Synthesis of 16α-Hydroxyandrost-4-ene-3,17,19-trione and 3β,16α-Dihydroxyandrost-5-ene-17,19-dione: Potential Intermediates of Estriol Biosynthesis

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16α-Hydroxyandrost-4-ene-3,17,19-trione (10) was synthesized from the 16α-hydroxy-6β,19-epoxy-17-one 3 *via* protection of the 16α-hydroxy function as its *tert*-butyldimethylsilyl ether or acetate. Reductive cleavage of the epoxy ring of the silyl ether 4 or the acetate 5 with zinc dust gave the 19-alcohol 6 or 7, which was treated with pyridinium dichromate or Jones reagent, respectively, and then hydrolyzed with diluted sulfuric acid, yielding the desired steroid 10. 3β,16α-Dihydroxyandrost-5-ene-17,19-dione (14) was also synthesized from 5α-bromo-3β,16α-diacetoxy-6β,19-epoxyandrostan-17-one (11) through the intermediates 12 and 13 with the 3β- and 16α-hydroxy functions protected as their acetates in a reaction sequence similar to that above.

Keywords 16α-hydroxy-6β,19-epoxy steroid; 16α-hydroxy-19-oxo steroid; acetyl ester; *tert*-butyldimethylsilyl ether; zinc dust reduction; Jones oxidation; pyridinium dichromate oxidation; hydrolysis

Estriol is the most abundant estrogen in pregnant women,[1] originating primarily in the placenta during aromatization of 16α-hydroxylated C_{19}-steroid precursors,[2] 16α-hydroxyandrost-4-ene-3,17-dione (**1**) and 16α,17β-dihydroxyandrost-4-en-3-one (**2**), the former being aromatized several times more efficiently than the latter.[3] The overall similarity of the formation of 16α-hydroxyestrogens, estriol and 16α-hydroxyestrone, to that of estrone–estradiol suggests that the 19-hydroxy and 19-oxo derivatives of the 16α-hydroxy C_{19}-steroids may be involved in an intermediary role. Akhtar's group[4] recently has synthesized the 19-oxygenated derivatives of the 16α,17β-dihydroxy steroid **2**, and demonstrated that these 19-oxygenated steroids are involved as intermediates in the estriol biosynthesis. On the other hand, we[5] have reported the synthesis of the 19-hydroxy derivative of the 17-oxo steroid **1**. To know the kinetic and biological properties and the mechanistic aspects of the formation of 16α-hydroxyestrogens, the still unknown 19-oxo derivatives of the 16α-ketol **1** and its 3β-hydroxy-5-ene derivative were required.

We report here the synthesis of the potential endogenous 16α-hydroxy-19-oxo C_{19}-steroids, 16α-hydroxyandrost-4-ene-3,17,19-trione (**10**) and 3β,16α-dihydroxyandrost-5-ene-17,19-dione (**14**).

Results and Discussion

The key intermediates for the synthesis of the 16α-hydroxy-19-oxo steroids **10** and **14** were the 16α-hydroxy-6β,19-epoxy-17-oxo derivatives **3** and **11**, respectively. These compounds were obtained from 3β-hydroxyandrost-5-en-17-one, as previously reported,[5] through the addition of hypobromous acid (*N*-bromoacetamide, $HClO_4$), the hypoiodate reaction [$Pb(OAc)_4$, I_2], the bromination at C-16α with $CuBr_2$, and the controlled alkaline hydrolysis of the 16α-bromoketone (NaOH, aqueous pyridine) as key reactions. The 16α-hydroxy group of compound **3** was protected before cleavage of the 6β,19-epoxy ring. We initially employed *tert*-butyldimethylsilyl (TBDMS) ether as the protecting group. Reaction of the 16α-hydroxy-17-ketol **3** with TBDMS chloride in the presence of imidazole produced the silyl ether **4**. Reduction of the 6β,19-epoxy ring of compound **4** with zinc dust in isopropanol containing acetic acid[6] yielded the 19-alcohol **6** in high quantity. Oxidation of compound **6** with pyridinium dichromate gave the 19-oxo steroid **8**, of

