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SYNTHESIS OF MULTIPLY DEUTERIUM-LABELLED PREDNISONE AND PREDNISOLONE

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SUMMARY

We have synthesised prednisone and prednisolone labelled with four deuterium atoms at chemically stable sites ([1,19,19,19-$^2H_4$]prednisone and [1,19,19,19-$^2H_4$]prednisolone), starting from [1,1,19,19,19-$^2H_5$]cortisone. The isotopic purities were demonstrated to be >98 atom%. There were no indications of deuterium loss from the C-19 and C-1 positions during the synthetic sequence involving an oxidation reaction step using selenium dioxide in *tert*-butanol.

Key Words: [1,19,19,19-$^2H_4$]prednisone, [1,19,19,19-$^2H_4$]prednisolone, [1,1,19,19,19-$^2H_5$]cortisone, deuterium labelling, deuterium labelled prednisone, deuterium labelled prednisolone

INTRODUCTION

Stable isotope methodology has provided a useful tool for clinical and pharmacokinetic investigations on steroid hormones in humans.[1-8] Several methods have been reported for the synthesis of ^2H-labelled corticoids for use as internal standards in stable isotope dilution mass spectrometry, including catalytic deuteriation[9] and hydrogen-deuterium exchange reactions.[10,11] It has recently been demonstrated that considerable losses of label during derivatization towards the GC-MS assays were observed for prednisolone, dexamethasone and betamethasone labelled with deuterium by a simple hydrogen-deuterium exchange reaction.[12] The difficulties encountered in the accurate and precise analysis of these compounds were apparently due

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to the fact that ^2H-atoms had been originally incorporated into the labile positions at C-2, C-4 and C-6 in the steroidal skeleton. Successful application of methodology with stable isotopes is dependent upon the availability of compounds which are labelled at predesignated positions.

We now report the synthesis of multiply labelled prednisone and prednisolone with deuterium at the non-exchangeable C-1 and C-19 positions.

EXPERIMENTAL

^1H N.m.r. spectra were determined on Varian Gemini-300 300 MHz and Brucker AM-400 400 MHz spectrometers for solutions in $CDCl_3$ and CD_3OD (Me_4Si as internal standard). E.i. mass spectra were recorded on a Hitachi M-80 mass spectrometer and on a Shimadzu GCMS-QP1000 gas chromatograph-mass spectrometer at 70 eV. Preparative t.l.c. was performed on glass plates coated with a 0.5-mm layer of silica gel 60 F_{254} (Merck). Column chromatography was performed on silica gel C-200 (74-149 μm, Wakogel). Tetrahydrofuran (THF), CH_2Cl_2, and MeOH were redistilled before use and all other chemicals and reagents were of analytical reagent grade and were used without further purification.

[1,1,19,19,19-$^2H_5$]-17α,20;20,21-*bismethylenedioxypregn*-4-*ene*-3,11,20-*trione* (*Cortisone-d$_5$-BMD*) (**2**).—35% HCl (0.25 mL) and 37% HCHO (0.25 mL) were added to a $CHCl_3$ suspension (0.5 mL) of cortisone-d_5 (**1**) (25 mg) synthesised in this laboratory[13] and the reaction mixture was stirred at room temperature for 15 h. After the aqueous layer was discarded, the organic layer was washed with saturated $NaHCO_3$ and then with water. The solution was dried with Na_2SO_4 and evaporated to dryness under reduced pressure. The resultant material was triturated with ether, filtered, and washed with cold ether to give colourless crystals of cortisone-d_5-BMD (**2**) (17.5 mg). Cortisone-d_5-BMD (**2**); δ_H (400 MHz, $CDCl_3$) 0.83 (3H, s, 13-Me), 3.94-4.00 (2H, d, 21-CH_2), 5.01-5.20 (4H, m, -OCH_2O-), and 5.73 (1H, s, 4-H); *m/z* 407 (M^+). A proton signal at δ_H 1.42 (10-Me) disappeared in cortisone-d_5-BMD. Cortisone-BMD; δ_H (400 MHz, $CDCl_3$) 0.83 (3H, s, 13-Me), 1.42 (3H, s, 10-Me), 3.94-4.00 (2H, d, 21-CH_2), 5.01-5.20 (4H, m, -OCH_2O-), and 5.73 (1H, s, 4-H); *m/z* 402 (M^+).

