SYNTHESIS OF TRIDEUTERATED STANOZOLOL LABELLED SELECTIVELY AT THE 17-METHYL GROUP

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SUMMARY

A synthetic procedure for stanozolol-d_3 is described. Deuterium labelling was achieved by the Grignard reaction of androstan-3β - ol - 17 - one with deuterium labelled methyl magnesium iodide (CD_3 Mg I). The synthesis was achieved in five steps from Δ5-androstan-3-β-ol-17-one in an overall yield of 15%. The procedure resulted in a product which was selectively deuterated and chemically pure.

Key Words : Stanozolol-d_3, Deuterium, Mass Fragmentography, Mass Spectrum.

INTRODUCTION

The gas chromatograph - mass spectrometer equipped with a multiple ion detector is being used increasingly in biological assays because of its high specificity. In this technique carriers which have isotopes already built into the parent compound serve as the ideal internal standard to correct for losses of the compound under study in the initial isolation procedures.

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Stanozolol (17α-hydroxy-17-methylandrostano [3,2-c] - pyrazole) is a steroid used in the treatment of anabolic disorders. In connection with an attempt[1] to establish a gas chromatographic - mass spectrometric method for the determination of stanozolol we have synthesised the title compound for use as in internal standard. The 17-methyl group was chosen not only because it offered the possibility of introducing three deuterium atoms but also because this position did not seem to suffer from any problems due to extensive isotope scrambling or loss of labelling atoms.

EXPERIMENTAL

Infra-red spectra were recorded from dispersions in potassium bromide using a Perkin Elmer 177 spectrophotometer. PMR spectra were obtained using a Varian 60 MHz spectrometer for solutions in deuterochloroform with tetramethylsilane as internal standard. Mass spectra were recorded on an AEI MS 9 mass spectrometer.

Iodomethane - d_3 (99 + atom % D) gold label was supplied by the Aldrich Chemical Company, Gillingham, Dorset.

Δ 5-Androstan 3β-ol-17 one (1)

This compound was purchased from Sigma, London, Kingston-upon Thames, Surrey : m.p. 143 - 145^{o}C (Lit 2 140 - 141^{o}C); n.m.r. δ 5.36 (one olefinic proton); i.r. 3420, 1750 cm^{-1} ; MS m/z 288 (M^{+}) .

Androstan-3β-ol-17-one (2)

The preparation of this unlabelled compound was carried out by catalytic hydrogenation of (1) using Pd/C in ethanol in a standard procedure, to give the product (2) in 92% yield . m.p. 161 - 164°C (lit.[3] 161 - 162°C), n.m.r. no olefinic protons i.r. 3460, 1750 cm^{-1}; MS m/z 290 (M^+).

17 - (d_3) - Methylandrostane-3, 17 - diol (3)

Iodomethane - d_3 (15 g, 0.16 mole) in dry ether (60 ml) was added slowly to magnesium turnings (2.68 g ; 0.11 mole) under a nitrogen atmosphere at such a rate that the reaction mixture refluxed gently. When this addition was completed and the reaction mixture had cooled, the ketone (2) (4 g , 14 mmole) in dry ether (300 ml) was added and the resulting mixture heated under reflux for seven hours. The reaction mixture was poured carefully on to a mixture of ice (300 g) and excess 2 M hydrochloric acid and the product collected by filtration, washed with 2 M hydrochloric acid, water and finally ether. Recrystallisation of the solid from ethyl acetate gave (3) in 46% yield. m.p. 200 - 201.5°C i.r. : 2220 cm^{-1} C - D stretch; MS m/z 309 (M^+).

17 - (d_3) Methylandrostan-17β-ol-3-one (4)

The diol (3) (1.7 g ; 5.5 mmole) was dissolved in glacial acetic acid (40 ml) and a solution of chromium trioxide (559 mg) in glacial acetic acid (23 ml) and water (4 drops) was added slowly with stirring. The mixture was stirred for a further 3 hours at room temperature and then diluted with water. The aqueous mixture was extracted with ether (2 x 200 ml). The ethereal extracts were washed with water and dilute sodium hydroxide before being dried ($MgSO_4$) and concentrated under reduced pressure to give a white solid (4) in 60% yield (1.0 g) m.p. 168 - 170°C. The product was shown to be homogeneous by t.l.c.; i.r. 3420, 2220, 1700 cm^{-1}. MS m/z 307 (M^+).

