SYNTHESIS OF CARBON-14 LABELLED C_{19}-STEROIDS

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SUMMARY

The carbon-14 labelled steroids, androsterone-4-^{14}C, isoandrosterone-4-^{14}C, 5α-androstanedione-4-^{14}C, 5α-androstane-3β,17β-diol-4-^{14}C, and 5α-androstane-3α,17β-diol-4-^{14}C were synthesized from dihydrotestosterone-4-^{14}C for use in studies of steroid metabolism.

Key Words: Dihydrotestosterone-4-^{14}C, Androsterone-4-^{14}C, Isoandrosterone-4-^{14}C, 5α-Androstane-3,17-dione-4-^{14}C, 5α-Androstane-3β,17β-diol-4-^{14}C, 5α-Androstane-3α,17β-diol-4-^{14}C.

INTRODUCTION

In metabolic studies involving incubations of mammalian tissues with tritium-labelled C_{19}-steroid precursors, e.g., testosterone-^{3}H**, androstenedione-^{3}H, dehydroisoandrosterone-^{3}H, etc, the addition of carbon-14 labelled steroids corresponding to the metabolites as recovery standards and to monitor their purification has been found to be most useful[1]. Since androsterone-4-^{14}C, isoandrosterone-4-^{14}C, 5α-androstanedione-4-^{14}C, 5α-androstane-3β,17β-diol-4-^{14}C and 5α-androstane-3α,17β-diol-4-^{14}C are not available commercially, these

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** Trivial names and abbreviations are as follows: testosterone, 17β-hydroxy-4-androsten-3-one; androstenedione, 4-androstene-3,17-dione; dehydroisoandrosterone, 3β-hydroxy-5-androsten-17-one; androsterone, 3α-hydroxy-5α-androstan-17-one; isoandrosterone, 3β-hydroxy-5α-androstan-17-one; 5α-androstanedione, 5α-androstane-3,17-dione; dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

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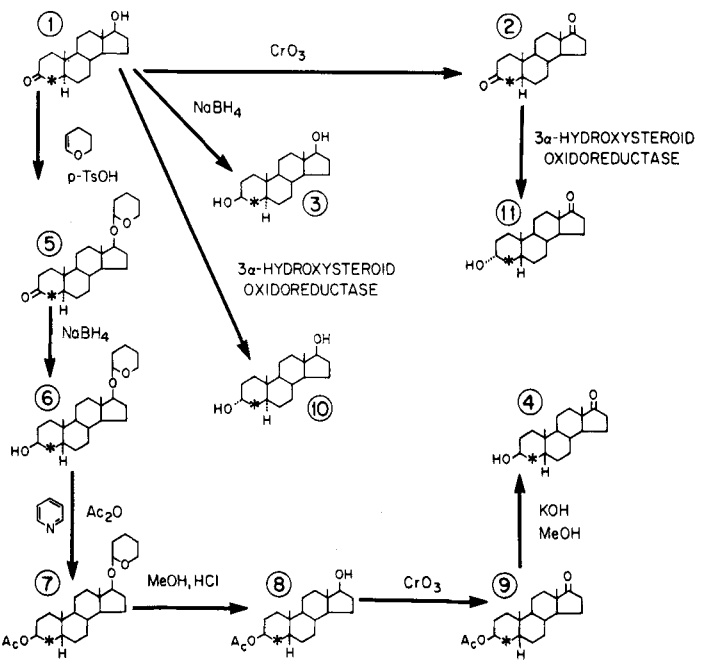
steroids were synthesized from dihydrotestosterone-4-^{14}C (1) using known chemical and biological procedures. Thus, 5α-androstanedione-4-^{14}C (2) was prepared by oxidizing dihydrotestosterone-4-^{14}C with chromic acid[2]; and 5α-androstane-3β-17β-diol-4-^{14}C (3) was synthesized by reduction of dihydrotestosterone-4-^{14}C with sodium borohydride[3]. A sequence of consecutive reactions were utilized to synthesize isoandrosterone-4-^{14}C (4) from dihydrotestosterone-4-^{14}C as follows: the 17β-hydroxy group was protected as the tetrahydropyranylether[4] (5), and this was followed by reduction of the 3-oxo group with sodium borohydride[3] (6) to give the 3β-hydroxysteroid which was then acetylated[5] (7). After acid hydrolysis of the tetrahydropyranylether[6], the corresponding 17β-hydroxysteroid was obtained (8), and this compound was oxidized with chromic acid[2] to give isoandrosterone-4-^{14}C acetate (9). After alkaline hydrolysis[6] isoandrosterone-4-^{14}C (4) was obtained. The 5α-androstane-3α,17β-diol-4-^{14}C (10) was synthesized from dihydrotestosterone-4-^{14}C by enzymatic reduction using rat ventral prostate 3α-hydroxysteroid oxidoreductase[7] and NADPH as cofactor. The androsterone-4-^{14}C (11) was prepared from 5α-androstanedione-4-^{14}C (2) by enzymatic reduction as above. The labelled products isolated by thin layer chromatography had mobilities that corresponded to those of authentic nonradioactive steroid standards. These products were further characterized by crystallization to constant specific activity after addition of corresponding nonradioactive steroids.

