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Stereospecific Introduction of Deuterium into Ring D of Estrogens[1]

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The synthetic routes for introducing deuterium stereospecifically into the C-14, C-15, and C-16 positions of estrone and estradiol have been developed. Epimeric 15-deuterioestriols and 14- and 16α-deuterated 15α-hydroxyestradiols have also been prepared. These methods will be conveniently applicable to labeling with tritium.

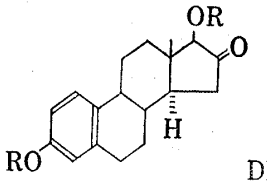
In connection with our studies on the metabolism of female hormone, the preparation of estrogens stereospecifically labeled in Ring D with hydrogen-isotope was requisite. This paper deals with the methods for introducing deuterium into the 14, 15α, 15β, and 16α positions of estrone and estradiol, which are substantially applicable to tritiation. In addition, the preparation of epimeric 15-deuterioestriols, 14- and 16α-deuterated 15α-hydroxyestradiols is also reported. Of the numerous deuteration methods so far available reductive cleavage of the epoxide, displacement of the sulfonate ester with lithium aluminum deuteride ($LiAlD_4$), and deuterioboration of the double bond appeared to be more suitable for the present purpose.

Introduction of deuterium into the 16α position of 17-oxygenated steroids is somewhat difficult, since undesirable regiospecificity would be anticipated in reductive cleavage of the 16β,17β-epoxide with metal hydride and hydroboration of the Δ^{16}-olefin. Accordingly, the Δ^{16}-17-ol acetate was reduced catalytically with deuterium gas or treated with lithium aluminum hydride ($LiAlH_4$) and then with deuterioacetic acid.[3] Recently, reductive cleavage of the 16β-deuterio-16α,17α-epoxide with metal hydride was also undertaken.[4] These methods, however, are not necessarily pertinent for tritium labeling and therefore development of an alternative synthetic route was required. Substitution of the 16β,17β-glycol 16-tosylate with $LiAlD_4$ seemed to be promising, when the 17-hydroxyl function could be appropriately protected. 3,17β-Dihydroxyestra-1,3,5(10)-trien-16-one (**1**), derivable from estrone in three steps, was treated with dimethyl-*tert*-butylsilyl chloride in dimethylformamide in the presence of imidazole to provide the 3,17-disilyl ether (**2**) almost quantitatively.[5] Reduction of **2** with sodium borohydride in aqueous tetrahydrofuran yielded the 3,16β,17β-triol 3,17-disilyl ether (**3**), which in turn was led to the 16-tosylate (**4**) by treatment with *p*-toluenesulfonyl chloride in pyridine in a good yield. Being refluxed with $LiAlD_4$ in ether, **4** underwent displacement to afford 16α-deuterioestradiol 3,17-disilyl ether (**5**) without formation of the unsaturated product and hydrolysis of the O-S bond. It was evident from the doublet pattern (J=6.5 Hz) due to 17α proton in the nuclear magnetic resonance (NMR) spectrum that deuterium was incorporated into the 16α position with configurational inversion at the reaction center. Upon exposure to 5N hydrochloric acid in acetone[6] elimination of the silyl group in

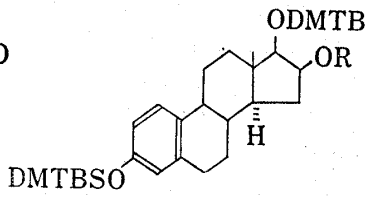
1) Part CVII of "Studies on Steroids" by T. Nambara; Part CVI: T. Nambara and M. Nokubo, *Chem. Pharm. Bull.* (Tokyo), **24**, 162 (1976). In this paper the following trivial names were used: estrone, 3-hydroxyestra-1,3,5(10)-trien-17-one; estradiol, estra-1,3,5(10)-triene-3,17β-diol; estriol, estra-1,3,5(10)-triene-3,16α,17β-triol.
2) Location: *Aobayama, Sendai.*
3) J. Fishman, *J. Am. Chem. Soc.*, **87**, 3455 (1965).
4) R. Robbiani and J. Seibl, *Helv. Chim. Acta*, **57**, 674 (1974).
5) E.J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94**, 6190 (1972).
6) H. Hosoda, K. Yamashita, H. Sagae, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **23**, 2118 (1975).

