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SYNTHESIS OF [4-METHYL-^{14}C]EPOSTANE

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Summary

A synthetic procedure for producing [4-methyl-^{14}C]epostane is described. The radiolabel is introduced using [^{14}C]formaldehyde as shown in the scheme.

Key words: Carbon-14, [^{14}C]formaldehyde, [4-methyl-^{14}C]epostane

Introduction

Epostane[1] (4α,5α-epoxy-3,17β-dihydroxy-4,17α-dimethylandrost-2-ene-2-carbonitrile, 7) is a synthetic steroid which has been shown to be a competitive inhibitor of the 3β-hydroxysteroid dehydrogenase-Δ^5-3-oxo-steroid isomerase (3β-HSD) enzyme system.[2,3,4] As part of our drug development programme it was necessary to prepare carbon-14 labelled drug for use in metabolism and drug disposition studies.

Discussion

The synthesis of epostane[5,6] involved the reaction of 4,17-dimethyltestosterone 3 to give the hydroxymethylene compound 4. Reaction of 4 with hydroxylamine hydrochloride gave the isoxazolosteroid 5 which was epoxidised to give 6. Treatment of 6 with base gave the required epostane 7. The pivotal step in the synthesis of [4-methyl-^{14}C]epostane involved the reaction of 17-methyltestosterone with [^{14}C]formaldehyde and

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thiophenol in a modified Mannich reaction by the procedure due to Kirk and Petrow[7] to give the 4-phenylthiomethyl compound 2. Careful reaction of 2 with Raney nickel gave [4-methyl-^{14}C]-4,17-dimethyltestosterone 3[8] which was taken through the remaining steps as shown in the scheme. Initial experiments were carried out using unlabelled materials to optimise the reaction conditions. The identities of the final product and labelled intermediates were confirmed by comparison of their IR and TLC properties with those of unlabelled authentic samples.

