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Synthesis of Multideuterated Dehydroepiandrosterone and Related 16, 17-Ketols[1]

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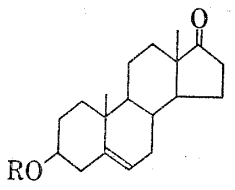
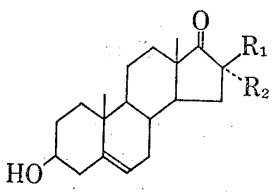
Two synthetic routes leading to C-2, C-4 and C-6 deuterium-labeled dehydroepiandrosterone have been developed. d_4-Dehydroepiandrosterone was prepared from testosterone by way of the 2,2,4,6-d_4-$\Delta^{3,5}$-dien-3-ol acetate. Alternatively, synthesis of d_5-dehydroepiandrosterone was carried out employing 3β-hydroxy-5α-androstane-6,17-dione silyl ether as a key intermediate. Deuterium labeling was attained by reduction of the 6-oxo group with lithium aluminum deuteride and perdeuteration of active methylene groups adjacent to the 3-oxo group. In addition, d_5-dehydroepiandrosterone was transformed into the epimeric 16-hydroxy-17-ketones and 17β-hydroxy-16-ketone.

Keywords——deuterium labeling; 2,2,4,6-d_4-dehydroepiandrosterone; 2,2,4,4,6-d_5-dehydroepiandrosterone; epimeric d_5-16-hydroxydehydroepiandrosterones; d_5-3β,17β-dihydroxy-5-androsten-16-one; lithium aluminum deuteride; *tert*-butyldimethylsilylation

In recent years development of gas chromatography-mass spectrometry (GC–MS) has made it possible to determine a trace amount of steroids in biological materials. The use of a deuterated compound as an internal standard is advantageous for the analysis of steroids by this technique. In 1975 Sennett *et al.* reported that an excreted amount of 16β-hydroxydehydroepiandrosterone (**2**) in urine was markedly elevated in patients with low-renin essential hypertension.[3] It is well known that dehydroepiandrosterone (**1**) and the isomeric 16,17-ketols (**3**, **4**) are also important from the metabolic and physiological points of view. These findings prompted us to prepare multideuterated dehydroepiandrosterone and related 16,17-ketols for the use of GC–MS as internal standards. The present paper deals with two different routes leading to C-2, C-4 and C-6 deuterated dehydroepiandrosterone whose labels are stable during treatments even under drastic conditions.



1 : R=H
13 : R=Si(CH$_3$)$_2$-*t*-Bu



2 : R_1=OH, R_2=H
3 : R_1=H, R_2=OH

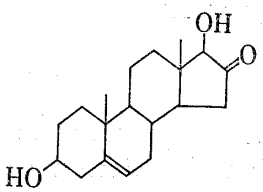


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Chart 1

An initial effort was directed to the preparation of 2,2,4,6-d_4-dehydroepiandrosterone (**12**) starting from readily available testosterone (**5**). Being treated with 20% sodium deuterium oxide in deuteromethanol, **5** was converted into 2,2,4,6,6-d_5-testosterone (**6**). Treatment of **6** with isopropenyl acetate in the presence of deuterosulfuric acid as a catalyst provided the

1) Part CXXXV of "Studies on Steroids" by T. Nambara; Part CXXXIV : J. Goto, M. Hasegawa, H. Kato, and T. Nambara, *Clin. Chim. Acta*, **87**, 141 (1978).
2) Location: *Aobayama, Sendai 980, Japan.*
3) J.A. Sennett, R.D. Brown, D.P. Island, L.R. Yarbro, J.T. Watson, P.E. Slaton, J.W. Hollifield, and G.W. Liddle, *Circ. Res.* (Suppl. 1), **36**—**37**, 1 (1975).

enol acetate (**7**). Hydrolysis and reduction of the oxygen function at C-3 without disturbance of the 17β-acetoxyl group were simultaneously effected by treatment with sodium borohydride in aq. ethanol–tetrahydrofuran (THF) to yield 2,2,4,6-d_4-5-androstene-3β,17β-diol 17-acetate (**8**). Subsequently, silylation with *tert*-butyldimethylsilyl chloride and imidazole in pyridine–dimethylformamide furnished the 3-silyl ether-17-acetate (**9**), which on saponification was led to the 3β,17β-diol 3-monosilyl ether (**10**). Oxidation of **10** with the chromic anhydride-pyridine complex afforded 2,2,4,6-d_4-dehydroepiandrosterone 3-*tert*-butyldimethylsilyl ether (**11**) in a fairly good yield. Removal of the silyl group of **11** with hydrogen chloride in acetone–methanol gave desired 2,2,4,6-d_4-dehydroepiandrosterone (**12**). The overall yield from **5** to **12** was 26%. Inspection of the molecular ion peak on the mass spectrum revealed that the isotopic purity of **12** was 53.1% d_4. It should be noted that introduction of deuterium at C-3 or C-4 is also possible when the reaction from **7** to **8** is carried out by the use of sodium borodeuteride or deuterium oxide.

