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Synthesis of Deuterium-Labelled 16α-Hydroxy-4-androstene-3,17-dione and 16α-Hydroxydehydroepiandrosterone

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16α-Hydroxy-4-androstene-3,17-dione-d_5 (**4**) and 3β,16α-dihydroxy-5-androsten-17-one-d_6 (**10**) were synthesized. Treatment of 16α-bromo-5-androstene-3,17-dione (**2**) with deuterium oxide and methanol-OD gave the 4-en-3-oxo derivative-*d* **3**. The bromide **3** was converted into the 16α-hydroxide-d_5 **4** *via* controlled alkaline hydrolysis using sodium hydroxide-OD in deuterium oxide–pyridine. 3β-Hydroxy-5-androsten-17-one-d_5 (**8**), which was obtained from 17,17-ethylenedioxy-5-androsten-3-one (**5**) *via* treatment with potassium *tert*-butoxide in *tert*-butanol-OD, followed by lithium aluminum tri-*tert*-butoxydeuteride reduction and then acid hydrolysis, was derivatized to the 16α-bromide **9** by treatment with cupric bromide. The bromide **9** was similarly hydrolyzed to yield the 16α-hydroxide-d_6 **10**.

Keywords——controlled alkaline hydrolysis; deuterium labelling; 16α-bromodehydroepiandrosterone-d_5; 16α-bromoandrostenedione-d_4; 16α-hydroxydehydroepiandrosterone-d_6; 16α-hydroxyandrostenedione-d_5

We recently discovered a controlled stereospecific alkaline hydrolysis of 16-bromo-17-oxo androgens[1] and also developed a hydrolytic method for the synthesis of several 16α-hydroxy-17-oxo steroids.[2] A stereospecific deuterium incorporation at the 16β-position of the 16α-hydroxides by hydrolysis using a medium containing deuterium oxide was also reported by us.[2a,d] In view of the quantitative importance[3] of estriol in pregnancy, it is of interest to know the concentrations of 16α-hydroxylated C_{19} steroid precursors in the feto-placental unit.

We report here an efficient synthesis of deuterium-labelled 16α-hydroxy-4-androstene-3,17-dione (**4**) and 16α-hydroxydehydroepiandrosterone[4] (**10**) with good isotopic purity. These compounds are required as carriers and internal standards for quantitative evaluation of the steroids by combined gas chromatography-mass spectrometry (GC-MS). Labelling experiments were undertaken with the intention of placing deuterium atoms at positions 2, 3, 4, 6 and 16 of the steroids, involving the controlled alkaline hydrolysis of the 16α-bromo-17-ones **3** and **9** as a key reaction.

Results and Discussion

When 16α-bromo-5- androstene-3,17-dione (**2**), obtained by 8 N chromic acid oxidation[5] of the corresponding 3β-hydroxide **1**, was heated under reflux in deuterium oxide and diglyme according to Malhotra and Ringold,[6] a debrominated and isomerized product, 4-androstene-3,17-dione-d_6 (d_3 13%, d_4 17%, d_5 20%, d_6 50%), was unexpectedly obtained in high yield. On the other hand, the use of methanol-OD instead of diglyme in the reaction gave a deuterated and isomerized product, 16α-bromo-4-en-3-one derivative **3**. The mechanism involved in the efficient debromination of the 16α-bromide **2** under the former conditions is not clear at present. However, hydrobromic acid liberated by partial decomposition of the bromide **2**-4, 4-d_2, initially produced,[6] should catalyze the isomerization of the 5-en-3-one to the 4-en-3-

one. Previous studies[6,7] have demonstrated that deuterium atoms are incorporated at positions 2, 4 and 6 of the product **3** by the above reaction. The labelled positions were further checked by proton nuclear magnetic resonance (^{1}H-NMR) analysis showing that hydrogens at the C-2 and -6 positions and hydrogen at C-4 had been exchanged for deuterium to the extents of about 50 and 80%, respectively.

