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Synthesis of 4-d_1-Testosterone and 4-d_1-Androstenedione[1]

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In order to clarify the stereochemistry at C-4 in enzymatic saturation of Δ^4-3-ketosteroids synthesis of 4-deuterated testosterone and androst-4-ene-3,17-dione (XVIII, XIX) has been undertaken. The key intermediate leading to the required substrate, 4α-d_1-5α-androstane-3β,4β,17β-triol 3,17-bis(dimethyl-*tert*-butylsilyl) ether (XVI), was prepared by stereospecific reduction with lithium aluminum deuteride from 3β,17β-dihydroxy-5α-androstan-4-one disilyl ether (XIII), which was readily obtainable from androst-4-ene-3β,17β-diol disilyl ether (XI) by hydroboration, followed by oxidation with chromium trioxide–pyridine complex. Dehydration of XVI with phosphorus oxychloride in pyridine provided the Δ^4 olefine (XVIIb) which on chromium trioxide oxidation was led to the desired compounds. Reductive dehalogenation of 4-bromotestosterone silyl ether (II) with lithium aluminum deuteride and subsequent oxidation with chromium trioxide-pyridine complex afforded 4-d_1-testosterone silyl ether (VII) in which the deuterium incorporation, however, proved to be unsatisfactory.

In a series of our studies on the microbial transformation of steroids the stereochemistry of dehydrogenation in ring A has previously been investigated.[3–8] Existence of the enzyme system, which is capable of saturating the Δ^4 double bond, was also demonstrated with microorganisms,[9,10] mammalian liver[11] and testis.[12] The steric mechanism of Δ^4 saturation appears to be an attractive problem to be solved, but still remains unclear.[13–16] The experimental design for elucidating this point requires the Δ^4-3-ketosteroid labeled with hydrogen isotope at C-4 as a substrate. The present paper describes the synthesis of 4-deuterated testosterone and androst-4-ene-3,17-dione starting from readily available testosterone.

An initial effort was focused on the preparation of desired compounds by reductive elimination of halogen at C-4. Preliminary examinations indicated that 4-chlorotestosterone resisted to reductive dehalogenation to some extent. Accordingly 4-bromotestosterone (I) was chosen as a starting material. In order to facilitate the separation of products (I) was first converted to the 17-dimethyl-*tert*-butylsilyl ether (II) in the usual manner.[17] Reduction with lithium aluminum deuteride provided 3α,4-d_2-androst-4-ene-3β,17β-diol 17-silyl ether (VI) and 3α-d_1-4-

1) Part CXIII of "Studies on Steroids" by T. Nambara; Part CXII: H. Hosoda, K. Yamashita, N. Chino, and T. Nambara, *Chem Pharm Bull.* (Tokyo), **24**, 1860 (1976).
2) Location: *Aobayama, Sendai.*
3) T. Anjyo, M. Ito, H. Hosoda, and T. Nambara, *Chem. Ind.* (London), **1972**, 384.
4) T. Nambara, T. Anjyo, M. Ito, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 1938 (1973).
5) S. Ikegawa and T. Nambara, *Chem. Ind.* (London), **1973**, 230.
6) T. Nambara, S. Ikegawa, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 2794 (1973).
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12) N. Bruchovsky and J.D. Wilson, *J. Biol. Chem.*, **243**, 2012 (1968).
13) J.S. Mcguire, Jr. and G.M. Tomkins, *Federation Proc.*, **19**, A-29 (1960).
14) Y.J. Abul-Hajj, *Steroids*, **20**, 215 (1972).
15) K. Nozu and B. Tamaoki, *Acta Endocrinol.*, **76**, 608 (1974).
16) M.K. Sanyal, J.C. Orr, and L.L. Engel, *Eur. J. Biochem.*, **48**, 21 (1974).
17) E.J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94**, 6190 (1972).

bromoandrost-4-ene-3β,17β-diol 17-silyl ether (VIIIb) together with a small amount of each C-3 epimer, whose separation was accomplished by preparative thin-layer chromatography (TLC). Among these the desired compound (VI) was unequivocally characterized by direct comparison with the non-labeled compound (IIIa),[18] prepared from II by metal hydride reduction. Being treated with chromium trioxide-pyridine complex, VI was led to 4-d_1-testosterone 17-silyl ether (VII) in a satisfactory yield. Inspection of the mass and nuclear magnetic resonance (NMR) spectra, however, disclosed that the deuterium incorporation into the C-4 position was 85%.

