Synthesis of Deuterium-Labeled Androst-5-ene-17β,19-diol and Its 4-Ene Isomer as Internal Standards for the Determination of the 19-Oxygenation of Aromatase Inhibitors Using GC-MS

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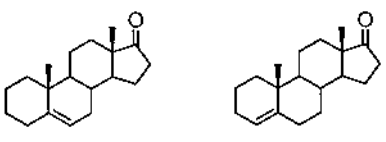
[3β,7,7,17α-$^2H_4$]Androst-5-ene-17β,19-diol (6) and [3,3,7,7,-$^2H_4$]androst-4-ene-17β,19-diol (15-d_4) were synthesized as internal standards for gas chromatographic-mass spectrometric analysis of the 19-hydroxylation of androst-5-en-17-one (1) and its 4-ene isomer 2, inhibitors of estrogen biosynthesis, using human placental aromatase. Treatment of [7,7-$^2H_2$]3β-tosylate 3 with Zn–NaI–D_2O, followed by reduction with $NaBD_4$, gave compound 6 (d_4, 79.8 atom%). Compound 15-d_4 was synthesized *via* 3β,17β-dihydroxy-5-en-7-one 10 as a key intermediate. Deoxygenation of the 5-en-7-one 10 and [7,7-$^2H_2$]17β-hydroxy-4-en-3-one 13-d_2, produced from compound 10 in two steps, with $AlCl_2D_2$ was used for the deuterium labeling reaction, producing compound 15-d_4 (d_4, 93.5 atom%). The 19-oxygenation products of aromatase inhibitors 1 and 2 could be analyzed, after $NaBH_4$ reduction, as the corresponding 17β,19-diol bis-trimethylsilyl ethers using the internal standards 6 and 15-d_4.

Key words aromatase inhibitor; 19-oxygenation; deuterium-labeled steroid; synthesis; internal standard; GC-MS

Aromatase is a cytochrome P-450 enzyme which catalyzes the conversion of androst-4-ene-3,17-dione (androstenedione) to estrone through three sequential oxygenations of the 19-methyl group.[1)] Inhibitors of aromatase have recently attracted interest not only in the treatment of advanced estrogen-dependent breast cancer[2)] but also in the elucidation of the spatial aspects of the active-site of the enzyme as well as the still unsolved mechanism of the aromatase reaction.[3)]

We have previously reported that the androst-4-en-17-one (**2**),[4)] 3-deoxy analog of the natural substrate androstenedione, and its 5-ene isomer **1**[5)] (Chart 1) are very potent competitive inhibitors of aromatase, although they have no carbonyl group at C-3 which is thought to be essential for tight binding of the substrate to the active site of aromatase. We have recently reported that some competitive inhibitors of aromatase, 6-alkyl[6)] and 6-oxo[7)] androstenediones, can serve as substrates for the enzyme to afford estrogens when they are incubated with human placental microsomes. Thus, it was of interest to investigate the relationship between the aromatase inhibitory activity of the 3-deoxy compounds, **1** and **2**, and their ability to act as substrates. It was postulated that the aromatase products of compounds **1** and **2**, obtained by incubation with placental microsomes, would be a mixture of the 19-hydroxy and 19-oxo derivatives[1)] as well as their 17β-alcohol analogs produced by the action of 17β-hydroxysteroid dehydrogenase[8)] in each incubation system. The aromatase-catalyzed 19-oxygenation activity could be determined by GC-MS analysis of the 17β,19-dihydroxy steroids obtained after $NaBH_4$ reduction of the mixture of 19-oxygenated products.[8b)] Thus, we synthesized the deuterium labeled 17β,19-diols **6** and **15**-d_4 as internal standards.



1 **2**

Chart 1. Structures of 3-Deoxy Steroids

Results and Discussion

We initially focused on the preparation of [3β,7,7,17α-$^2H_4$] androst-5-ene-17β,19-diol (**6**). [7,7-$^2H_2$]3β-Acetoxyandrost-5-en-17-one[9)] (d_2, 98.6 atom%) was converted [7,7-$^2H_2$]3β-*p*-toluenesulfonyloxy-19-(*tert*-butyldimethyl-siloxy)androst-5-en-17-one (**3**) according to a previously reported method[5)] involving the addition of hypobromous acid to a double–bond at C-5, followed by the "hypoiodate reaction" (lead tetraacetate, iodine, and hν) and subsequent zinc dust reduction (Fig. 1).[10)] Reductive elimination of the 3β-tosylate, **3**, with zinc dust–D_2O in the presence of NaI[11)] produced the 3β-deuterio compound **4** (d_3, 89.5 atom%) (Table 1). Deprotection of the 19-*tert*-butyldimethylsilyl (TBDMS) group of compound **4** with acid followed by reduction of the 17-oxo function with $NaBD_4$ afforded the deuterated 17β,19-diol **6** (d_4, 79.8 atom%).

