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SYNTHESIS [4-^{14}C]- AND [6,7-D_2]TRILOSTANE

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Summary

A synthetic procedure for producing [6,7-d_2]trilostane is described. The synthesis was accomplished in 6 steps from androsta-4,6-dien-3,17-dione with the label being introduced by catalytic deuteration. The synthetic procedure, using [4-^{14}C]testosterone, gave the required [4-^{14}C]trilostane in four steps.

Key words: Deuteration, [6,7-d_2]trilostane, [4-^{14}C]trilostane.

Introduction

Trilostane [(4α, 5α, 17β)-4,5-epoxy-3,17-dihydroxyandrost-2-ene-2-carbonitrile 7a] is a novel synthetic steroid which has been shown to be a competitive inhibitor of 3β-hydroxysteroid dehydrogenase-Δ^5-3-oxosteroid isomerase (3β-HSD) enzyme system in laboratory animals[1-3] and humans.[4] The drug has been used to modify adrenal steroidogenesis in conditions such as Cushing's Syndrome,[4] primary aldosteronism,[5] various forms of hypertension,[6] and recently it has been found to be of benefit in the treatment of some forms of cancer of the breast.[7] As part of our drug development programme it was necessary to prepare deuterium labelled drug.

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Discussion

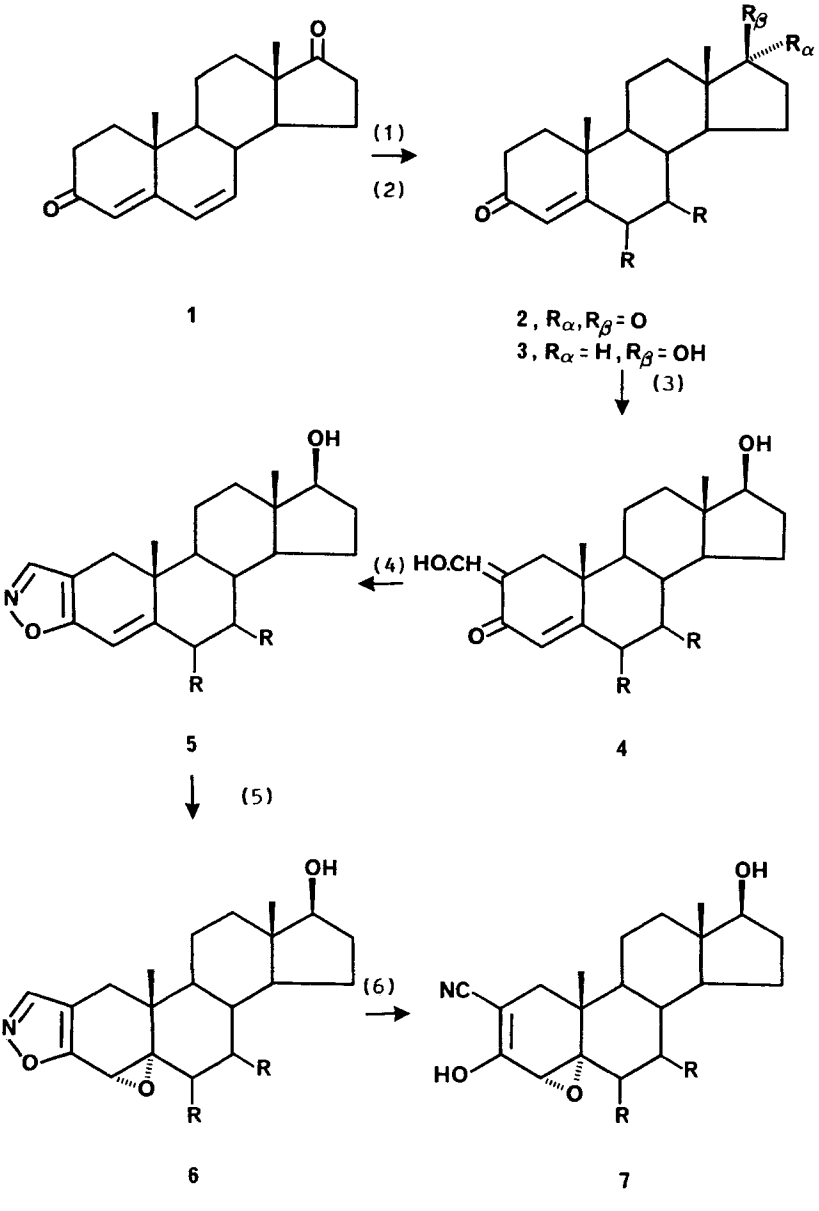
The synthesis of trilostane in the literature[8] is based on testosterone 3a as shown in the Scheme. It involves the reaction of 3a with methyl formate to give the hydroxymethylene compound 4a, the subsequent generation of the isoxazole derivative 5a, epoxidation of 5a to give 6a and isomerisation of 6a to give the desired product 7a. Bearing in mind the need to locate the label in a stable position we decided to use the approach outlined in the Scheme to prepare deuterated trilostane 7b. We synthesised androsta-4,6-dien-3,17-dione 1 using a literature method,[9] and catalytic reduction of this compound with deuterium gas gave [6,7-d_2]androst-4-en-3,17-dione 2b. Selective reduction of 2b with DIBAL gave [6,7-d_2]testosterone 3b in good yield. This deuterated testosterone was successfully taken through the reaction sequence outlined in the Scheme to give the required [6,7-d_2]trilostane. The catalytic reduction of 1 gave no products of over reduction of the conjugated dienone system.