5 was readily attained to provide the desired 16α-deuterioestradiol (**6**). Subsequent oxidation with Jones reagent afforded 16α-deuterioestrone (**7**) in a reasonable yield. As judged from the infrared (IR) spectra 16α-deuterioestrongens (**6**, **7**) hereby obtained were obviously distinguishable from the 16β-epimers (**8**, **9**), which were prepared from 16α,17α-epoxyestra-1,3,5(10)-trien-3-ol by reductive cleavage with $LiAlD_4$ according to the method of Fishman.[3]

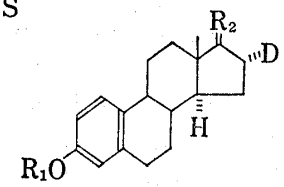
The next project was directed to the syntheses of estrone, estradiol, and estriol stereospecifically labeled with isotope at C-15. Levitz, *et al.* have already disclosed the preparation



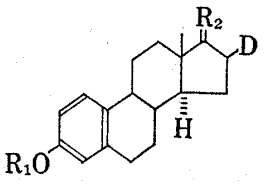
1 : R=H
2 : R=DMTBS



3 : R=H
4 : R=Ts



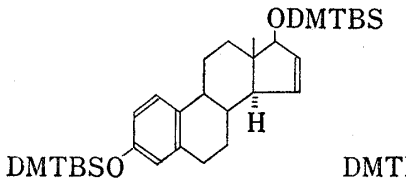
5 : R_1=DMTBS, R_2=⁄ODMTBS H
6 : R_1=H, R_2=⁄OH H
7 : R_1=H, R_2=O



8 : R_1=H, R_2=⁄OH H
9 : R_1=H, R_2=O

DMTBS=$-Si(CH_3)_2$-*t*-Bu
Ts=$-SO_2C_6H_4$-*p*-CH_3

Chart 1



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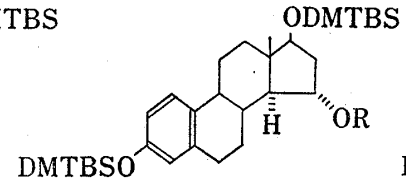
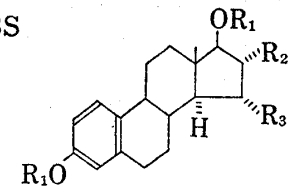
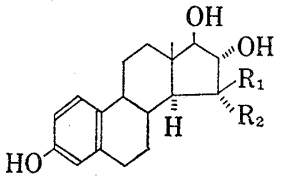


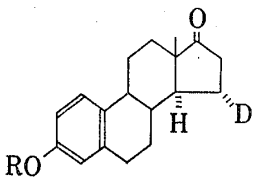
11 : R=H
26 : R=Ts



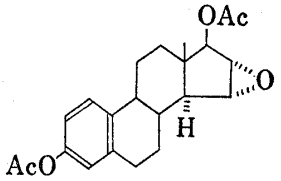
12 : R_1=DMTBS, R_2=OH, R_3=D
13 : R_1=DMTBS, R_2=D, R_3=OH
15 : R_1=H, R_2=D, R_3=OH



14 : R_1=H, R_2=D
18 : R_1=D, R_2=H



16 : R=H
20 : R=DMTBS



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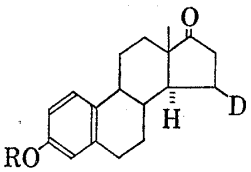
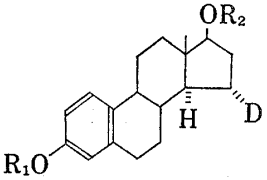
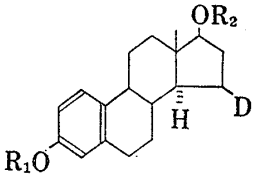


19 : R=H
21 : R=DMTBS



22 : R_1=DMTBS, R_2=H
24 : R_1=R_2=H



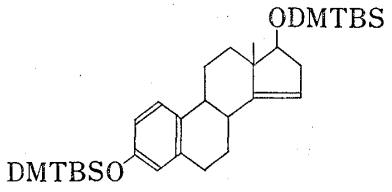
23 : R_1=DMTBS, R_2=H
25 : R_1=R_2=H
27 : R_1=R_2=DMTBS