[1,19,19,19-$^2H_4$]-17α,21-*Dihydroxypregna*-1,4-*diene*-3,11,20-*trione* (*prednisone-d$_4$*) (**4**).— A suspension of cortisone-d_5-BMD (**2**) (9.2 mg) and selenium dioxide (11 mg) in *tert*-butyl alcohol (1.0 ml) was refluxed for 24 h under the argon atmosphere. After filtration with aid of MeOH, the solvent was evaporated to dryness under reduced

pressure. To the residue was added MeOH (1 ml) and deactivated Raney nickel catalyst (15 mg), and the mixture was stirred for 2 h at room temperature. After filtration with aid of charcoal to remove excess selenium dioxide and Raney nickel catalyst, the solvent was evaporated to dryness under reduced pressure. The residue was then dissolved in $CHCl_3$ (3 x 3 ml), washed with water, and dried with anhydrous Na_2SO_4. The solvent was evaporated to dryness under reduced pressure and the residue was purified by preparative t.l.c. (R_F 0.25, *n*-hexane-AcOEt 1:1 v/v as developing solvent) to give the pure prednisone-d_4-BMD (**3**) (*ca.* 2 mg, 17%); non-labelled compound *m/z* 358 (M^+) and labelled compound *m/z* 362 (M^+).

A solution of prednisone-d_4-BMD (**3**) (*ca.* 1 mg) in EtOH-THF (1:1 v/v, 0.3 ml) was added dropwise to 46% HF (1.2 ml) in a polyethylene test-tube at 0 °C with stirring, and the mixture was stirred vigorously for 90 min and kept at 2-5 °C for 12 h. The mixture was neutralised to pH *ca.* 7 by careful addition of chilled saturated Na_2CO_3 and then extracted with EtOAc (4 x 10 ml). The extracts were washed with water and the solvent evaporated under reduced pressure to give a crude product. Purification by t.l.c. (R_F 0.33 $CHCl_3$-MeOH 9:1 as developing solvent) gave the pure prednisone-d_4 (**4**) (*ca.* 0.80 mg, 89%) as colourless crystals: δ_H (400 MHz; CD_3OD) of authentic prednisone 0.69 (3H, s, 18-Me), 1.51 (3H, s, 19-Me), 6.13 (1H, s, 4-H), 6.22 (1H, d, 2-H), and 7.80 (1H, d, 1-H); *m/z* 358 (M^+). Synthesised prednisone: δ_H (400 MHz; CD_3OD) 0.69 (3H, s, 18-Me), 1.51 (3H, s, 19-Me), 6.13 (1H, s, 4-H), 6.22 (1H, d, 2-H), and 7.80 (1H, d, 1-H); *m/z* 400 (M^+). Prednisone-d_4 (**4**): δ_H (400 MHz; CD_3OD) 0.69 (3H, s, 18-Me), 6.13 (1H, s, 4-H), and 6.22 (1H, s, 2-H); *m/z* 404 (M^+). Proton signals at δ_H 1.51 (19-Me) and δ_H 7.80 (1-H) disappeared in the labelled compound.

[1,19,19,19-$^2H_4$]-11β,17α,21-*Trihydroxypregna*-1,4-*diene*-3,20-*dione* (*prednisolone-d*$_4$) (**7**).— To a solution of prednisone-d_4-BMD (**3**) (*ca.* 1 mg) in CH_2Cl_2-MeOH-pyridine (10:40:1, 0.5 ml) was added semicarbazide hydrochloride (10 mg) in water (50 μl). The reaction mixture was then stirred at room temperature for 40 h. The solution was concentrated under reduced pressure. The mixture extracted with $CHCl_3$ (3 x 3 ml) was washed with water, and evaporated to dryness under reduced pressure to give almost pure prednisone-d_4-BMD 3-semicarbazone (**5**).

A mixture of prednisone-d_4-BMD 3-semicarbazone (**5**) and KBH_4 (5 mg) in THF-H_2O (5:1, 1.2 ml) was stirred at room temperature for 16 h. After addition of 2% AcOH (1.5 ml) (pH 5), the resulting suspension was extracted with $CHCl_3$ (3 x 2 ml). The extracts were washed with water and evaporated to dryness under reduced pressure to give

prednisolone-d_4-BMD 3-semicarbazone (**6**).