We then synthesized another deuterium-labeled steroid,

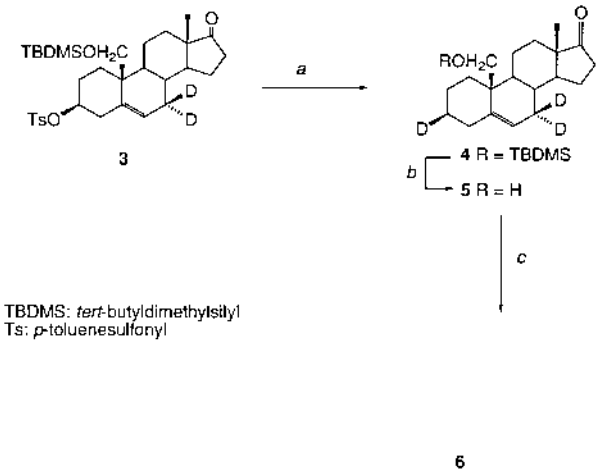


Fig. 1. Synthesis of Deuterated 17β,19-dihydroxy-5-ene **6**

a) Zn dust, NaI, D_2O, diglyme. *b*) dil. HCl, THF, 2-propanol. *c*) $NaBD_4$, MeOD.

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[3,3,7,7-$^2H_4$]androst-4-ene-17β,19-diol (**15**-d_4). Non-labeled compound **15** has been synthesized previously using desulfurization of the 3,3-ethylenedithio derivative of 19-(*tert*-butyldimethylsiloxy)androst-4-ene-3,17-dione with a sodium-liquid ammonia–MeOH system.[4] This synthesis was unsuitable for introduction of deuterium atoms at C-3 because deuterated liquid ammonia is not readily available. We therefor employed another route to synthesize the deuterated compound **15**. 3β-Acetoxy-19-(*tert*-butyldimethylsiloxy)androst-5-en-17β-ol (**7**), obtained by $NaBH_4$ reduction of the known 17-oxo derivative,[12] was converted to 3β,17β-diacetate, **8**. Treatment of this with *tert*-butylhydroperoxide and pyridinium dichromate in the presence of Celite 545[13] gave the 7-oxo compound, **9**, in 63% yield from compound **7** (Fig. 2). After hydrolysis of acetate **9** with K_2CO_3, the reductive deoxygenation with $AlCl_2H_2$ or $AlCl_2D_2$ (generated from $LiAlH_4$ and $AlCl_3$ or $LiAlD_4$ and $AlCl_3$ in ether, respectively) yielded the 5-ene compound, **11**, or its 7,7-deuterio analog (**11**-d_2, d_2>99 atom%) in good yield. This 3β,17β-diol, **11** or **11**-d_2, was oxidized with Jones reagent to the 3,17-dione, **12** or **12**-d_2, followed by treatment with 0.48 mol eq of $NaBH_4$ to give the 17β-ol, **13**, or its 7,7-deuterio analog, **13**-d_2, in 15% yield from compound **11** or **11**-d_2. Treatment of 4-en-3-one steroid, **13** or **13**-d_2, with $AlCl_2H_2$ or $AlCl_2D_2$ as described above, and subsequent hydrolysis of the 19-siloxy group gave the 3-deoxy compound, **15**, or its 3,3,7,7-deuterio analog, **15**-d_4, (d_4, 93.3 atom%) respectively in good yield.

Table 1. Deuterium Content of Labeled Compounds

Compound	Molecular or fragment ion (m/z)	Relative intensity				
		d_0	d_1	d_2	d_3	d_4
[7,7-$^2H_2$]3β-Acetoxy-androst-5-en-17-one	M^+-60 (270)[a]	0.7	0.2	98.6	0	0
4	M^+-57 (345)[a]	0	0.6	9.9	89.5	0
5	M^+ (288)[b]	0	0	5.8	94.2	0
6	M^+ (290)[b]	0	0	7.0	13.2	79.8
6-bis TMS	M^+-90 (344)[a]	0	0	0	61.0	39.0
11-bis TMS	M^+-57(570)[a]	0	0	100	0	0
15-d_4	M^+ (290)[b]	0	0	3.6	2.9	93.5
15-d_4-bis TMS	M^+-90(344)[a]	0	0	0	64.8	33.2

a) Analysis by GC-MS. *b*) Analysis by direct MS.