Since the carbon-14 is stably incorporated into the steroid nucleus at the C-4 position, and since no unlabelled steroid was added, the specific activity of the precursor and products are identical.



EXPERIMENTAL SECTION

Acetone, ethyl acetate, methanol, petroleum ether (30-60°C), chloroform, 2-propanol, pyridine and dihydropyran of analytical reagent grade were purchased from Mallinckrodt Chemical Works. Sodium borohydride and *p*-toluenesulfonic acid were obtained from Matheson Coleman and Bell. The dihydrotestosterone-4-^{14}C (50 mCi/mmole) was purchased from New England Nuclear, and nonradioactive steroids were obtained from Steraloids, Inc. NADPH was purchased from P-L Biochemicals, Inc. Thin layer chromatography (TLC) was performed on silica gel precoated plates (Polygram Sil G-HY, Macherey-Nagel & Co) except when otherwise stated, and the plates were developed with one or two solvent systems. Crystallizations were carried out after the addition of

60 mg of the corresponding authentic nonradioactive steroid. The samples were crystallized 5 times; the specific activities corresponding to the mother liquors (ML) and crystals (CR) remaining after the last crystallization are given for each isolated product. The following solvent systems were used for crystallization: 5α-androstanedione, ethyl ether-petroleum ether (20-40°C); androsterone and isoandrosterone, acetone-petroleum ether (20-40°C); 5α-androstane-3β,17β-diol and 5α-androstane-3α,17β-diol, acetone.

5α-Androstanedione-4-^{14}C (2). Jones reagent[2] (0.1 ml) was added to a solution of dihydrotestosterone-4-^{14}C (1) (1.5 μCi) in 1 ml of acetone and the mixture was left for 30 min at room temperature with occasional stirring. Water (2 ml) was added, and the mixture was extracted with ethyl ether (4x7 ml). The organic layer was washed with water (3x1 ml) and then evaporated to dryness at 45°C under nitrogen. Product 2 was purified by TLC using chloroform-methanol (98.5:2.5, v/v) and then methylene chloride-ethyl acetate (8:2, v/v). 5α-Androstanedione-4-^{14}C had a R_f = 0.72, and dihydrotestosterone-4-^{14}C a R_f = 0.48. The yield of 5α-androstanedione-4-^{14}C was 1.3 μCi. An aliquot (10.3nCi) was used for crystallization to constant specific activity (nCi/mmol: ML1 48.6, ML2 47.3, ML3 47.3, ML4 47.3, ML5 46.4, CR 47.7).