For the purpose of improving the isotopic purity of the product, the reaction sequence to the final goal was somewhat changed. Reduction of II with lithium aluminum tri(*tert*-butoxy) hydride provided 4-bromoandrost-4-ene-3β,17β-diol 17-monosilyl ether (IIIb) accompanied with a trace amount of its C-3 epimer. Subsequent dehalogenation with lithium aluminum deuteride yielded 4-d_1-androst-4-ene-3β,17β-diol 17-monosilyl ether (IX), which exhibited only 70% deuterium content.

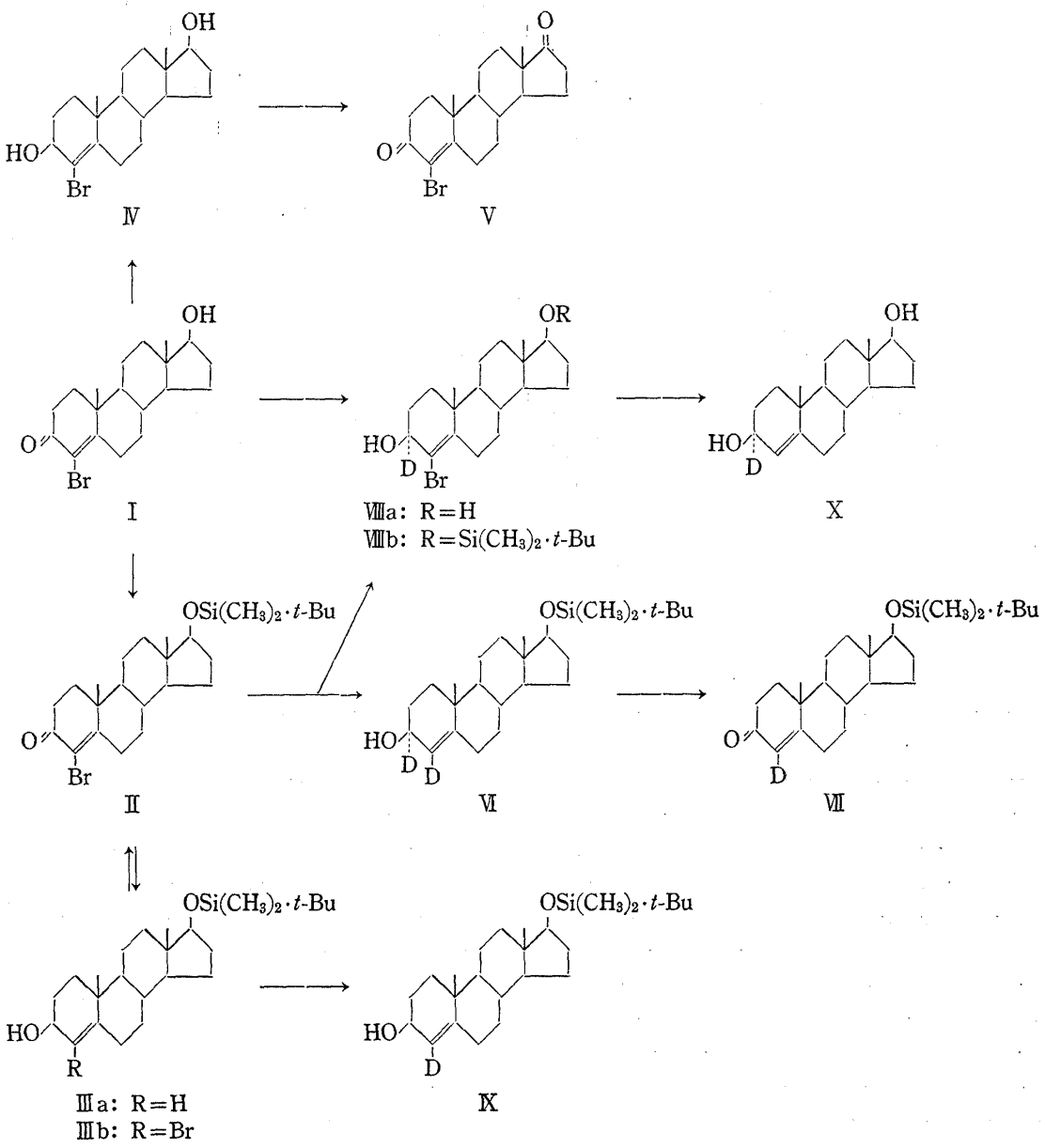

Chart 1

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

Hereupon, it seemed to be of interest to clarify whether or not the loss of the label at C-4 would be ascribable to the 1,2-shift between C-3 and C-4. Brief treatment of I with lithium aluminum deuteride under the mild conditions afforded 3α-d_1-4-bromoandrost-4-ene-3β,17β-diol (VIIIa), which was identified by comparison with the non-labeled compound (IV), obtainable from I by metal hydride reduction. When refluxed with lithium aluminum hydride in tetrahydrofuran, VIIIa was transformed into 3α-d_1-androst-4-ene-3β,17β-diol (X) in a reasonable yield. Inspection of the mass and NMR spectra showed that labeled deuterium at C-3 retained almost intact in X indicating the lack of the 1,2-shift during the debromination process.