Chart 2

of 15-tritiated estriol, where the labeling was carried out under the non-stereospecific conditions.[7] In a preceding paper of this series it has been reported that hydration of estra-1,3,5(10),15-tetraene-3,17β-diol bis(dimethyl-*tert*-butylsilyl) ether (**10**) with diborane and alkaline hydrogen peroxide resulted in formation of estriol 3,17-disilyl ether and 15α-hydroxyestradiol 3,17-disilyl ether (**11**).[8] This synthetic route seemed to be convenient for the preparation of 15α-deuterioestrone as well as 15α-deuterioestriol and 16α-deuterio-15α-hydroxyestradiol, since transformation of estriol into estrone has fully been established.[9] The silyl ether (**10**) was treated with deuteriodiborane and subsequently with alkaline hydrogen peroxide to yield the 3,17-bis(dimethyl-*tert*-butylsilyl) ethers of 15α-deuterioestriol and 16α-deuterio-15α-hydroxyestradiol (**12, 13**). In the NMR spectrum **13** exhibited the signals of 17α proton at 3.88 ppm as a doublet (J=9 Hz) and 15β proton at 4.16 ppm as a triplet (J=9 Hz) showing incorporation of deuterium at the 16α position by *cis*-addition. Removal of the silyl group in **12** and **13** with hydrochloric acid provided the desired 15α-deuterioestriol (**14**) and 16α-deuterio-15α-hydroxyestradiol (**15**), respectively. Dehydration of **14** without loss of isotope was effected by fusion with pyridine hydrochloride to furnish 15α-deuterioestrone (**16**) in a satisfactory yield.

Labeling of deuterium at the 15β position was then performed from the 15α,16α-epoxide by utilizing the *trans*-diaxial opening reaction with $LiAlD_4$. Reductive cleavage of 15α,16α-epoxyestradiol 3,17-diacetate (**17**)[10] with $LiAlD_4$ in tetrahydrofuran proceeded regiospecifically to afford 15β-deuterioestriol (**18**) as a sole product. Upon dehydration with pyridine hydrochloride **18** was converted to the desired 15β-deuterioestrone (**19**). In order to confirm the configuration of labeled deuterium by the NMR study using the lanthanide shift reagent **16** and **19** were derivatized into the 3-dimethyl-*tert*-butylsilyl ethers (**20, 21**) which in turn were reduced to the estradiol derivatives (**22, 23**) as suitable substrates.[11] Removal of the silyl group at C-3 was effected by brief exposure to hydrochloric acid in acetone yielding the desired epimeric 15-deuterioestradiols (**24, 25**). 15β-Deuterioestradiol (**25**) could also be obtained from 15α-tosyloxyestradiol 3,17-disilyl ether (**26**) by reduction with $LiAlD_4$, followed by acid hydrolysis.

Finally, introduction of deuterium into C-14 was undertaken employing estra-1,3,5(10),14-tetraene-3,17β-diol bis(dimethyl-*tert*-butylsilyl) ether (**28**)[8] as a starting compound. Deuterioboration and subsequent oxidation of the organoborane with alkaline hydrogen peroxide furnished 14-deuterio-15α-hydroxyestradiol 3,17-bis(dimethyl-*tert*-butylsilyl) ether (**29**), which was transformed into the 15-tosylate (**30**). Being treated with $LiAlH_4$ in ether, **30**



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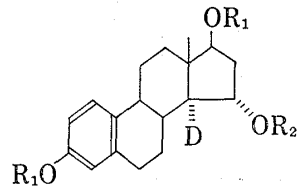
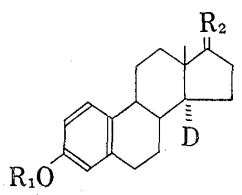