5α-Androstane-3β,17β-diol-4-^{14}C (3). Dihydrotestosterone-4-^{14}C (1) (0.5 μCi) was dissolved in 1 ml of 2-propanol and 5 mg of sodium borohydride was added[3]. After standing at room temperature for 96 h, the mixture was evaporated to dryness and redissolved in ethyl acetate (7 ml) and water (1 ml). The organic phase was transferred to a clean tube, and the aqueous phase was reextracted with ethyl acetate (3x7 ml). The pooled organic extract was then washed with water (3x2 ml) and dried at 45°C under nitrogen. 5α-Androstane-3β,17β-diol-4-^{14}C was purified by TLC using chloroform-methanol (98.5:2.5,v/v)

and then methylene chloride-ethyl acetate (8:2, v/v). After development in both solvent systems the R_f of this product was 0.28 and the yield was 0.44 μCi. An aliquot (38 nCi) was used for crystallization to constant specific activity (nCi/mmol: ML1 200, ML2 191, ML3 181, ML4 176, ML5 178, CR 180).

Isoandrosterone-4-^{14}C (4). Dihydropyran (0.2 ml) and 3.0 mg of *p*-toluenesulfonic acid were added to dihydrotestosterone-4-^{14}C (1) (2.0 μCi) and the mixture was left for 15 h at room temperature[4]. A solution of 5% sodium bicarbonate in water (6 ml) and benzene (3 ml) were added to the resultant dark, viscous material, and the mixture was shaken periodically for 24 h. The benzene layer was transferred to a 25 x 150 mm tube (teflon cap) and the aqueous layer was reextracted with benzene (3x3 ml). Pyridine (50 μl) was added to the benzene extract, and evaporated to dryness at 45°C under nitrogen. The residue contained dihydrotestosterone-17β-tetrahydropyranylether-4-^{14}C (5) which was not isolated. To the residue, pyridine (25 μl), 2-propanol (1 ml) and sodium borohydride (16 mg) were added, and the mixture was left at room temperature for 96 h. The solvent was evaporated under nitrogen at 45°C. The product, 3β-hydroxy-5α-androstane-17β-tetrahydropyranylether-4-^{14}C (6), was acetylated[5] by adding pyridine (1 ml) and acetic anhydride (0.3 ml) and allowing the mixture to stand at room temperature for 15 h. The reagents were evaporated under nitrogen at 45°C. The product 3β-acetoxy-5α-androstane-17β-tetrahydropyranylether-4-^{14}C (7), was not isolated but used for acid hydrolysis[6] of the tetrahydropyranylether. Methanol (7 ml) containing 0.2 ml of 37% hydrochloric acid was added to the residue and left at room temperature for 15 min. The solution was chilled, and 10 ml of 5% sodium bicarbonate solution in water was added to neutralize the acid. The solvent was partially evaporated at 45°C under nitrogen, and the aqueous

residue was extracted with ethyl acetate (4x7 ml). The pooled ethyl acetate layers were washed with water (2x1 ml), and evaporated to dryness at 45°C under nitrogen. This extract contained 3β-acetoxy-5α-androstan-17β-ol-4-^{14}C (**8**), which was not purified. The residue was dissolved in acetone and 1 ml of Jones reagent[2] was added dropwise over 10 min. to maintain the orange color, which is an indication of reagent availability. After chilling, a 5% sodium bicarbonate solution was added very slowly to neutralize the acid (litmus paper was used to follow the reaction). The residue was extracted with ethyl acetate (4x7 ml), and the organic solvent was washed with water (2x1 ml) and evaporated at 45°C under nitrogen. An oily residue containing isoandrosterone-4-^{14}C acetate (**9**) was obtained. Most of the oily contaminant was removed by dissolving the residue in a mixture of water (0.5 ml) and ethyl acetate (0.5 ml) and extracting with 10 ml of petroleum ether (30-60°C). Extraction with petroleum ether was repeated 3 times. The petroleum ether was evaporated to a final volume of 5 ml and left at room temperature. Under these conditions, a residue separated on the walls of the capped tube. The petroleum ether solution containing the labelled steroid was transferred to another tube and evaporated. Product 9 was purified by TLC on aluminum oxide GF_{254} Type E (Merck, Darmstadt) [benzene-ethyl ether (8:2, v/v, 2 ascents). Isoandrosterone-4-^{14}C acetate had a R_f = 0.81]. For comparison of chromatographic mobility, 10 μg of authentic isoandrosterone acetate was spotted on both sides of the plate, and the authentic standards were detected using a spray of rhodamine 6-G (0.2 mg) in ethanol (200 ml); the compound was visualized under long wave ultraviolet light. The area corresponding to product 9 was scraped and the adsorbent was transferred to a disposable pipette containing a glass wool plug, and eluted with 5 ml ethyl acetate; 95% of the total radio-

