SYNTHESIS OF DEUTERIUM LABELED 17-METHYL-TESTOSTERONE

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ABSTRACT

The synthesis of two forms of selectively deuterated 17-methyl-testosterone is described. 17-Methyl-d_3-testosterone was prepared by the Grignard reaction of dehydroepiandrosterone with deuterium labeled methyl magnesium iodide followed by an Oppenauer oxidation. 17-Methyl-d_3-testosterone-19,19,19-d_3 was prepared by treating 3,3-ethylenedioxy-5,10-epoxy-5α,10α-estran-17-one with deuterium labeled methyl magnesium bromide followed by hydrolysis and dehydration of the 5α-hydroxyandrostane derivative.

INTRODUCTION

Methyltestosterone (17β-hydroxy-17-methyl-4-androsten-3-one) is a synthetic androgen which has been used in the treatment of eunuchism, eunuchoidism, male impotence and female breast cancer. It is an orally effective hormone analog and has been shown to have considerable higher oral activity than testosterone (1-3).

The bioavailability / bioequivalency regulations of the United States Food and Drug Administration (FDA) listed 110 drugs and drug dosage forms which were known or suspected of having potential bioavailability / bioequivalency problems (4). Although methyltestosterone is one of the drugs listed, there appears to be little information on its pharmacokinetic or bioavailability

characteristics.

The use of stable isotopes has become accepted in drug metabolism, pharmacokinetic and bioavailability studies (5-7). Studies on the relative bioavailability of several different formulations of the same drug have been performed effectively by use of a stable isotope labeled variant as the reference with which the test unlabeled formulations are compared (8-10).

We have undertaken studies to assess the relative bioavailability of methyltestosterone tablet formulations with coadministration of a stable isotope labeled methyltestosterone solution as an internal biological standard. In the present paper, we describe the synthese of 17-methyl-d_3-testosterone (methyltestosterone-d_3) and 17-methyl-d_3-testosterone-19,19,19-d_3 (methyltestosterone-d_6).

EXPERIMENTAL

All melting points are uncorrected. Optical rotations were determined on a JASCO DIP-4 digital polarimeter with ethanol as solvent. IR spectra were recorded with a Shimadzu IR-400 spectrometer for KBr. ^{1}H-NMR spectra were determined on a Varian EM-390 90 MHz NMR spectrometer for solutions in deuteriochloroform with tetramethylsilane as an internal standard (chemical shifts in δ ppm). Mass spectra were recorded on a Shimadzu QP-1000 gas chromatograph-mass spectrometer (EI: 20 eV. CI: 200 eV, reagent gas: isobutane).

17α-methyl-d_3-5-androstene-3β,17β-diol (II)

The deuterium labeled Grignard reagent (0.83 M of CD_3MgI-dry ether solution) was prepared by using methyl iodide-d_3 (CD_3I, 99.5 atom%d, Merck). To 36 mL (29.7 mmol) of the above Grignard reagent was added dropwise over 100 min a solution of 1.5 g (5.20 mmol) of dehydroepiandrosterone [3β-hydroxy-5-androsten-17-one](I) (Sigma) in 30 mL of dry ether. The solution was refluxed for 7 hr. After cooling, 50 mL of 1.5 M ammonium chloride solution was added dropwise.

Dilution with H_2O, extraction with ether, washing with 3M HCl, 5% $NaHCO_3$, 10% $Na_2S_2O_3$ and H_2O, followed by drying over $MgSO_4$ and evaporation gave 1.49 g (93%) of crystalline residue. It was recrystallized from ethyl acetate. mp 202 - 203°; IR ν_{C-D} 2250 cm^{-1}; MS m/z 307(M^+).