29 : R_1=DMTBS, R_2=H
30 : R_1=DMTBS, R_2=Ts
34 : R_1=R_2=H



31 : R_1=DMTBS, R_2= 
32 : R_1=H, R_2= 
33 : R_1=H, R_2=O

Chart 3

7) M. Levitz and J. Katz, *Steroids*, **5**, 11 (1965).
8) H. Hosoda, K. Yamashita, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **23**, 3141 (1975).
9) J.C. Sheehan, W.F. Erman, and P.A. Cruickshank, *J. Am. Chem. Soc.*, **79**, 147 (1957).
10) J. Fishman and H. Guzik, *J. Org. Chem.*, 33, 3133 (1968).
11) H. Hosoda, K. Yamashita, and T. Nambara, *Chem. Ind.* (London), **1975**, 650.

was readily converted to 14-deuterioestradiol disilyl ether (**31**). The desired 14-deuterated estradiol (**32**) and estrone (**33**) were obtained in the usual manner. Acid hydrolysis of **29** furnished 14-deuterio-15α-hydroxyestradiol (**34**) in a fairly good yield.

Inspection of the molecular ion peak in the mass spectra revealed that the isotopic purity of these deuterated compounds except **7** was 97 to 99%. Therefore, the synthetic routes thus established may be applicable for the preparation of the multideuterated steroid used for gas chromatography–mass spectrometry as an internal standard. Furthermore, the stereospecifically tritiated estrogens prepared by the present methods will serve as substrates for the studies on estrogen metabolism. The locality of deuterium in the labeled compounds (**7**, **9**, **16**, **19**, **33**) could be ascertained with the 3-dimethyl-*tert*-butylsilyl ethers by means of the NMR spectroscopy using the lanthanide shift reagent, and the details will be published elsewhere in the near future.

Experimental[12]

3,17β-Dihydroxyestra-1,3,5(10)-trien-16-one Bis(dimethyl-*tert*-butyl-silyl) Ether (**2**)——To a solution of 3,17β-dihydroxyestra-1,3,5(10)-trien-16-one (**1**) (180 mg) in dimethylformamide (2 ml) were added imidazole (700 mg) and dimethyl-*tert*-butylsilyl chloride (620 mg) and allowed to stand at room temperature for 3 hr. The resulting solution was diluted with ether, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **2** (250 mg) as colorless plates. mp 167—168°. $[\alpha]_D^{21}$ −49.7° (c=0.13). NMR (CCl_4) δ: 0.04, 0.14 (each 3H, s, 17-OSi$(CH_3)_2$), 0.17 (6H, s, 3-OSi$(CH_3)_2$), 0.78 (3H, s, 18-CH_3), 0.91 (9H, s, 17-OSi-*t*-Bu), 0.98 (9H, s, 3-OSi-*t*-Bu), 3.66 (1H, s, 17α-H), 6.33—6.68 (2H, 2- and 4-H), 7.00 (1H, d, J=8 Hz, 1-H). *Anal.* Calcd. for $C_{30}H_{50}O_3Si_2$: C, 69.99; H, 9.79. Found: C, 69.77; H, 9.76.

Estra-1,3,5(10)-triene-3,16β,17β-triol 3,17-Bis(dimethyl-*tert*-butylsilyl) Ether (**3**)——To a solution of **2** (240 mg) in tetrahydrofuran (8 ml) was added $NaBH_4$ (300 mg) in H_2O (2 ml) at 0° and stirred at room temperature for 2 hr. After addition of 10% AcOH (3 ml) to decompose the excess reagent, the resulting solution was extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **3** (180 mg) as colorless leaflets. mp 129.5—131°. $[\alpha]_D^{21}$ +39.7° (c=0.10). NMR (CCl_4) δ: 0.11 (6H, s, 17-OSi$(CH_3)_2$), 0.16 (6H, s, 3-OSi$(CH_3)_2$), 0.82 (3H, s, 18-CH_3), 0.97 (18H, s, 3- and 17-OSi-*t*-Bu), 3.41 (1H, d, J=7.5 Hz, 17α-H), 3.88 (1H, m, 16α-H), 6.30—6.60 (2H, 2- and 4-H), 6.96 (1H, d, J=8 Hz, 1-H). *Anal.* Calcd. for $C_{30}H_{52}O_3Si_2$: C, 69.71; H, 10.14. Found: C, 69.51; H, 10.25.

