Deuterated anabolic drugs. Part 2

Synthesis of (R,S)-3,4-bis(4-hydroxyphenyl)-[2,2,5,5-d_4]hexane (HEX-d_4) and (E,E)-3,4-bis(4-hydroxyphenyl)-[2,5-d_2]hexa-2,4-diene (DE-d_2)

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Summary

Hexestrol-d_4 was synthesized from 1-hydroxy-1p-methoxyphenyl-[2,2-d_2]propane (7) by reductive coupling. The preparation of 7 involved reduction of p-methoxy-[2,2-d_2]propiophenone (2b) obtained by exchange deuteration. Dienestrol-d_2 was synthesized from p-hydroxy-[2,2-d_2]propiophenone by pinacol coupling followed by dehydratation of the coupling product using acetic anhydride/acetyl chloride.

Introduction

As part of a continuing program concerning the monitoring of illegal use of anabolic drugs as growth rate improving agents, sensitive and reliable methods are required in order to control the use of these anabolics. Although radioimmunoassay or enzyme linked immunoassay offer sensitive and fast screening methods, a

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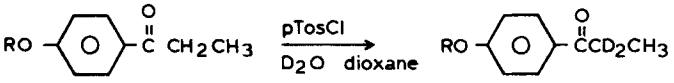
reliable back-up system is needed to verify the results obtained with these methods. Isotope-dilution-gas-chromatography-mass-spectroscopy (ID-GC-MS) offers a sensitive and reliable tool excellently suited for this purpose. In this connection deuterated internal standards are required. Recently we described the synthesis of hexadeuterated diethylstilbestrol (DES-d_6) (1). The preparations of deuterated hexestrol (HEX-d_4) and deuterated dienestrol (DE-d_2) are reported in this paper.

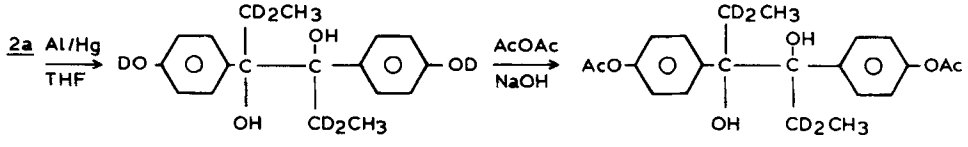
Results and discussion

The synthetic route leading to dienestrol-d_2 is shown in scheme 1 (2).



1a R=H
1b R=CH3

2a R=D
2b R=CH3



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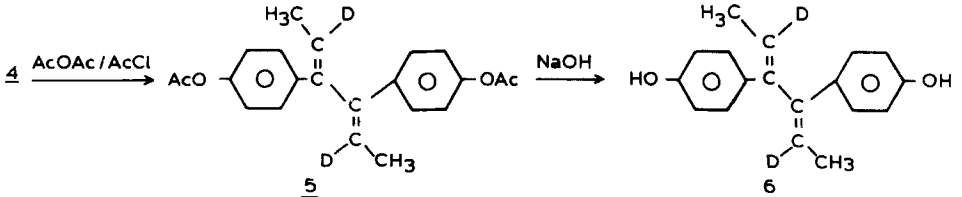

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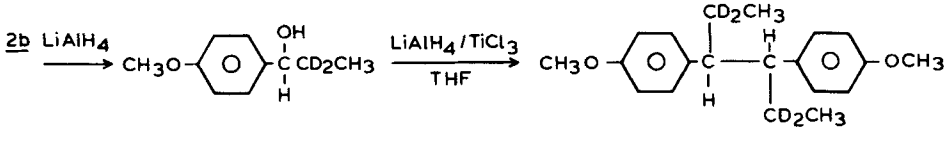
Scheme 1

The starting material, p.hydroxypropiophenone (1a), exchanged its alpha protons in a mixture of dioxane/D_2O containing p-toluenesulphonylchloride. The product (2a), obtained in 90% yield, was treated with aluminium amalgam in tetrahydrofuran (THF) to afford the pinacol 3 (as a mixture of dl and meso isomeric forms). Acylation using acetic anhydride in aqueous base gave the diacetoxy compound 4. Crude 4 was dehydrated by heating it under reflux in a mixture of acetic anhydride and acetyl chloride. The product mixture, containing dienestrol-d_2-acetate (5) was saponified with NaOH in aqueous methanol to yield dienestrol-d_2 (6) in an overall yield of 30% after purification (chromatography followed by crystallization). The compound was characterized by chromatographic (TLC and GC) and spectroscopic (NMR and MS) methods.

Scheme 2 shows the synthetic route leading to deuterated hexestrol (HEX-d_4).



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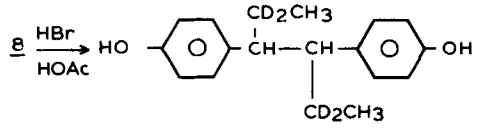

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Scheme 2

p-Methoxy-[2,2-d_2]propiophenone (2b) was prepared by exchange of the alpha protons of 1b using the same system as described for the preparation of deuterated p-hydroxypropiophenone. Reduction of 2b with lithium aluminium hydride in THF gave the deuterated hydroxy compound 7 in 90% yield. Reductive coupling of 7 was accomplished using the lithium aluminium hydride/ titanium trichloride system (3) in refluxing THF. The meso product 8 was readily obtained from the reaction mixture by crystallization. Demethylation of 8 was effected using HBr in boiling acetic acid to afford the key compound meso-hexestrol-d_4 (9) in 25% yield from 7. The product was characterized by TLC, GC, NMR and MS.