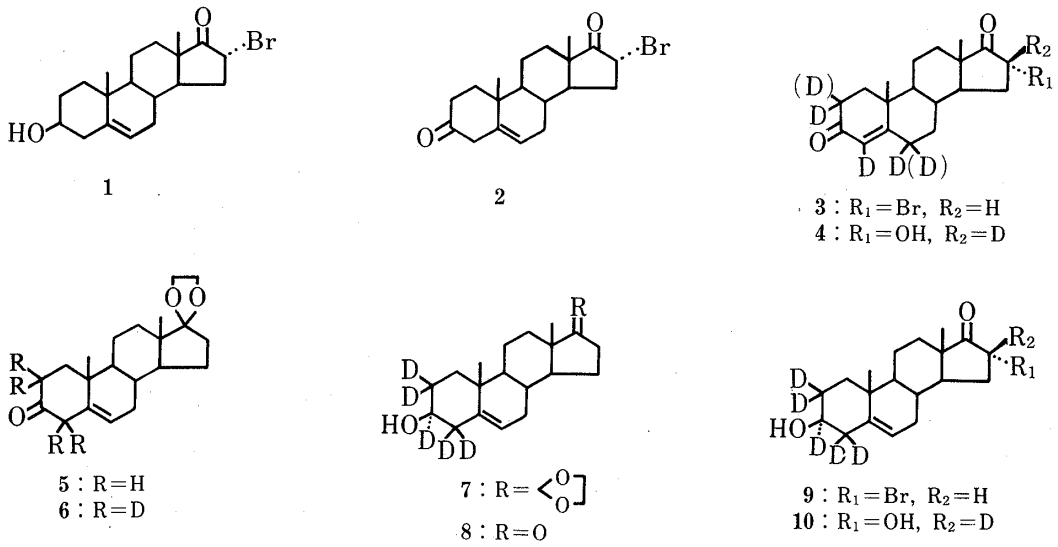

Chart 1

Treatment of deuterio 16α-bromo-17-one **3** with 1.2 eq of sodium hydroxide-OD in deuterium oxide and pyridine[2a] gave the corresponding 16α-hydroxide **4** (d_3 16%, d_4 27%, d_5 52%, d_6 5%) with more than 98% deuterium at C-16β in quantitative yield. Equilibration of the unsaturated ketones **3** and **4** in the basic medium might cause enrichment of the isotope contents at C-2, -4 and -6.[8]

In order to obtain the 16α-hydroxy-17-one-d_6 **10** having a 5-en-3β-ol system, we initially synthesized dehydroepiandrosterone-d_5 (**8**) from 17,17-ethylenedioxy-5-androsten-3-one (**5**). Treatment of compound **5** with potassium *tert*-butoxide in *tert*-butanol-OD, followed by acetic acid protonation[9] gave the deuterio derivative **6** along with the 4-en-3-oxo isomer. The deuterated mixture was subjected to reduction with lithium aluminum tri-*tert*-butoxydeuteride. The product, 3β-hydroxy-17,17-ethyleneacetal-d_5 **7**, was then treated with acid (without isolation) to give compound **8**. The yield (30%) was much improved, compared to that previously reported (4%) which was obtained using 4-en-3-one derivatives as substrates. Deuterium was also efficiently incorporated at C-2, -3α and -4 as expected (d_2 6%, d_3 10%, d_4 25%, d_5 59%).

Bromination of the 17-oxo derivative **8** with 3 eq[2a] of cupric bromide gave the 16α-bromo-17-one **9** in quantitative yield. Treatment of the bromide **9** with sodium hydroxide-OD in deuterium oxide and pyridine[2a] afforded the 16α-hydroxide [10] (d_3 5%, d_4 11%, d_5 26%, d_6 58%) in high yield.

The structures of deuterio compounds **3**, **4**, **9** and **10** were confirmed by the ^{1}H-NMR spectra. This synthesis offers the advantage of permitting a high deuterium content without contamination by the natural form (d_0-species). The deuterio androgens should be suitable as internal standards for mass fragmentography. A quantitative GC-MS analysis of the androgens in biological fluid is under way to further investigate their physiological importance.

Experimental

Melting points were measured on a Yanagimoto melting-point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR 400 spectrometer as KBr pellets. NMR spectra were obtained with a JEOL PMX 60 spectrometer at 60 MHz with tetramethylsilane as an internal standard. Mass spectra were measured on a Hitachi RMU-7 spectrometer.

16α-Bromo-5-androstene-3,17-dione (2)——A solution of 16α-bromo-3β-hydroxy-5-androsten-17-one (**1**) (1.0 g) in acetone (80 ml) was treated with 8 N chromic acid[5] (4 ml) at 0 °C for 5 min. The reaction mixture was poured into ice-water (500 ml). The precipitate was collected by filtration and crystallized from acetone to give **2** (630 mg, 63%) as colorless needles, mp 147—148 °C. IR ν_{max}^{KBr} cm^{-1}: 1745 and 1710. ^{1}H-NMR ($CDCl_3$): δ 0.95 (3H, s, 18-CH_3), 1.21 (3H, s, 19-CH_3), 4.56 (1H, t, J=8 Hz, 16β-H), 5.40 (1H, m, 6-H). *Anal.* Calcd for $C_{19}H_{25}BrO_2$: C, 62.47; H, 6.90; Br, 21.87. Found: C, 62.45; H, 6.66; Br, 21.99.

Treatment of Compound 2 with D_2O——A) A solution of **2** (288 mg) in D_2O (0.7 ml) and diglyme (7 ml) was heated under reflux for 4.5 d. Water was added and the precipitate was collected by filtration and crystallized from acetone to give 4-androstene-3,17-dione-d_6 (130 mg, 45%) as colorless needles, mp 169—171 °C (lit.[10] 168—170 °C). MS: d_3 13%, d_4 17%, d_5 20%, d_6 50%.