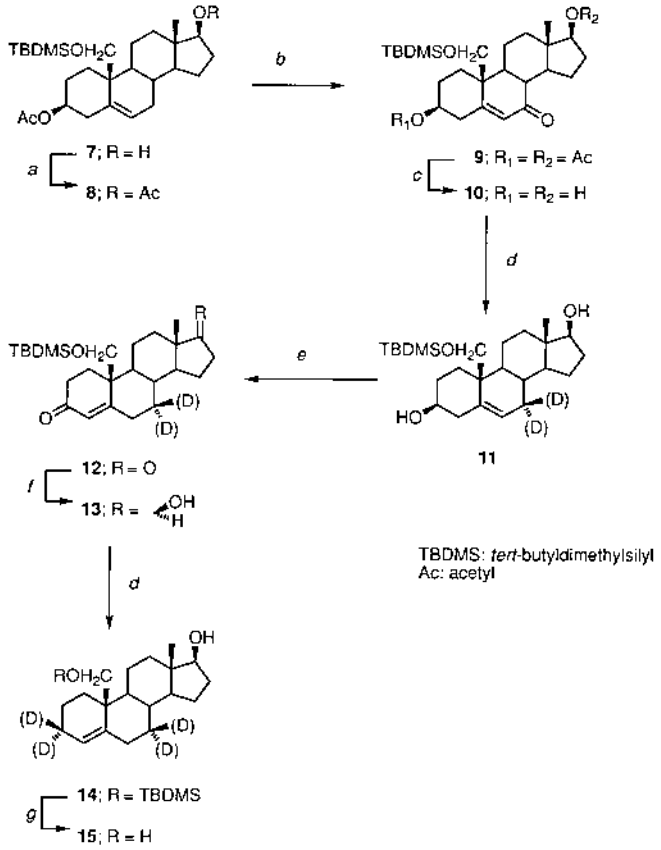

Fig. 2. Synthesis of 17β,19-Dihydroxy-4-ene **15** and Its [3,3,7,7-$^2H_4$] Analog

a) $(CH_3CO)_2O$, pyridine. *b*) *tert*-BuOOH, pyridinium dichromate, Celite, benzene. *c*) K_2CO_3, MeOH, H_2O. *d*) $LiAlH_4$ or $LiAlD_4$, $AlCl_3$, Et_2O. *e*) Jones reagent, acetone. *f*) $NaBH_4$, MeOH. *g*) dil. HCl, THF, 2-propanol.

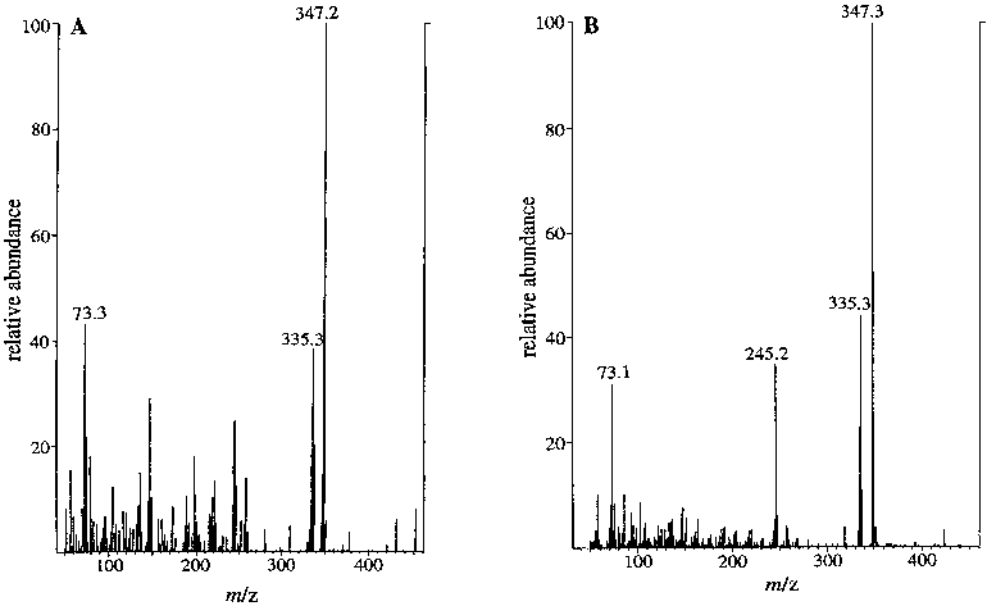

Fig. 3. Mass Spectra of the Trimethylsilyl Ether Derivatives of Compounds **6** (A) and **15**-d_4 (B)

The deuterium content of compounds **6** and **15**-d_4 was very high making them suitable internal standards for GC-MS analysis.

The mass spectra and deuterium content of the trimethylsilyl derivatives of the deuterated steroids **6** and **15**-d_4 are shown in Fig. 3 and Table 1. In every case, no molecular ion (M^+, m/z 434) was observed and the base ion peak (m/z 344) was a fragment ion corresponding to M^+-90, with a deuterium content different from that of the M^+ ion obtained by the direct MS method. During fragmentation of the trimethylsilyl (TMS)-derivative, a deuterium incorporated at C-3 of the 4-ene steroid, **15**-d_4, or C-7 of the 5-ene steroid, **6**, would be lost. However, the deuterium content (d_3, 61.0 and 64.8 atom% for **6** and **15**-d_4, respectively) is still sufficient for use as an internal standard for GC-MS analysis.

