SYNTHESIS OF CARBON-14 LABELLED 1-[4-METHYL-3-OXO-4-AZA-5α-ANDROSTANE-17β-CARBONYL]-1,3-DIISOPROPYLUREA (TUROSTERIDE), A NEW 5α-REDUCTASE INHIBITOR.

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SUMMARY

The synthesis in eight steps of the title compound from the commercially available[20-^{14}C]pregnenolone 1 is described. The expected 1-[4-methyl-3-oxo-4-aza-5α-androstane-17β-[^{14}C]carbonyl]-1,3-diisopropylurea ([^{14}C]FCE 26073) was obtained with a radiochemical purity higher than 97% and a specific activity of 2.03 GBq/mmol. An overall radiochemical yield of 21% was achieved from 1.

Key words : Turosteride, Testosterone 5α-reductase inhibitors, FCE 26073, 14-C Labelled steroids, [^{14}C] Turosteride, [^{14}C]FCE 26073.

INTRODUCTION

During recent years, the possibility of controlling androgen-dependent diseases such as benign prostatic hyperplasia, prostatic cancer, acne, female hirsutism and male pattern baldness [1-4] by inhibition of 5α-reductase, the enzyme catalyzing the conversion of testosterone to 5α-dihydrotestosterone, has attracted increasing attention.

The ability of 4-azasteroids to inhibit testosterone 5α-reductase has been reported [5], and a compound of this class, finasteride, has recently been introduced on the market for the treatment of benign prostatic hyperplasia [6]. As a part of a research program aimed at finding new 5α-reductase inhibitors, several 17β-acylureas of 4-azasteroids were synthesized and tested for their 5α-reductase inhibitory properties [7,8]. Among them, turosteride (1-[4-methyl-3-oxo-4-aza-5α-androstane-17β-carbonyl]-1,3-diisopropylurea ; code name FCE 26073) was found to be very effective and selective both *in vitro* and *in vivo* [8-10]. The compound showed no binding to androgen receptors and, at variance with finasteride, it

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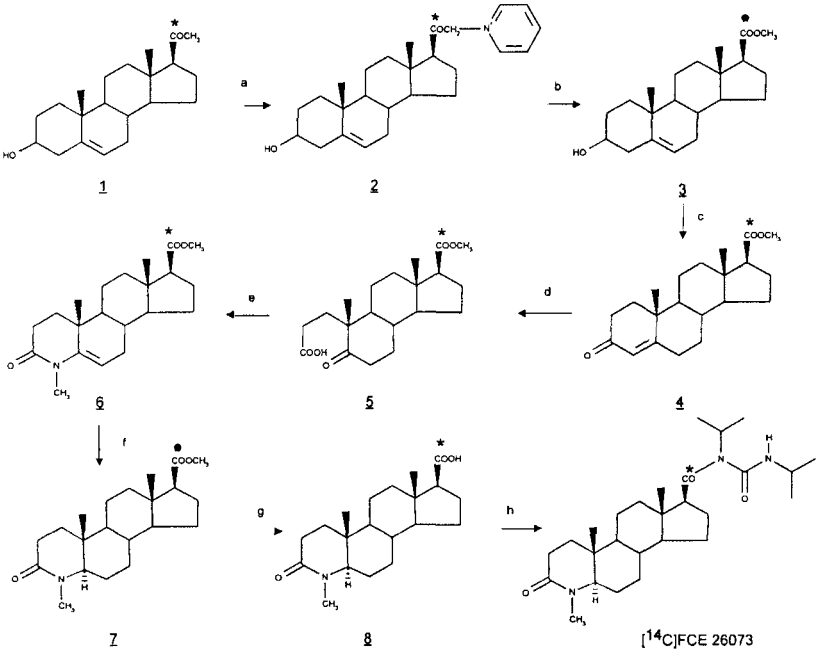
does not change the intraprostatic testosterone content [9]. Turosteride is now under phase I clinical evaluation. In order to fully investigate the absorption, pharmacokinetics and metabolism of this novel 5α-reductase inhibitor, the preparation of the ^{14}C-labelled material was required. A possible labelling site was the carbonyl group attached to the C-17 position of the steroid skeleton, which was unlikely to be lost through metabolic activity. In the present paper we describe the preparation of 1-[4-methyl-3-oxo-4-aza-5α-androstane-17β-[^{14}C]carbonyl]-1,3-diisopropylurea ([^{14}C]FCE 26073) from the commercially available [20-^{14}C]pregnenolone.

