SYNTHESIS OF [^{2}H]$_5$-EPITESTOSTERONE

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SUMMARY

Isotopically pure d_5-epitestosterone has been synthesized for the first time. This polydeuterated 17α-hydroxyandrosterone was prepared by H/D exchange reaction with MeOD-D_2O under basic conditions. Its characteristics are described in detail, including its enolization into a d_4-heterodienic silyl ether derivative upon silylation.

Keywords: [^{2}H]$_5$-17α-Hydroxyandrost-4-en-3-one, d_5-epitestosterone, deuteration, labeling, synthesis, dienolsilyl ether

INTRODUCTION

Deuterated epitestosterone (17α-hydroxyandrost-4-en-3-one, 17α-*cis*-testosterone) was required as a standard for the determination of urinary epitestosterone in doping analysis. The quantitation is performed by gas chromatography - mass spectrometry selected ion monitoring (GC/MS SIM) using the standard screening procedure for anabolic steroids.[1] In this procedure, the urine sample is extracted using either XAD-2 resin or bonded phase column (C18) followed by enzymatic hydrolysis of steroid glucuronides, extraction with diethyl ether and derivatization of steroids to trimethylsilyl ethers.

For quantitation, the use of a polydeuterated analog as an internal standard is highly recommended. It is desirable that the internal standard is labelled by at least 3-4 deuterium atoms in order to obtain a straight line calibration curve free of interference from the compound to be measured. In addition, the deuterated internal standard should be compatible with all sample preparation steps because any re-exchanges of C—D to C—H can not be tolerated.

d_1- And d_2- labeled derivatives of epitestosterone have been synthesized previously from 3-methoxy-3,5-androstadiene-17-one.[2] The latter was reduced by $NaBD_4$ to give a mixture of 17-d_1-testosterone and 17-d_1-epitestosterone in an unspecified yield and ratio in about 90% isotopic purity, the remainder consisting of unlabeled products. 16,16-d_2-Epitestosterone was prepared by deuteration of 3-methoxy-3,5-androstadiene-17-one with NaOD/CD_3OD followed by $NaBH_4$ reduction, hydrolysis and HPLC purification to afford a 0.7% yield of 16,16-d_2-epitestosterone.[2] It is not clear why the expensive CD_3OD had to be used here instead of CH_3OD as the methanol methyl group is not involved in any reactions.

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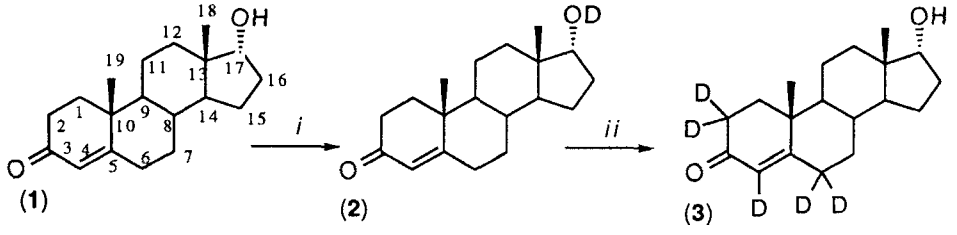
RESULTS AND DISCUSSION

Since we required epitestosterone with a minimum of three D atoms we envisaged a Mitsunobu inversion[3] of the commercially available 16,16,17-d_3-testosterone. Unexpectedly, this reaction was unsuccessful and we will report a study of this failure separately. However, a base catalyzed H/D exchange reaction[4] of 17-*O*-*d*-epitestosterone (**2**) in MeOD-D_2O furnished 2,2,4,6,6-[^{2}H]$_5$-epitestosterone (**3**) in quantitative yield and with a d_5 isotopic purity over 90% according to ^{13}C-NMR and MS data. After the exchange reaction the product was treated with a large excess of H_2O to reinstate the protic hydroxy group so as to avoid ambiguities resulting from uncontrolled OD/OH exchange.



Scheme 1. Synthesis of d_5-epitestosterone (**3**) from predeuterated epitestosterone (**2**). *i* : D_2O, *ii* MeONa, MeOD, D_2O, then H_2O.

As expected, the d_5-deuterated epitestosterone (**3**) performs very satisfactorily in GC/MS SIM analysis, despite the fact that one D atom is lost when the standard is trimethylsilylated using trimethyliodosilane[5] as a catalyst. This is due to enolization[5] of the 3-keto group to give, after silylation, the heterodienol silyl ether (**4**) in 99% yield and pure by GC. This enol ether (**4**) produces an abundant molecular ion (M^+ 436, 100%) in the mass spectrum increasing the sensitivity for the selected ion monitoring (SIM) quantitation. The structure of this dienol silyl ether was established by NMR, UV and MS spectroscopy, all the spectral data being completely incompatible with the alternative homodienic silyl ether structure. To our knowledge there is no literature report of the exact structure of the dienol silyl ether of testosterone or epitestosterone although the enolization is known to occur on silylation with a number of catalysts.