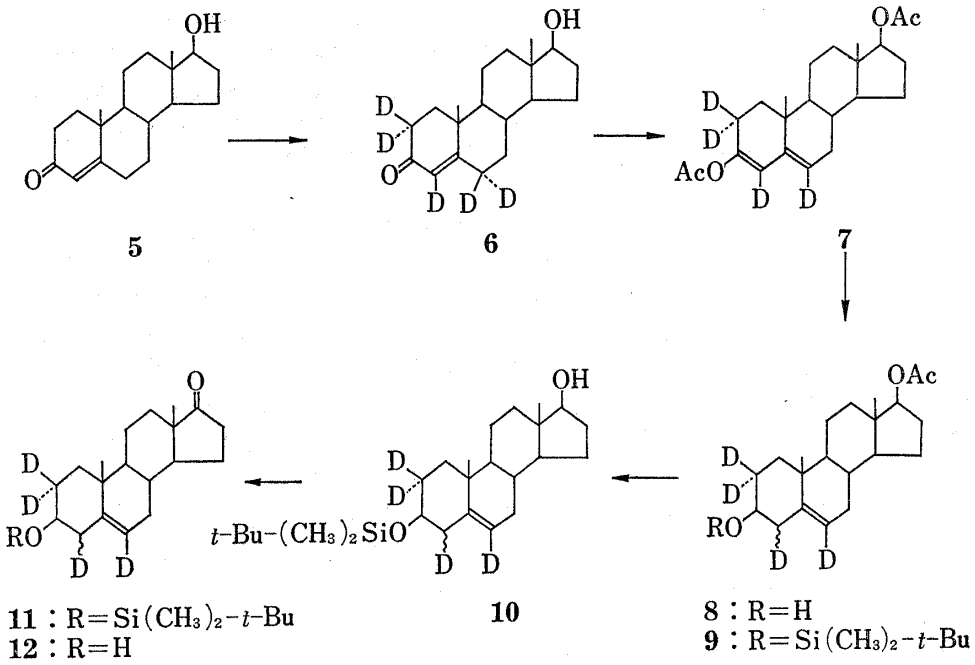

Chart 2

In order to improve the isotopic purity an alternative method involving two steps for incorporation of deuterium was undertaken starting from dehydroepiandrosterone *tert*-butyldimethylsilyl ether (**13**). Hydroboration of **13** followed by oxidation of the organoborane with alkaline hydrogen peroxide and subsequent oxidation of the product with pyridinium chlorochromate gave the 6,17-diketone (**14**). Upon reduction with lithium aluminum deuteride in THF **14** was converted into the 6α,17α-d_2-3β,6β,17β-triol 3-monosilyl ether (**15**). When the diacetate (**16**) obtainable from **15** by usual acetylation was treated with Jones reagent, desilylation and oxidation of the silyl ether at C-3 took place simultaneously to provide the 3-ketone (**17**), which on saponification was led to the 6β,17β-dihydroxy-3-ketone (**18**). Being submitted to deuteration with 20% sodium deuterium oxide in deuteromethanol followed by reduction *in situ* with sodium borohydride and *tert*-butyldimethylsilylation, **18** was transformed into the 2,2,4,4,6,17-d_6-3β,6β,17β-triol 3,17-disilyl ether (**19**). It is to be noted that the 6β-hydroxyl group resisted to silylation probably due to 1,3-diaxial interaction with the 19-methyl group. Dehydration of **19** with phosphorus oxychloride in pyridine proceeded regiospecifically to give 2,2,4,4,6,17-d_6-5-androstene-3β,17β-diol disilyl ether (**20**). For transformation of **20** into 2,2,4,4,6-d_5-dehydroepiandrosterone acetate (**23**), selective protection of