RESULTS AND DISCUSSION

The synthetic route used to introduce carbon-14 at the C-20 position of the title compound is outlined in the scheme.

SCHEME



* = ^{14}C

Reagents and Conditions

a - I_2, Pyridine. b - $NaOCH_3$, CH_3OH. c - $(iPrO)_3Al$, Cyclohexanone. d - $KMnO_4$, $NaIO_4$.

e - CH_3NH_2, Diglyme. f -. H_2/PtO_2, Acetic acid. g - KOH, CH_3OH. h - 1,3-Diisopropylcarbodiimide, CH_2Cl_2

The synthesis is based on known methods. [20-^{14}C]Pregnenolone 1, , was converted to the methyl ester 3 via the pyridinium iodide 2 according to the Ortoleva-King procedure [11]. The Oppenauer oxidation of the intermediate 3 with aluminum isopropoxide in the presence of cyclohexanone, gave the Δ^4-3-keto compound 4. The oxidative cleavage of 4 with potassium permanganate/sodium periodate [12] followed by ring closure with methylamine at high temperature,

afforded the ene-lactam 6. The intermediate 6 was then hydrogenated over Adam's platinum oxide catalyst in acetic acid and the 5α-4-azasteroid 7 was selectively obtained [13]. Alkaline hydrolysis of the carboxymethyl group attached at the C-17 position of the steroid group gave the corresponding carboxylic acid 8. The reaction of 8 with 1,3-diisopropylcarbodiimide in methylene chloride at reflux [14], afforded the crude [^{14}C]FCE 26073 which was purified by preparative HPLC. [^{14}C]FCE 26073 was obtained with a radiochemical purity >97% and a specific activity of 2.03 Gbq/mmol. The method of synthesis here described enables the preparation of the radiochemically pure [^{14}C]FCE 26073 with an overall radiochemical yield of 21% from 1.

EXPERIMENTAL

General methods.

[20-^{14}C]Pregnenolone was purchased from Amersham International p.l.c.. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Radioactivity was measured by a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail.

High performance liquid chromatography (HPLC) was performed at ambient using a Perkin-Elmer pump mod 2/2 equipped with a Perkin-Elmer LC 75 UV/VIS spechtrophotometer (λ=206 nm). HPLC purification was carried out on a Waters μBondapack C18 column (300x7.8 mm I.D.; particle size 10 μm) eluted with acetonitrile:methanol:water (4:2:4 by volume) at 5 ml/min. Radiochemical purities were determined by TLC with a Packard Bioscan System 200 imaging scanner , on silica gel Merck F254 plates (20x5 cm ; 0.25 mm thick) in toluene:ethyl acetate:methanol:glacial acetic acid (55:40:3:2 by volume) and by HPLC using a Packard Trace model 7130 radioactivity flow detector, under the following conditions : Waters Novapack C18 column (150x3.9 mm I.D.; particle size 4 μm) eluted with A) 0.05 M KH_2PO_4 buffer pH4.5:methanol (7:3 by volume) at 1 ml/min or B) acetonitrile:methanol:water (4:2:4 by volume) at 1 ml/min and Merck Lichrospher 100 RP8 column (240x4 mm I.D.; particle size 5 μm) eluted with acetonitrile:methanol:water (4:2:4 by volume) at 1 ml/min. All labelled materials were characterized by chromatographic comparison with authentic unlabelled samples.

3β-Hydroxy-pregn-5-en-[20-^{14}C]one 21-pyridinium iodide (2)

Pyridine (1.7 ml; distilled on KOH) and iodine (408 mg; 1.6 mmol) were added to [20-^{14}C]pregnenolone 1 (1.85 MBq; 0.958 mmol) and the solution was refluxed under stirring for 4.5 hours. At the end of the reaction (determined by radio HPLC; system A) the solution was cooled at 10°C and stirred for 20 minutes. The resulting mixture was filtered through a D4 sintered-glass filter. The solid was washed with pyridine (4x2 ml) and with ethyl ether (4x2 ml) until the filtrate was colourless. The powder was suspended in ethyl ether and transferred into a 25 ml round bottom flask. Evaporation of the

solvent to dryness, afforded compound 2 as a brown powder (360.7 mg; 0.690 mmol) 97% radiochemically pure (by radio-HPLC; system A: Retention Time (RT) = 2.4 min).