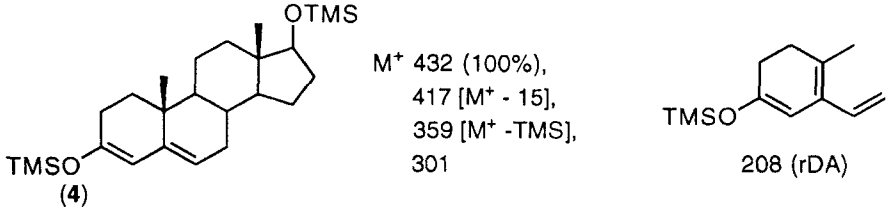


Figure 1. The dienol silyl ether of epitestosterone (**4**) and its main fragments in the mass spectrum.

EXPERIMENTAL

The m.p. was determined in an open capillary tube with an Electrothermal apparatus, and is uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian GEMINI-200 FT NMR spectrometer for solutions in $CDCl_3$ and CD_3COCD_3 (Me_4Si as the internal standard). Mass spectra were obtained with a Jeol JMS SX102 mass spectrometer operating at 70 eV, or 10-20 eV when determining the deuterium content. Samples were introduced at 120-150°C by a direct-inlect probe. TLC was conducted on Merck silica gel 60 F_{254} plates with CH_2Cl_2-EtOAc (7:2) elution. *d_5*-Epitestosterone was fully characterized by ^1H, ^{13}C NMR and DEPT spectra, LRMS and HRMS analysis and the compound was homogenous by TLC. In the ^{13}C NMR spectrum the deuterated methine carbon appears as a low-intensity triplet (t) in the proton noise decoupled spectrum as compared to the singlet in the spectrum of undeuterated material.

2,2,4,6,6-[$^2H]_5$-epitestosterone

Epitestosterone (**1**) was suspended in D_2O and the heavy water was evaporated by rotary evaporator to give the predeuterated epitestosterone (**2**). Slices of clean solid sodium (0.2 g, 8.7 mmol) were added slowly into MeOD (10 ml) under argon followed by **2** (0.1 g, 0.35 mmol). The yellowish reaction mixture was heated to reflux, D_2O (1.5 ml) added and the refluxing continued for 1.5 h. After the reaction MeOD and D_2O were evaporated off

. The residue was dissolved in D_2O and acidified with 2 N H_2SO_4. The precipitated crude product (100 %) was collected by filtration and recrystallized from methanol, m.p. 210-212 °C. In ^1H-NMR the undeuterated signals at δ 5.73 (H-4, 1H), 2.15 (H-2, 2H) are missing; ^{13}C- NMR δ 17.3 (C-18), 17.8 (C-19), 20.9 (C-11), 24.9 (C-15), 31.5 (C-16), 32.3 (C-7), 32.5 (C-6)^t, 32.7 (C-2)^t, 35.9 (C-1 & C-8), 36.1 (C-12), 38.9 (C-10), 45.5 (C-13), 48.5 (C-14), 54.0 (C-9), 80.0 (C-17), 129.6 (C-4)^t, 171.8 (C-5), 200.3 (C-3); *m/z* 293 (M^+, 46%), 291 (35), 276 (32), 260 (14), 247 (19), 231 (19), 147 (65), 129 (41), 105 (17), 93 (27) (Found: M+ 293.2408. $C_{19}H_{23}O_2D_5$ requires 293.2413).

Silylation

Epitestosterone was derivatized by 50µl of a solution of *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide, trimethyliodosilane and dithioerythritol (1000:2:4), at 60 °C for 15 min yielding 99 % of the corresponding dienol silyl ether (**4**), λ_{max} (hexane)/nm 242, 290; ^1H-NMR δ 5.27 (H-4, 1H), 5.16 (H-6, 1H), 3.63 (H-17, 3-H); ^{13}C- NMR δ 151.2 (C-3), 143.5 (C-5), 120.9 (C-6), 111.4 (C-4), 84.3 (C-17), 53.8 (C-9), 51.3 (C-14), 45.8 (C-13), 39.6 (C-2), 36.7 (C-12), 34.7 (C-8), 34.0 (C-1), 33.5 (C-7), 32.9 (C-10), 30.3 (C-16), 24.0 (C-15), 23.5 (C-11), 21.2 (C-19), 20.7 (C-18).

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