16β-*p*-Toluenesulfonyloxyestra-1,3,5(10)-triene-3,17β-diol Bis(dimethyl-*tert*-butylsilyl) Ether (**4**)——To a solution of **3** (120 mg) in pyridine was added *p*-toluenesulfonyl chloride (1.2 g) and stirred at 0° for 48 hr. The resulting solution was diluted with ether, washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–AcOEt (20: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **4** (110 mg) as colorless leaflets. mp 65—72°. $[\alpha]_D^{21}$ +34.3° (c=0.10). NMR (CCl_4) δ: 0, 0.02 (each 3H, s, 17-OSi$(CH_3)_2$), 0.12 (6H, s, 3-OSi$(CH_3)_2$), 0.83 (3H, s, 18-CH_3), 0.88 (9H, s, 17-OSi-*t*-Bu), 0.96 (9H, s, 3-OSi-*t*-Bu), 2.44 (3H, s, 16-$OSO_2C_6H_4C\underline{H}_3$), 3.48 (1H, d, J=8 Hz, 17α-H), 4.76 (1H, m, 16α-H), 6.32—6.52 (2H, 2- and 4-H), 6.91 (1H, d, J=8 Hz, 1-H), 7.24, 7.68 (each 2H, d, J=8 Hz, 16-$OSO_2C_6\underline{H}_4CH_3$). *Anal.* Calcd. for $C_{37}H_{58}O_5SSi_2$: C, 66.22; H, 8.71. Found: C, 66.01; H, 9.12.

16α-Deuterioestradiol Bis(dimethyl-*tert*-butylsilyl) Ether (**5**)——To a solution of **4** (80 mg) in anhydrous ether was added $LiAlD_4$ (100 mg) and refluxed for 12 hr. After addition of moist ether to decompose the excess reagent the resulting solution was diluted with 25% Rochelle salt and extracted with ether. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane as developing solvent. Recrystallization of the eluate from MeOH gave **5** (35 mg) as colorless needles. mp 127—128°. NMR (CCl_4) δ: 0 (6H, s, 17-OSi$(CH_3)_2$),

12) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. IR spectra were obtained on a JASCO Model IR-S spectrometer. NMR spectra were recorded on a Hitachi Model R-20A spectrometer at 60 MHz or a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet. Mass spectra were measured by a Hitachi Model RMU-7 spectrometer. For preparative TLC silica gel HF_{254} (E. Merck AG, Darmstadt) was used as an adsorbent. Isotopic purity of $LiAlD_4$ used was over 99%. The deuterated compounds obtained were submitted to mixed melting point measurement on admixture with the nonlabeled authentic sample,[6,8] and no depression was observed in all the cases.

0.13 (6H, s, 3-OSi$(CH_3)_2$), 0.72 (3H, s, 18-CH_3), 0.88 (9H, s, 17-OSi-*t*-Bu), 0.96 (9H, s, 3-OSi-*t*-Bu), 3.59 (1H, d, $J=6.5$ Hz, 17α-H), 6.32—6.54 (2H, 2- and 4-H), 6.94 (1H, d, $J=8$ Hz, 1-H).

16α-Deuterioestradiol (6)——To a solution of **5** (35 mg) in acetone (5 ml) was added 5N HCl (0.8 ml) and allowed to stand at room temperature for 5 hr. The resulting solution was neutralized with 5% $NaHCO_3$, concentrated to its half volume under the reduced pressure, and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using benzene–ether (1: 1) as developing solvent. Recrystallization of the eluate from aq. MeOH gave **6** (17 mg) as colorless prisms. mp 172—174°. Mass Spectrum *m/e*: 273 (M^+) (98% d_1).

16α-Deuterioestrone (7)——To an ice-cooled solution of **6** (16 mg) in acetone (1.5 ml) was added Jones reagent (50 μl) and allowed to stand at 0° for 15 min. After addition of MeOH to decompose the excess reagent the resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using benzene–ether (3: 1) as developing solvent. Recrystallization of the eluate from benzene–EtOH gave **7** (15 mg) as colorless prisms. mp 251—255°. Mass Spectrum *m/e*: 271 (M^+) (90% d_1).