the hydroxyl function at C-3 or C-17 was required. Previously, it has been postulated that selective desilylation at C-3 in the 3,17-disilyl ether is attained efficiently by acid treatment.[4] Thus, **20** was hydrolyzed with hydrogen chloride in acetone to give the 17-monosilyl ether (**21**), which in turn was converted to the 3-acetate (**22**). Desilylation and simultaneous oxidation of the silyl ether at C-17 in **22** was effected under Jones oxidation conditions to furnish the desired **23**. The overall yield from **13** to **23** was *ca.* 20%. Mass spectrum of 2,2,4,4,6-d_5-dehydroepiandrosterone (**24**) derivable from **23** showed the isotopic purity of 67.0% d_5. The use of sodium borodeuteride in the transformation of **18** to **19** is capable of providing 2,2,3,4,4,6-d_6-dehydroepiandrosterone.

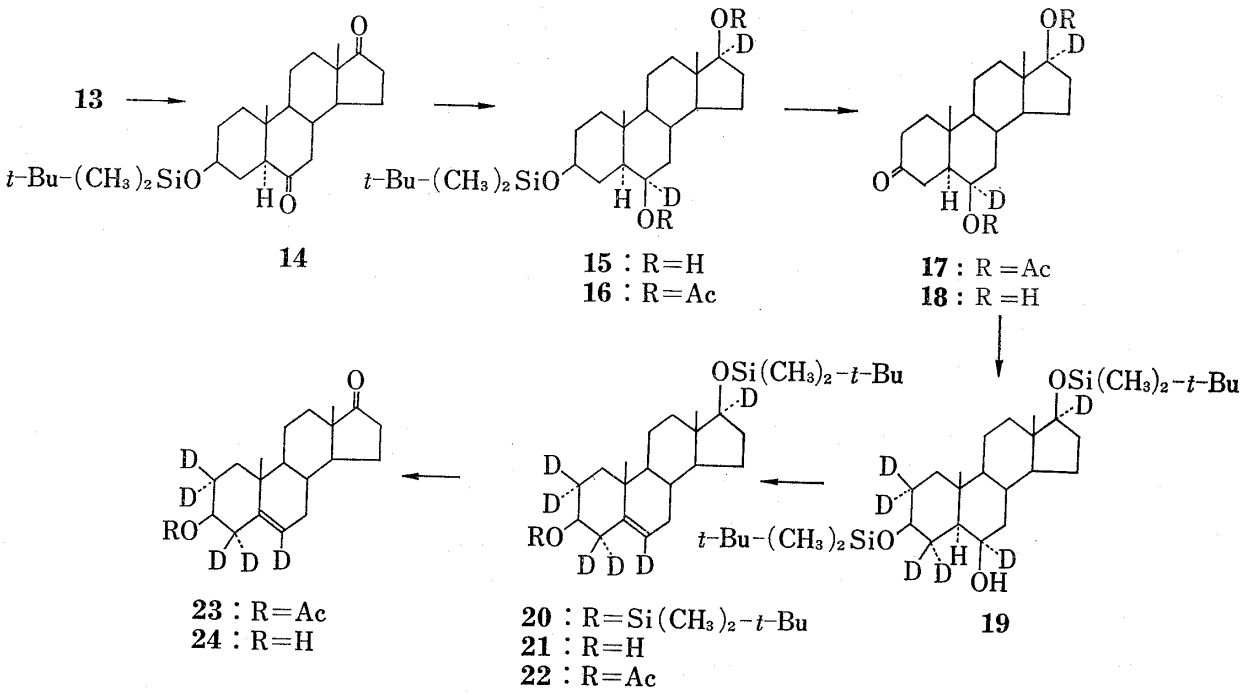

Chart 3

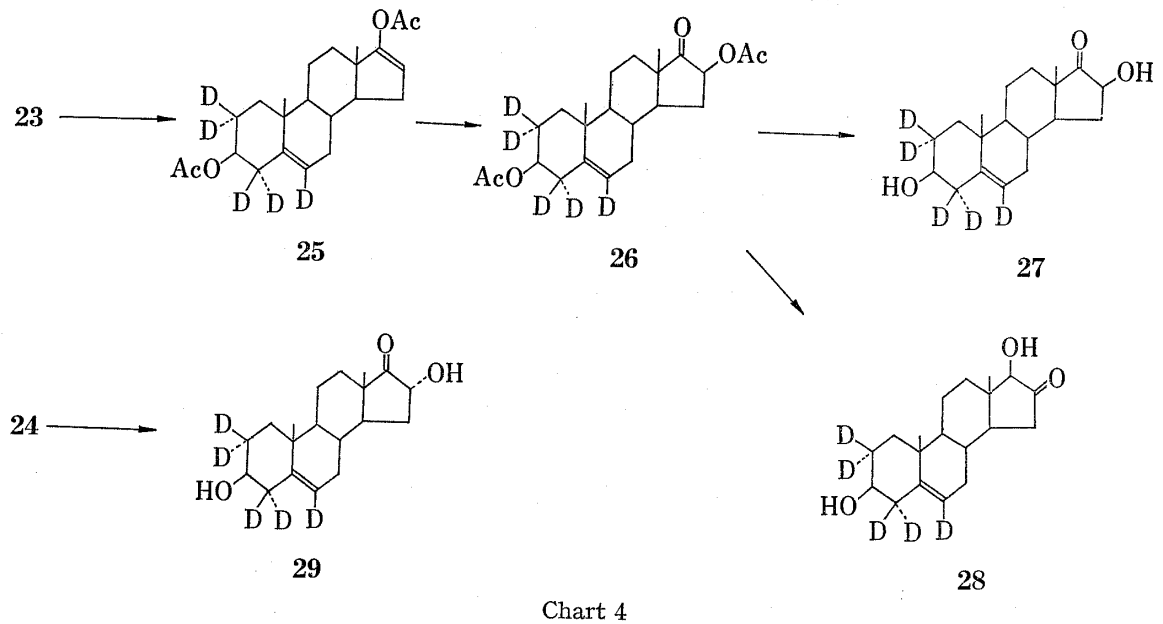

Chart 4

4) H. Hosoda, K. Yamashita, H. Sagae, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), 23, 2118 (1975).

The d_5-labeled 16,17-ketols were then prepared starting from **23** according to the known methods with slight modifications. For the preparation of 2,2,4,4,6-d_5-16β-hydroxydehydroepiandrosterone (**27**) and 2,2,4,4,6-d_5-16-oxoandrostenediol (**28**) the 17-ketone (**23**) was converted into the enol acetate (**25**). Treatment of **25** with lead tetraacetate in acetic acid–acetic anhydride gave d_5-3β,16β-diacetoxy-5-androsten-17-one (**26**). Enzymic deacetylation[5] of **26** yielded the desired 16β-hydroxy-17-ketone (**27**) while the isomeric 17β-hydroxy-16-one (**28**) could be obtained by saponification and concomitant rearrangement of **26** with base. 2,2,4,4,6-d_5-16α-Hydroxydehydroepiandrosterone (**29**) was prepared from **24** according to the method of Hassner and Catsoulacos.