SYNTHESIS OF TRITIUM-LABELLED 7α-HYDROXY AND 7β-HYDROXY DEHYDROEPIANDROSTERONE

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SUMMARY

Tritium labelled 7α-hydroxy and 7β-hydroxy dehydroepiandrosterone were synthesised by the allylic acyloxylation of [1,2-$^{3}H(n)$] dehydroepiandrosterone-3β-acetate with t-butylperbenzoate. The 7α epimer was of comparable specific activity to the substrate.

Key Words: Dehydroepiandrosterone, 7α-Hydroxy dehydroepiandrosterone, Tritium, Allylic acyloxylation

INTRODUCTION

The hydroxylation of dehydroepiandrosterone (DHA) at the C_7 carbon atom has been demonstrated in various human and animal tissues *in vitro*[1,2,3]. In order to investigate the biological role of such compounds, high specific activity 7α and 7β hydroxy DHA were required for radioimmunoassay and metabolism studies.

The authors have successfully used the method of Stárka[4] for the synthesis of milligram quantities of 7α and 7β-hydroxy DHA. This procedure involves acyloxylation of the Δ^5-steroid 3β-acetate with t-butylperbenzoate in acetic acid, catalysed by cuprous bromide. Application of this same technique was inadequate when applied to the synthesis of microgram quantities of tritium and ^{14}C labelled 7α-hydroxy DHA (approximate yield, 1%). Major degradation products were not identified.

The present investigation describes the synthesis of high specific activity tritium labelled 7α-hydroxy DHA (approximate yield,10%) from

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[1,2-^{3}H(n)]DHA using the acyloxylation reaction with toluene as the solvent. [^{3}H] 7β-hydroxy DHA was also produced (approximate yield, 1%). The procedure was also used in the synthesis of ^{14}C labelled 7α and 7β-hydroxy DHA. Preliminary experiments using [7(n)-^{3}H] DHA 3β-acetate as substrate produced [7-^{3}H] 7α and 7β-hydroxy DHA.

MATERIALS AND METHODS

STEROIDS

Non-radioactive steroids were obtained from Steraloids, Wilton, New Hampshire, U.S.A. and Sigma, St Louis, U.S.A. The 7-hydroxy epimers of DHA were synthesised by the method of Stárka[4].

[1,2-^{3}H(n)] DHA (specific activity 58.6 Ci/mmol) was obtained from New England Nuclear, Mass., U.S.A. [7(n)-^{3}H] DHA (specific activity 16.6 Ci/mmol) and [4-^{14}C] DHA (specific activity 52mCi/mmol) were obtained from the Radiochemical Centre, Amersham, England.

REAGENTS

Solvents were of analytical grade and chemicals used were of laboratory reagent grade.

Aluminium oxide (Woelm neutral, activity grade 1) was obtained from ICN Pharmaceuticals, GmbH and Co, West Germany and deactivated with 6% water to activity III[5]. Precoated silica gel layers (0.25 mm) were obtained from E.Merck, Darmstadt, Germany. Chromatography paper (Whatman Chr.1) and Phase Separating paper (No.1 PS) were obtained from W. and R. Balston, Kent, England.

The liquid scintillation counting fluid was 2,5-diphenyl oxazole [4 gm] and 1,4-bis [2(5-phenyloxazolyl)]benzene [100 mg] per litre of toluene. All evaporations were carried out at <45^{o} with oxygen-free nitrogen.

CHROMATOGRAPHY, LOCALISATION AND ESTIMATION OF RADIOACTIVE STEROIDS

The following systems were used for thin layer chromatography

(t.l.c.) and paper partition chromatography (p.p.c.)

I. Chloroform-ethanol (9:1, v/v)

II. Development in dichloromethane-methanol (93:7, v/v) followed by diethyl ether[6].

III. The Bush A system[7] for 6½ hours.

IV. Heptane-toluene-methanol-water (9:11:16:4 by vol) for 48 hours.

The reference steroid DHA and its 7-hydroxylated epimers were visualised with phosphomolybdate and antimony trichloride reagents[8] respectively. 7-Keto DHA was located by observation under ultra violet light. Radioactivity was detected using a Radiochromatograph Scanner [Packard 7200] and quantitated on a Packard Liquid Scintillation Spectrometer [Model 2425]. Methanol [0.5 ml] was added to the scintillation fluid [10 ml] when measuring carrier 7α and 7β-hydroxy DHA during crystallisation experiments.

EXPERIMENTAL

ALLYLIC OXIDATION OF [1,2-^{3}H(n)] DHA-3β-ACETATE

DHA-3β-acetate[2] (500μCi) and cuprous bromide (3 mg) in toluene (2 ml) were gently refluxed with stirring in a nitrogen atmosphere. t-Butylperbenzoate (0.5 ml) in toluene (1 ml) was added dropwise to the mixture over a period of 15 minutes and the reaction continued for a further 2 hours. After cooling, the mixture was washed with saturated sodium bisulphite solution (2 ml), the supernatent filtered through phase separating paper and the filtrate evaporated to dryness. The residue was dissolved in 2.5% (w/v) methanolic KOH (5 ml) and hydrolysed at 30^{o}C with shaking overnight. After neutralisation with 1 M-HCl the products were extracted with benzene (2 x 10 ml), and the combined extracts filtered through phase separating paper and evaporated to dryness.