The synthetic route involving reductive dehalogenation with metal deuteride was found to be unsuitable for obtaining a deuterated substrate with satisfactory isotopic abundance, although no plausible explanation for the significant loss of the label is available. The present method, however, may be applicable for preparation of the tritiated substrate.

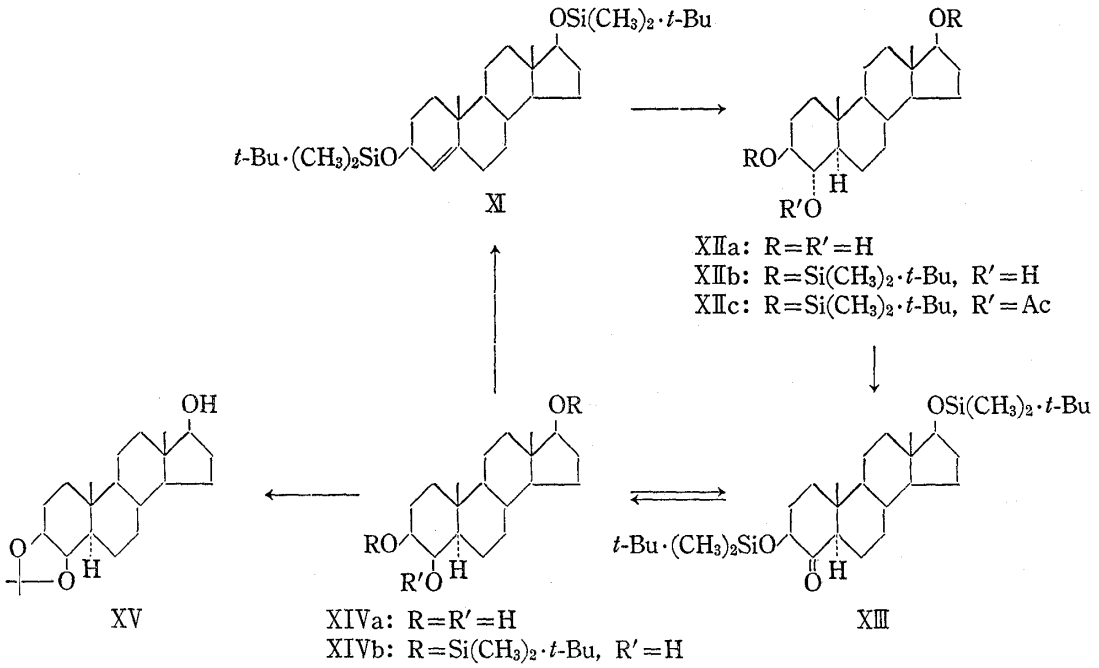

Chart 2

Therefore, the next project was directed to the development of an alternative route to obtain desired substrates. Introduction of an oxygen function into the C-4 position was undertaken employing the Δ^4 unsaturated compound. Hydroboration of androst-4-ene-3β,17β-diol bis(dimethyl-*tert*-butylsilyl) ether (XI) and subsequent oxidation of the organoborane with alkaline hydrogen peroxide provided solely a *cis*-addition product, 5α-androstane-3β,4α,17β-triol 3,17-disilyl ether (XIIb). The configurational assignment of the *cis*-adduct was rationalized by leading to the known 3β,4α,17β-triol (XIIa) by acid hydrolysis. Oxidation of XIIb with chromium trioxide afforded the 4-ketone (XIII) as a single product without disturbing the silyl ether at C-3 and C-17. It is sufficiently substantiated that the A/B-*trans* fusion is thermodynamically more stable than the A/B-*cis* juncture. In actuality the stereochemistry at C-5 retained to be α as judged from the chemical shift of 19-methyl proton in the NMR spectrum. Reduction of the oxo function with lithium aluminum hydride occurred stereoselectively to give the 4β-hydroxyl derivative (XIVb) in a satisfactory yield. The product was obviously differentiated from the epimeric 4α-hydroxyl compound (XIIb) and was oxidized back to the 4-ketone (XIII). The *cis*-glycol structure was rationalized by the formation of the 3,4-acetonide (XV) from the 3β,4β,17β-triol (XIVa), derivable from the 3,17-disilyl ether (XIVb) by acid hydrolysis. Configuration of the newly introduced hydroxyl function at C-4 was thus unambiguously