This approach[8] has also been successfully applied to the preparation of [4-^{14}C]trilostane (specific activity 95 uCi/g), radiochemical purity greater than 98%, in four steps from [4-^{14}C]testosterone (specific activity 250 uCi/g, supplied by Amersham International, Amersham, Bucks.) in overall chemical yield of 57%.

Experimental

Infra-red (IR) spectra (KBr dispersions) were recorded with a Perkin Elmer 177 spectrophotometer. Thin layer chromatography (TLC) was carried out on 0.25 mm G-60 F_{254} silica gel plates (Merck). Mass spectra were supplied by Mr. P. Kelly, Newcastle University. Preparative high performance liquid chromatography (HPLC) was carried out with a Waters LC500 system, with a refractive index detector, using Prep PAK 500 silica columns and an eluent flow rate of 200 ml min^{-1}.

Androst-4-ene-3,17-dione was purchased from Sigma and converted



REAGENTS: (1), a) Pd/C and H_2; b) Pd/C and D_2; (2) DIBAL; (3) NaH/HCO_2Me; (4) $NH_2OH.HCl$/NaOAc; (5) m-CPBA; (6) KOH/THF/H_2O

SCHEME (a, R = H; b, R = D)

to androsta-4,6-diene-3,17-dione 1 using established literature methods.[9] The melting point of 1 was 168-169°C in good agreement with the reported value of 169-170°C.

[6,7-d_2]Androst-4-ene-3,17-dione 2b

A slurry of 5% palladium on charcoal (0.20 g) in methanol (80 ml) was added to a solution of potassium hydroxide (0.14 g, 2.50 mmol) in methanol (200 ml). The catalyst was saturated with deuterium for 1 hour and to this suspension was added a solution of androsta-4,6-diene-3,17-dione, 1 (2.5 g, 8.79 mmol), in methanol (70 ml). The mixture was deuterated until the required amount of deuterium (1.0 - 1.1 equivalents) was absorbed. The catalyst was removed by filtration through a bed of celite, the filtrate was neutralised with glacial acetic acid (0.2 ml, 2.50 mmol), and then evaporated to dryness under reduced pressure. The crude product was purified by preparative HPLC, as described under the experimental section, on silica using chloroform : ethyl acetate (80:20) as eluent. The fractions containing 2b were evaporated to dryness under reduced pressure to give an off-white solid 2.0 g (79%) m.p. 169-172°C IR υ_{max} 2260-2230 cm^{-1} (C-D stretch) 1730 cm^{-1} (C=O), 1659 cm^{-1} (C=O).

[6,7-d_2]Testosterone 3b

A solution of [6,7-d_2]androst-4-ene-3,17-dione, 2b, (1.95 g, 6.81 mmol) in toluene (52.80 ml) was cooled to 0°C in an ice/methanol bath and purged with a steady stream of dry nitrogen. A solution of diisobutylaluminium hydride (DIBAL-H) in toluene (25% v/v, 8.85 ml) was added to the reaction mixture via a syringe. The temperature was maintained at 0°C throughout the 15 minute addition period. The resultant light-orange coloured solution was stirred at 0°C for 1 hour and at room temperature for a further 1 hour.

