SYNTHESIS OF DEXAMETHASONE-4-^{14}C

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SUMMARY

The bismethylenedioxy (BMD) derivative of dexamethasone 2 was silylated with trimethylchlorosilane and imidazole in dimethylformamide to give the 11β-trimethylsilyloxy BMD derivative 3. The Δ^1-double bond in 3 was hydrogenated over 5% palladium on carbon to give the Δ^4-3-oxo steroid 4. Oxidation of 4 with potassium permanganate-sodium metaperiodate gave the seco-acid 5 which on subsequent treatment with acetic anhydride; sodium acetate and triethylamine gave the enol-lactone 6. The enol-lactone 6 was reacted with ^{14}C-methylmagnesium iodide to give an adduct 7a which on heating at reflux with lithium 2,6-di-*t*-butylphenoxide in dioxane gave the Δ^4-3-oxo derivative 8. Compound 8 was heated at reflux with *m*-iodylbenzoic acid and diphenyl diselenide in toluene to give the $\Delta^{1,4}$-3-oxo steroid 9. The protecting BMD and silyl groups were removed in a single step by reaction with aqueous trifluoroacetic acid containing 2N hydrochloric acid at room temperature to give dexamethasone-4-^{14}C 10.

Key Words: 4-^{14}C-dexamethasone, *m*-iodylbenzoic acid, diphenyl diselenide, lithium 2,6-di-*t*-butylphenoxide

INTRODUCTION

Synthetic glucocorticoids, which are widely used as anti-inflammatory agents and for the treatment of allergic conditions in humans, have been implicated to cause some teratogenic effects in experimental animals. Walker [1] observed that among the different glucocorticoids, triamcinolone and dexamethasone are most teratogenic and can induce cleft palate in rabbits. Hendrickx *et al.* [2,3] found that triamcinolone acetonide can cause teratogenic effects on the skeletal and lymphoid systems in nonhuman primates. In order to study the metabolism and the role of different maternal and fetoplacental metabolites on the cause of teratogenic effects, it is desirable to have suitably labeled substrates. In this publication we describe an efficient synthesis of dexamethasone-4-^{14}C.

DISCUSSION

Earlier Mertel *et al.* [4] have described the synthesis of ^{14}C-labeled dexamethasone with the

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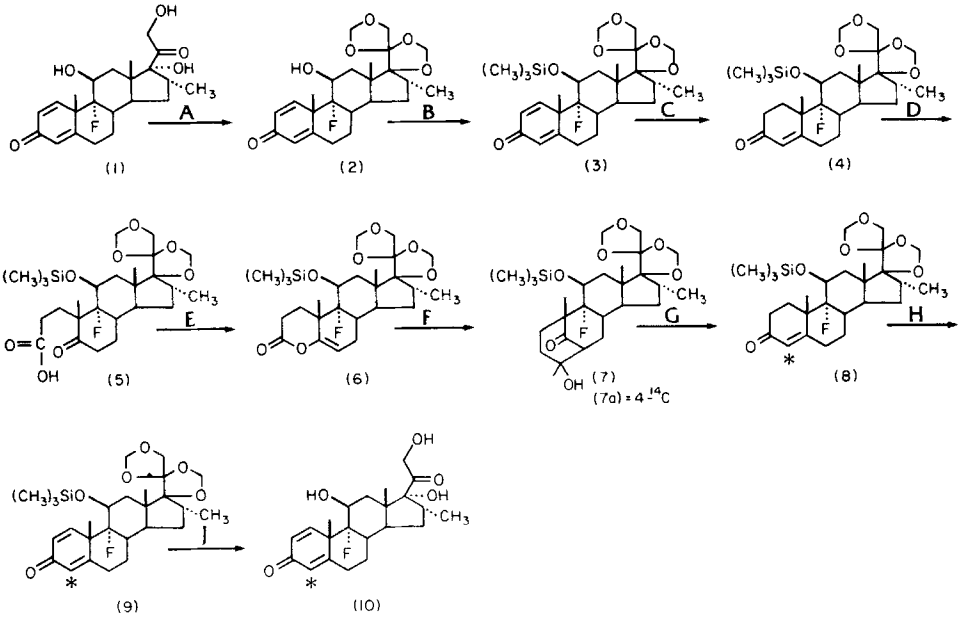
Scheme I