17β-Hydroxy-17-methyl-d_3-4-androsten-3-one (III)

A solution of 0.65 g (2.11 mmol) of the diol (II) in 15 mL of toluene and 3 mL of cyclohexanone was heated to 106° and 2 mL of the solvent was removed by distillation. To this hot solution, 2.3 g of aluminum isoproxide was added rapidly and the mixture stirred and heated under reflux for 3 hr. The reaction mixture was then poured into an ice bath and 0.5 mL of acetic acid was added to ensure decomposition of the alkoxide-steroid complex. After steam distillation, the residual aqueous solution was extracted with benzene, and washed with 3M HCl, 5% $NaHCO_3$, and H_2O. After drying over $MgSO_4$ and evaporating the solvent, a yellow residue was obtained. After silica gel column chromatography of the residue using dichloromethane-acetone (9:1) as an eluting solvent, the purified product (III)(0.46 g, 72%) was obtained as colorless crystals following evaporation of the solvent under reduced pressure. The crystals were recrystallized from n-hexane-AcOEt (5:1). mp 162° (The mmp with 17-methyl-testosterone showed no depression.); IR ν_{O-H} 3430 cm^{-1}, ν_{C-D} 2230 cm^{-1}; NMR 0.92 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 5.76 (1H, s, 4H); MS m/z 305(M^+); $[\alpha]_D$ +80°; Anal. Calcd. for $C_{20}H_{27}D_3O_2$: C, 78.64; H, 9.90. Found: C, 78.55; H, 9.85.

3,3-Ethylenedioxy-5,10-epoxy-5α,10α-estran-17-one (V)

To a solution of 1.00 g (3 mmol) of 3,3-ethylenedioxy-5,10-epoxy-5α,10α-estran-17β-ol (IV) (11) in 12 mL of dry pyridine was added 1.00 g (10 mmol) of chromium trioxide (reagent grade) in small portions with efficient stirring and cooling (internal temperature 15 - 20°) over a period of 30 min under nitrogen. The mixture was then stirred at room temperature for 20 hr. Afterwards, water was added to the reaction mixture; extraction with dichloromethane followed by washing with H_2O, drying over Na_2SO_4 and evaporation gave a yellow residue. After silica gel column chromatography of the residue using dichloromethane-acetone (9:1) as an eluting solvent, the purified product (V) (0.58 g, 58%) was obtained as colorless crystals following evaporation of the solvent under reduced pressure. IR $\nu_{C=O}$ 1730 cm^{-1}; NMR 0.90 (3H, s, 18-CH_3), 3.88 (4H, s, 3-O-CH_2-CH_2-O-); MS (CI) m/z 333 (M+1).

3,3-Ethylenedioxy-17-methyl-d_3-5α-androstane-19-d_3-5,17β-diol (VI)

The deuterium labeled Grignard reagent (0.84 M of CD_3MgBr-dry tetrahydrofuran solution) was prepared by using methyl bromide-d_3 (CD_3Br, 99.5 atom%d, Merck). To 18 mL (15.14 mmol) of the above Grignard reagent was added dropwise a solution of 0.385 g (1.16 mmol) of the epoxide (V) in 6 mL of dry tetrahydrofuran. The solution was reflux for 2 hr. After cooling, 30 mL of 1.5 M ammonium chloride solution was added dropwise. Dilution with H_2O, extraction with dichloromethane, washing with 3M HCl, 5% $NaHCO_3$ and H_2O, followed by drying over $MgSO_4$ and evaporation gave 0.393 g of crystalline residue. Purification of the residue by silica gel column chromatography using benzene-AcOEt (1:1) as an eluting solvent furnished 0.120 g (28%) of the product (VI). IR ν_{O-H} 3500 - 3350 cm^{-1}, ν_{C-D} 2225 cm^{-1}; NMR 0.89 (3H, s, 18-CH_3), 3.99 (4H, s, 3-O-CH_2-CH_2-O-); MS m/z 370 (M^+).