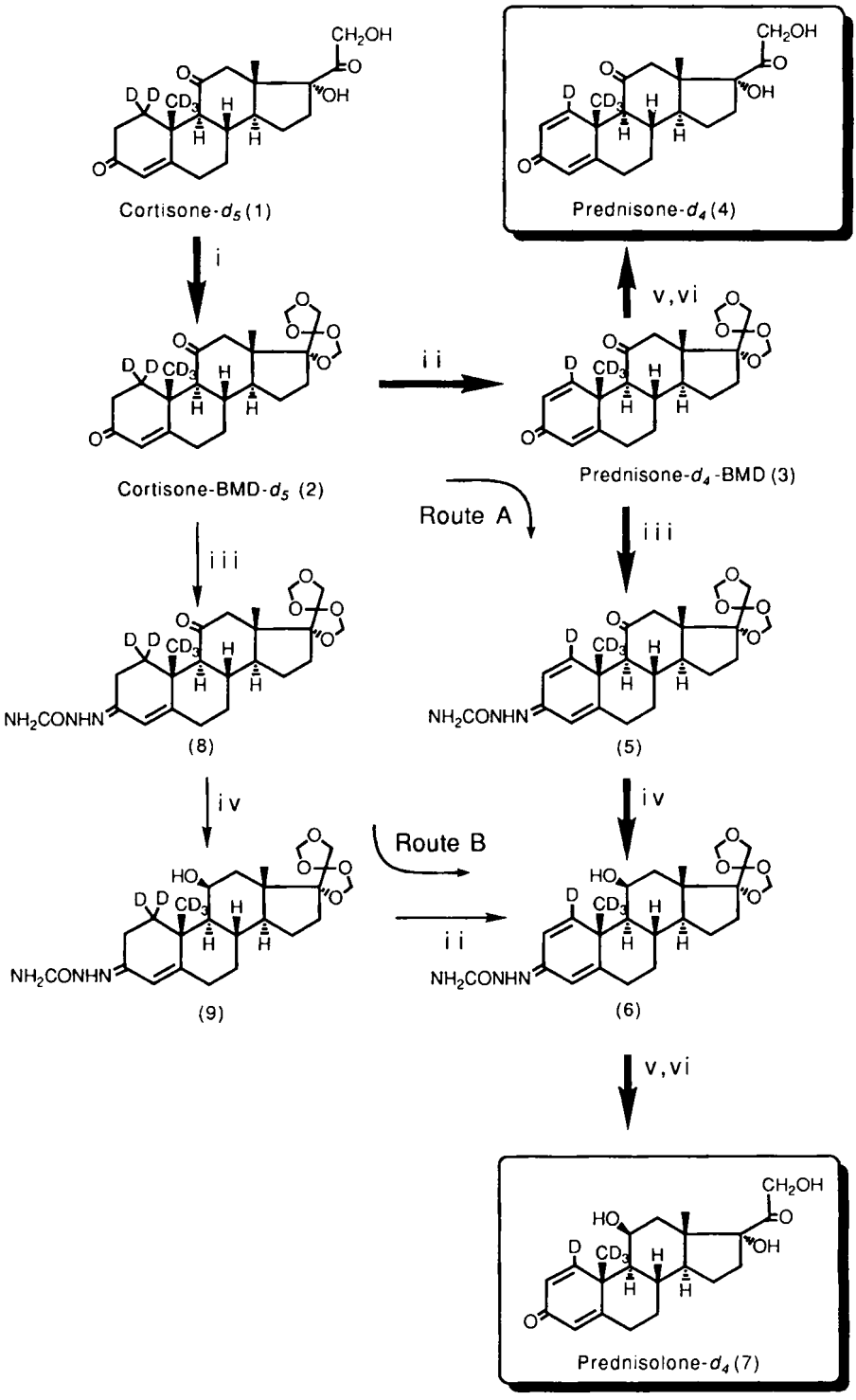
A solution of compound (**6**) in pyruvic acid-H_2O-AcOH (1:1:1 v/v, 0.5 ml) was stirred at room temperature for 15 h, then diluted with water (1 ml), and extracted with EtOAc (3 x 3 ml). The extracts were washed with 3% Na_2CO_3 and then with water. Evaporation of the solvent gave prednisolone-d_4-BMD.

