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

MITSUTERU NUMAZAWA* and MIEKO OGATA

*Tohoku College of Pharmacy, 4-1 Komatsushima-4-chome,
Sendai, Miyagi 983, Japan*

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16 α -Hydroxy-4-androstene-3,17-dione- d_5 (**4**) and 3 β ,16 α -dihydroxy-5-androsten-17-one- d_6 (**10**) were synthesized. Treatment of 16 α -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 16 α -hydroxide- d_5 **4** via controlled alkaline hydrolysis using sodium hydroxide-OD in deuterium oxide-pyridine. 3 β -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 16 α -bromide **9** by treatment with cupric bromide. The bromide **9** was similarly hydrolyzed to yield the 16 α -hydroxide- d_6 **10**.

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

We recently discovered a controlled stereospecific alkaline hydrolysis of 16-bromo-17-oxo androgens¹⁾ and also developed a hydrolytic method for the synthesis of several 16 α -hydroxy-17-oxo steroids.²⁾ A stereospecific deuterium incorporation at the 16 β -position of the 16 α -hydroxides by hydrolysis using a medium containing deuterium oxide was also reported by us.^{2a,d)} In view of the quantitative importance³⁾ of estriol in pregnancy, it is of interest to know the concentrations of 16 α -hydroxylated C₁₉ steroid precursors in the feto-placental unit.

We report here an efficient synthesis of deuterium-labelled 16 α -hydroxy-4-androstene-3,17-dione (**4**) and 16 α -hydroxydehydroepiandrosterone⁴⁾ (**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 16 α -bromo-17-ones **3** and **9** as a key reaction.

Results and Discussion

When 16 α -bromo-5-androstene-3,17-dione (**2**), obtained by 8 N chromic acid oxidation⁵⁾ of the corresponding 3 β -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, 16 α -bromo-4-en-3-one derivative **3**. The mechanism involved in the efficient debromination of the 16 α -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 (¹H-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 16 α -bromo-17-one **3** with 1.2 eq of sodium hydroxide-OD in deuterium oxide and pyridine^{2a)} gave the corresponding 16 α -hydroxide **4** (d_3 16%, d_4 27%, d_5 52%, d_6 5%) with more than 98% deuterium at C-16 β 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 16 α -hydroxy-17-one- d_6 **10** having a 5-en-3 β -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 protonation⁹⁾ 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, 3 β -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, -3 α 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 16 α -bromo-17-one **9** in quantitative yield. Treatment of the bromide **9** with sodium hydroxide-OD in deuterium oxide and pyridine^{2a)} afforded the 16 α -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 ¹H-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.

16 α -Bromo-5-androstene-3,17-dione (2)—A solution of 16 α -bromo-3 β -hydroxy-5-androsten-17-one (1) (1.0 g) in acetone (80 ml) was treated with 8 N chromic acid³⁾ (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 KBr, cm^{-1} : 1745 and 1710. ¹H-NMR (CDCl₃): δ 0.95 (3H, s, 18-CH₃), 1.21 (3H, s, 19-CH₃), 4.56 (1H, t, J = 8 Hz, 16 β -H), 5.40 (1H, m, 6-H). *Anal.* Calcd for C₁₉H₂₅BrO₂: C, 62.47; H, 6.90; Br, 21.87. Found: C, 62.45; H, 6.66; Br, 21.99.

Treatment of Compound 2 with D₂O—A) A solution of **2** (288 mg) in D₂O (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 D₂O (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–H₂O to give 16 α -bromo-4-androstene-3,17-dione-*d* (**3**) (242 mg, 78%) as colorless needles, mp 170–171°C (lit.¹¹⁾ 172–174°C). ¹H-NMR (CDCl₃): δ 0.98 (3H, s, 18-CH₃), 1.23 (3H, s, 19-CH₃), 2.30–2.70 (*ca.* 5H), 5.76 (0.2H, s, 4-H).

16 α -Hydroxy-4-androstene-3,17-dione-2,2,4,6,16 β - or 2,4,6,6,16 β -*d*₅ (4)—Sodium metal (18 mg) was carefully added to a mixture of D₂O (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). ¹H-NMR (CDCl₃): δ 1.00 (3H, s, 18-CH₃), 1.21 (3H, s, 19-CH₃), 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).

3 β -Hydroxy-5-androsten-17-one-2,2,3 α ,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 $\times$ 2), the organic layer was washed with 5% NaHCO₃ solution and water, dried (Na₂SO₄), 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).

LiAlD₄ (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)₃D 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–H₂O to yield **8** (120 mg, 30%), mp 150–153°C (lit.¹³⁾ 153°C). ¹H-NMR (CDCl₃): δ 0.90 (3H, s, 18-CH₃), 1.03 (3H, s, 19-CH₃), 5.43 (1H, m, 6-H). MS: *d*₂ 6%, *d*₃ 10%, *d*₄ 25%, *d*₅ 59%.

16 α -Bromo-3 β -hydroxy-5-androsten-17-one-2,2,3 α ,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). ¹H-NMR (CDCl₃): δ 0.93 (3H, s, 18-CH₃), 1.00 (3H, s, 19-CH₃), 4.40 (1H, m, 16 β -H), 5.40 (1H, m, 6-H).

3 β ,16 α -Dihydroxy-5-androsten-17-one-2,2,3 α ,4,4,16 β -*d*₆ (10)—Compound **9** (100 mg) was hydrolyzed with NaOD in D₂O 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). ¹H-NMR (CDCl₃): δ 0.98 (3H, s, 18-CH₃), 1.00 (3H, s, 19-CH₃), 5.41 (1H, m, 6-H). MS: *d*₃ 5%, *d*₄ 11%, *d*₅ 26%, *d*₆ 58%.

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