17β-hydroxy-17-methyl-d_3-4-androsten-19-d_3-3-one (VIII)

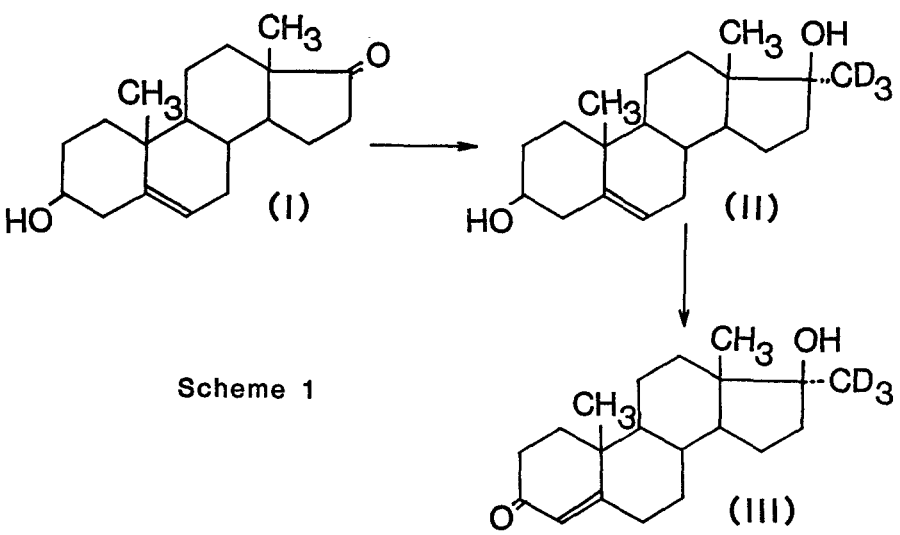
A solution of 0.113 g (0.305 mmol) of the 5α-hydroxy-3-cycloethyleneketal (VI) in 5 mL of MeOH was treated with 0.5 mL 1 M H_2SO_4 under reflux for 20 min. After cooling, ether and H_2O were added, and the aqueous layer was separated and re-extracted with ether. The combined extract was washed with 5% $NaHCO_3$ and H_2O and dried over $MgSO_4$. Removal of the ether by evaporation resulted in 0.070 g (0.215 mmol) of 5,17β-dihydroxy-17-methyl-d_3-5α-androstan-19-d_3-3-one (VII) as colorless crystals.

The above product (VII) was dissolved in 5 mL of MeOH, treated with 0.5 mL of 5% methanolic KOH, heated under reflux for 1 hr, and cooled. After dilution with H_2O and extraction with ether, the combined ether solution was washed with 1 M HCl, 5% $NaHCO_3$ and H_2O, followed by drying over $MgSO_4$. The ether was then evaporated to give 0.042 g of a crystalline residue. After silica gel column chromatography of the residue using benzene-AcOEt (1:1) as an eluting solvent, the purified product (VIII) (0.032 g , 34%) was obtained as colorless crystals. Recrystallization from n-hexane-AcOEt (5:1) led to 0.020 g (0.065 mmol) of the final product (VIII) as colorless needles. IR ν_{C-D} 2225 cm^{-1}, $\nu_{C=O}$ 1670 cm^{-1}, ν_{C-D} 1041 cm^{-1}; NMR 0.92 (3H, s, 18-CH_3), 5.76 (1H, s, 4H); MS m/z 308 (M^+).

RESULTS AND DISCUSSION

In the synthesis of deuterium labeled methyltestosterone for use as an internal biological standard, special attention was paid to the selective deuterium labeling of the 17α-methyl group. The 17α-methyl position was chosen not only because it offered the possibility of introducing three deuterium atoms but also because this position seemed not to suffer from any primary isotope effect. The main metabolic pathways of methyltestosterone (12-15), i.e., 3-keto reduction, C-16 oxygenation and glucuronidation, do not involve any reactions at the 17-methyl position.