15α-Deuterioestriol 3,17-Bis(dimethyl-*tert*-butylsilyl)Ether (12), 16α-Deuterio-15α-hydroxyestradiol 3,17-Bis(dimethyl-*tert*-butylsilyl) Ether (13)——To a stirred solution of estra-1,3,5(10),15-tetraene-3,17β-diol bis-(dimethyl-*tert*-butylsilyl) ether (**10**) (353 mg) and $LiAlD_4$ (650 mg) in anhydrous ether (10 ml) was added BF_3–etherate (3 g) in anhydrous ether (10 ml) dropwise at 0° over a period of 30 min under a N_2 gas stream. The ice-bath was then removed and the reaction mixture was stirred at room temperature for 1 hr. After addition of moist ether to decompose the excess reagent the resulting solution was extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. To the residue dissolved in tetrahydrofuran (12 ml) were added 30% H_2O_2 (4 ml) and 10% NaOH (6 ml) and stirred at 0° for 1 hr. The resulting solution was diluted with ether, washed with 5% $NaHSO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–AcOEt (10: 1) as developing solvent. Recrystallization of the major product from MeOH gave **12** (156 mg) as colorless leaflets. mp 140—143.5°. NMR ($CDCl_3$) δ: 0.12 (6H, s, 17-OSi$(CH_3)_2$), 0.20 (6H, s, 3-OSi$(CH_3)_2$), 0.78 (3H, s, 18-CH_3), 0.92 (9H, s, 17-OSi-*t*-Bu), 0.97 (9H, s, 3-OSi-*t*-Bu), 3.51 (1H, d, $J=6$ Hz, 17α-H), 4.09 (1H, q, $J=8$, 6 Hz, 16β-H), 6.48—6.72 (2H, 2- and 4-H), 7.13 (1H, d, $J=8$ Hz, 1-H). The minor product obtained from the more polar fraction was recrystallized from MeOH to give **13** (23 mg) as colorless needles. mp 146—149°. NMR ($CDCl_3$) δ: 0.05 (6H, s, 17-OSi$(CH_3)_2$), 0.20 (6H, s, 3-OSi$(CH_3)_2$), 0.78 (3H, s, 18-CH_3), 0.90 (9H, s, 17-OSi-*t*-Bu), 0.98 (9H, s, 3-OSi-*t*-Bu), 3.88 (1H, d, $J=9$ Hz, 17α-H), 4.16 (1H, t, $J=9$ Hz, 15β-H), 6.50—6.70 (2H, 2- and 4-H), 7.12 (1H, d, $J=8$ Hz, 1-H).

15α-Deuterioestriol (14)——Hydrolysis of **12** (148 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. The crude product obtained was purified by preparative TLC using $CHCl_3$–EtOH (15: 1) as developing solvent. Recrystallization of the eluate from acetone gave **14** (52 mg) as colorless prisms. mp 281—284.5°. Mass Spectrum *m/e*: 289 (M^+) (99% d_1).

16α-Deuterio-15α-hydroxyestradiol (15)——Hydrolysis of **13** (10 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. The crude product obtained was purified by preparative TLC using $CHCl_3$–EtOH (10: 1) as developing solvent. Recrystallization of the eluate from acetone gave **15** (4 mg) as colorless prisms. mp 246—248.5°. Mass Spectrum *m/e*: 289 (M^+) (98% d_1).

15α-Deuterioestrone (16)——**14** (52 mg) was fused with anhydrous pyridine·HCl (2 g) at 200—220° for 3 hr. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–AcOEt (1: 1) as developing solvent. Recrystallization of the eluate from benzene–EtOH gave **16** (38 mg) as colorless prisms. mp 252—258.5°. Mass Spectrum *m/e*: 271 (M^+) (97% d_1).

15β-Deuterioestriol (18)——To a solution of 15α,16α-epoxyestradiol diacetate (**17**)[10] (82 mg) in anhydrous tetrahydrofuran (10 ml) was added $LiAlD_4$ (160 mg) and refluxed for 9 hr. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 25% Rochelle salt and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using $CHCl_3$–EtOH (8: 1) as developing solvent. Recrystallization of the eluate from acetone gave **18** (29 mg) as colorless prisms. mp 279.5—281°. Mass Spectrum *m/e*: 289 (M^+) (97% d_1).