Using a selected-ion monitoring method with the fragment ion M^+-90 (m/z 344 and 348 for non-labeled steroid and internal standard, respectively), compounds **6** and **15**, potential metabolites of inhibitors **1** and **2** of aromatase, could be detected in amounts as low as 50—80 pg ($s/n=10$).

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Fourier transform (FT)-IR 1725 spectrophotometer and UV spectra in 95% EtOH solution on a Hitachi 150-20 spectrophotometer. ^{1}H-NMR spectra were obtained in $CDCl_3$ solution with a JEOL EX 270 (270 MHz) spectrometer using tetramethylsilane as an internal standard, and direct MS with a JEOL JMS-DX 303 spectrometer. GC-MS was carried out with a Finnigan MAT SSQ 710 GC-MS instrument or HP 5970B GC-MS system. TLC was performed on E. Merk pre-coated silica-gel plates. Column chromatography was conducted with silica-gel (E. Merk, 70—230 mesh). Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was obtained from Tokyo Kasei Kogyo Co.

[7,7-^{2}H$_2$]3β-Hydroxyandrost-5-en-17-one This was obtained from 3β-(*tert*-butyldimethylsiloxy)androst-5-en-7-one according to the published method[9] in which deoxygenation of the C-7 carbonyl function was carried out using $LiAlD_4$ and $AlCl_3$ instead of $LiAlH_4$ and $AlCl_3$, mp 139—140 °C (lit.[14] mp 140—141 °C and 152—153 °C, dimorphous, for non-labeled form). ^{1}H-NMR δ: 0.89 (3H, s, 18-Me), 1.04 (3H, s, 19-Me), 3.54 (1H, m, 3α-H), 5.38 (1H, d, J=1.8 Hz, 6-H).

[7,7-^{2}H$_2$]19-(*tert*-Butyldimethylsiloxy)-3β-(*p*-tolylsulfonyloxy)androst-5-en-17-one (3) This was obtained from [7,7-^{2}H$_2$]3β-hydroxyandrost-5-en-17-one in 6 steps according to the published methods,[5] mp 135—136 °C (lit.[5] mp 139—141 °C for the non-labeled form). ^{1}H-NMR δ: 0.02 and 0.09 (3H each, s, 19-OSiMe$_2$), 0.84 (9H, s, 19-OSiCMe$_3$), 0.88 (3H, s, 18-Me), 2.44 (3H, s, 3β-OSO$_2$PhMe), 3.54 and 3.70 (1H each, d, J=10.7 Hz, 19-CH$_2$), 4.31 (1H, m, 3α-H), 5.54 (1H, s, 6-H). FT-IR (KBr): 1739 (C=O), 1360 (SO) cm^{-1}.

[3β,7,7-^{2}H$_3$]19-(*tert*-Butyldimethylsiloxy)androst-5-en-17-one (4) To a solution of the 3β-tosylate, **3** (0.80 g, 1.39 mmol), in ethyleneglycol dimethyl ether (24 ml), deuterium oxide (2.9 ml), NaI (1.12 g, 7.46 mmol) and zinc dust (1.07 g, 16.4 mmol) were added and the suspension was refluxed under Ar with stirring for 8 h. The reaction mixture was filtered and the residue was washed with AcOEt (50 ml). The combined filtrate was diluted with AcOEt (150 ml), washed with 5% $NaHCO_3$ solution and brine, and dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by column chromatography. Elution with hexane–AcOEt (7 : 1) gave a crude product which was recrystallized from acetone to give compound **4** as colorless needles (410 mg, 75%), mp 80—83 °C (lit.[5] mp 85—87 °C for non-labeled form). ^{1}H-NMR δ: 0.01 and 0.03 (3H each, s, 19-OSiMe$_2$), 0.86 (9H, s, 19-OSiCMe$_3$), 0.89 (3H, s, 18-Me), 3.49 and 3.70 (1H each, d, J=10.9 Hz, 19-CH$_2$), 5.29 (1H, s, 6-H). FT-IR (KBr): 1743 (C=O) cm^{-1}.

[3β,7,7-^{2}H$_3$]19-Hydroxyandrost-5-en-17-one (5) To a solution of compound **4** (0.30 g, 0.74 mmol) in THF (5.3 ml)-2-propanol (8.3 ml), 3 mol/l HCl (5.3 ml) was added and the mixture was stirred at room temperature for 2 d. After this time, the reaction mixture was diluted with AcOEt (100 ml), washed with 5% $NaHCO_3$ and brine, and dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by column chromatography. Elution with hexane–AcOEt (3 : 1) gave a crude product which was recrystallized from acetone to give compound **5** (94 mg, 75%), mp 120—121 °C (lit.[5] mp 120—124 °C for non-labeled form). ^{1}H-NMR δ: 0.94 (3H, s, 18-Me), 3.59 and 3.89 (1H each, d, J=11.4 Hz, 19-CH$_2$), 5.71 (1H, s, 6-H). FT-IR (KBr): 3480 (OH), 1743 (C=O) cm^{-1}.