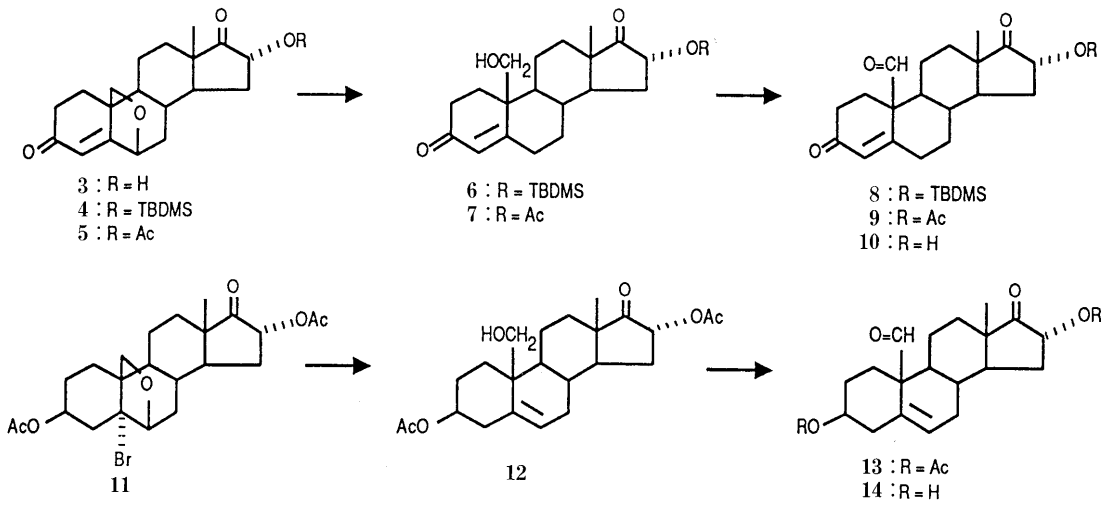

Fig. 1

which treatment with tetra-*n*-butylammonium fluoride or diluted HCl resulted in a complex mixture of products in every case. Finally, deprotection of the silyl ether at C-16 of compound **8** was efficiently achieved by using diluted H_2SO_4 in aqueous dioxane (room temperature, 1.75 h) according to the previous method,[7] to give the 16α-alcohol **10** in 33% yield. To obtain the desired steroid **10** more efficiently, we next employed acetate for the protection of the 16α-hydroxyl group. Since the 19-oxo-4-en-3-one system loses formic acid very rapidly on treatment with a base to give 19-nor steroid in high yield,[8] hydrolysis of the 16α-acetoxy-19-oxo-4-en-3-one **9**, obtained from the 16α-acetate **5** through the same procedure as **4**→**8** was carried out similarly using diluted H_2SO_4 (room temperature, 2 d) to afford the ketol **10** in much improved yield (66%).

Since the 19-oxo-3β-hydroxy-5-ene steroid is relatively stable toward treatment with a base compared to the 19-oxo-4-en-3-one,[8] the 3β,16α-diacetate **11**, was used for the intermediate of the synthesis of another 19-oxo steroid **14**. Reduction of compound **11** with zinc dust in ethanol and subsequent Jones oxidation produced the protected 19-oxo derivative **13**, of which brief treatment with K_2CO_3 in aqueous methanol at room temperature under nitrogen gave compound **14** in excellent yield.

The structures of the 16α-hydroxy-19-oxo steroids **10** and **14** were proved by spectral data and elemental analysis. The formation of their corresponding thermodynamically more stable 17β-hydroxy-16-oxo isomers[9] was not detected in the above reaction sequence.