[6] The 16α-bromo-17-keto derivative obtainable from **24** by bromination with cupric bromide in ethanol was treated with sodium methoxide in methanol and then with hydrochloric acid to provide the desired ketol (**29**) in a satisfactory yield. The isotopic purity of the d_5–16,17-ketols was nearly identical with that of the starting material (**24**).

Although in the present work 16β-hydroxydehydroepiandrosterone was prepared by enzymic deacetylation from the 3,16-diacetate, the method is not necessarily suitable for the large scale preparation. Development of a new method for this purpose is now in progress and the details will be reported elsewhere. It is hoped that the availability of these multideuterated compounds will serve for the metabolic study of steroids by GC–MS.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. 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, t=triplet, and m=multiplet. Mass spectra were measured by a Hitachi Model RMU-7 spectrometer. Isotopic purity of $LiAlD_4$, MeOD, and NaOD used was over 99%. All the deuterated compounds obtained were cheracterized by mixed melting point measurement on admixture with the non-deuterated authentic sample.

Deuteration of Testosterone (5)——To a solution of testosterone (**5**) (500 mg) in MeOD (8 ml) was added 20% NaOD (1.6 ml), and the solution was refluxed for 22 hr under a N_2 gas stream. The reaction mixture was diluted with $CHCl_3$, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product, 2,2,4,6,6-d_5-testosterone (**6**), was submitted to the next reaction without purification.