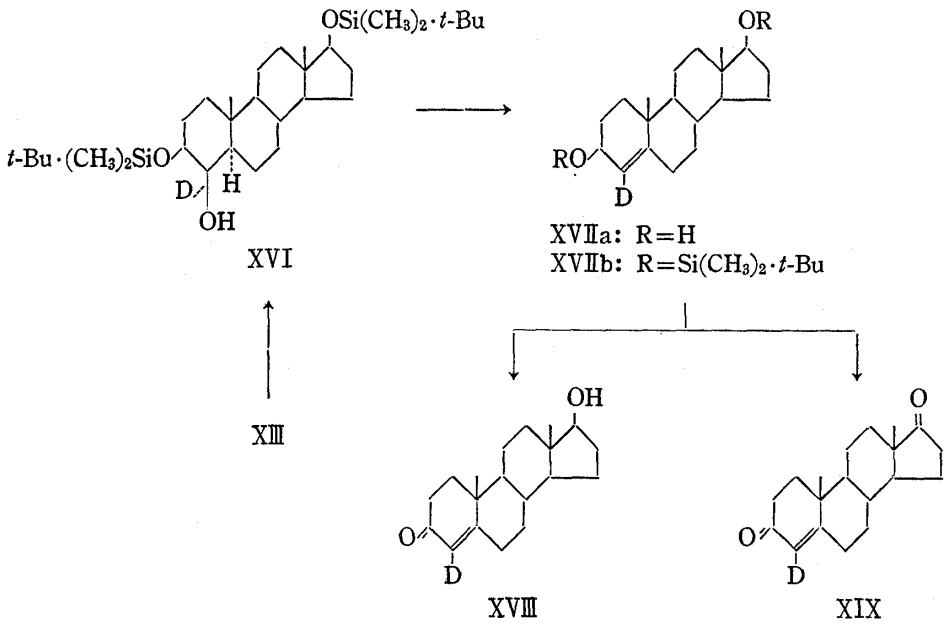

Chart 3

characterized. It is to be noted that the presence of a bulky substituent at 3β would favor the preferential attack of the reagent toward the 4-ketone from the α-side of a molecule. When XIVb was treated with phosphorus oxychloride in pyridine, dehydration reaction proceeded to the desired direction to yield the Δ^4 olefine (XI).

The synthetic route thus established proved to be promising to introduce the deuterium label into the C-4 position of Δ^4-3-ketosteroids. Reduction of XIII with lithium aluminum deuteride in anhydrous ether under the mild conditions, followed by resilylation in the usual manner yielded 4α-d_1-5α-androstane-3β,4β,17β-triol 3,17-disilyl ether (XVI). Dehydration with phosphorus oxychloride in pyridine and subsequent removal of the protecting group at C-3 and C-17 provided 4-d_1-androst-4-ene-3β,17β-diol (XVIIa) in a reasonable yield.

There are several methods for selective oxidation of the allyl alcohol in the field of steroids. Among these N-bromoacetamide is a relatively suitable reagent for allylic oxidation. However, it is anticipated that acidic condition due to the liberation of bromine during the reaction process may be unfavorable for the retention of deuterium at the position adjacent to the ketone. 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ) is also widely used for selective oxidation of a secondary allyl alcohol to the corresponding ketone.[19] The probable mechanism in which the protonated ketone formed by abstraction of the hydride is associated with enolization, may result in the loss of the isotope labeled at the α-position. Therefore, oxidation with chromium trioxide-pyridine complex under the mild conditions was undertaken. On brief exposure to a limited amount of this oxidant XVIIa was converted to the desired 4-d_1-testosterone (XVIII) and 4-d_1-androstenedione (XIX) in a ratio of *ca.* 2 to 1 whose separation was effected by TLC.

The locality and quantity of deuterium in these labeled substrates were determined by means of mass and NMR spectrometry. Examination of molecular ion peaks, which appeared at *m/e* 289 and 287 with an increment of one mass unit, elucidated that the isotopic purity was *ca.* 98% with these labeled compounds.

It is hoped that the facile availability of specifically labeled substrates may serve to clarify the reduction mechanism of Δ^4-3-keto-steroids.

19) S.H. Burstein and H.J. Ringold, *J. Am. Chem. Soc.*, **86**, 4952 (1964).