ISOLATION AND CHARACTERISATION

Column chromatography of the residue on aluminium oxide (activity

III; 6.6 gm, height of column 5 cm) was carried out collecting fractions (3 ml) and examining the eluates for radioactivity by scintillation counting.

Fraction	Solvent	Product	Radioactivity (μCi)
1 - 9	Benzene	-	negligible
10 - 20	Benzene with 0.5% ethanol	-	negligible
21 - 26	" with 1.0% "	A	119.2
27 - 31	" with 1.0% "	-	negligible
32 - 37	" with 3.0% "	B	18.2
38 - 43	" with 3.0% "	C	8.1
44 - 51	" with 4.0% "	D	48.6
52 - 60	" with 5.0% "	E	4.5

(i) Product A cochromatographed with DHA in systems I (Rf 0.61) and III (migration distance 19 cm) but the majority of the radioactivity did not cocrystallise to constant specific activity with carrier DHA (Table I). The identity of the contaminant is unresolved.

(ii) Product B, in system I, gave a main peak at the locality of 7-keto DHA, R_{DHA} 0.84. However, the radioactivity in this region did not cocrystallise with carrier 7-keto DHA. Other unidentified peaks, of lower magnitude, at R_{DHA} 0.93 and R_{DHA} 0.70, were not examined further.

(iii) Product C had the same mobility as 7β-hydroxy DHA in system II (Rf 0.41) and system IV (migration distance 11.3 cm). The product purified in these systems (5.3 μCi) cocrystallised to constant specific activity with carrier 7β-hydroxy DHA (Table I).

(iv) Products D and E cochromatographed with 7α-hydroxy DHA in system II (Rf 0.34) and system IV (migration distance 14.2 cm). The combined radioactivity from products D and E was purified in system IV, to yield [1,2-^{3}H(n)] 7α-hydroxy DHA (51.0μCi). A portion was cocrystallised to constant specific activity with

carrier 7α-hydroxy DHA (Table I).

Table I. The Specific Activities of Carrier Steroids after Recrystallisation

Carrier	Solvent System	Specific Activities (d.p.m./mg)		
		Co	CR	ML
7α-hydroxy DHA	B/H	3172	3337	2922
	"		3135	3431
	M/W		3202	
7β-hydroxy DHA	A/H	2417	2626	2095
	B/H		2561	2292
	D/H		2445	
DHA	B/H	3835	1915	6515
	A/H		1531	3458
	A/H		1283	1622

B,benzene; H,hexane; M,methanol; A,acetone; D,diethylether; W,water. Co,starting material; CR,crystals; ML,mother liquor.

DISCUSSION

The use of the acyloxylation reaction involving peroxides has been reviewed[9]. The generally accepted mechanism involves homolysis of the peroxyester by cuprous ions in a suitable inert solvent. The low yields and degradation observed using acetic acid in the acyloxylation reaction prompted a comparison of benzene[9,10] and toluene as reaction solvents. Toluene was the most effective, possibly because of its similar boiling point (110°C) to acetic acid (118°C)

Preliminary synthesis was performed using [7(n)-^{3}H] DHA since, at the time, this was the highest specific activity tritium labelled DHA commercially available. Tritium nuclear magnetic resonance studies of [7(n)-^{3}H] DHA suggest that, of the batches examined, greater than 90% of the tritium was at the 7-position[11]. The tritium label was approximately equally distributed between the 7β and 7α positions, the 7β position being preferred. In one batch the 16α position was also labelled. Acyloxylation of the 7α position would cause a loss of the tritium substituent. Similarly the alkaline hydrolysis step can be expected to displace some label from the labile 16α position. Thus the maximum specific activity likely in a 7α-hydroxylated reaction product would be about half that of the starting material. The conversion of this substrate to [7β-^{3}H] 7α-hydroxy DHA and [7α-^{3}H] 7β-hydroxy DHA was 4.6 and 0.6 per cent respectively. The possibility of a primary isotope effect, caused by involvement of the tritium labelled protons at C_7, has not been studied by the authors.

A competitive protein binding method using antisera raised in rabbits immunised with 3β,7α-dihydroxy androst-5-ene-17β-carboxy-bovine serum albumin (unpublished results) was used to estimate the specific activity of the [^{3}H] 7α-hydroxy DHA produced. The specific activity of the synthesised [1,2-^{3}H(n)] 7α-hydroxy DHA was 49-56 Ci/mmol (84-95% of the specific activity of the starting material).

The specific activity of the [7β-^{3}H] 7α-hydroxy DHA synthesised from [7(n)-^{3}H] DHA (specific activity 16.6 Ci/mmol) was 4.9 Ci/mmol.

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