15β-Deuterioestrone (19)——Dehydration of **18** (25 mg) by fusion with anhydrous pyridine·HCl was carried out in the manner as described with **14**. The crude product obtained was purified by preparative TLC using hexane–AcOEt (2: 1) as developing solvent. Recrystallization of the eluate from benzene–EtOH gave **19** (17 mg) as colorless prisms. mp 254—258°. Mass Spectrum *m/e*: 271 (M^+) (97% d_1).

15α-Deuterioestrone Dimethyl-*tert*-butylsilyl Ether (20)——To a solution of **16** (36 mg) in dimethylformamide (0.7 ml) were added imidazole (150 mg) and dimethyl-*tert*-butylsilyl chloride (120 mg) and allowed to stand at room temperature for 2 hr. The resulting solution was diluted with ether, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–AcOEt (10: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **20** (39 mg) as colorless needles. mp 172—174.5°.

15β-Deuterioestrone Dimethyl-*tert*-butylsilyl Ether (21)——Silylation of **19** (25 mg) with dimethyl-*tert*-butylsilyl chloride and imidazole, followed by purification by preparative TLC was carried out in the manner as described with **16**. Recrystallization from MeOH gave **21** (28 mg) as colorless needles. mp 172—174°.

15α-Deuterioestradiol 3-Dimethyl-*tert*-butylsilyl Ether (22)——Reduction of **20** (30 mg) with $NaBH_4$ was carried out in the manner as described with **2**. The crude product obtained was purified by preparative TLC using hexane–AcOEt (4: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **22** (29 mg) as colorless needles. mp 149—151°. The non-labeled authentic sample was prepared from estrone dimethyl-*tert*-butylsilyl ether.[6] $[\alpha]_D^{22}$ +49.5° (c=0.12). *Anal.* Calcd. for $C_{24}H_{38}O_2Si$: C, 74.56; H, 9.91. Found: C, 74.43; H, 9.86.

15β-Deuterioestradiol 3-Dimethyl-*tert*-butylsilyl Ether (23)——Reduction of **21** (20 mg) with $NaBH_4$ was carried out in the manner as described with **2**. Purification by preparative TLC, followed by recrystallization of the eluate from MeOH gave **23** (20 mg) as colorless needles. mp 149—150°.

15α-Deuterioestradiol (24)——Hydrolysis of **22** (8 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. The crude product obtained was purified by preparative TLC using benzene–ether (2: 1) as developing solvent. Recrystallization of the eluate from aq. MeOH gave **24** (5 mg) as colorless prisms. mp 169.5—172°. Mass Spectrum m/e: 273 (M^+) (97% d_1).

15β-Deuterioestradiol (25)——i) Hydrolysis of **23** (8 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. The crude product obtained was purified by preparative TLC using benzene–ether (2: 1) as developing solvent. Recrystallization of the eluate from aq. MeOH gave **25** (4 mg) as colorless prisms. mp 170—174°. Mass Spectrum m/e: 273 (M^+) (97% d_1).

ii) Hydrolysis of **27** (15 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. Purification by preparative TLC, followed by recrystallization of the eluate from aq. MeOH gave **25** (7.5 mg) as colorless prisms. mp 173—176°. IR spectra of the two samples obtained in i) and ii) were entirely identical in every respect.

15α-*p*-Toluenesulfonyloxyestradiol Bis(dimethyl-*tert*-butylsilyl) Ether (26)——Tosylation of **11** (50 mg) with *p*-tolyenesulfonyl chloride and pyridine was carried out in the manner as described with **3**. The crude product obtained was purified by preparative TLC using hexane–AcOEt (20: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **26** (60 mg) as colorless needles. mp 142—143°. $[\alpha]_D^{20}$ +90.8° (c=0.11). NMR (CCl_4) δ: 0.02, 0.04 (each 3H, s, 17–OSi$(CH_3)_2$), 0.16 (6H, s, 3–OSi–$(CH_3)_2$), 0.73 (3H, s, 18–CH_3), 0.84 (9H, s, 17–OSi–*t*–Bu), 0.97 (9H, s, 3–OSi–*t*–Bu), 2.44 (3H, s, 15–$OSO_2C_6H_4CH_3$), 3.73 (1H, t, J= 8 Hz, 17α-H), 4.74 (1H, m, 15β-H), 6.30—6.58 (2H, 2- and 4-H), 6.93 (1H, d, J=8 Hz, 1-H), 7.27, 7.74 (each 2H, d, J=8 Hz, 15–$OSO_2C_6H_4CH_3$). *Anal.* Calcd. for $C_{37}H_{58}O_5SSi_2$: C, 66.22; H, 8.71. Found: C, 65.96; H, 8.86.