Experimental[20]

4-Bromotestosterone Dimethyl-*tert*-butylsilyl Ether (II)——To a solution of 4-bromotestosterone (I)[21] (146 mg) in DMF (0.5 ml) were added dimethyl-*tert*-butylsilyl chloride (150 mg) and imidazole (300 mg) and allowed to stand at room temperature for 15 min. The resulting solution was diluted with H_2O and extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was submitted to preparative TLC using hexane–AcOEt (20: 1) as developing solvent. Recrystallization of the eluate from ether–MeOH gave II (200 mg) as colorless plates. mp 173—174.5°. $[\alpha]_D^{18}$ +88.9° (c= 0.18). *Anal.* Calcd. for $C_{25}H_{41}O_2BrSi$: C, 62.35; H, 8.58. Found: C, 62.67; H, 8.87. NMR ($CDCl_3$) δ: 0 (6H, s, Si-CH_3), 0.72 (3H, s, 18-CH_3), 0.87 (9H, s, *tert*-C_4H_9), 1.22 (3H, s, 19-CH_3), 3.54 (1H, t, J=7Hz, 17α-H).

Reduction of II with $LiAlH_4$——To a solution of II (30 mg) in anhydrous ether (8 ml) was added $LiAlH_4$ (50 mg) and refluxed for 5 hr. After addition of moist ether to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–AcOEt (10: 1) as developing solvent. Recrystallization of the eluate from MeOH gave androst-4-ene-3β,17β-diol 17-dimethyl-*tert*-butylsilyl ether (IIIa) (10 mg) as colorless leaflets. mp 140—142°. Mixed melting point on admixture with the authentic sample[18] showed no depression and IR spectra of two samples were entirely identical.

4-d_1-Testosterone Dimethyl-*tert*-butylsilyl Ether (VII)——To a solution of II (200 mg) in anhydrous ether (20 ml) was added $LiAlD_4$ (200 mg) and refluxed for 10 hr. After addition of moist ether to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was submitted to preparative TLC using hexane–AcOEt (10: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.4) gave 3α-d_1-4-bromoandrost-4-ene-3β,17β-diol 17-dimethyl-*tert*-butylsilyl ether (VIIIb) (90 mg). Elution of the adsorbent corresponding to the spot (Rf 0.3) and recrystallization of the eluate from MeOH gave 3α,4-d_2-androst-4-ene-3β,17β-diol 17-dimethyl-*tert*-butylsilyl ether (VI) (30 mg) as colorless leaflets. mp 141—142°. Mixed melting point on admixture with the non-labeled authentic sample[18] showed no depression.

To a solution of VI (27 mg) in pyridine (0.5 ml) was added CrO_3–pyridine complex (1:10 w/v)(1 ml) and allowed to stand at room temperature for 1.5 hr. The reaction mixture was diluted with AcOEt, washed successively with 10% AcOH, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product was recrystallized from MeOH to give VII (22 mg) as colorless leaflets. mp 140—140.5°. Mixed melting point on admixture with the non-labeled authentic sample[18] showed no depression. Mass Spectrum m/e: 403 (M^+) (85% d_1). In the NMR spectrum 85% of the C-4 proton area disappeared.

4-Bromoandrost-4-ene-3β,17β-diol 17-Dimethyl-*tert*-butylsilyl Ether (IIIb)——To a solution of LiAl(*tert*-BuO)$_3$H freshly prepared from $LiAlH_4$ (400 mg) and *tert*-BuOH (3.5 ml) in anhydrous ether (35 ml) was added II (300 mg) in anhydrous ether (10 ml) and the solution was stirred at room temperature for 30 min. After addition of moist ether and 20% Rochelle salt solution the resulting solution was extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was recrystallized from MeOH to give IIIb (210 mg) as colorless leaflets. mp 121—123°. $[\alpha]_D^{21}$ +50.0° (c=0.14). *Anal.* Calcd. for $C_{25}H_{43}O_2BrSi$: C, 62.09; H, 8.96. Found: C, 62.31; H, 9.00. NMR ($CDCl_3$)δ: 0 (6H, s, Si-CH_3), 0.72 (3H, s, 18-CH_3), 0.87 (9H, s, *tert*-C_4H_9), 1.09 (3H, s, 19-CH_3), 3.51 (1H, t, J=7 Hz, 17α-H), 3.90—4.20 (1H, m, 3α-H).