[3β,7,7,17α-^{2}H$_4$]Androst-5-ene-17β,19-diol (6) To a solution of 17-ketone **5** (20 mg, 0.068 mmol) in MeOD (3 ml), $NaBD_4$ (6 mg, 0.14 mmol) was added at 0 °C and the mixture was stirred at 0 °C for 30 min. After this time, the reaction mixture was diluted with AcOEt (30 ml), washed with 5% HCl, 5% $NaHCO_3$, and brine, sequentially, and dried (Na_2SO_4). After evaporation of the solvent, the residue was recrystallized from AcOEt–Et_2O to give compound **6** as colorless prisms (15 mg, 73%), mp 133—134 °C. (lit.[5] mp 137—138 °C for the non-labeled form). ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 3.59 and 3.86 (1H each, d, J=11.2 Hz, 19-CH$_2$), 5.67 (1H, s, 6-H). FT-IR (KBr): 3433 (OH) cm^{-1}.

3β-Acetoxy-19-(*tert*-butyldimethylsiloxy)androst-5-en-17β-ol (7) To a solution of 3β-acetoxy-19-(*tert*-butyldimethylsiloxy)androst-5-en-17-one[12] (244 mg, 0.53 mmol) in EtOH (12 ml), $NaBH_4$ (10 mg, 0.26 mmol) was added under ice-cooling. The reaction mixture was stirred at 0 °C for 4 h. After extraction with AcOEt (60 ml×2), the organic layer was washed with 5% HCl, 5% $NaHCO_3$ and brine, sequentially, and dried (Na_2SO_4). After evaporation of the solvent, the oily product obtained was purified by column chromatography. Elution with hexane–AcOEt (3 : 1) gave compound **7** as colorless prisms (199 mg, 81%), mp 123—124 °C (acetone). ^{1}H-NMR δ: 0.04 and 0.05 (3H each, s, 19-OSiMe$_2$), 0.78 (3H, s, 18-Me), 0.88 (9H, s, 19-OSiCMe$_3$), 2.06 (3H, s, 3β-OAc), 3.63 (1H, m, 17α-H), 3.59 and 3.74 (1H each, d, J=10.6 Hz, 19-CH$_2$), 4.62 (1H, m, 3α-H), 5.56 (1H, m, 6-H). FT-IR (KBr): 3449 (OH), 1734 (C=O) cm^{-1}. *Anal*. Calcd for $C_{27}H_{46}O_4Si$: C, 70.08; H, 10.02. Found: C, 70.37; H, 10.35.

19-(*tert*-Butyldimethylsiloxy)androst-5-en-3β,17β-diyl Diacetate (8) A solution of compound **7** (200 mg, 0.30 mmol) in pyridine (2 ml) and Ac_2O (1 ml) was allowed to stand at room temperature for 12 h. After this time, the reaction mixture was poured into ice-water (20 ml), and then extracted with AcOEt (50 ml). The organic layer was washed with 5% HCl, 5% $NaHCO_3$, and brine, sequentially, and dried (Na_2SO_4). After evaporation of the solvent, the crystalline solid obtained was recrystallized from hexane to give compound **8** as colorless needles (191 mg, 88%), mp 121—123 °C. ^{1}H-NMR δ: 0.03 and 0.04 (3H each, s, 19-OSiMe$_2$), 0.83 (3H, s, 18-Me), 0.86 (9H, s, 19-OSiCMe$_3$), 2.03 (6H, s, 3β- and 17β-OAc), 3.59 and 3.74 (1H each, d, J=10.6 Hz, 19-CH$_2$), 4.58 (1H, m, 17α-H), 4.64 (1H, m, 3α-H), 5.56 (1H, m, 6-H). FT-IR (KBr): 1736 (C=O) cm^{-1}. *Anal*. Calcd for $C_{29}H_{48}O_5Si$: C, 69.00; H, 9.58. Found: C, 68.80; H, 9.94.