Methyl 3β-hydroxy-androst-5-ene-17β-[^{14}C]carboxylate (3)

Methanol (3.5 ml) and sodium methoxide (87.7 mg;1.62 mmol) were added under nitrogen to the intermediate 2 (360.7 mg; 0.690 mmol) and the reaction mixture was heated at reflux for 90 minutes. At the end of reaction (determined by radio HPLC; system A) the solution was cooled at room temperature in 60 minutes then water (2 ml) was added. The solution was cooled at 10°C, 1N HCl (1 ml) was introduced into the flask and the mixture was stirred for 1 hour. The resulting precipitate was filtered through a D4 sintered-glass filter and washed with a solution water:methanol (1:1 by volume) until the filtrate was colourless. The solid was dissolved in ethyl acetate (20 ml) and the solution was transferred into a 25 ml round bottom flask. After solvent evaporation to dryness, compound 3 was obtained (1.24 GBq; 0.609 mmol) 96% radiochemically pure (by radio-HPLC; system A: RT = 21 min). The radiochemical yield was 67% from 1.

Methyl 3-oxo-androst-4-ene-17β-[^{14}C]carboxylate (4)

The intermediate 3 (1.24 GBq; 0.609 mmol), toluene (3.2 ml) and cyclohexanone (0.32 ml) were refluxed under stirring for 10 minutes. After solvent evaporation to dryness in a "vacuum manifold", the residue was suspended in toluene (3.2 ml) and then cyclohexanone (0.32 ml) and aluminum isopropoxide (141 mg; 0.69 mmol) in toluene (0.7 ml) were added. The mixture was stirred and heated at reflux for 90 minutes. At the end of reaction (determined by radio-HPLC; system A), carbon black (50.7 mg) in toluene (0.25 ml), dicalite (23.8 mg) and distilled water (0.02 ml) were added. The suspension was stirred for 15 minutes, filtered through a D4 sintered-glass filter and washed with toluene (3x5 ml). The filtrate was evaporated to dryness in a "vacuum manifold". The yellow residue was dissolved in ethyl acetate (20 ml), transferred into a 50 ml separating funnel and washed with water (3x7 ml). The organic phase was dried (Na_2SO_4) and, after solvent evaporation, gave the intermediate 4 (1.135 GBq; 0.558 mmol) 98% radiochemically pure (by radio-HPLC; system A: RT = 12.3 min) with a radiochemical yield of 91.5% from 3.

17β-Methoxy-[^{14}C]carbonyl-5-oxo-4-nor-3,5-secoandrostan-3-oic acid (5)

A solution of 2M Na_2CO_3 (0.41 ml) was added to the intermediate 4 (1.135 GBq; 0.5576 mmol) dissolved in tert-butanol (4 ml) and the solution was heated to 40°C. Two separate solution of 2% $KMnO_4$ (0.42 ml) and of $NaIO_4$ (5.8 ml; 0.16 g/ml) respectively, were slowly and contemporary dropped (1 hour) into the reaction flask so that the reaction mixture colour was mantained pink. The mixture was stirred at 40°C for two hours. At this time the conversion of 4 to 5 was not complete and the amount of 4 (17% by radio HPLC; system A) did not reduce after stirring the reaction mixture one additional hour at 40°C. The suspension was then filtered through a D4 sintered-glass filter and the solid was washed

with 0.02M Na_2CO_3 (30 ml) until the filtrate was colourless. The filtrate was evaporated to dryness, dissolved in water (10 ml), made alkaline up to pH=8 with 2N Na_2CO_3 and transferred into a 50 ml separating funnel. The solution was extracted with ethyl acetate (3x5 ml). The organic phase was dried (Na_2SO_4) and, after solvent evaporation, the precursor 4 was recovered (320 MBq; 0.158 mmol). The aqueous phase was then acidified up to pH=2 with 1N HCl and extracted with ethyl acetate (3x5 ml). After drying (Na_2SO_4), evaporation of the organic phase gave the crude 5 (699 MBq; 0.344 mmoles). A further amount of crude 5 (309 MBq; 0.151 mmol) was obtained by oxidation, according to the reaction conditions above described, from the recovered precursor 4. The combination of the two batches yielded compound 5 (1.008 GBq; 0.495 mmol), 82% radiochemically pure (by radio-HPLC; system A: RT = 2.8 min), which was used without further purification in the next step. The radiochemical yield was 89% from 4 .