A solution of prednisolone-d_4-BMD in EtOH-THF (1:1 v/v, 0.3 ml) was added dropwise to 46% HF (0.6 ml) in a polyethylene test-tube at 0 °C with stirring, and the mixture was stirred vigorously for 90 min and kept at 2-5 °C for 12 h. The mixture was neutralised to pH *ca.* 7 by careful addition of chilled saturated Na_2CO_3 and then extracted with EtOAc (4 x 10 ml). The extracts were washed with water and the solvent evaporated under reduced pressure to give a crude product. Purification by t.l.c. (R_F 0.20 $CHCl_3$-MeOH 9:1 as developing solvent) gave the pure prednisolone-d_4 (**7**) (*ca.* 0.55 mg, 55%) as colourless crystals: δ_H (400 MHz; CD_3OD) of authentic prednisolone 0.95 (3H, s, 18-Me), 1.53 (3H, s, 19-Me), 6.04 (1H, s, 4-H), 6.27 (1H, d, 2-H), and 7.48 (1H, d, 1-H); *m/z* 360 (M^+). Synthesised prednisolone: δ_H (400 MHz; CD_3OD) 0.95 (3H, s, 18-Me), 1.53 (3H, s, 19-Me), 6.04 (1H, s, 4-H), 6.27 (1H, d, 2-H), and 7.48 (1H, d, 1-H); *m/z* 360 (M^+). Prednisolone-d_4 (**7**): δ_H (400 MHz; CD_3OD) 0.95 (3H, s, 18-Me), 6.04 (1H, s, 4-H), and 6.27 (1H, s, 2-H); *m/z* 364 (M^+). Proton signals at δ_H 1.53 (19-Me) and δ_H 7.48 (1-H) disappeared in the labelled compound.

RESULTS AND DISCUSSION

We have previously described a concise 11-step total synthesis of cortisol and its application to selective deuteriation at the C-19 methyl and the C-1 methylene.[13] In our synthesis of ^{2}H-labelled cortisol, commercially available acetone-d_6 was an attractive precursor for a pentadeuterioisopropenyl anion which was suitable for introducing five ^{2}H-atoms during the reconstruction of rings *A* and *B* of cortisol. With the label selectively at the C-19 and C-1 positions, the incorporated ^{2}H-label was chemically and biologically stable and suitable for use in stable isotope methodology coupled with GC-MS.[14,15] We were willing to adapt this approach towards the synthesis of other ^{2}H-labelled corticoids.

A very short synthesis of multiply ^{2}H-labelled prednisone and prednisolone would be possible if an oxidative step of introducing C-1/C-2 double bond could be set after the preparation of ^{2}H-labelled cortisone-BMD (Scheme 1). Dihydroxyacetone moiety of cortisone-d_5 (**1**) was first protected as bismethylenedioxy (BMD) with 37% HCHO to give cortisone-d_5-BMD (**2**) in 77% yield. The introduction of double bond at



Scheme 1. *Reagents* i, HCHO/HCl; ii, SeO_2; iii, $NH_2CONHNH_2$•HCl; iv, KBH_4; v, CH_3COCO_2H; vi, HF

C-1/C-2 was conducted by refluxing (**2**) in *tert*-butanol for 24 h in the presence of selenium dioxide to give prednisone-d_4-BMD (**3**) in 17% yield. Hydrolysis of the BMD acetals with 46% hydrogen fluoride afforded the ^{2}H-labelled prednisone (prednisone-d_4, **4**) in 90% yield.

The conversion of prednisone-d_4-BMD (**3**) to the ^{2}H-labelled prednisolone (**7**) was achieved by the following route (**route A**) in 55% overall yield (Scheme 1). The 3-ketone of (**3**) was selectively protected as the semicarbazone (**5**) and subsequent stereoselective reduction of the C-11 carbonyl group with potassium borohydride gave the semicarbazone 11β-alcohol (**6**). Sequential deprotection of (**6**) *via* hydrolysis of the semicarbazone at C-3 with pyruvic acid and the BMD acetals with 46% hydrogen fluoride gave the desired prednisolone-d_4 (**7**). The ^{2}H-labelled prednisolone (**7**) was also prepared by an alternative route (**route B**, Scheme 1). The 3-ketone of cortisone-d_5-BMD (**2**) was first protected as the semicarbazone (**8**) and subsequent reduction of the C-11 carbonyl group with potassium borohydride to give cortisol-d_5-BMD semicarbazone (**9**). Oxidation of (**9**) with selenium dioxide and subsequent deprotections at C-3 and C-17 gave prednisolone-d_4 (**7**). This route, however, was less satisfactory because of the formation of byproducts, especially in the oxidation of (**9**).