2,2,4,6-d_4-3,5-Androstadiene-3,17β-diol Diacetate (7)——A solution of **6** (500 mg) in isopropenyl acetate (8 ml) containing conc. D_2SO_4 (20 μl) was refluxed for 1 hr. The resulting solution was diluted with ether, washed with ice-cooled 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the residue obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt (5:1) and recrystallization of the eluate from MeOH gave **7** (430 mg) as colorless needles. mp 141—145° (lit. mp 143—147°).[7]

2,2,4,6-d_4-5-Androstene-3β,17β-diol 17-Acetate (8)——To a solution of **7** (430 mg) in THF (2 ml)–EtOH (5 ml) was added $NaBH_4$ (700 mg) in EtOH (1.5 ml)–H_2O (2.5 ml) at 0°, and the solution was allowed to stand at room temperature for 3.5 hr. After addition of 10% AcOH to decompose the excess reagent the resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt (5:2) and recrystallization of the eluate from MeOH gave **8** (250 mg) as colorless needles. mp 145—147° (lit. mp 146—147°).[8]

2,2,4,6-d_4-17β-Acetoxy-5-androsten-3β-ol *tert*-Butyldimethylsilyl Ether (9)——To a solution of **8** (250 mg) in dimethylformamide (1.4 ml)–pyridine (0.7 ml) were added imidazole (500 mg) and *tert*-butyldimethylsilyl chloride (250 mg), and the solution was stirred at room temperature for 4 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 **9** (330 mg) as colorless leaflets. mp 141—142°. The non-deuterated compound; mp 141.5—143°. $[\alpha]_D^{25}$ −50.0° (c=0.24). NMR ($CDCl_3$) δ: 0.05 (6H, s, 3-OSi$(CH_3)_2$), 0.78 (3H, s, 18-CH_3),

5) K.N. Wynne and A.G.C. Renwick, *Steroids*, **19**, 293 (1972).
6) A. Hassner and P. Catsoulacos, *J. Org. Chem.*, **31**, 3149 (1966).
7) A.J. Liston and P. Toft, *J. Org. Chem.*, **33**, 3109 (1968).
8) P. Wieland and K. Miescher, *Helv. Chim. Acta*, **32**, 1768 (1949).

0.88 (9H, s, 3-OSi-*t*-Bu), 0.99 (3H, s, 19-CH_3), 2.02 (3H, s, 17-OCOCH_3), 3.50 (1H, m, 3α-H), 4.60 (1H, t, J=8 Hz, 17α-H), 5.32 (1H, m, 6-H). *Anal.* Calcd. for $C_{27}H_{46}O_3Si$: C, 72.59; H, 10.38. Found: C, 72.16; H, 10.07.

2,2,4,6-d_4-5-Androstene-3β,17β-diol 3-*tert*-Butyldimethylsilyl Ether (10)——To a solution of **9** (330 mg) in THF (3 ml)–MeOH (6 ml) was added 10% NaOH (2 ml), and the solution was stirred at room temperature for 1 hr. The reaction mixture was diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the residue obtained was recrystallized from MeOH to give **10** (280 mg) as colorless needles. mp 170—171° (lit. mp 171—172°).

2,2,4,6-d_4-Dehydroepiandrosterone *tert*-Butyldimethylsilyl Ether (11)——To a solution of **10** (280 mg) in pyridine (1.5 ml) was added CrO_3–pyridine complex (1: 10 w/v) (3 ml), and the resulting solotion was allowed to stand at room temperature for 20 hr. The reaction mixture was diluted with ether, washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was recrystallized from MeOH to give **11** (200 mg) as colorless needles. mp 146—147° (lit. mp 146—147°).[9]

2,2,4,6-d_4-Dehydroepiandrosterone (12)——To a solution of **11** (200 mg) in acetone (3 ml) –MeOH (1 ml) was added 5 N HCl (1.5 ml), and the resulting solution was allowed to stand at room temperature for 1 hr. The reaction mixture was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **12** (130 mg) as colorless plates. mp 145—146°. Mass spectrometric analysis indicated 1.0% d_0, 1.8% d_1, 7.9% d_2, 33.7% d_3, 53.1% d_4, 1.4% d_5 and 1.1% d_6 isotope composition.