4-d_1-Androst-4-ene-3β,17β-diol 17-Dimethyl-*tert*-butylsilyl Ether (IX)——To a solution of IIIb (50 mg) in anhydrous ether (10 ml) was added $LiAlD_4$ (50 mg) and refluxed for 28 hr. After addition of moist ether and 20 % Rochelle salt solution the resulting solution was extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–AcOEt (10: 1) as developing solvent. Recrystallization of the eluate from MeOH gave IX (24 mg) as colorless leaflets. mp 141.5—142.5°. Mixed melting point on admixture with the non-labeled authentic sample[18] showed no depression. Mass Spectrum m/e: 405 (M^+) (70% d_1). In the NMR spectrum 70% of the C-4 proton area disappeared.

4-Bromoandrost-4-ene-3β,17β-diol (IV)——To a solution of LiAl(*tert*-BuO)$_3$H freshly prepared from $LiAlH_4$ (300 mg) and *tert*-BuOH (2.5 ml) in anhydrous ether (20 ml) was added I (100 mg) in anhydrous ether (10 ml)

20) All melting points were taken on a hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. IR spectra were recorded on a JASCO Model IRA-1 spectrometer. NMR spectra were obtained on a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, and m=multiplet. Mass spectral measurements were run on a Hitachi Model RMU-6E spectrometer under the following conditions: ionization voltage 80 eV, accelerator voltage 1.4 kV, temperature of ionization chamber 160°, and width of collector slit 0.4 mm. For preparative TLC silica gel H, silica gel G, and silica gel HF_{254} (E. Merck AG, Darmstadt) were used as an adsorbent.

21) H.J. Ringold, E. Batres, O. Mancera, and G. Rosenkranz, *J. Org. Chem.*, **21**, 1432 (1956).

and the solution was stirred at room temperature for 30 min. After addition of moist AcOEt and 20% Rochelle salt solution the resulting solution was extracted with AcOEt. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was recrystallized from acetone to give IV (50 mg) as colorless needles. mp 216—218°. $[\alpha]_D^{18}$ +57.1° (c=0.21). *Anal.* Calcd. for $C_{19}H_{29}O_2Br$: C, 61.78; H, 7.91. Found: C, 61.90; H, 7.94. NMR ($CDCl_3$) δ: 0.78 (3H, s, 18-CH_3), 1.12 (3H, s, 19-CH_3), 3.65 (1H, t, J=7 Hz, 17α-H), 4.08—4.32 (1H, m, 3α-H).

4-Bromoandrost-4-ene-3,17-dione (V)——To a solution of IV (10 mg) in pyridine (0.2 ml) was added CrO_3-pyridine complex (1: 10 w/v) (0.5 ml) and stirred at room temperature for 4 hr. The resulting solution was diluted with AcOEt, washed successively with 10% AcOH, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product was purified by preparative TLC using hexane–AcOEt (2: 1) as developing solvent. Recrystallization of the eluate from CH_2Cl_2–hexane gave V (8 mg) as colorless needles. mp 151—152°. $[\alpha]_D^{14}$ +183.3° (c=0.12). *Anal.* Calcd. for $C_{19}H_{25}O_2Br$: C, 62.46; H, 6.90. Found: C, 62.38; H, 6.81. NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-CH_3), 1.26 (3H, s, 19-CH_3).

3α-d_1-4-Bromoandrost-4-ene-3β,17β-diol (VIIIa)——To a solution of I (50mg) in tetrahydrofuran (THF) (4 ml) was added $LiAlD_4$ (50 mg) and allowed to stand at room temperature for 8 hr. After addition of moist AcOEt and 20% Rochelle salt solution the resulting solution was extracted with AcOEt. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was submitted to preparative TLC using benzene–ether (2: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.6) and recrystallization of the eluate from acetone gave VIIIa (23 mg) as colorless needles. mp 212—215°. Mixed melting point on admixture with IV showed no depression. Mass Spectrum *m/e*: 370 (M^+) (98% d_1). In the NMR spectrum the 3α-proton signal disappeared completely.

3α-d_1-Androst-4-ene-3β,17β-diol (X)——To a solution of VIIIa (20 mg) in THF (6 ml) was added $LiAlH_4$ (30 mg) and refluxed for 16 hr. After addition of moist AcOEt and 20% Rochelle salt solution the resulting solution was extracted with AcOEt. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was submitted to preparative TLC using benzene–ether (2: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.5) and recrystallization of the eluate from MeOH gave X (10 mg) as colorless plates. mp 150—153°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass Spectrum *m/e*: 291 (M^+) (98% d_1). In the NMR spectrum the 3α-proton signal disappeared completely.