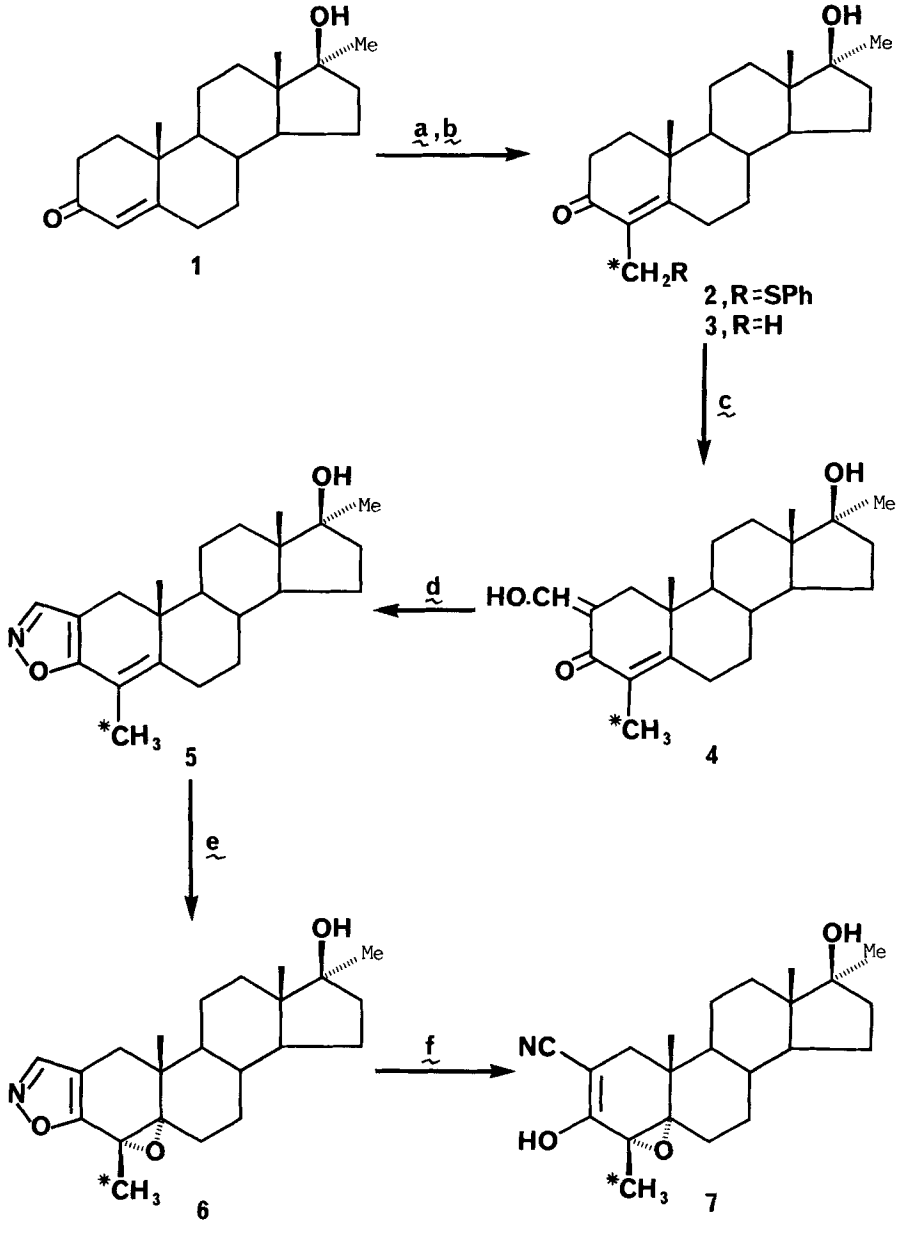
Experimental

Infra-red (IR) spectra (KBr dispersions) were recorded with a Perkin Elmer 177 spectrophotometer. Radioactivity measurements were performed on a Packard TRICARB 300C Counter using Instagel (Packard) as counting medium. Thin layer chromatography (TLC) was carried out on 0.25 mm G-60F_{254} silica gel plates (Merck). Plates were scanned on a Panax scanner. All stages of the reaction were monitored using the solvent system cyclohexane : ethyl acetate (50:50) unless otherwise indicated.

[^{14}C]Formaldehyde (20 mCi) was purchased from Amersham International plc and had a nominal activity of 15 mCi/mmol and a radiochemical purity of 95.2%.

Preparation of 17β-hydroxy-17α-methyl-4-(phenylthio[^{14}C]methyl)androst-4-en-3-one, 2

To a 5 ml round bottomed flask was added 17-methyltestosterone (500 mg, 1.653 mmol) and formaldehyde (38% solution) (0.100 ml, 1.356 mmol), followed by thiophenol (0.26 ml, 2.45 mmol), 20 mCi of [^{14}C]formaldehyde (1.27 mmol) washed in with ethanol (2 ml), then a further portion of formaldehyde (0.115 ml, 1.559 mmol) and triethylamine (0.28 ml, 2.02 mmol) were added. The resulting mixture was heated at reflux for 68 hours then cooled to room temperature. The yellow solution was quenched in 0.5M potassium hydroxide solution (10 ml) and stirred for one hour. The product which precipitated was collected by filtration under suction and the cake washed successively with water (3 x 10 ml) and n-hexane (10 ml) then air dried under suction for 3 hours to yield 730.8 mg of crude product. The crude product was pre-adsorbed onto silica gel (5 g) by evaporation of a dichloromethane (20 ml) slurry under reduced pressure, and then added to the top of a dry silica column (200 g). The column was eluted with cyclohexane : ethyl acetate (70:30, 500 ml; 60:40, 500 ml; 50:50, 500 ml) with 50 ml fractions being collected. The fractions were monitored by TLC. Fractions containing the desired product were combined



REAGENTS: a, [^{14}C]Formaldehyde, thiophenol, triethylamine; b, Raney nickel; c, Methyl formate, sodium hydride; d, Hydroxylamine; e, m-Chloroperbenzoic acid; f, Sodium methoxide.