3β-*tert*-Butyldimethylsilyloxy-5α-androstane-6,17-dione (14)——To a stirred solution of dehydroepiandrosterone *tert*-butyldimethylsilyl ether (13)[9] (2.8 g) and $NaBH_4$ (850 mg) in anhydrous THF (30 ml) was added a solution of dimethyl sulfate (2.7 g) in anhydrous THF (10 ml) dropwise at 0° over a period of 10 min under a N_2 gas stream, and the reaction mixture was stirred at room temperature for 1 hr. After addition of 10% NaOH (10 ml) and 30% H_2O_2 (8 ml) under ice-cooling the resulting solution was stirred at 0° for 1 hr. The reaction mixture was diluted with AcOEt, washed with 5% $NaHSO_3$, 5% $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, and evaporated. To the residue dissolved in dry methylene chloride (10 ml) were added AcONa (1.5 g) and pyridinium chlorochromate (6 g), and the solution was stirred at room temperature overnight. The resulting solution was diluted with anhydrous ether and filtered through a column of Florisil. After evaporation of the solvent the residue obtained was chromatographed on alumina (100 g). Elution with hexane–AcOEt (5: 1) and recrystallization of the eluate from MeOH gave **14** (1.9 g) as colorless leaflets. mp 161.5—162.5°. $[\alpha]_D^{24}$ +35.8° (c=0.21). NMR ($CDCl_3$) δ: 0.05 (6H, s, 3-OSi$(CH_3)_2$), 0.76 (3H, s, 18-CH_3), 0.88 (12H, s, 19-CH_3 and 3-OSi-*t*-Bu), 3.50 (1H, m, 3α-H). *Anal.* Calcd. for $C_{25}H_{42}O_3Si$: C, 71.72; H, 10.11. Found: C, 71.85; H, 10.33.

6α,17α-d_2-5α-Androstane-3β,6β,7β-triol 3-*tert*-Butyldimethylsilyl Ether (15)——To a solution of **14** (1.8 g) in anhydrous THF (12 ml) was added $LiAlD_4$ (330 mg), and the reaction mixture was allowed to stand at room temperature overnight. 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 and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was chromatographed on silica gel (70 g). Elution with hexane-AcOEt (2: 1) and recrystallization of the eluate from aq. acetone gave **15** (1.65 g) as colorless needles. mp 173—173.5°. The non-deuterated compound: mp 173—174°. $[\alpha]_D^{21}$ −13.2° (c=0.19). NMR ($CDCl_3$) δ: 0.05 (6H, s, 3-OSi$(CH_3)_2$), 0.78 (3H, s, 18-CH_3), 0.88 (9H, s, 3-OSi-*t*-Bu), 1.05 (3H, s, 19-CH_3), 3.1—3.7 (3H, 3α-, 6α- and 17α-H). *Anal.* Calcd. for $C_{25}H_{46}O_3Si$: C, 71.05; H, 10.97. Found: C, 70.80; H,11.19.

6α,17α-d_2-3β-*tert*-Butyldimethylsilyloxy-5α-androstane-6β,17β-diol Diacetate (16)——A solution of **15** (1.6 g) in Ac_2O (2 ml)–pyridine (4 ml) was allowed to stand at room temperature for 2 days. To the resulting solution was added H_2O, and the precipitate formed was collected by filtration and dried. Recrystallization of the crude product from MeOH gave **16** (1.6 g) as colorless plates. mp 130.5—132°. The non-deuterated compound: mp 134—135°. $[\alpha]_D^{23}$ −38.1° (c=0.21). NMR ($CDCl_3$) δ: 0.05 (6H, s, 3-OSi$(CH_3)_2$), 0.79 (3H, s, 18-CH_3), 0.88 (9H, s, 3-OSi-*t*-Bu), 0.98 (3H, s, 19-CH_3), 2.02 (6H, s, 6- and 17-OCOCH_3), 3.50 (1H, m, 3α-H), 4.57 (1H, t, J=8 Hz, 17α-H), 4.88 (1H, m, 6α-H). *Anal.* Calcd. for $C_{29}H_{50}O_5Si$: C, 68.73; H, 9.95. Found: C, 68.67; H, 10.31.

6α,17α-d_2-6β,17β-Dihydroxy-5α-androstan-3-one Diacetate (17)——To a solution of **16** (1.0 g) in acetone (7 ml) was added Jones reagent (2 ml), and the solution was allowed to stand at room temperature for 2 hr. After addition of MeOH to decompose the excess reagent the resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the residue obtained was chromatographed on silica gel (25 g). Elution with hexane–AcOEt (3: 1) and recrystallization of the eluate from hexane gave **17** (630 mg) as colorless needles. mp 133—135°. The non-deuterated compound: mp 133—134.5°/145.5—146° (lit. mp 129—130°).[10] $[\alpha]_D^{23}$ −32.6° (c=0.20). NMR ($CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 1.19 (3H, s, 19-CH_3), 2.02 (3H, s, 17-OCOCH_3), 2.05 (3H, s, 6-OCOCH_3), 4.62 (1H, t, J=8 Hz,

9) H. Hosoda, D.K. Fukushima, and J. Fishman, *J. Org. Chem.*, **38**, 4209 (1973).
10) G. Rosenkranz, M. Velasco, and F. Sondheimer, *J. Am. Chem. Soc.*, **76**, 5024 (1954).