Recently, 19-oxygenated androst-4-ene-3,17-dione, an intermediate of estrone biosynthesis, was found to be significantly higher in hypertensive pregnant women than in normotensive women,[10] and it essentially originates in the placenta. By inference from the pathway of the estriol biosynthesis, it is presumed that the 16α-hydroxy-19-oxo C_{19}-steroids may be found as steroid metabolites in the plasma of pregnant women and may be involved in the regulation of blood pressure in pregnant subjects. Their role in pregnancy and identification of biological properties are now underway.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with JEOL PMX 60 (60 MHz) and JEOL GX 400 (400 MHz) spectrometers using tetramethylsilane as an internal standard, and mass spectra (MS) on a JEOL JMS-DX 303 spectrometer. Ultraviolet (UV) spectra were determined on a Hitachi UV 150-20 spectrophotometer, and infrared (IR) spectra on a Shimadzu IR-430 spectrophotometer.

16α-(*tert*-Butyldimethylsiloxy)-6β,19-epoxyandrost-4-ene-3,17-dione (4) 16α-Hydroxy-6β,19-epoxyandrost-4-ene-3,17-dione (**3**) (500 mg, 1.58 mmol) which was obtained by the previous method[5] was dissolved in 6 ml of dimethylformamide. Imidazole (108 mg, 1.58 mmol) and *tert*-butyldimethylsilyl chloride (238 mg, 1.58 mmol) were added to this solution and the mixture was stirred at room temperature for 24 h, diluted with AcOEt (200 ml), washed with 5% HCl, sat. $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent afforded a solid, which was recrystallized from MeOH to give **4** (460 mg, 61%) as colorless prisms, mp 127—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.08 (6H, s, 16α-Si(Me)$_2$), 0.87 (9H, s, 16α-Si-*tert*-butyl), 0.97 (3H, s, 18-Me), 3.50 (1H, d, J=8 Hz, 19-H$_a$), 4.20 (1H, d, J=8 Hz, 19-H$_b$), 4.26 (1H, m, 16β-H), 4.72 (1H, d, J=6 Hz, 6α-H), 5.85 (1H, s, 4-H). IR (KBr): 1748, 1678 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 238 (1.19×10^4). *Anal.* Calcd for $C_{25}H_{38}O_4Si$: C, 69.72; H, 8.89. Found: C, 69.72; H, 8.81.

16α-(*tert*-Butyldimethylsiloxy)-19-hydroxyandrost-4-ene-3,17-dione (6) Zinc dust (2.5 g) was added to a solution of compound **4** (393 mg, 0.92 mmol) in isopropanol (21 ml) and acetic acid (1.7 ml) and the mixture was heated under reflux with stirring for 24 h. The suspension was filtered and the residue was washed with isopropanol. The combined filtrates were condensed to about 5 ml under reduced pressure, diluted with AcOEt (200 ml), washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). A solid product, obtained by evaporation of the solvent, was purified by silica gel column chromatography (silica gel, 25 g). The fractions eluted with hexane–AcOEt (1 : 1) were combined and then recrystallized from ethyl ether to give **6** (296 mg, 74%) as colorless needles, mp 150—152 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.10 (6H, s, 16α-Si(Me)$_2$), 0.90 (9H, s, 16α-Si-*tert*-butyl), 0.94 (3H, s, 18-Me), 3.96 (1H, d, J=12 Hz, 19-H$_a$), 4.20 (1H, d, J=12 Hz, 19-H$_b$), 4.33 (1H, m, 16β-H), 5.95 (1H, s, 4-H). IR (KBr): 3400, 1754, 1666 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 243 (1.38×10^4). *Anal.* Calcd for $C_{25}H_{40}O_4Si$: C, 69.39; H, 9.31. Found: C, 69.65; H, 9.14.