15β-Deuterioestradiol Bis(dimethyl-*tert*-butylsilyl) Ether (27)——Reduction of **26** (40 mg) with $LiAlD_4$ was carried out in the manner as described with **4**. The crude product obtained was purified by preparative TLC using hexane as developing solvent. Recrystallization of the eluate from MeOH gave **27** (15 mg) as colorless needles. mp 126—127°.

14-Deuterio-15α-hydroxyestradiol 3,17-Bis(dimethyl-*tert*-butylsilyl) Ether (29)——Deuterioboration of estra-1,3,5(10),14-tetraene-3,17β-diol bis(dimethyl-*tert*-butylsilyl) ether (**28**)[8] (300 mg) with B_2D_6 and subsequent oxidation of the organoborane with H_2O_2–NaOH were carried out in the manner as described with **10**. The crude product obtained was purified by preparative TLC using hexane–AcOEt (10: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **29** (185 mg) as colorless needles. mp 145—147°. NMR (CCl_4) δ: 0.03 (6H, s, 17–OSi$(CH_3)_2$), 0.15 (6H, s, 3–OSi$(CH_3)_2$), 0.75 (3H, s, 18–CH_3), 0.88 (9H, s, 17–OSi–*t*–Bu), 0.97 (9H, s, 3–OSi–*t*–Bu), 3.84 (1H, t, J=8 Hz, 17α-H), 4.06 (1H, q, J=3.5, 9 Hz, 15β-H), 6.32—6.56 (2H, 2- and 4-H), 6.97 (1H, d, J=8 Hz, 1-H).

14-Deuterio-15α-*p*-toluenesulfonyloxyestradiol Bis(dimethyl-*tert*-butylsilyl) Ether (30)——Tosylation of **29** (65 mg) with *p*-toluenesulfonyl chloride and pyridine, followed by purification by preparative TLC was carried out in the manner as described with **11**. Recrystallization of the eluate from MeOH gave **30** (80 mg) as colorless needles. mp 142—143°.

14-Deuterioestradiol Bis(dimethyl-*tert*-butylsilyl) Ether (31)——Reduction of **30** (80 mg) with $LiAlH_4$ was carried out in the manner as described with **4**. The crude product obtained was purified by preparative TLC using hexane as developing solvent. Recrystallization of the eluate from MeOH gave **31** (43 mg) as colorless needles. mp 126—127°.

14-Deuterioestradiol (32)——Hydrolysis of **31** (40 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. The crude product obtained was purified by preparative TLC using $CHCl_3$–EtOH (15: 1) as developing solvent. Recrystallization of the eluate from acetone gave **32** (20 mg) as colorless prisms. mp 170—173°. Mass Spectrum m/e: 273 (M^+) (97% d_1).

14-Deuterioestrone (33)——Oxidation of **32** (20 mg) with Jones reagent was carried out in the manner as described with **6**. The crude product obtained was purified by preparative TLC using benzene–ether (3: 1) as developing solvent. Recrystallization of the eluate from benzene–EtOH gave **33** (18 mg) as colorless prisms. mp 252—256°. Mass Spectrum m/e: 271 (M^+) (97% d_1).

14-Deuterio-15α-hydroxyestradiol (34)——Hydrolysis of **29** (10 mg) with 5N HCl in acetone was carried out in the manner as described with **5**. The crude product obtained was purified by preparative TLC using $CHCl_3$–EtOH (10: 1) as developing solvent. Recrystallization of the eluate from acetone gave **34** (4 mg) as colorless prisms. mp 246—249°. Mass Spectrum *m/e*: 289 (M^+) (97% d_1).

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