17α-H), 4.88 (1H, m, 6α-H).

6α,17α-d_2-6β,17β-Dihydroxy-5α-androstan-3-one (18)——A solution of **17** (710 mg) in MeOH (10 ml)–10% NaOH (7 ml) was allowed to stand at room temperature for 2 hr. The resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was recrystallized from aq. acetone to give **18** (550 mg) as colorless needles. mp 234—237° (lit. mp 242—244°).[10]

2,2,4,4,6α,17α-d_6-5α-Androstane-3β,6β,17β-triol 3,17-Bis(*tert*-butyldimethylsilyl) Ether (19)——A solution of **18** (550 mg) in MeOD (8.5 ml)–20% NaOD (1 ml) was refluxed for 21 hr. To the resulting solution was added $NaBH_4$ (500 mg), and the reaction mixture was stirred at room temperature for 30 min. After addition of 10% AcOH to decompose the excess reagent the resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. To the residue dissolved in dimethylformamide (2 ml)–pyridine (1 ml) were added imidazole (2 g) and *tert*-butyldimethylsilyl chloride (1 g), and the reaction mixture was stirred at room temperature for 15 hr. The resulting solution was diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was chromatographed on silica gel (60 g). Elution with hexane–AcOEt (10:1) and recrystallization of the eluate from MeOH gave **19** (880 mg) as colorless plates. mp 158—161°. The non-deuterated compound was prepared from 5α-androstane-3β,6β,17β-triol 3-*tert*-butyldimethylsilyl ether by silylation. mp 163—163.5°. $[\alpha]_D^{24}$ −7.5° (c=0.20). NMR ($CDCl_3$) δ: 0 (6H, s, 17-OSi$(CH_3)_2$), 0.05 (6H, s, 3-OSi$(CH_3)_2$), 0.72 (3H, s, 18-CH_3), 0.88 (18H, s, 3- and 17-OSi-*t*-Bu), 1.02 (3H, s, 19-CH_3), 3.44—3.85 (3H, 3α-, 6α- and 17α-H). *Anal.* Calcd. for $C_{31}H_{60}O_3Si_2$: C, 69.34; H, 11.26. Found: C, 69.06; H, 11.57.

2,2,4,4,6,17α-d_6-5-Androstene-3β,17β-diol Bis(*tert*-butyldimethylsilyl) Ether (20)——To a solution of **19** (650 mg) in pyridine (4 ml) was added phosphorus oxychloride (0.5 ml) at 0°, and the reaction mixture was stirred at room temperature for 1 hr. After addition of moist ether at 0° the resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue obtained from MeOH gave **20** (590 mg) as colorless leaflets. mp 116—118° (lit. mp 113—115°).[9]

2,2,4,4,6,17α-d_6-5-Androstene-3β,17β-diol 17-*tert*-Butyldimethylsilyl Ether (21)——To a solution of **20** (500 mg) in acetone (40 ml) was added 5 N HCl (400 μl), and the reaction mixture was allowed to stand at room temperature for 40 min. After neutralization with 5% $NaHCO_3$ the resulting solution was concentrated, diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was chromatographed on silica gel (13 g). Elution with hexane–AcOEt (7:1) and recrystallization of the eluate from MeOH gave **21** (280 mg) as colorless leaflets. mp 142—142.5° (lit. mp 142—143°).[11]

2,2,4,4,6,17α-d_6-3β-Acetoxy-5-androsten-17β-ol *tert*-Butyldimethylsilyl Ether (22)——A solution of **21** (240 mg) in Ac_2O (1.2 ml)–pyridine (2.5 ml) was allowed to stand at room temperature overnight. The resulting solution was diluted with ether, washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was recrystallized from MeOH to give **22** (260 mg) as colorless needles. mp 133—134° (lit. mp 133.5—134°).[11]

2,2,4,4,6-d_5-Dehydroepiandrosterone Acetate (23)——To a solution of **22** (102 mg) in acetone (3 ml) was added Jones reagent (0.3 ml), and the solution was allowed to stand at room temperature for 1.5 hr. After addition of MeOH the resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue obtained from MeOH gave **23** (65 mg) as colorless needles. mp 168—169°.