5α-Androstane-3β,4α,17β-triol 3,17-Bis(dimethyl-*tert*-butylsilyl) Ether (XIIb)——To a stirred solution of androst-4-ene-3β,17β-diol bis(dimethyl-*tert*-butylsilyl) ether (XI)[18] (250 mg) and $LiAlH_4$ (400 mg) in anhydrous ether (42 ml) was added BF_3-etherate (3 g) in anhydrous ether (18 ml) at 0° over a period of 15 min under a stream of N_2 gas and then stirred at room temperature for 1 hr. After addition of moist ether the resulting solution was extracted with ether. The organic phase was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. To a solution of the residue in THF (10 ml) were added dropwise 10% NaOH (4 ml) and 30% H_2O_2 (3 ml) under ice-cooling and stirred at 0° for 1 hr. The resulting solution was diluted with H_2O and extracted with ether. The organic phase was washed with 5% $NaHSO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–AcOEt (30: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XIIb (200 mg) as colorless leaflets. mp 151.5—152°. $[\alpha]_D^{21}$ +3.1° (c=0.16). *Anal.* Calcd. for $C_{31}H_{60}O_3Si_2$: C, 69.34; H, 11.26. Found: C, 69.15; H, 11.27. NMR ($CDCl_3$) δ: 0 (6H, s, 17-OSi(CH_3)$_2$), 0.08 (6H, s, 3-OSi(CH_3)$_2$), 0.68 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 0.88 (9H, s, 17-OSi-*tert*-C_4H_9), 0.91 (9H, s, 3-OSi-*tert*-C_4H_9), 3.24—3.68 (3H, m, 3α-, 4β-, and 17α-H).

5α-Androstane-3β,4α,17β-triol (XIIa)——To a solution of XIIb (20 mg) in MeOH (2 ml) was added 20% HCl (0.3 ml) and allowed to stand at room temperature for 15 min. 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 was recrystallized from aq. MeOH to give XIIa (7 mg) as colorless needles. mp 245—248°. Nakata[22] prepared this compound by the different method (reported mp 248—250°).

4α-Acetoxy-5α-androstane-3β,17β-diol Bis(dimethyl-*tert*-butylsilyl) Ether (XIIc)——Treatment of XIIb (30 mg) with Ac_2O (0.5 ml) and pyridine (1 ml) in the usual manner, followed by purification by preparative TLC using hexane–AcOEt (30: 1) as developing solvent gave XIIc (17 mg) as semi-crystalline product. $[\alpha]_D^{16}$ +27.3° (c=0.22). *Anal.* Calcd. for $C_{33}H_{62}O_4Si_2$: C, 68.46; H, 10.80. Found: C, 68.61; H, 10.89. NMR ($CDCl_3$) δ: 0 (6H, s, 17-OSi(CH_3)$_2$), 0.01, 0.03 (6H, each s, 3-OSi(CH_3)$_2$), 0.66 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.83, 0.86 (18H, each s, *tert*-C_4H_9), 2.01 (3H, s, -OCOCH_3), 3.27—3.70 (2H, m, 3α- and 17α-H), 4.60—5.00 (1H, m, 4β-H).

3β,17β-Dihydroxy-5α-androstan-4-one Bis(dimethyl-*tert*-butylsilyl) Ether (XIII)——To a solution of XIIb (140 mg) in pyridine (2 ml) was added CrO_3-pyridine complex (1: 10 w/v) (10 ml) and stirred at room temperature for 5 days. The resulting solution was diluted with ether, washed successively with 10% AcOH, 10% Na_2CO_3, and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product was purified by preparative TLC using hexane–benzene (2: 1) as developing solvent. Recrystallization of the eluate from acetone gave XIII (85 mg) as colorless leaflets. mp 158—160°. $[\alpha]_D^{21}$ −11.6° (c=0.43). *Anal.* Calcd. for $C_{32}H_{58}O_3Si_2$: C, 69.61; H, 10.93. Found: C, 69.68; H, 10.73. NMR ($CDCl_3$)δ: 0, 0.15 (12H, each s, Si-CH_3), 0.72 (3H, s, 18-CH_3),

22) H. Nakata, *Bull. Chem. Soc. Japan*, 38, 378 (1965).

0.76 (3H, s, 19-CH_3), 0.88, 0.92 (18H, each s, *tert*-C_4H_9), 3.62 (1H, t, $J=7$ Hz, 17α-H), 4.12—4.36 (1H, m, 3α-H).