The above solution was cooled in an ice/methanol bath and a mixture of propan-2-ol (2.84 ml) and acetone (2.84 ml) was slowly

added. The temperature was maintained at 0°C throughout the 10 minute addition period. The reaction mixture was then stirred at room temperature for a further 16 hours. After this time the solution was acidified with 0.5M sulphuric acid and the precipitated aluminium salts were removed by filtration. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2 x 100 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a colourless oil. The crude product was purified by preparative HPLC, as described in experimental section, on silica gel using chloroform : methanol (97:3) as eluent. The appropriate fractions were combined and evaporated to dryness under reduced pressure to give [6,7-d_2]testosterone 3b as an off-white solid 1.2 g (61%) m.p. 152-154°C IR ν_{max} 3420-336 cm^{-1} (OH), 2170-2140 cm^{-1} (C-D stretch), 1660 cm^{-1} (C=O).

2-Methylenehydroxy-[6,7-d_2]androst-4-en-3-one-17β-ol 4b

[6,7-d_2]Testosterone 3b, (0.89 g, 3.06 mmol) was dissolved in dry pyridine (5.00 ml) and the solution was purged with dry nitrogen gas. Sodium hydride (57% w/w suspension in oil, 0.5 g, 11.87 mmol) was carefully added to the stirred reaction mixture. The resultant mixture was cooled to 0°C in an ice/methanol bath, methyl formate (2.00 ml) was added over a 30 minute period and the mixture was stirred for a further 16 hours at room temperature. After this time the solution was diluted with distilled water (2 ml) and slowly acidified to pH 1 by the addition of concentrated hydrochloric acid. The resultant orange coloured solution was extracted with ether (3 x 50 ml), the combined extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a light red oil, 4b (0.60 g, 62%). The TLC and IR properties of this material were consistent with an authentic sample and it was used without further purification.

[6,7-d_2]Androst-4-eno[2,3-d]isoxazole-17β-ol 5b

A solution of sodium acetate trihydrate (0.20 g, 1.90 mmol) and hydroxylamine hydrochloride (0.15 g, 2.16 mmol) in water (0.80 ml) was added over a 10 minute period to a solution of hydroxymethylene-[6,7-d_2]androst-4-en-3-one-17β-ol, 4b (0.60 g, 1.88 mmol), in glacial acetic acid (5.85 g, 1.90 mmol). The orange coloured solution was stirred for 16 hours at room temperature. After this time, distilled water (10 ml) was added and the aqueous solution was extracted with ether (3 x 10 ml), the combined extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give an orange oil, 5b (0.58 g, 98%), which was used in the next stage without further purification.

4,5-Epoxy-[6,7-d_2]androstano[2,3-d]isoxazole-17β-ol 6b

3-Chloroperbenzoic acid (0.35 g, 2.03 mmol) was carefully added to a solution of [6,7-d_2]androst-4-eno[2,3-d]isoxazole-17β-ol 5b (0.60 g, 1.90 mmol) in chloroform (3 ml) and the reaction mixture was stirred for 16 hours at room temperature. After this time the solution was washed with a 1M sodium bicarbonate solution (3 x 10 ml), the chloroform layer was separated and dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a light yellow oil, 6b (0.60 stirred for a further 16 hours at room temperature. After this time the solution was diluted with distilled water (2 ml) and slowly acidified to pH 1 by the addition of concentrated hydrochloric acid. The resultant orange coloured solution was extracted with ether (3 x 50 ml), the combined extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a light red oil, 4b (0.60 g, 62%). The TLC and IR properties of this material were consistent with an authentic sample and it was used without further purification.

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[6,7-d_2]Trilostane 7b

A solution of 4,5-epoxy-[6,7-d_2]androstano[2,3-d]isoxazole-17β-ol, 6b (0.60 g, 1.81 mmol), in tetrahydrofuran (2.30 ml) was added over a 30 minute period to a solution of potassium hydroxide (0.21 g, 3.75 mmol) in tetrahydrofuran/water (4.50 : 1.85 v/v, 6.35 ml). The orange coloured solution was stirred for 16 hours at room temperature; after this time the solution was added to a cold stirred solution of concentrated hydrochloric acid (0.39 ml) in water (50.0 ml) over a 30 minute period. The precipitated solid formed a gum and the aqueous layer was removed by decantation and the residual gum was triturated with ether to give [6,7-d_2]trilostane as an off-white solid, 7b (0.25 g, 42%), m.p. 255.7-256.7°C (trilostane lit.[8] m.p. 258 - 270°C dec.).

The chemical identity was confirmed by TLC on silica gel using the following systems in which no impurities were detected.

i) Ethyl acetate : benzene (1:1) Rf : 0.3

ii) Chloroform : methanol : acetic acid (94 : 4 : 2) Rf : 0.4

The TLC and IR properties were shown to be identical to an authentic sample of trilostane. Mass spectral analysis of the product 7b indicated 63% d_2 37% d_1.

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