2,2,4,4,6-d_5-Dehydroepiandrosterone (24)——A solution of **23** (50 mg) in MeOH (5 ml)–10% NaOH (0.5 ml) was refluxed for 1 hr. The reaction mixture was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue obtained from MeOH gave **24** (30 mg) as colorless plates. mp 145—146°. Mass spectrometric analysis indicated 1.4% d_2, 4.1% d_3, 24.9% d_4, 67.1% d_5, 2.2% d_6, and 0.3% d_7 isotope composition.

Enolacetylation of 23——A solution of **23** (570 mg) in isopropenyl acetate (5.5 ml) containing anhydrous *p*-TsOH (50 mg) was refluxed for 3 hr and then concentrated to its half volume. Isopropenyl acetate (1.5 ml) and anhydrous *p*-TsOH (25 mg) were added, and the resulting solution was concentrated again to its half volume during 1 hr. The reaction mixture was diluted with ether, washed with ice-cooled 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was chromatographed on silica gel (25 g). Elution with hexane–AcOEt (5:1) gave 2,2,4,4,6-d_5-5,16-androstadiene-3β,17-diol diacetate (**25**) (450 mg), which was submitted to the next reaction without recrystallization.

2,2,4,4,6-d_5-3β,16β-Diacetoxy-5-androsten-17-one (26)——To a solution of **25** (450 mg) in AcOH (8.0 ml)-Ac_2O (0.65 ml) was added $Pb(OAc)_4$ (1.5 g), and the reaction mixture was stirred at room temperature for 7 hr. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **26** (470 mg) as colorless needles.

11) H. Hosoda, K. Yamashita, S. Ikegawa, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **25**, 2545 (1977).

mp 174—175°. (lit. mp 172—173°).[12]

2,2,4,4,6-d_5-16β-Hydroxydehydroepiandrosterone (27)——Prepared by the method of Wynne *et al.*[5] with a slight modification. To a solution of **26** (100 mg) in propylene glycol (50 ml) was added a suspended solution of hog pancreatic amylase (6 g) in H_2O (300 ml), and the solution was incubated at 23° for 2 days. The incubation mixture was extracted with AcOEt, and the organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue obtained was rinsed with hexane to give a crystalline product. Recrystallization of the crude product from MeOH–AcOEt gave **27** (40 mg) as colorless needles. mp 205—208° (lit. mp 207—210°).[5]

2,2,4,4,6-d_5-3β,17β-Dihydroxy-5-androsten-16-one (28)——A solution of **26** (50 mg) in 0.5 N KOH (1.2 ml)–MeOH (4.2 ml) was allowed to stand at room temperature overnight under a N_2 gas stream. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **28** (30 mg) as colorless needes. mp 199—200.5° (lit. mp 197°).[13]

2,2,4,4,6-d_5-16α-Hydroxydehydroepiandrosterone (29)——Prepared by the method of Hassner *et al.*[6] with a slight modification. A solution of **24** (110 mg) and $CuBr_2$ (280 mg) in EtOH (1.7 ml) was refluxed for 1 hr. The reaction mixture was diluted with AcOEt, washed with 5% HCl, 5% $NaHCO_3$, and H_2O successively, dried over anhydrous Na_2SO_4, and evaporated. To the residue was added a solution of Na (120 mg) in MeOH (3 ml), and the resulting solution was refluxed for 1.5 hr. The solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. To the residue dissolved in MeOH (2 ml) was added 5% HCl (0.1 ml), and the solution was allowed to stand at room temperature for 15 min. The reaction mixture was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the residue obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt (1 : 2) and recrystallization of the eluate from MeOH gave **29** (40 mg) as colorless needles. mp 187—188° (lit. mp 177—180°, 177—181°).[6,14]

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12) T. Aoki, H. Yamamura, K. Takei, and H. Mori, *Chem. Pharm. Bull.* (Tokyo) **12**, 808 (1964).
13) A. Butenandt, J. Schmidt-Thomé, and T. Weiss, *Chem. Ber.*, **72**, 417 (1939).
14) K. Fotherby, A. Colas, S.M. Atherden, and G.F. Marrian, *Biochem. J.*, **66**, 664 (1957).