3β,17β-Diacetoxy-19-(*tert*-butyldimethylsiloxy)androst-5-en-7-one (9) *tert*-BuOOH (0.26 ml) was added dropwise to a mixture of compound **8** (252 mg, 0.50 mmol), benzene (7.7 ml), Celite 545 (774 mg), and pyridinium dichromate (952 mg, 0.40 mmol) under ice-cooling and the resulting mixture was stirred at room temperature for 3 d, diluted with AcOEt (50 ml), and filtered through a pad of Celite 545 which was washed with AcOEt (50 ml). The combined filtrate was washed with 5% HCl, 5% $NaHCO_3$ and brine, sequentially, and dried (Na_2SO_4). The solvent was evaporated to dryness to give an oily product, which was purified by column chromatography. Elution with hexane–AcOEt (5 : 1) gave unchanged starting material (53 mg, 21%) as a less polar steroid and compound **9** (163 mg, 63%) as a more polar steroid, mp 118—120 °C (hexane) as colorless needles. ^{1}H-NMR δ: 0.04 and 0.07 (3H each, s, 19-OSiMe$_2$), 0.84 (3H, s, 18-Me), 0.86 (9H, s, 19-OSiCMe$_3$), 2.04 and 2.05 (3H each, s, 3β- and 17β-OAc), 3.83 and 3.92 (1H each, d, J=10.7 Hz, 19-CH$_2$), 4.62 (1H, dd, J=7.2, 9.2 Hz, 17α-H), 4.73 (1H, m, 3α-H), 5.88 (1H, d, J=1.7 Hz, 6-H). FT-IR (KBr): 1736, 1672 (C=O) cm^{-1}. UV λ_{max} nm (ε): 237 (12000). *Anal*. Calcd for $C_{29}H_{46}O_6Si$: C, 67.14; H, 8.94. Found: C, 67.24; H, 8.96.

3β,17β-Dihydroxy-19-(*tert*-butyldimethylsiloxy)androst-5-en-7-one (10) To a solution of compound **9** (170 mg, 0.33 mmol) in MeOH (6 ml) and water (1 ml), K_2CO_3 (90 mg, 0.65 mmol) was added and the mixture was heated at 60 °C for 3 h. After this time, the reaction mixture was concentrated to about 2 ml and extracted with AcOEt (50 ml). The organic layer was washed with brine, and dried (Na_2SO_4). After evaporation of the solvent, the crystalline solid obtained was recrystallized from acetone to give compound **10** (140 mg, 98%) as colorless prisms, mp 185—186.5 °C. ^{1}H-NMR δ: 0.03 and 0.06 (3H each, s, 19-OSiMe$_2$), 0.79 (3H, s, 18-Me), 0.85 (9H, s, 19-OSiCMe$_3$), 3.65 (1H, m, 17α-H), 3.72 (1H, m, 3α-H), 3.83 and 3.93 (1H each, d, J=10.6 Hz, 19-CH$_2$), 5.87 (1H, d, J=1.7 Hz, 6-H). FT-IR (KBr): 3297 (OH), 1676 (C=O) cm^{-1}. UV λ_{max} nm (ε): 240 (15000). *Anal*. Calcd for $C_{25}H_{42}O_4Si$: C, 69.07; H, 9.74. Found: C, 69.22; H, 9.92.

19-(*tert*-Butyldimethylsiloxy)androst-5-ene-3β,17β-diol (11) and Its [7,7-^{2}H$_2$] Analog Aluminum chloride (277.5 mg, 2.08 mmol) was carefully added to dry Et_2O (4 ml) at 0 °C, then, a solution of $LiAlH_4$ (22.8 mg,

0.60 mmol) in dry Et_2O (4 ml) was added dropwise at 0 °C to this suspension. The mixture was heated under reflux for 30 min under N_2, and then cooled to room temperature. A solution of compound **10** (100 mg, 0.23 mmol) in dry THF (2 ml) was added to the mixture, and then heated under reflux for 2.5 h and cooled. Water was carefully added to the reaction mixture, followed by extraction with AcOEt (100 ml). The organic layer was washed with 5% HCl, 5% $NaHCO_3$ and brine, sequentially, and dried (Na_2SO_4). Evaporation of the solvent afforded a solid which was recrystallized from acetone to give compound **11** (77 mg, 79%) as colorless prisms, mp 205—206 °C. ^{1}H-NMR δ: 0.03 and 0.04 (3H each, s, 19-OSiMe_2), 0.78 (3H, s, 18-Me), 0.87 (9H, s, 19-OSiCMe_3), 3.56 (1H, m, 3α-H), 3.62 (1H, m, 17α-H), 3.58 and 3.74 (1H each, d, J=10.6 Hz, 19-CH_2), 5.54 (1H, m, 6-H). FT-IR (KBr): 3402 (OH), 1637 (C=C) cm^{-1}. *Anal*. Calcd for $C_{25}H_{44}O_3Si$: C, 71.37; H, 10.54. Found: C, 71.29; H, 11.09.

[7,7-$^2H_2$]Compound **11**-d_2, mp 187—191 °C, was synthesized from compound **10** essentially according to the method described above, with $LiAlD_4$ being used instead of $LiAlH_4$.