A: Paraformaldehyde, HCl, H_2O, CH_2Cl_2

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

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

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

E: Ac_2O - NaOAc, NEt_3, 145°C

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

G: Dioxane, lithium 2,6-di-t-butylphenoxide, 102°C

H: Toluene, m-iodylbenzoic acid, diphenyl diselenide, 110°C

I: Trifluoroacetic acid, HCl (2N), room temp

label located in the 16α-methyl group. Their synthesis was very lengthy and involved more than fifteen steps after the carbon-14 was incorporated. Ideally, a radiochemical synthesis should consist of only a few steps after the desired label has been incorporated in order to achieve high overall yield. Our strategy for the synthesis of the title compound was to employ dexamethasone itself as starting material, suitably protect the sensitive functional groups, and convert the compound to a Δ^4-3-oxo steroid before adapting the classical Turner procedure [5], which was further modified by Fujimoto [6], for the preparation of 4-^{14}C-labeled steroids. Accordingly, in our present synthesis of dexamethasone-4-^{14}C there are only three steps after carbon-14 is introduced, and we obtained the labeled compound with high specific activity (50.6 mCi/mmol).

The dihydroxyacetone side chain of dexamethasone (1, Scheme I) was protected as the bismethylenedioxy (BMD) derivative 2 [7]. The 11β-hydroxyl group in 2 was then silylated with trimethylchlorosilane and imidazole in dimethylformamide to give the trimethylsilyloxy (TMS) derivative 3. The Δ^1-double bond present in 3 was catalytically reduced with hydrogen over 5% palladium on carbon to give a mixture of products from which the Δ^4-3-oxo derivative 4 was separated by preparative liquid chromatography. Compound 4 is now suitably disposed to adapt the general procedure of Turner [5]. Compound 4 was subjected to a modified potassium permanganate-sodium metaperiodate oxidation [8] to give the seco-acid 5 in good yield. The seco-acid 5 was treated with a mixture of acetic anhydride, sodium acetate, and triethylamine [9] to give the desired enol-lactone 6. In the next step the reaction conditions were optimized with the enol-lactone 6 employing unlabeled methylmagnesium iodide to obtain the abeo derivative 7. In the actual radio-synthesis, the enol-lactone 6 was subjected to Grignard reaction with ^{14}C-methylmagnesium iodide (50 mCi, 51.0 mCi/mmol) to give the ^{14}C-abeo derivative 7a. Reaction of the abeo compound 7a with lithium 2,6-di-*t*-butylphenoxide in dioxane at reflux [10] gave the Δ^4-3-oxo derivative 8. Attempts to obtain the $\Delta^{1,4}$-3-oxo compound with selenium dioxide oxidation gave inferior results. In a recent publication Barton *et al.* [11] described an excellent procedure for the preparation of $\Delta^{1,4}$-3-oxo steroids by employing *m*-iodylbenzoic acid and diphenyl diselenide. When the Δ^4-3-oxo steroid 8 was heated with *m*-iodylbenzoic acid and diphenyl diselenide in toluene the $\Delta^{1,4}$-3-oxo derivative 9 was obtained. Normally the BMD protecting group is hydrolyzed to the dihydroxy-acetone side chain under acidic conditions by heating with dilute formic acid or acetic acid [7]. When we attempted hydrolysis of the unlabeled BMD derivative 3 with 50% acetic acid or 50% dichloroacetic acid, only partial hydrolysis occurred. Other acidic catalysts, such as Nafion-H® [12] or dilute hydrochloric acid in tetrahydrofuran, hydrolyzed only the TMS ether function. Finally, we discovered that when compound 3 was treated at room temperature with aqueous trifluoroacetic acid containing 2N hydrochloric acid, both the TMS and BMD protecting groups were hydrolyzed to give dexamethasone in excellent yield. Accordingly, the labeled compound 9 was hydrolyzed with a mixture of aqueous trifluoroacetic acid and 2N hydrochloric acid at room temperature to give 4-^{14}C-dexamethasone 10, with a specific activity of 50.6 mCi/mmol.