Experimental

NMR spectra were taken using a Varian EM 360 NMR spectrometer. Absorptions were recorded in δ-values. GC analyses were performed on a Varian 3700 gas chromatograph, equiped with a 3 % OV-1 on Supelcoport (100-120 mesh) column (2 m x 2 mm) operated at a temperature of 200 °C and a flow-rate (nitrogen) of 25 ml/min. HPLC analysis were performed with the aid of a Kipp Analytica 9208 HPLC pump on an 150 x 4.6 mm Hypersil ODS column. (Eluent 65 % (v/v) methanol in water, UV detection at 254 nm). Mass spectra were recorded on a MAT mass spectrometer (direct inlet).

Deuterated p-hydroxy-propiophenone (2a)

p-Hydroxy-propiophenone (5 g) in a mixture of dioxane/ D_2O (1/1, 100 ml) was heated under reflux in a nitrogen atmosphere with p-toluenesulphonylchloride (1 g) overnight. After cooling to room temperature the mixture was saturated with salt and the aqueous

layer separated and extracted with diethyl ether. The organic layers were dried and evaporated to give 4.8 g of deuterated p-hydroxy-propiophenone (NMR ($CDCl_3$) : δ 1.2 (s,3H), 6.8 and 7.8 ppm (AB-system, 4H)).

Deuterated p-methoxy-propiophenone (2b)

p-Methoxy-propiophenone-d_2 was prepared from p-methoxy-propiophenone using the same conditions as described for the preparation of deuterated p-mhydroxy-propiophenone; NMR ($CDCl_3$) : δ 1.2 (s,3H), 3.8 (s,3H), 6.9 and 7.9 ppm (AB-system, 4H).

Dienestrol-d_2 (6)

Aluminium foil (4.5 g) was treated with a 2% $HgCl_2$ solution (150 ml). After 3 minutes the amalgam was washed with water, absolute ethanol and diethyl ether. The dry amalgam was covered with dry THF (ca. 100 ml). To this mixture 2.5 g of deuterated p-hydroxy-propiophenone (2a) were added. After one hour the mixture was gently heated under reflux in a nitrogen atmosphere for 3 hours. The reaction mixture was filtered, dried over Na_2SO_4 and evaporated to afford the crude pinacol (2 g). The product was dissolved in 10% NaOH (20 ml) and treated with acetic anhydride (10 ml). The diacetylated product (4) consisted of a mixture of dl- and meso-pinacol. This mixture was heated under reflux with a mixture of acetic anhydride/ acetyl chloride (2/1, 100 ml) for 2 hours. The reaction mixture was poured into water and neutralized with powdered potassium carbonate. Extraction with dichloromethane, drying over Na_2SO_4 and evaporation of the solvent yielded crude dienestrol-d_2. Deacetylation was achieved by treatment of the product with 10% NaOH in ethanol. The product was purified by flash chromatography (SiO_2, hexane/ EtOAc, 2/1)

followed by recrystallization from benzene. The product thus obtained had a purity > 95% as determined by NMR, HPLC and GLC. NMR (CD_3COCD_3) : δ 1.4 (s,6H), 6.8-7.1 (AB-system, 8H), 8.0 ppm (s,2H). MS m/z (%) : 268 (100), 253 (55), 238 (60), 146 (35), 122 (52)

1-Hydroxy-1-p-methoxyphenyl-[2,2-d_2]propane (7)

Deuterated p-methoxy-propiophenone (1.64 g) in 10 ml of absolute THF was added to lithium aluminium hydride (0.12 g) in 20 ml of THF. After stirring for 15 minutes the mixture was cautiously treated with excess water. Filtration and removal of the solvent by distillation afforded 7 as an oil (1.5 g); NMR ($CDCl_3$) : δ 0.8 (bs,3H), 2.8 (bs,1H), 3.7 (s,3H), 4.4 (s,1H), 6.8-7.3 ppm (AB-system,4H).

Hexestrol-d_4 (9)

To a stirred slurry of $TiCl_3$ (3 g) in 60 ml of THF was added $LiAlH_4$ (0.4 g) followed by 15 ml of THF. After the initial vigorous reaction subsided the mixture was stirred for 30 minutes. 1-Hydroxy-1-p-methoxyphenyl[2,2-d_2]propane (1.04 g) in 10 ml of THF was added over 5 minutes. The mixture was stirred and heated under reflux in a nitrogen atmosphere for 2 hours. After cooling to room temperature the mixture was poured into water and extracted with dichloromethane (3 x 40 ml). The organic layers were washed with water, dried over Na_2SO_4 and evaporated to yield deuterated hexestrol dimethyl ether as a mixture of meso and dl isomers. The meso compound crystallized from 15 ml of methanol (yield 0.4 g; NMR ($CDCl_3$) : δ 0.5 (s,6H), 2.47(s,2H), 3.8 (s,6H), 6.7-7.3 ppm (AB-system,8H)). A solution of 8 (400mg) in 8 ml of hydrobromic acid in acetic acid (40%) was heated under reflux for

3 hours. The reaction mixture was cooled to room temperature, poured on ice and extracted with diethyl ether (3 x 50 ml). The combined organic layers were washed with water (4 x 40 ml) and with 5% $NaHCO_3$ solution. The ether solution was extracted with 5% NaOH (3 x 20 ml) and the aqueous extracts washed with diethyl ether (2 x 30 ml). The aqueous layer was acidified with diluted sulfuric acid and then extracted with diethyl ether (3 x 30 ml). The combined extracts were washed with water, dried over Na_2SO_4 and the diethyl ether evaporated yielding meso-hexestrol-d_4. Recrystallization from aqueous ethanol afforded 250 mg of white needles (NMR (CD_3COCD_3) : δ 0.49 (s,6H), 2.40 (s,2H), 5.0 (bs,2H), 6.6-7.1 ppm (AB-system,8H)). MS m/z (%) : 137 (100)

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