The mass spectra of the labelled prednisone (**4**) and prednisolone (**7**) showed that the molecular ions at m/z 362 and at m/z 364, respectively, were four mass units higher than those of the unlabelled compounds. The isotopic purities were demonstrated to be >98 atom%. As shown in Figure 1, ^{1}H n.m.r. spectra of the labelled (**4**) and (**7**) showed that the C-19 methyl signals at δ_H 1.51 for prednisone and δ_H 1.53 for prednisolone disappeared. The C-1 methylene doublets at δ_H 7.80 (prednisone) and δ_H 7.48 (prednisolone) also disappeared and the doublet proton signals at C-2 of unlabelled prednisone (δ_H 6.23) and prednisolone (δ_H 6.27) became singlets in the labelled compounds. There were no indications of deuterium loss from the C-19 and C-1 positions during the synthetic sequence involving the oxidation reaction with selenium dioxide in *tert*-butanol.

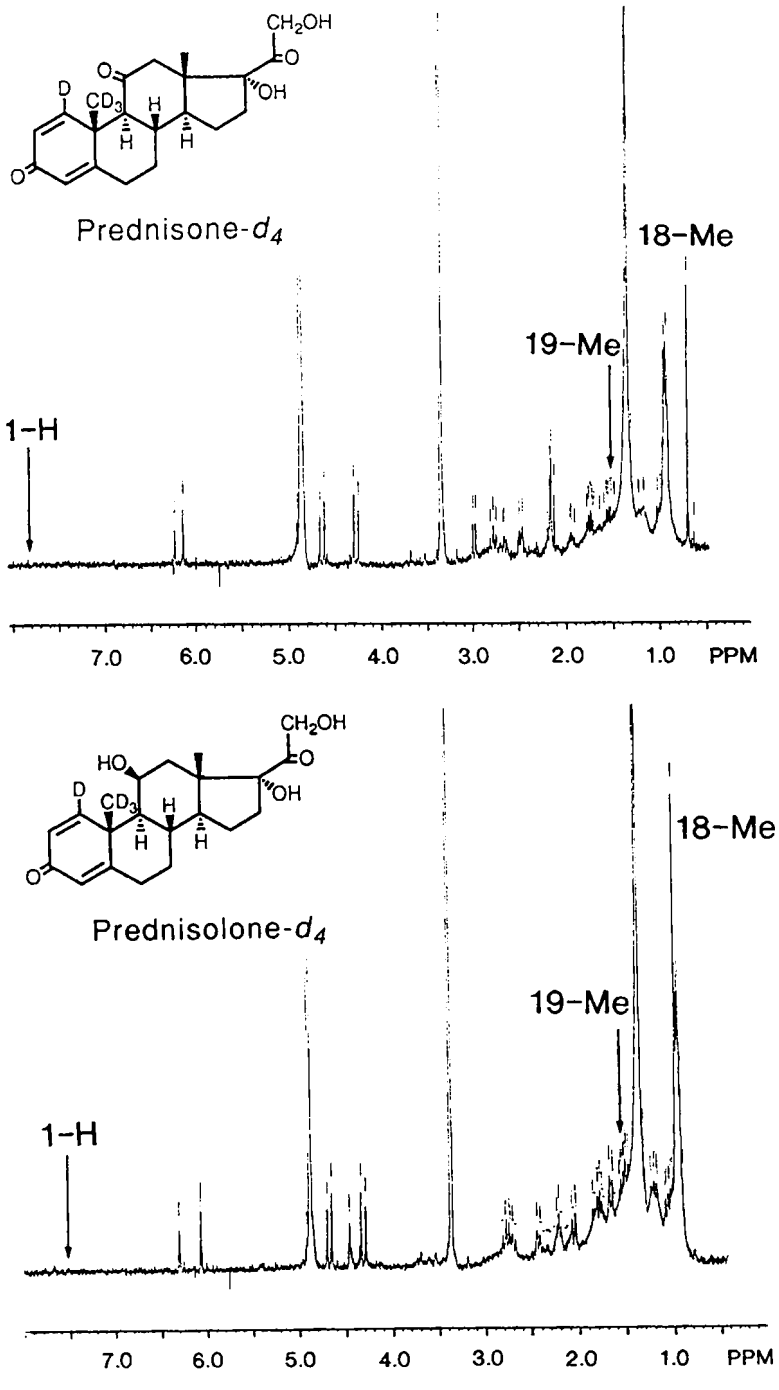


Figure 1. 400 MHz ^{1}H n.m.r. Spectra of [1,19,19,19-$^2H_4$]Prednisone (Prednisone-d_4, Upper) and [1,19,19,19-$^2H_4$]Prednisolone (Prednisolone-d_4, Lower).

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