EXPERIMENTAL

Most chemicals and solvents were analytical reagent grade and were used without further purification. Some reagents and solvents, such as acetic anhydride, dioxane, and triethylamine, were purified according to standard laboratory procedures. Methyl iodide-^{14}C (50 mCi; 51 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 determined on a Finigan quadrupole mass spectrometer. 'Dry column' chromatography was performed on Woelm silica gel in a nylon column as described by Loev and Goodman [13]. Thin layer chromatographic (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 materials 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-362 radiochromatogram scanner. Preparative high pressure liquid chromatography (HPLC) was carried out on a Waters Associates Prep LC/System 500 employing PrepPak 500/silica cartridge. HPLC analysis of ^{14}C-labeled material was carried out on Waters Associates HPLC equipment (Model 6000A pump) employing a reverse phase Partisil PXS 10/25 ODS-3 column (Whatman), and monitored by an LDC Spectromonitor III and a Beckman LS 7500 scintillation counter. Microanalyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-hydroxy-16α-methyl-1,4-pregnadien-3-one (2)

Paraformaldehyde (100 g), water (300 ml), and concentrated hydrochloric acid (38%, 300 ml) were combined and stirred at room temperature for 4 hr. A suspension of dexamethasone (1) (10 g, 25.5 mmol) in dichloromethane (500 ml) was then added and the reaction mixture was vigorously stirred for an additional 4 hr. The mixture was diluted with dichloromethane (500 ml), washed once with saturated sodium bicarbonate solution, and once with brine. Removal of the solvent, followed by recystallization of the residue from methanol:dichloromethane, afforded pure compound 2 (8.9 g): mp 310-313°C (dec.); Lit. mp 310-320°C [7]. IR: ν_{max} 3415, 1665, 1620, 1605, and 1090 cm^{-1}; UV: λ_{max} = 239 nm (ϵ = 15,900); NMR: 0.93(d, J = 6.4 Hz, 3H, 16-CH_3), 1.16(s, 3H, 18-CH_3) 1.52(s, 3H, 19-CH_3), 3.95(s, 2H, 21-H_2), 4.27(m, 1H, 11-H), 4.99(d of d, J = 11.2 Hz, J = 1.2 Hz, 2H, dioxymethylene), 5.1(d, J = 14.4 Hz, 2H, dioxymethylene), 6.07(broad s, 1H, 4-H),

6.28(d of d, J = 9.6 Hz, J = 2 Hz, 1H, 2-H), 7.16(d, J = 9.6 Hz, 1-H)ppm; Analysis: Calc'd for $C_{24}H_{31}FO_6$: C, 66.34; H, 7.19. Found: C, 66.28; H, 7.19.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-1,4-pregnadien-3-one (3)