Scheme

and evaporated to dryness under reduced pressure to yield 573 mg (82%) of 2 as a white solid [IR ν_{max} 3600-3100 (OH stretch) cm^{-1}, 1677 (conjugated C=O stretch) cm^{-1}; Partial ^{1}H-NMR ($CDCl_3$): δ 7.5-7.1 (5H, m), 3.85 (2H, s, SCH_2) ppm].

Preparation of [4-methyl-^{14}C]-4,17-dimethyltestosterone, 3

Raney nickel catalyst (5 ml) was successively washed with water (5 x 40 ml) and acetone (3 x 40 ml) under a nitrogen atmosphere. The catalyst was resuspended in acetone (20 ml) and a solution of 2 (573 mg, 1.35 mmol) in acetone (20 ml) added. The mixture was stirred at room temperature under nitrogen for 3 hours after which time TLC showed complete absence of starting material. The acetone solution was decanted from the catalyst and the catalyst washed with acetone (6 x 20 ml). The original acetone solution plus washings were combined, filtered through Celite filter aid, then concentrated under reduced pressure to give a colourless oil. The oil was dissolved in cyclohexane : ethyl acetate (70:30, 10 ml) and added to the top of a column comprising silica (150 g) preconditioned with the same solvent. The column was eluted with cyclohexane : ethyl acetate (70:30) and 50 ml fractions collected. The fractions were monitored by TLC and fractions containing the desired product were collected. These fractions were combined and evaporated to dryness under reduced pressure to give 373 mg (87%) of a white crystalline solid, [4-methyl-^{14}C]-4,17-dimethyltestosterone 3.

Preparation of [4-methyl-^{14}C]-17β-hydroxy-2-hydroxymethylene-4,17α-dimethylandrost-4-en-3-one, 4

A solution of [4-methyl-^{14}C]-4,17-dimethyltestosterone (373 mg, 1.18 mmol) in dried distilled tetrahydrofuran (3.5 ml) and methanol (7 ul) was stirred at room temperature and sodium hydride (60% dispersion in oil, 173 mg, 2.6 mmol) added, followed by methyl formate (375 ul, 6 mmol) in one portion. The resulting suspension was stirred at room temperature for 24 hours under nitrogen. The reddish brown mixture was quenched by the addition of water (5 ml) and the tetrahydrofuran carefully removed under reduced pressure. The slightly cloudy solution was filtered through a glass fibre filter paper and the pH of the filtrate adjusted to 3 using 5M hydrochloric acid.

The resulting yellow slurry was stirred for a further hour, the solid removed by filtration under suction, washed with water (3 x 5 ml) then dried under reduced pressure at 60^o for 24 hours. This yielded 312 mg (77%) of 4 as a yellow solid.

Preparation of [4-methyl-^{14}C]-4,17α-dimethylandrosta-2,4-dieno[2,3-d]-isoxazol-17β-ol, 5

A mixture of 4 (312 mg, 0.906 mmol) and glacial acetic acid (2.7 ml) was stirred while an aqueous solution (420 ul) containing sodium acetate trihydrate (141 mg, 1.04 mmol) and hydroxylamine hydrochloride (72 mg, 1.04 mmol) was added. The mixture was stirred for 6 hours at room temperature, then distilled water (2 ml) was slowly added and the stirring continued for a further hour. The resulting solid was collected by filtration under suction, washed with water (2 x 5 ml) and n-hexane (5 ml) then dried overnight at 50°C under reduced pressure. This yielded 210 mg (68%) of 5 as a yellow crystalline solid.