19-(*tert*-Butyldimethylsiloxy)androst-4-ene-3,17-dione (12) and Its [7,7-$^2H_2$]Analog Jones reagent (0.17 ml) was added to a solution of compound **11** (100 mg, 0.24 mmol) in acetone under ice-cooling. The resulting mixture was stirred for 25 min. After this time, the reaction mixture was poured into ice-water (50 ml), extracted with AcOEt (100 ml×2), washed with 5% $NaHCO_3$ and brine, and dried (Na_2SO_4). Evaporation of the solvent afforded an oily product (105 mg) which was dissolved in acetone (2 ml). *p*-Toluenesulfonic acid monohydrate (3.7 mg, 0.02 mmol) was added to this solution and then allowed to stand at room temperature for 3 h, diluted with AcOEt (50 ml), and washed with 5% $NaHCO_3$ and brine, and dried (Na_2SO_4). Evaporation of the solvent gave an oily product which was purified by column chromatography. Elution with hexane–AcOEt (3 : 1) gave compound **12** (21 mg, 21%) as colorless needles, mp 156—157.5 °C. ^{1}H-NMR δ: 0.04 and 0.05 (3H each, s, 19-OSiMe_2), 0.87 (9H, s, 19-OSiCMe_3), 0.92 (3H, s, 18-Me), 3.87 and 3.91 (1H each, d, J=10.0 Hz, 19-CH_2), 5.87 (1H, s, 4-H). FT-IR (KBr): 1737, 1667 (C=O) cm^{-1}. UV λ_{max} nm (ε): 242 (14900). *Anal*. Calcd for $C_{25}H_{40}O_3Si$: C, 72.08; H, 9.66. Found: C, 72.27; H, 9.52.

[7,7-$^2H_2$]Compound **12**-d_2, mp 135—138 °C, was obtained from [7,7-$^2H_2$]compound **11**-d_2 essentially according to the above method used for the synthesis of compound **12**.

17β-Hydroxy-19-(*tert*-butyldimethylsiloxy)androst-4-en-3-one (13) and Its [7,7-$^2H_2$]Analog $NaBH_4$ (2.1 mg, 0.056 mmol) was added to a solution of compound **12** (49 mg, 0.12 mmol) in MeOH (2 ml) under ice-cooling, and the resulting mixture was stirred for 3 h, diluted with AcOEt (50 ml), washed with 5% HCl, 5% $NaHCO_3$ and brine, sequentially, and dried (Na_2SO_4). Evaporation of the solvent yielded an oily product which was purified by preparative silica-gel TLC (two developments with hexane–AcOEt (1 : 1)) to give compound **13** as colorless prisms (35 mg, 70%), mp 141—143 °C. ^{1}H-NMR δ: 0.03 and 0.05 (3H each, s, 19-OSiMe_2), 0.79 (3H, s, 18-Me), 0.85 (9H, s, 19-OSiCMe_3), 3.64 (1H, m, 17α-H), 3.86 and 3.91 (1H each, d, J=10.0 Hz, 19-CH_2), 5.85 (1H, s, 4-H). FT-IR (KBr): 3464 (OH), 1653 (C=O) cm^{-1}. UV λ_{max} nm (ε): 244 (14700). *Anal*. Calcd for $C_{25}H_{42}O_3Si$: C, 71.71; H, 10.11. Found: C, 71.71; H, 10.41.

[7,7-$^2H_2$]Compound **13**-d_2, mp 135—138 °C, was synthesized basically according to the above method used for the synthesis of non-labeled **13**.

19-(*tert*-Butyldimethylsiloxy)androst-4-en-17β-ol (14) and Its [3,3,7,7-$^2H_4$]Analog Compound **13** (36 mg, 0.085 mmol) in dry THF (0.7 ml) was treated with $AlCl_3$ (102 mg, 0.77 mmol) in dry Et_2O (1.5 ml) and $LiAlH_4$ (8.4 mg, 0.22 mmol) in dry Et_2O (0.3 ml) as in the preparation of compound **11** described above. After heating under reflux for 3.5 h, water was added to the mixture, followed by extraction with AcOEt (50 ml), then washed with 5% HCl, 5% $NaHCO_3$ and brine, sequentially, and dried (Na_2SO_4). Evaporation of the solvent afforded an oily product which was purified by preparative silica-gel TLC (two developments with hexane–AcOEt (2 : 1)) to give compound **14** (27 mg, 76%) as colorless prisms, mp 99—101 °C (hexane) (lit.[4b] mp 105.5—106 °C). ^{1}H-NMR δ: 0.03 and 0.04 (3H each, s, 19-OSiMe_2), 0.77 (3H, s, 18-Me), 0.88 (9H, s, 19-OSiCMe_3), 3.61 (1H, dd, J=8.1, 8.7 Hz, 17α-H), 3.67 and 3.79 (1H each, d, J=9.9 Hz, 19-CH_2), 5.40 (1H, m, 4-H). FT-IR (KBr): 3318 (OH), 1655 (C=O) cm^{-1}.

[3,3,7,7-$^2H_4$]Compound **14**-d_4, mp 67—69 °C, was synthesized from [7,7-$^2H_2$]-labeled steroid **13**-d_2, as described above, with $LiAlD_4$ being used instead of $LiAlH_4$.