Trimethylchlorosilane (7 ml, 55 mmol) was added under nitrogen atmosphere to a solution of compound 2 (5 g, 11.5 mmol) and imidazole (5 g, 73.4 mmol) in dry dimethylformamide (200 ml). The mixture was stirred at room temperature for 16 hr, poured into an ice cold phosphate buffer (pH 7), and extracted with dichloromethane (3x). Evaporation of the solvent followed by recrystallization of the residue from ether:hexanes afforded pure compound 3 (4.9 g); mp 215.5-217°C. IR: ν_{max} 1665, 1630, 1608, 1080, 845, and 755 cm^{-1}; UV: λ_{max} 238.5 nm (ϵ = 14,800); NMR: 0.16(s, 9H, $Si(CH_3)_3$), 0.88(d, J = 6.3 Hz, 3H, 16-CH_3), 1.08(s, 3H, 18-CH_3), 1.43(s, 3H, 19-CH_3), 3.93(s, 2H, 21-H_2), 4.25(d of t, J_{HF} = 9 Hz, J_{HH} = 2.7 Hz, 1H, 11-H), 4.97(d of d, J = 13.5 Hz, J = 1.2 Hz, 2H, dioxymethylene), 5.08(d, J = 14.4 Hz, 2H, dioxymethylene), 6.06(broad s, 1H, 4-H), 6.29(d of d, J = 9.9 Hz, J = 1.8 Hz, 1H, 2-H), 7.0(d, J = 9.9 Hz, 1H, 1-H)ppm; Analysis: Calc'd for $C_{27}H_{39}FO_6Si$: C, 64.00; H, 7.76. Found: C, 63.87; H, 7.86.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-4-pregnen-3-one (4)

Palladium on carbon (5%, 3.0 g) was suspended in dioxane (200 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 minutes. The manometer was then charged with hydrogen and the catalyst was reduced with vigorous stirring to a constant manometer reading. Compound 3 (3.0 g, 5.9 mmol) was dissolved in dioxane (50 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 1.15 equivalents of hydrogen was utilized, the reaction mixture was filtered from the catalyst and the solvent was removed under reduced pressure to give 3.0 g of crude material. NMR analysis of the crude material indicated a product mixture consisting of the Δ^1, Δ^4, and saturated 3-oxo derivatives of 3. A total amount of 24.0 g of 3 was reduced in 3.0 g batches giving rise to 25.1 g of crude product mixture. The product mixture was separated by means of preparative liquid chromatography (one PrepPak column, hexanes:ethyl acetate:dichloromethane, 7:2:1, 200 ml/min). The combined yield of 4 was 8.9 g; mp 231-233°C. IR: ν_{max} 1670, 1625, 1085, 845, and 755 cm^{-1}; UV: λ_{max} 238.5 nm (ϵ = 17,300); NMR: 0.09(s, 9H, $Si(CH_3)_3$), 0.85(d, J = 6.3 Hz, 3H, 16-CH_3), 1.04(s, 3H, 18-CH_3), 1.4(s, 3H, 19-CH_3), 3.91(s, 2H, 21-H_2), 4.2(m, 1H, 11-H), 4.97(d of d, J = 9.9 Hz, J = 1.2 Hz, 2H, dioxymethylene) 5.07(d, J = 11.7 Hz, 2H, dioxymethylene), 5.71(s, 1H, 4-H)ppm; MS: m/e = 508 (M^+); Analysis: Calc'd for $C_{27}H_{41}FO_6Si$: C, 63.75; H, 8.12. Found: C, 63.99; H, 8.31.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-3,5-seco-4-nor-pregna-5-one-3-carboxylic acid (5)

Solutions of anhydrous potassium carbonate (4.5 g) in water (45 ml), sodium metaperiodate (21.5 g) in water (175 ml), and potassium permanganate in water (0.8%) were prepared prior to the reaction. A solution of enone 4 (4.5 g, 14.7 mmol) in dichloromethane (50 ml) was diluted with *t*-butanol (175 ml). The potassium carbonate solution (4.5 ml) and a portion of the sodium metaperiodate solution (25 ml) were added to the reaction mixture with vigorous stirring, followed by the addition of a sufficient quantity of potassium permanganate solution to maintain a constant purple color. The remaining portion of the sodium metaperiodate solution was added over the next 30 min, with the addition of permanganate when needed to maintain the color. The reaction mixture was then vigorously stirred at room temperature for 3 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 to 0°C in an ice bath, and adjusted to approximately pH 4 with sulfuric acid (50%). The mixture was then quickly extracted (3x) with dichloromethane and the organic extract washed with brine. Removal of the solvent under reduced pressure followed by trituration of the residue with ether:hexanes at -78°C afforded 5 (6.9 g); mp 123-128°C. IR: ν_{max} 3480, 1710, 1090, 845 and 755 cm^{-1}; NMR: 0.18(s, 9H, $Si(CH_3)_3$), 0.93(d, J = 5.4 Hz, 3H, 16-CH_3), 1.15(s, 3H, 18-CH_3), 1.33(s, 3H, 19-CH_3), 3.98(s, 2H, 21-H_2), 4.26(m, 1H, 11-H), 5.03(d of d, J = 13 Hz, J = 1.2 Hz, 2H, dioxymethylene), 5.13(d, J = 13 Hz, 2H, dioxymethylene)ppm; MS: m/e = 508 (M^+ -20, HF); Analysis: Calc'd for: $C_{26}H_{41}FO_8Si$: C, 59.07; H, 7.82. Found: C, 58.80; H, 7.95.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-3,5-seco-4-nor-5-pregnen-3-oic acid 3,5-lactone (6)