Stanozolol - d_3 (6)

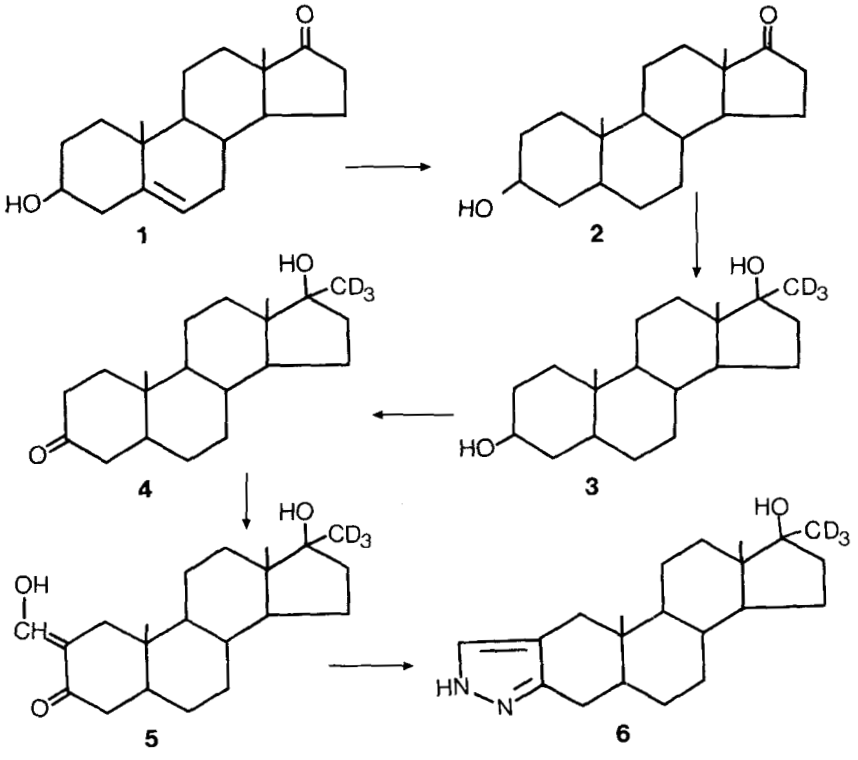
Sodium hydride (50% disp. in oil ; 3 g ; 62 mmole) was added to a solution of the ketone (4) (2 g ; 6.5 mmole) and ethyl formate (2 ml) in dry benzene under an atmosphere of nitrogen. Immediate effervescence was observed and the solution became yellow in colour. The solution was stirred for eighteen hours at room temperature and then methanol (10 ml) was added. The mixture was poured into water (300 ml) and extracted with ether (2 x 200 ml). The aqueous mixture was then acidified and the resulting precipitate was extracted with ether. The ether extract was dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow foam. This was crude hydroxymethylene product (5).

The hydroxymethylene compound (5) was dissolved in ethanol (25 ml), hydrazine hydrate (1 g ; 20 mmole) added, and the resulting mixture heated under reflux for 90 minutes. The solvent was removed under reduced pressure and the resulting product purified by chromatography on silica gel using first chloroform (800 ml) and then a chloroform-methanol mixture (9 : 1). The resulting solid was recrystallised from ethanol to give stanozolol-d_3 (6) in 50% yield (1.0 g) m.p. 149 - 170°C ; t.l.c. identical with unlabelled stanozolol. MS m/z 331 (M^+).

DISCUSSION

The choice of the 17-methyl group for deuterium labelling was based primarily on a desire to introduce three or more deuterium atoms into the steroid molecule. The preparation of stanozolol-d_3 was accomplished as shown in the scheme. Introduction of three deuterium atoms was achieved by treatment of androstan-3β-ol-17-one (2) with deuterium labelled methyl magnesium iodide to give (3).

Oxidation of (3) with chromium trioxide followed by reaction with methyl formate and then hydrazine gives stanozolol - d_3 (6). For precise evaluation of the labelling results the deuterium labelled stanozolol had to be pure. Conventional purification procedures by chromatography and recrystallisation proved to be quite effective for this purpose.



Mass spectrometric analysis of the stanozolol - d_3 obtained in this synthesis demonstrated very high isotopic purity.

In mass spectrometry the use of trideuterated stanozolol as an internal standard will greatly increase the sensitivity of the analytical method and should lead to a more successful method than before.[4,5] The application of the stanozolol - d_3 obtained in this synthesis for the determination of stanozolol in biological fluids by mass fragmentography has been described elsewhere.[1]

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