activity on the plate was found in this area. The labelled product 9 was rechromatographed and used for alkaline hydrolysis[6] as follows: the dried product was dissolved in 9 ml of 1% potassium hydroxide solution in methanol-water (9:1), gassed with nitrogen (and the tube capped) and kept for 15 h at 4°C. A 5% sodium bicarbonate solution (4 ml) was added. Gas developed and a gel formed. The mixture was evaporated at 45°C under nitrogen to a final volume of 2 ml, extracted with ethyl acetate (4x7 ml), and the organic layer was washed with water (2x2 ml) and evaporated to dryness at 45°C under nitrogen. The product, isoandrostrone-4-^{14}C was purified by TLC on aluminum oxide GF_{254} [benzene-ethanol (96:2, v/v). Isoandrosterone-4-^{14}C had a R_f = 0.49]; the location of the compound was detected as indicated above. The overall yield of isoandrosterone-4-^{14}C was 0.9 μCi. An aliquot of isoandrosterone-4-^{14}C (9.5 nCi) was used for crystallization to constant specific activity (nCi/mmol: ML1 49.1, ML2 49.1, ML3 48.6, ML4 49.1, ML5 49.0, CR 49.5).

5α-Androstane-3α,17β-diol-4-^{14}C (10). Dihydrotestosterone-4-^{14}C (1) (0.5 μCi) was dissolved in 9 ml of 0.1 M potassium phosphate buffer, pH 7.4. A cytosol fraction (4.8 ml), prepared from rat ventral prostate containing 3α-hydroxysteroid oxidoreductase[7], and NADPH (3.7 mM final concentration) were added, and the mixture was incubated for 72 h at 24°C. The reaction was terminated by the addition of 30 ml of chloroform-methanol (2:1). The chloroform layer was transferred to a clean tube and the aqueous residue was reextracted twice with 10 ml chloroform. The pooled chloroform layers were washed with water (3x5 ml) and evaporated to dryness at 45°C under nitrogen. Product 10 was purified by TLC [a) chloroform-methanol (98.5:2.5, v/v), and b) methylene chloride-ethyl acetate (8:2, v/v). 5α-Androstane-3α,17β-diol-4-^{14}C had a R_f = 0.26] . The yield of 5α-androstane-3α,17β-

diol-4-^{14}C was 0.41 μCi. An aliquot of product 10 (29.4 nCi) was used for crystallization to constant specific activity (nCi/mmol: ML1 145, ML2 148, ML3 153, ML4 145, ML5 147, CR 144).

Androsterone-4-^{14}C (11). 5α-Androstanedione-4-^{14}C (2) (0.8 μCi) was dissolved in 9 ml of potassium phosphate buffer pH 7.4; 4.8 ml of rat prostate cytosol containing 3α-hydroxysteroid oxidoreductase[7] and NADPH (3.7 mM final concentration) were added, and the mixture was incubated for 72 h at 24°C. Steroids in the reaction mixture were extracted as described above. Product 11 was purified by TLC [a) chloroform-methanol (98.5:2.5,v/v), and b) methylene chloride-ethyl acetate (8:2, v/v). Androsterone-4-^{14}C had a R_f = 0.48]. The yield of purified androsterone-4-^{14}C was 0.63 μCi. An aliquot (17.5 nCi) was used for crystallization to constant specific activity (nCi/mmol: ML1 78.8, ML2 79.3, ML3 78.8, ML4 78.8, ML5 79.3, CR 78.8).

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