Methyl 4-methyl-3-oxo-4-aza-androst-5-ene-17β-[^{14}C]carboxylate (6)

2-Methoxyethyl ether (1.5 ml) and methylamine dissolved in 2-methoxyethyl ether (1.4 ml; 7.27 mmol) were added to the intermediate 5 (1.008 Gbq; 0.4954 mmol). The temperature of the stirred solution was slowly raised up to 180°C (1 hour). Then the mixture was refluxed for 90 minutes. At the end of the reaction (determined by radio HPLC; system A), the solution was evaporated to dryness in a "vacuum manifold". The yellow residue was dissolved in ethyl acetate (20 ml), the solution transferred into a 50 ml separating funnel and washed with 1M Na_2CO_3 (3x5 ml), then with water to neutrality. The organic phase, after drying (Na_2SO_4), was evaporated affording the crude 6 (902 MBq; 0.444 mmol) 94% radiochemically pure (determined by radio-HPLC; system A: RT = 13.5 min) with a radiochemical yield of 89% from 6.

Methyl 4-methyl-3-oxo-4-aza-5α-androstane-17β-[^{14}C]carboxylate (7)

The intermediate 6 (902 MBq; 0.444 mmol), glacial acetic acid (3 ml) and platinum oxide (135.7 mg; 0.487 mmol) were stirred at 40°C under hydrogen atmosphere for 90 minutes (23.75 ml of the gas were consumed). At the end of the reaction (determined by radio HPLC; system A), the catalyst was filtered off and washed with acetic acid (10x5 ml), then with water (4x5 ml). The combined filtrates were evaporated to dryness by adding cyclohexane (3x5 ml).

The resulting yellow gum was dissolved in methylene chloride (20 ml), the solution was transferred into a 50 ml separating funnel and washed with 1N sulfuric acid (2x5 ml), brine (3x5 ml), Na_2CO_3 saturated solution (3x5 ml), and water (3x5 ml). The organic phase, after drying (Na_2SO_4), was evaporated to dryness to give compound 6 (810 MBq; 0.399 mmol), >97% radiochemically pure (determined by radio-HPLC; system A: RT = 9.7 min), with a radiochemical yield of 90% from 5.

4-Methyl-3-oxo-4-aza-5α-androstane-17β-[^{14}C]carboxylic acid (8)

The intermediate 7 (810 MBq; 0.399 mmol), methanol (2 ml) and potassium hydroxide (0.16 g) in a mixture (0.32 ml) methanol:water (1:1 by volume) were stirred at reflux for four hours. At the end of the reaction (determined by radio

HPLC; system A) the solution was cooled at 5°C and acidified up to pH 2 with 1N HCl. The precipitate was filtered through a D4 sintered-glass filter, then washed with water (2x0.5 ml) and with acetone (3x0.5 ml). The solid was dissolved with methanol (10 ml) and methylene chloride (15 ml). Then the solution was evaporated to dryness yielding the intermediate 8 (564 MBq; 0.277 mmol) 92% radiochemically pure (by radio-HPLC; system A: RT = 3.8 min). The radiochemical yield was 69% from 7.

1-[4-Methyl-3-oxo-4-aza-5α-androstane-17β-[^{14}C]carbonyl]-1,3-diisopropylurea ([^{14}C]FCE 26073)

Diisopropylcarbodiimide (0.0212 mmol) in methylene chloride (1 ml) was added to the intermediate 8 (44.55 MBq; 0.0198 mmol) suspended in methylene chloride (1 ml). The mixture was heated to reflux under stirring for three hours. At the end of the reaction (determined by radio-HPLC; system B) the solution was transferred into a 50 ml separating funnel and washed with 0.1M $NaHCO_3$ (3x5 ml), 1M HCl (3x5 ml), brine (3x5 ml) and water (3x5 ml). The organic phase was dried (Na_2SO_4) and then evaporated to dryness to give the crude [^{14}C]FCE 26073 (33.57 MBq; 0.0165 mmol), 91% radiochemically pure (by radio-HPLC; system B). Purification by preparative HPLC (see General methods) yielded [^{14}C]FCE 26073 (21.47 MBq; 0.0106 mmol) with a radiochemical purity >97% (by radio-HPLC; system B: RT = 6.6 min and system C: RT = 14 min - by radio-TLC : Rf = 0.22) and a radiochemical yield of 48% from 8. The overall radiochemical yield was 21% from 1.

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