The keto acid 5 (1.0 g, 1.9 mmol) and anhydrous sodium acetate (2.5 g, 30.5 mmol) were dissolved in freshly distilled acetic anhydride (50 ml). The reaction mixture was then heated to reflux under nitrogen. Five min after initiation of reflux, dry triethylamine (5 ml) was added. The reaction mixture was then stirred at reflux and monitored by TLC (ether:hexanes, 1:1), which indicated complete reaction after 45 min. The reaction mixture was cooled to room temperature and the solvent removed under reduced pressure. The residue was taken up in ethyl acetate, filtered, and washed with brine. Removal of the solvent, purification of the residue by 'dry column' chromatography (ether:hexanes, 1:2), and crystallization from ether:hexanes, afforded pure 6 (0.66 g); mp 178-181°C. IR: ν_{max} 1772, 1758, 1690, 1090, 845, and 755 cm^{-1}; NMR: 0.15(s, 9H, $Si(CH_3)_3$), 0.94(d, J = 6.3 Hz, 16-CH_3), 1.10(s, 3H, 18-CH_3), 1.38(s, 3H, 19-CH_3), 3.96(s, 2H, 21-H_2), 4.3(d of t, J_{HF} = 10.8 Hz, J = 3.15 Hz, 1H, 11-H), 5.02(d of d, J = 12.6 Hz, J = 1.2 Hz, 2H, dioxymethylene), 5.11(d, J = 12.6 Hz, 2H, dioxymethylene), 5.23(t, J = 2.7 Hz, 1H, 6-H)ppm; MS: m/e = 510 (M^+); Analysis: Calc'd for $C_{26}H_{39}FO_7Si$: C, 61.15; H, 7.70. Found: C, 61.11; H, 7.85.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-3-hydroxy-3-methyl-3-(5→6βH)abeo-A-norpregna-5-one (7)

Magnesium turnings (0.034 g, 1.4 mmol) were added to a 50-ml, two-necked flask equipped with a reflux condenser and a rubber septum. The contents were flushed with dry nitrogen, stirred magnetically and dried by flaming the flask. The flask was allowed to cool to room temperature and ice water was circulated through the condenser. Anhydrous diethyl ether (2 ml) and ethylene dibromide (0.02 nl, 0.043 g, 0.23 mmol) were added. Evidence of reaction (ethylene evolution, turbidity, etc.) indicated the system was dry and ready for the introduction of methyl iodide. Methyl iodide (0.06 ml, 0.142 g, 1 mmol) was added. Initiation of reaction was immediate as indicated by solvent reflux. Reflux was maintained to the completion of the reaction by means of a warm water bath. At the end of this time enol lactone 6 (0.51 g, 1 mmol) in anhydrous benzene (2 ml) was added. After 1 hr 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 followed by crystallization of the residue from ether:hexanes gave pure 7 (0.45 g); mp 188-190°C. IR : ν_{max} 3450, 1730, 845, and 755 cm^{-1}; NMR: 0.15(s, 9H, $Si(CH_3)_3$), 0.95(d, J = 7.5 Hz, 3H, 16-CH_3), 1.04(s, 3H, 18-CH_3), 1.2(s, 3H, 19-CH_3), 1.27(s, 3H, 3-CH_3), 3.97(s, 2H, 21-H_2), 4.02(m, 1H, 11-H), 5.06(d of d, J = 12.6 Hz, J = 1.4 Hz, 2H, dioxymethylene), 5.16(d, J = 10.8, 2H, dioxymethylene)ppm; MS: m/e = 526 (M^+); Analysis: Calc'd for $C_{27}H_{43}FO_7Si$: C, 61.57; H, 8.23. Found: C, 61.29; H, 8.37.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-3-hydroxy-3-methyl-3-(5→6βH)abeo-A-norpregna-5-one-4-^{14}C (7a)