Androst-4-ene-17β,19-diol (15) and Its [3,3,7,7-$^2H_4$]Analog To a solution of compound **14** (50 mg, 0.123 mmol) in THF (0.9 ml) and 2-propanol (1.4 ml), 3 mol/l HCl (0.9 ml) was added, and the resulting mixture was allowed to stand at room temperature. After 3 d, the reaction mixture was diluted with AcOEt (50 ml), washed with 5% $NaHCO_3$ and brine, and dried (Na_2SO_4). Evaporation of the solvent yielded an oily product which was purified by preparative silica-gel TLC (three developments with hexane : AcOEt=2 : 1) to give compound **15** as colorless prisms (27 mg, 75%), mp 176—179 °C (acetone) (lit.[4b] mp 97—101 °C). ^{1}H-NMR δ: 0.75 (3H, s, 18-Me), 3.53 and 3.95 (1H, d, J=10.4 Hz, 19-CH_2), 3.62 (1H, t, J=8.4 Hz, 17α-H), 5.72 (1H, m, 4-H).

[3,3,7,7-$^2H_4$]Compound **15**-d_4, mp 138—140 °C, was obtained as described above from [3,3,7,7-$^2H_4$]compound **14**-d_4.

GC-MS Gas chromatographic conditions: column, 30 m×0.25 mm i.d. fused-silica DB5 MS (J & W Scientific, CA, U.S.A.); column temperature, from 50 °C at 25 °C/min to 250 °C and then at 10 °C/min to 280 °C; carrier gas, He at a flow rate of 1 ml/min. Mass spectrometric conditions: ionization energy, 70 eV; ion source temperature, 150 °C.

Derivatization of Compounds 6 and 15 with BSTFA BSTFA (20 μl) was added separately to a solution of the steroid **6** and **15**-d_4 in pyridine (50 μl). The mixture was heated at 60 °C for 30 min and then the solvent was removed under a stream of N_2. The residue was dissolved in anhydrous hexane containing 0.5% pyridine (50 μl), and 1 μl of the solution was subjected to analysis.

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References

1) Thompson E. A., Jr., Siiteri P. K., *J. Biol. Chem*., **249**, 5373—5378 (1974); Kellis J., Jr., Vickery L. E., *ibid*., **262**, 4413—4420 (1987); Yoshida N., Osawa Y., *Biochemistry*, **30**, 3003—3010 (1991).
2) For example see: Harvey H. A., Lipton A., Sante R. J., *Cancer Res*. (Suppl.), **42**, 3261s—3269s (1982).
3) For example see: Covey D. F., "Steroid Biosynthesis Inhibitors: Pharmaceutical and Agrochemical Aspects," ed. by Berg D., Plemel M., Ellis Horwood Ltd., Chichester, 1988, pp. 534—571.
4) *a*) Numazawa M., Mutsumi A., Hoshi K., Koike R., *Biochem. Biophys. Res. Commun*., **160**, 1009—1014 (1989); *b*) Numazawa M., Mutsumi A., Hoshi. K., Oshibe M., Ishikawa E., Kigawa H., *J. Med. Chem*., **34**, 2496—2504 (1991).
5) Numazawa M., Mutsumi A., Tachibana M., Hoshi K., *J. Med. Chem*., **37**, 2198—2205 (1994).
6) Numazawa M., Yoshimura A., Oshibe M., *Biochem. J*., **329**, 151—156 (1998).
7) Numazawa M., Midzuhashi K., Nagaoka M., *Biochem. Pharmacol*., **47**, 717—726 (1994); Numazawa M., Sugiyama T., Nagaoka M., *Biol. Pharm. Bull*., **21**, 289—292 (1998).
8) *a*) Ryan K. J., "Maternal-Fetal Endocrinology," ed. by Tulchinsky D., Ryan K. J., Saunders, Philadelphia, 1980, pp. 3—16; *b*) Numazawa M., Satoh S., Ogura Y., Nagaoka M., *Anal. Biochem*., **149**, 409—414 (1985).
9) Numazawa M., Satoh S., Osawa Y., *Chem. Pharm. Bull*., **40**, 2759—2763 (1992).
10) Hersler K., Kalvoda J., "Organic Reactions in Steroid Chemistry," Vol. II, ed. by Fried J., Edwards J. A., Van Nostrand Reinhold, New York, 1972, pp. 237—287.
11) Turecek F., Veres F., Kocovsky P., Pouzar V., Fajkos J., *J. Org. Chem*., **48**, 2233—2237 (1983).
12) Numazawa M., Mutsumi A., Hoshi K., Kigawa H., Oshibe M., *J. Steroid Biochem. Mol. Biol*., **39**, 959—966 (1991).
13) Chidambaram N., Chandrosekaran S., *J. Org. Chem*., **52**, 5048—5051 (1987).
14) "The Merk Index 11th Edition," ed. by Budavari S., O'Neil M. J., Smith A., Heckelman P. E., Merk & Co., Inc., Rahway, 1989, p. 1221.