Preparation of [4-methyl-^{14}C]-4α,5α-epoxy-4,17α-dimethylandrost-2-eno-[2,3-d]isoxazol-17β-ol, 6

A solution of 5 (210 mg, 0.615 mmol) in dichloromethane (2.5 ml) was cooled to 5°C in an ice/water bath, m-chloroperbenzoic acid (80%, 145 mg, 0.672 mmol) was added and the mixture stirred at 5°C for one hour, then overnight at room temperature. The excess peracid was decomposed by the addition of a solution of sodium sulphite (20 mg) in water (0.5 ml). The two phase mixture was stirred for 15 minutes, filtered through a glass fibre filter paper under suction and the cake washed with dichloromethane (2 ml). The filtrate was washed with saturated sodium bicarbonate solution (3 x 10 ml) followed by water (2 x 10 ml) and brine (10 ml). The aqueous portions were combined and extracted with dichloromethane (10 ml). The organic portions were combined, charcoal (20 mg) and anhydrous sodium sulphate added, and the slurry was stirred for 15 minutes before being filtered under suction through a glass fibre filter paper and the cake washed with dichloromethane (2 ml). The filtrate was evaporated to dryness under reduced pressure to yield 170 mg of crude product. This was pre-adsorbed by dissolving it in dichloromethane (5 ml), silica (1.5 g) was added and the mixture evaporated to dryness under reduced pressure. The residual solid was added to a silica (60 g) column, eluted with cyclohexane : ethyl acetate (70:30) and 10 ml fractions collected. The fractions shown by TLC to contain the product as single spot material were combined and evaporated to dryness under reduced pressure to yield 137 mg (62%) of 6 as a white solid.

Preparation of [4-methyl-^{14}C]-4α,5α-epoxy-3,17β-dihydroxy-4,17α-dimethyl-androst-2-ene-2-carbonitrile ([4-Methyl-^{14}C]epostane) 7

To a round bottomed flask (5 ml) was added 6 (60 mg, 0.168 mmol), dried

distilled tetrahydrofuran (2 ml) and sodium methoxide (20 mg, 0.270 mmol). The mixture was stirred under nitrogen at room temperature for 3 hours then added dropwise to a stirred solution of 1M hydrochloric acid (3 ml). The flask was washed with water (2 ml) and this was added to the hydrochloric acid. The mixture was stirred under nitrogen for 2 hours at room temperature then cooled to 0°C in an ice bath. The resulting white product was collected by filtration under suction, washed with water (3 x 1 ml) then dried under reduced pressure at 50°C for 24 hours. This yielded 41 mg (68%) of [4-methyl-^{14}C]epostane, specific activity 9.5 uCi/mg, with a radiochemical purity of greater than 98% and chemical purity of greater than 99% as determined by TLC in the following systems:-

i) Hexane : tetrahydrofuran : acetic acid (75:20:5), Rf 0.6

ii) Chloroform : acetone : cyclohexane : acetic acid (30:30:30:10), Rf 0.6

The TLC and IR properties of the product and intermediates were in full agreement with those of authentic samples. The overall radiochemical and chemical yields were 4.5% and 15.8%, respectively.

References

1. Christiansen R.G., U.S. Patent 4 160 027, "Cyano-4,5-epoxy-steroid derivatives and their 2,3-isoxazolo precursors - useful for inducing abortion".
2. Creange J.E., Anzalone A.J., Potts G.O., and Schane H.P., Contraception, 24 : 289 (1981).
3. Snyder B.W. and Schane H.P., Contraception, 31 : 479 (1985).
4. Snyder B.W., Beecham G.D., and Schane H.P., Proc. Soc. Exp. Biol., 176 : 238 (1984).
5. Christiansen R.G., B.P. 2 088 878B, "Isoxazole intermediates of 2 - cyano-3-oxo-steroids".
6. Christiansen R.G., Newmann H.C., Salvador U.J., Bell M.R., Schane H.P., Creange J.E., Potts G.O., and Anzalone A.J., J. Med. Chem., 27 : 928 (1984).
7. Kirk D.N. and Petrow V., U.S. Patent 2 989 552, "4-Organothio-methyl-3-oxo- Δ^3-steroids".
8. Kirk D.N. and Petrow V., J. Chem. Soc., 1091 (1962)