B) A solution of **2** (312 mg) in D_2O (0.8 ml) and MeOD (10 ml) was heated under reflux for 4 d. Evaporation of the solvent under reduced pressure gave a solid, which was crystallized from acetone–H_2O to give 16α-bromo-4-androstene-3,17-dione-*d* (**3**) (242 mg, 78%) as colorless needles, mp 170—171 °C (lit.[11] 172—174 °C). ^{1}H-NMR ($CDCl_3$): δ 0.98 (3H, s, 18-CH_3), 1.23 (3H, s, 19-CH_3), 2.30—2.70 (*ca.* 5H), 5.76 (0.2H, s, 4-H).

16α-Hydroxy-4-androstene-3,17-dione-2,2,4,6,16β- or 2,4,6,6,16β-d_5 (4)——Sodium metal (18 mg) was carefully added to a mixture of D_2O (1.5 ml) and pyridine (5.5 ml) and then **3** (242 mg) was dissolved in the solution. The mixture was allowed to stand at room temperature for 2 h. After the same work-up as reported previously,[2d] the crude product (200 mg) was obtained. Crystallization of the product from acetone yielded **4** (185 mg, 93%) as colorless needles, mp 187—188 °C (lit.[2a] 188—190 °C). ^{1}H-NMR ($CDCl_3$): δ 1.00 (3H, s, 18-CH_3), 1.21 (3H, s, 19-CH_3), 5.72 (0.2H, s, 4-H). MS: d_3 16%, d_4 27%, d_5 52%, d_6 5%.

17,17-Ethylenedioxy-5-androsten-3-one (5)——Compound **5** was synthesized according to Williams *et al.*[12] mp 142—146 °C (lit.[12] 141—146 °C).

3β-Hydroxy-5-androsten-17-one-2,2,3α,4,4-d_5(8)——Under a nitrogen atmosphere, *tert*-BuOH (12 ml) was added to **5** (454 mg) and *tert*-BuOK (1.15 g), and the yellow solution was allowed to stand at room temperature for 2 h. Cold AcOD (22 ml, 10%) was rapidly added and the mixture was poured into ice-water. After extraction with ether (300 ml × 2), the organic layer was washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to give an oily residue. Thin-layer chromatographic analysis (hexane–AcOEt, 3 : 1) indicated the residue to be a mixture of 5-en- and 4-en-3-oxo steroids (*ca.* 3 : 1).

$LiAlD_4$ (181 mg) was placed in dry tetrahydrofuran (THF) (14 ml) and the suspension was stirred in an ice bath for 10 min. While the slurry was being stirred, *tert*-BuOH (0.9 ml) was added dropwise, followed by the dropwise addition of a solution of the oily residue obtained above in THF (6 ml). The entire mixture was stirred for 30 min at 0 °C and for 2 h at room temperature. A few drops of water were added to decompose the excess LiAl (*tert*-BuO)$_3$D and the mixture was acidified with 10% HCl solution (10 ml) and then allowed to stand for 5 h at room temperature. The 17-oxo products were recovered by extraction with AcOEt and subjected to silica gel column chromatography (hexane–AcOEt) to give a solid, which was crystallized from acetone–H_2O to yield **8** (120 mg, 30%), mp 150—153 °C (lit.[13] 153 °C). ^{1}H-NMR ($CDCl_3$): δ 0.90 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 5.43 (1H, m, 6-H). MS: d_2 6%, d_3 10%, d_4 25%, d_5 59%.

16α-Bromo-3β-hydroxy-5-androsten-17-one-2,2,3α,4,4-d_5 (9)——A solution of **8** (100 mg) in dry MeOH (10 ml) was heated under reflux for 12 h. After the same work-up as reported previously,[14] a crude product was obtained. Crystallization of the product from MeOH gave **9** (120 mg, 94%), mp 176—178 °C (lit.[14] 175—176 °C). ^{1}H-NMR ($CDCl_3$): δ 0.93 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 4.40 (1H, m, 16β-H), 5.40 (1H, m, 6-H).

3β,16α-Dihydroxy-5-androsten-17-one-2,2,3α,4,4,16β-d_6 (10)——Compound **9** (100 mg) was hydrolyzed with NaOD in D_2O and pyridine essentially as described for the synthesis of **4** to afford **10** (76 mg, 92%) as colorless needles, mp 187—189 °C (MeOH) (lit.[2a] 188—190 °C). ^{1}H-NMR ($CDCl_3$): δ 0.98 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 5.41 (1H, m, 6-H). MS: d_3 5%, d_4 11%, d_5 26%, d_6 58%.

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