The above procedure was repeated with compound 6 (0.5 g, 1 mmol) and ^{14}C-methyl iodide (0.141 g, 0.98 mmol, 50 mCi) to give the C-4 labeled compound 7a, indicated by a TLC radiochromatographic scan to consist of a single radioactive product identical in Rf to that of the unlabeled material. The total Grignard product was used in the subsequent reaction without further purification.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-4-pregnen-3-one (4)

A solution of 2,6-di-*t*-butylphenol (5 g, 24.3 mmol) in dry dioxane (100 ml) was de-gassed for 30 min followed by the addition of *n*-butyllithium in hexanes (50 ml, 23 mmol). The mixture was then stirred at room temperature for 30 min prior to use.

To a solution of the crude Grignard product 7 in dry dioxane (4 ml) a portion (10 ml) of the lithium 2,6-di-*t*-butylphenoxide solution was added under nitrogen. The reaction mixture was then heated at reflux and stirred under nitrogen for 3 hr. At the end of this time the reaction mixture was cooled to room temperature, poured into an ice cold phosphate buffer(pH 7) and extracted (3x)

with ethyl acetate. The organic extract 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 4 (0.37 g).

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-4-pregnen-3-one-4-^{14}C (8)

The above procedure was repeated with compound 7a to afford 8 (0.34 g), indicated by a TLC radiochromatographic scan to consist of a single radioactive product identical in Rf to that of the unlabeled material.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-1,4-pregnadien-3-one (3)

I. *m*-Iodylbenzoic acid

A solution of sodium hydroxide (50 g, 1.25 moles) in water (200 ml) was cooled to 0°C followed by the addition of crushed ice (100 g). Chlorine gas was then bubbled through the solution until 41 g (0.58 mole) was absorbed. The resulting solution of sodium hypochlorite was kept in the dark at 0°C until needed. The concentration of the sodium hypochlorite solution was determined to be 1.9M by adding a sample to an acidified solution of potassium iodide and titrating the liberated iodine with a standard sodium thiosulfate solution [14].

The sodium hypochlorite solution (150 ml, 0.285 mole) was added dropwise to a solution of *m*-iodobenzoic acid (10 g, 0.04 mole) in acetic acid (100 ml). The reaction mixture was then stirred at room temperature until the precipitation of product was complete (15-20 min). The precipitate was filtered, washed with water and dried *in vacuo* to afford the *m*-iodylbenzoic acid (11.1 g): mp 225°C (deflagrates); Lit. mp 230°C [11].

II. Oxidation of 9α-Fluoro-17α,20;20,21-bismethylenedioxy-11β-trimethylsilyloxy-16α-methyl-4-pregnen-3-one (4)

m-Iodylbenzoic acid (0.675 g, 2.4 mmol), diphenyl diselenide (0.046 g, 0.15 mmol), and dry toluene (20 ml) were combined and heated at reflux under nitrogen until the yellow color of the diselenide disappeared (5-15 min). Compound 4 (0.37 g, 0.73 mmol) in toluene (3 ml) was added and heating was continued for an additional 3 hr. After cooling, the reaction mixture was taken up in ethyl acetate, washed (3x) with saturated sodium bicarbonate solution, and once with brine. Removal of the solvent followed by purification of the residue by 'dry column' chromatography gave pure 3 (0.26 g).