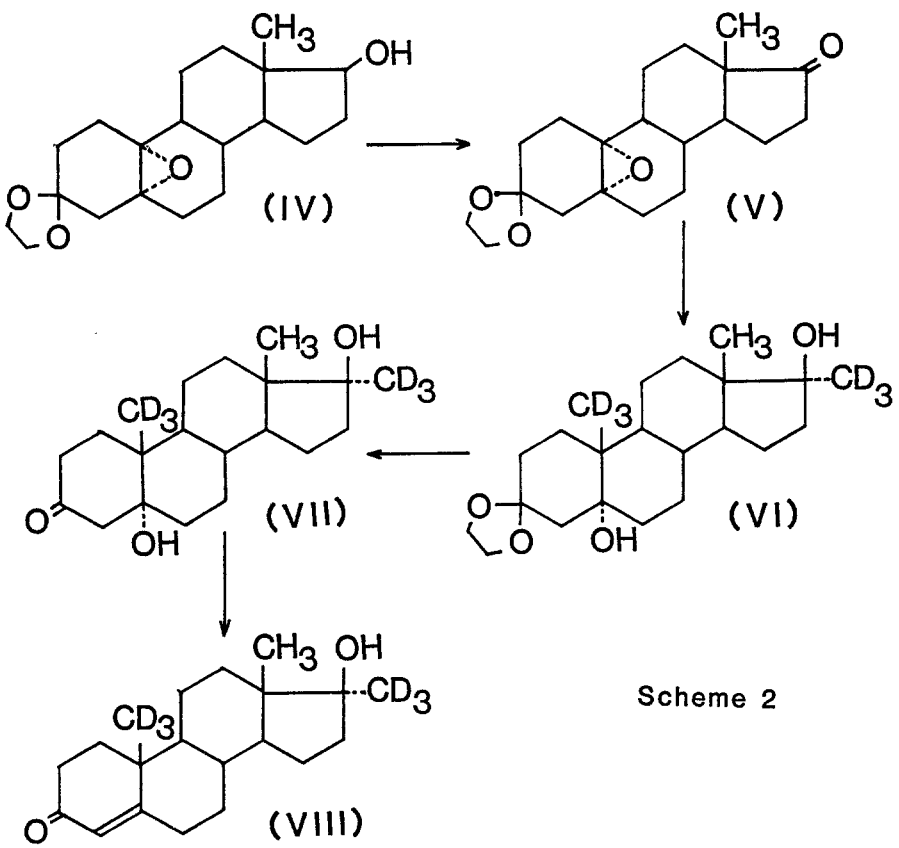
The method employed here for the synthesis of methyltestosterone-d_3 was a modification of the procedure of Hyde *et al.* (16,17) and the synthetic sequence is shown in Scheme 1. Introduction of three deuterium atoms was achieved by treatment of dehydroepiandrosterone with



Scheme 1

a six-fold excess of deuterium labeled methyl magnesium iodide followed by an Oppenauer oxidation with aluminium isoproxide.

The electron impact mass spectrum of the product (III) showed the molecular ion at m/z 305 (the unlabeled compound; m/z 302). The IR spectrum of the product (III) showed the C-D streching vibration band at 2230 cm^{-1}. These results confirmed that three deuterium atoms were incorporated into the 17α-methyl group of methyltestosterone. The chemical purity of methyltestosterone-d_3 was confirmed by gas chromatography, thin layer chromatography and an elemental analysis.



Scheme 2

Mass spectrometric analysis of methyltestosterone-d_3 obtained in this experiment demonstrated very high isotopic purity of the product (III) (d_3; 98.64%, d_2; 1.36%, d_1; 0.00%: 99.6 atom% d).

Methyltestosterone-d_6 (VIII) for use as an internal standard for mass fragmentographic assays was synthesized by the routes presented in Scheme 2. Formation of the 5α,10α-epoxy-17-keto-steroid (V) proceeded in 58% yield by a Sarett oxidation (18) of the 5α,10α-epoxide (IV) (11), wheres a Jones oxidation of (IV) resulted in opening of the epoxide ring. The Grignard reaction of the 5α,10α-epoxy-17-keto steroid (V) with CD_3MgBr successfully incorporated six deterium atoms in the steroid molecule, three at C-19 and three at the 17α-methyl group, because of the presence of two reactive functional groups in (V). The use of CD_3MgI instead of CD_3MgBr was unsuccessful in the synthesis of (VI) due to the formation of 19-nor-derivative.

Mass spectrometric determination of the final labeled product (VIII) revealed high isotopic purity of 99.3 atom% d (d_6; 96.88%, d_5; 2.26%, d_4; 0.86%). This purity is sufficient for use as an internal standard for mass fragmentographic assays.

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