16α-Acetoxy-19-hydroxyandrost-4-ene-3,17-dione (7) Compound **5** (1.0 g, 2.79 mmol), obtained according to the previous report,[5] was subjected similarly to the above reduction reaction with zinc dust (15 g) in isopropanol (100 ml) and acetic acid (12 ml) (6 h). After a similar workup to that above, the oily product obtained was triturated with ethyl ether and recrystallized from acetone to yield **7** (910 mg, 91%) as colorless leaflets, mp 194—194.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, s, 18-Me), 2.10 (3H, s, 16α-OCOMe), 3.90 (1H, d, J=8 Hz, 19-H$_a$), 4.13 (1H, d, J=8 Hz, 19-H$_b$), 5.43 (1H, m, 16β-H), 5.93 (1H, s, 4-H). IR (KBr): 3400, 1750, 1663 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 243 (1.46×10^4). *Anal.* Calcd for $C_{21}H_{28}O_5$: C, 69.97; H, 7.82. Found: C, 69.63; H, 7.87.

16α-(*tert*-Butyldimethylsiloxy)androst-4-ene-3,17,19-trione (8) A solution of compound **6** (200 mg, 0.46 mmol) in dichloromethane (10 ml) was stirred at room temperature while pyridinium dichromate (267 mg, 0.69 mmol) was added. After 4.5 h the mixture was diluted with AcOEt (100 ml), washed with 5% HCl, 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent left an oil which was subjected to silica gel column chromatography (silica gel, 15 g). Fractions eluted with hexane–AcOEt (2 : 1) were combined and **8** (61 mg, 31%) was obtained as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.10 (6H, s, 16α-Si(Me)$_2$), 0.89 (9H, s, 16α-Si-*tert*-butyl), 0.90 (3H, s, 18-Me), 4.36 (1H, m, 16β-H), 6.03 (1H, s, 4-H), 10.01 (1H, s, 19-H). IR (KBr): 1750, 1720, 1670 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 237 (1.10×10^4). Exact MS: found 430.1999, calcd for $C_{25}H_{38}O_4Si$: (M^+) 430.1945.

16α-Acetoxyandrost-4-ene-3,17,19-trione (9) Compound **7** (550 mg, 1.53 mmol) in acetone (60 ml) at 0 °C was stirred during dropwise addition of Jones reagent until there was a permanent orange color; then the excess of the reagent was destroyed by adding MeOH (0.5 ml), and the solution was poured into chilled water (500 ml) saturated with NaCl. The precipitates were collected by filtration, dried under vacuum and recrystallized from acetone to give **9** (480 mg, 88%) as colorless needles, mp 125—126 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, 18-Me), 2.10 (3H, s, 16α-OCOMe), 5.40 (1H, dd, J=2, 8 Hz, 16β-H), 6.00 (1H, s, 4-H), 9.93 (1H, s, 19-H). IR (KBr): 1750, 1740, 1665 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 235 (1.47×10^4). *Anal.* Calcd for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.12; H, 7.37.

16α-Hydroxyandrost-4-ene-3,17,19-trione (10) A) Compound **8** (27 mg, 0.062 mmol) in 80% dioxane (2 ml) was treated with 3 M H_2SO_4 (0.1 ml) at room temperature for 1.75 h, and then the mixture was diluted with AcOEt (50 ml), washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent afforded a solid, which was purified by flash silica gel column chromatography (silica gel, 10 g; hexane–AcOEt = 1 : 2) to give **10** (6.5 mg, 33%) as a colorless solid, mp 147—149 °C. ^{1}H-NMR ($CDCl_3$) (400 MHz) δ: 0.98 (3H, s, 18-Me), 4.38 (1H, dd, J=6.2, 7.0 Hz, 16β-H), 6.00 (1H, s, 4-H), 9.93 (1H, s, 19-H). IR (KBr): 3400, 1740, 1650 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 240 (8.3×10^3). Exact MS: found 316.1749, calcd for $C_{19}H_{24}O_4$ (M^+) 316.1756.

