NOTES

SYNTHESIS OF [20-^{14}C]-METHANDROSTENOLONE[1]

KEY WORDS

Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); methandrostenolone (17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one); [20-^{14}C]-methandrostenolone.

INTRODUCTION

During preliminary metabolism and excretion studies with unlabelled methandrostenolone (17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one), it was found that only about 30% of the dose fed to rats was recovered from urine and faeces. This low recovery prompted a desire for a supply of ^{14}C-labelled methandrostenolone.

A single stage synthesis of [4-^{14}C]-methandrostenolone was possible by dehydrogenation of [4-^{14}C]-methyltestosterone but this intermediate was very expensive and not readily available. It was found that a relatively inexpensive route was available to synthesize the title compound as an alternative to the same chemical labelled at C_4. A peripherally labelled compound would often be less dependable for biological studies. However, no mass spectral evidence has been seen of methyl exchange in either *in vivo* (rat) or *in vitro* experiments, despite a literature report of epimerisation at C_{17} in humans dosed with unlabelled methandrostenolone (1).

EXPERIMENTAL

Specific activities were determined in ScintiVerse®[2] in either a Nuclear Chicago Unilux I system (efficiency determined by the addition of internal standard [^{14}C]-benzoic acid) or in a Beckman LS-9000 system (efficiency determined by H number and subsequent computer data reduction). All counting was carried out in plastic vials.

Radiochromatographic analysis was done with a Nuclear Chicago Actigraph III equipped with a thin-layer plate conveyor system (Model 1006), scan speed

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30 cm/hr, slit width 1.5 mm. All plates were coated with 250 μm of silica gel G-F 254 and were developed twice in acetone/petroleum ether (60-90°) (1:4).

Gas-liquid chromatographic analyses were carried out with a Varian Chromatograph (Model 1840), using hydrogen flame ionisation detectors and glass columns (180 cm long, 2 mm internal diameter) packed with 2% OV-7 on Chromosorb G-HP (100-120 mesh). The carrier gas was nitrogen (30 ml/min). The column temperature was programmed to start at 240° (6 min) then allowed to rise to 275° at a rate of 6°/min.

The alumina used in column chromatography was supplied by British Drug Houses Ltd., Chemicals, Brockman Activity II and had 5% w/w water added 24 hr prior to use.

Synthesis of [20-^{14}C]-methyltestosterone

Starting from dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one) (25 mg, 0.087 mM), the [20-^{14}C]-methyltestosterone was prepared essentially as described by Hyde et al. (2,3), except that aluminum isopropoxide was used in the Oppenauer oxidation instead of aluminum t-butoxide. Following steam distillation, the reaction mixture was extracted with ether (3x10 ml) and the combined ether extracts were washed successively with saturated sodium potassium tartrate solution, water and brine. After concentration under reduced pressure, the extract was spread on thick layer (1000 μm) silica gel G-F 254 plates and developed in the acetone/petroleum ether solvent. The radioactive band corresponding to methyltestosterone was scraped off and eluted with acetone. Evaporation of the solvent under reduced pressure gave the desired crude product, which was carried to the next stage without further purification.

Synthesis of [20-^{14}C]-methandrostenolone (4)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (20 mg), benzoic acid (10 mg) and benzene (1 ml) were added to the crude [20-^{14}C]-methyltestosterone. The mixture was refluxed for 15 hr, then filtered through cotton wool and the filtrate was

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[2]Fisher Scientific Co. Ltd.

placed on top of an alumina column (5.0 x 0.5 cm). The column was eluted with methylene chloride (6 ml), then 25% v/v acetone in methylene chloride (10 ml). The solvents were evaporated under a stream of nitrogen and unlabelled methandrostenolone (200 mg) was added to the residue. Recrystallization from an acetone/petroleum ether mixture gave 190.9 mg of purified product, m.p. 163.5° - 164°C, specific activity 0.11 mCi/mmol. Radiochemical purity was very high (>99%) with the balance of the radioactivity due almost entirely to traces of [20-^{14}C]-methyltestosterone. Chemical purity was >99.5% in gas chromatographic analysis and was very similar to that of the commercial methandrostenolone used to dilute the crude reaction product (5).

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