9α-Fluoro-17α,20;20,21-bismethylenedioxy-11α-trimethylsilyloxy-16α-methyl-4-pregnen-3-one-4-^{14}C (9)

The above procedure was repeated with compound 8 (0.34 g, 0.67 mmol), *m*-iodylbenzoic acid (0.62 g, 2.2 mmol), diphenyl diselenide (0.042 g, 0.14 mmol), and toluene (20 ml) to afford 9 (0.19 g). The identification and purity of compound 9 was established by a radiochromatographic scan.

Dexamethasone (1)

All glassware was initially rinsed with EDTA solution (1%) followed by glass distilled water. Compound 3 (0.26 g, 0.51 mmol), trifluoroacetic acid (4 ml), and 2N hydrochloric acid (4 ml, prepared from Baker analyzed grade) were combined under nitrogen and stirred at room temperature for 3 hr. At the end of this time the reaction mixture was diluted with ethyl acetate, washed (3x) with water and once with brine. Removal of the solvent followed by crystallization from acetone: ether afforded pure 1 (0.15 g).

Dexamethasone-4-^{14}C (10)

The above procedure was repeated with compound 9 (0.19 g, 0.37 mmol) and identical amounts of trifluoroacetic acid and 2N hydrochloric acid for a period of 3-4 hr. Crystallization from acetone: ether afforded pure 10 (0.085 g). The mother liquors from the crystallization were concentrated and the residue was purified by 'dry column' chromatography to afford a second crop, slightly less pure, of 0.006 g. The specific activity of the first crop was determined as 50.6 mCi/mmol.

HPLC Analysis of Dexamethasone-4-^{14}C

The radiochemical purity was determined by co-injection of ^{14}C-labeled sample plus unlabeled dexamethasone on a reverse phase Partisil PXS 10/25 ODS-3 column (Whatman) using a methanol: water solvent system (55:45) at a flow rate of 1 ml/min. The unlabeled material was monitored by U.V. 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 a 1% EDTA solution followed by glass distilled water. A small amount ($\ll$ 1 mg, estimated) of the ^{14}C-labeled sample was dissolved in 5 ml of HPLC-grade (de-gassed) methanol containing unlabeled dexamethasone (1 mg/ml). This solution was then used for injection to give the HPLC scans shown in Fig. 1.

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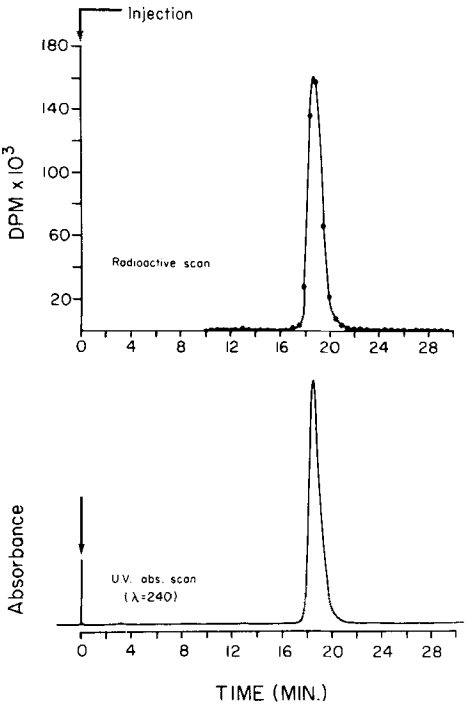

Fig. 1. HPLC analysis of a mixture of unlabeled and 4-^{14}C labeled dexamethasone on a reverse phase C_{18} column (Whatman, Partisil 10 ODS-3, 10 μm, 25 cm x 4.60 mm) using MeOH:H_2O (55:45) solvent system at a flow rate of 1 ml/min.

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