SYNTHESIS OF Δ^4-3-OXO-STEROIDS LABELLED SPECIFICALLY AT THE 6β-POSITION

P.Toft, A.J.Liston and A.Y.Viau
Research Laboratories
Health Protection Branch
Department of National Health and Welfare
Ottawa, Canada
K1A 0L2
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SUMMARY

[6β-^2H] Testosterone and [6β-^3H] Testosterone were each synthesized in good yield in three steps: Treatment of 5α, 6α-epoxy-3, 3-ethylene-dioxyandrostan-17β-ol with labelled lithium aluminum hydride is followed by deacetalation and dehydration under carefully controlled basic conditions.

INTRODUCTION

The 6β-position of Δ^4-3-oxo-steroids is one of the major sites of hydroxylation in several mammalian species (1). In connection with some studies on the mechanism of such hydroxylations, a steroid labelled specifically at the 6β-position was required, and no suitable literature method was available for its synthesis. The syntheses of [6β-^{2}H] and [6β-^{3}H] testosterone are described; the method is also applicable to other Δ^4-3-oxo-steroids.

EXPERIMENTAL

Melting points were recorded on an Electrothermal apparatus

and are corrected. N.m.r. spectra were determined with a Varian A-60 A spectrometer for solutions in deuterochloroform with tetramethylsilane as internal standard. Mass spectra were determined with a Hitachi-Perkin-Elmer RMU-6L spectrometer. G.L.C. was carried out with a model 5750 F & M gas chromatograph. The columns were 5% OV-210 (trifluorosilicone) on 60/80 Chromosorb W (8 ft. x 4 mm i.d.); carrier gas helium; flow rate 60 ml/min; column temperature 225°. Tetrahydrofuran was distilled from lithium aluminum hydride immediately before use. Lithium aluminum deuteride was obtained from International Chemical and Nuclear Corporation, Irvine, California, and lithium aluminum tritide was purchased from New England Nuclear, Boston, Mass.

[6β-^{2}H] 3,3-Ethylenedioxyandrostane-5α, 17β-diol (IIa):

5α, 6α-Epoxy-3,3-ethylenedioxyandrostan-17β-ol (1) (2,3) (1.40 g) in dry tetrahydrofuran (100 ml) was heated under reflux with stirring in an atmosphere of nitrogen with lithium aluminum deuteride (0.3 g, 99 atom % ^{2}H) for 1 hr. G.L.C. analysis of an aliquot indicated that reaction was complete and it was quenched with 1% aqueous sodium hydroxide. The reaction mixture was diluted with ether, washed with water, and dried (Na_2SO_4). The solvent was evaporated, and the residue was crystallized from acetone-hexane yielding the title compound IIa (1.21 g): m.p. 202-205°; molecular weight by mass spectrometry 351.

Unlabelled 3,3-ethylenedioxyandrostane-5α,17β-diol (II) was synthesized from compound I (1.12 g) using lithium aluminum hydride (0.5 g). The reaction was carried out as above, and the residue crystallized from acetone-hexane yielding 0.92 g; m.p. 202-204°; $[\alpha]_D^{24}$ -8° (c, 1.0, $CHCl_3$) (lit (4) m.p. 202-204°; $[\alpha]_D^{20}$ -8°); n.m.r. δ 0.74 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 3.65 (1H, t, J = 8 H_2, 17-H) and 3.97 (4H, s, acetal-H_4) p.p.m.; molecular weight by mass spectrometry 350.

[6β-^{2}H]Testosterone (IVa):

Compound IIa (980 mg) was heated on a steam bath for 15 min. with acetic acid (15 ml) and water (5 ml). The solution was extracted with ether. The ethereal solution was washed with saturated aqueous sodium bicarbonate and water, and dried (Na_2SO_4). The product (IIIa) was dissolved in methanol (60 ml) and water (39 ml) and treated with 0.1 N sodium hydroxide 1 ml. The solution was stirred at room temperature for 16 hr. and then treated with acetic acid (0.5 ml). The solution was evaporated to half volume on a rotary evaporator, and the product was isolated with ether, purified on a column of Florisil, and crystallized from acetone-hexane to yield [6β-^{2}H] testosterone (610 mg); m.p. 144-145°; $[\alpha]_D^{24}$ +71° (c, 1.0, EtOH); mass spectrum m/e (intensity) 289 (100) and 288 (4); n.m.r. δ 0.79 (3H, s, 18-H_3), 1.20 (3H, s, 18-H_3) and 5.73 (1H, s, 4-H)p.p.m.

Unlabelled material prepared by the same route was identical (m.p., rotation, i.r. and mass spectrum) with authentic testosterone.

[6β-^{3}H] Testosterone (IVb) - was obtained, in a manner analogous to that described above, from compound I (25 mg) and lithium aluminum tritide (1.9 mg, 37.8 mCi/mM). The final product (IVb) was purified on t.l.c. plates using benzene-ethanol (9:1) as eluant to yield 6 mg of pure material, which had a specific activity of 3.9 mCi/mM.

DISCUSSION

The reaction sequence followed is shown in the chart. Treatment of I with lithium aluminum hydride results in diaxial opening of the epoxide ring to yield the diol II (cf. ref. 5). The mass spectrum of compound IIa, prepared by using lithium aluminum deuteride, demonstrates the incorporation of one atom of deuterium per molecule.

The acetal group of IIa was hydrolyzed under mild acidic conditions so as to avoid subsequent acid catalyzed dehydration. The mass spectrum of the product IIIa again indicates that each molecule contains one atom of deuterium.

If suitably mild basic conditions are used, dehydration of steroidal 6β-alkyl-5α-hydroxy-3-ketones can be achieved without affecting the stereochemistry of the 6-substituent (2,3,6). The base used has to be strong enough to effect the dehydration reaction but sufficiently mild so as not to cause formation of the 3,5-dienol from the Δ^4-3-ketone produced. Enolization of 6β-alkyl-4-en-3-ones leads to epimerization of the 6-substituent via loss of the equatorial 6α-hydrogen followed by reprotonation of the 3,5-dienol. Enolization of unsubstituted 4-en-3-ones causes loss of either the 6α-equatorial or the 6β-axial hydrogen, with the latter being lost preferentially (7). Enolization of IVa or IVb would result therefore in either loss of 6β-label or its epimerization. Consequently the conditions required for the dehydration of compounds IIIa and IIIb are critical. 6β-Alkyl-5α-hydroxy-3-oxo-steroids can be dehydrated with complete retention of configuration at the 6-position by using 0.00475 N sodium hydroxide (2), i.e. enolization via loss of the 6α-equatorial hydrogen does not occur under these conditions. Compounds IVa and IVb were prepared by using 0.001 N sodium hydroxide. Compound IVa had 96% retention of deuterium as measured by mass spectrometry. Furthermore the n.m.r. spectrum of IVa has a singlet at δ 5.73 for the olefinic proton at C-4. In the spectrum of testosterone the corresponding signal is present as a doublet due to allylic coupling with the 6β-proton (8). Under these conditions for the dehydration step therefore, no epimerization at the 6-position would have occurred and all the label is present at the 6β-position.

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OH
O
O
O
I
LiAlH4*
OH
OH
H*
II
AcOH | H2O
III
0.001 N NaOH
IV
a H*= deuterium
b H*= tritium