B) 3 M H_2SO_4 (1.56 ml) was added to a solution of compound **9** (340 mg, 0.94 mmol) in dioxane (9.5 ml) and water (2.3 ml). The mixture was allowed to stand at room temperature for 2 d and then diluted with AcOEt (200 ml), washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). After evaporation of the solvent an oily product was triturated with ether and recrystallized from acetone to give **10** (192 mg, 66%) as colorless powder, mp 147—149 °C. *Anal.* Calcd for $C_{19}H_{24}O_4$: C, 72.13; H, 7.65. Found: C, 71.99; H, 7.70

Compound **10** obtained from compound **9** was identical with that obtained from compound **8** in every respect.

3β,16α-Diacetoxy-19-hydroxyandrost-5-en-17-one (12) Compound **11** (1.23 g, 3.11 mmol), obtained according to the previous method,[5] in EtOH (56 ml) was stirred and heated under reflux with zinc dust (2.7 g). The reaction was monitored by thin-layer chromatography and was essentially

complete after 3 h. The filtered solution was evaporated under reduced pressure and the product was extracted into AcOEt (300 ml), which was washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent afforded a solid which was recrystallized from acetone to give **12** (856 mg, 70%) as colorless needles, mp 145.5—146 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, s, 18-Me), 2.03 (3H, s, 3β-OCOMe), 2.10 (3H, s, 16α-OCOMe), 3.67 (1H, d, J=9 Hz, 19-H_a), 3.93 (1H, d, J=9 Hz, 19-H_b), 4.63 (1H, br m, 3α-H), 5.40 (1H, dd, J=2, 8 Hz, 16β-H), 5.80 (1H, m, 6-H). IR (KBr): 1758, 1740, 1726 cm^{-1}. *Anal.* Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 67.99; H, 7.96.

3β,16α-Diacetoxyandrost-5-ene-17,19-dione (13) Jones reagent was added dropwise to a stirred solution of compound **12** (650 mg, 1.64 mmol) in acetone (65 ml) at 0 °C until there was a permanent orange color. The excess of the reagent was destroyed by adding MeOH (0.5 ml), and the solution was poured into chilled water (500 ml) saturated with NaCl. The precipitates were collected by filtration, dried under vacuum, and recrystallized from aqueous acetone to give **13** (540 mg, 84%) as colorless needles, mp 137—138 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s, 18-Me), 2.03 (3H, s, 3β-OCOMe), 2.10 (3H, s, 16α-OCOMe), 4.76 (1H, br m, 3α-H), 5.45 (1H, dd, J=2, 8 Hz, 16β-H), 5.91 (1H, m, 6-H), 9.77 (1H, s, 19-H). IR (KBr): 1734 cm^{-1}. *Anal.* Calcd for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.61; H, 7.33.

3β,16α-Dihydroxyandrost-5-ene-17,19-dione (14) Compound **13** (58 mg, 0.147 mmol) in MeOH (6 ml) and water (0.6 ml) flushed with nitrogen was treated with K_2CO_3 (19 mg, 0.136 mmol) at room temperature for 1.5 h. After this time, the mixture was poured into chilled water (100 ml) saturated with NaCl, and the precipitates were collected by filtration, dried under vacuum, and then recrystallized from acetone to give **14** (45 mg, 96%) as colorless prisms, mp 163—164 °C. ^{1}H-NMR ($CDCl_3$–CD_3OD=10:0.5) (400 MHz): δ: 0.91 (3H, s, 18-Me), 3.50 (1H, m, 3α-H), 4.36 (1H, d, J=8.0 Hz, 16β-H), 5.87 (1H, d, J=5.9 Hz, 6-H), 9.69 (1H, d, J=1.1 Hz, 19-H). IR (KBr): 3450, 1750, 1740 cm^{-1}. *Anal.* Calcd for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found: C, 71.57; H, 8.19.

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