-labeled material was dissolved in 10 ml of HPLC-grade (de-gassed) methanol containing unlabeled triamcinolone acetonide (1 mg/ml). This solution was then used for injection to give the HPLC scans shown in Fig. 1 for the crystalline product.

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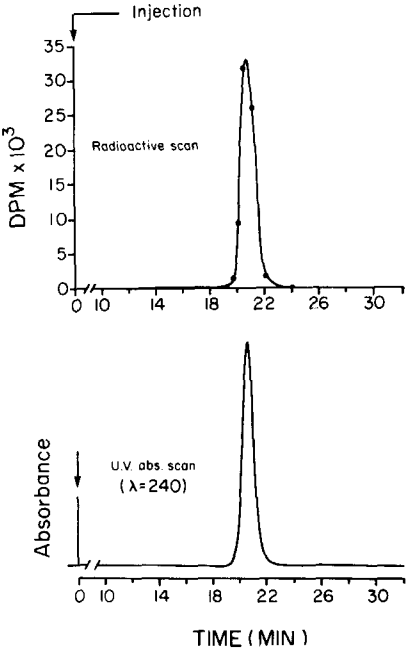

Fig. 1. HPLC analysis of a mixture of unlabeled and 4-^{14}C-labeled triamcinolone acetonide on a reverse phase C_{18} column (Chromega-bond MC-18, 10 μm, 30 cm x 4.6 mm) using MeOH:H_2O (55:45) solvent system at a flow rate of 1 ml/min.

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