5α-Androstane-3β,4β,17β-triol 3,17-Bis(dimethyl-*tert*-butylsilyl) Ether (XIVb)——To a solution of XIII (10 mg) in anhydrous ether (2 ml) was added $LiAlH_4$ (10 mg) and stirred at room temperature for 25 min. After addition of moist ether and 20% Rochelle salt solution the resulting solution was extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The residue was treated with dimethyl-*tert*-butylsilyl chloride (150 mg) and imidazole (300 mg) in DMF (0.5 ml) at room temperature for 1.5 hr. The resulting solution was diluted with H_2O and extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–benzene (2: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XIVb (7 mg) as colorless leaflets. mp 175—176°. $[\alpha]_D^{21}$ −30.0° ($c=0.15$). *Anal.* Calcd. for $C_{31}H_{60}O_3Si_2$: C, 69.34; H, 11.26. Found: C, 69.37; H, 11.42. NMR ($CDCl_3$) δ: 0 (6H, s, 17-OSi(CH_3)$_2$), 0.08 (6H, s, 3-OSi-(CH_3)$_2$), 0.70 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 0.88, 0.92 (18H, each s, *tert*-C_4H_9), 3.48—3.80 (3H, m, 3α-, 4α-, and 17α-H).

5α-Androstane-3β,4β,17β-triol (XIVa)——Treatment of XIVb (27 mg) with 20% HCl (0.5 ml) in MeOH (4 ml) in the manner as described in XIIa, followed by recrystallization from MeOH gave XIVa (15 mg) as colorless prisms. mp 258—261°. $[\alpha]_D^{18}$ −4.2° ($c=0.12$, MeOH). *Anal.* Calcd. for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 73.84; H, 10.56.

5α-Androstae-3β,4β,17β-triol 3,4-Acetonide (XV)——To a solution of XIVa (8 mg) in acetone (10 ml) was added anhydrous $CuSO_4$ (100 mg) and refluxed for 8 hr. The precipitate was removed by filtration and the filtrate was concentrated *in vacuo*. The crude product was purified by preparative TLC using hexane–AcOEt (1: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XV (6 mg) as colorless needles. mp 173—174°. $[\alpha]_D^{15}$ −19.2° ($c=0.13$). *Anal.* Calcd. for $C_{22}H_{36}O_3$: C, 75.81; H, 10.41. Found: C, 75.67; H, 10.49. NMR ($CDCl_3$) δ: 0.76 (3H, s, 18-CH_3), 1.08 (3H, s, 19-CH_3), 1.32, 1.53 (6H, each s, >C(CH_3)$_2$), 3.64 (1H, t, $J=$ 7 Hz, 17α-H), 3.92—4.08 (2H, m, 3α- and 4α-H).

Oxidation of XIVb with CrO_3-Pyridine Complex——To a solution of XIVb (10 mg) in pyridine (0.5 ml) was added CrO_3–pyridine complex (1: 10 w/v) (1 ml) and allowed to stand at room temperature for 22 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. Recrystallization of the crude product from acetone gave XIII (7 mg) as colorless leaflets. mp 155—157°. Mixed melting point on admixture with the authentic sample showed no depression and IR spectra of two samples were entirely identical.

Dehydration of XIVb——To a solution of XIVb (10 mg) in pyridine (1 ml) was added $POCl_3$ (0.2 ml) under ice-cooling and allowed to stand at room temperature for 24 hr. After addition of moist ether the resulting solution was extracted with ether. The organic phase was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–AcOEt (100: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XI (5 mg) as colorless leaflets. mp 100.5—102°. Mixed melting point on admixture with the authentic sample[18] showed no depression and IR spectra of two samples were entirely identical.

4α-d_1-5α-Androstane-3β,4β,17β-triol 3,17-Bis(dimethyl-*tert*-butylsilyl) Ether (XVI)——To a solution of XIII (500 mg) in anhydrous ether (20 ml) was added $LiAlD_4$ (300 mg) and stirred at room temperature for 2.5 hr. After addition of moist ether and 20% Rochelle salt solution the resulting solution was extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The residue was treated with dimethyl-*tert*-butylsilyl chloride (1 g) and imidazole (1.5 g) in DMF (6 ml) at room temperature for 30 min. The resulting solution was diluted with H_2O and extracted with ether. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–benzene (2: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XVI (400 mg) as colorless leaflets. IR ν_{max}^{KBr} cm^{-1}: 2100 (C-D). mp 175—176°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass Spectrum *m/e*: 537 (M^+) (98% d_1).

4-d_1-Androst-4-ene-3β,17β-diol Bis(dimethyl-*tert*-butylsilyl) Ether (XVIIb)——Treatment of XVI (200 mg) with $POCl_3$ (0.6 ml) in pyridine (6 ml) in the manner as described in dehydration of XIVb. The crude product was purified by preparative TLC using hexane–AcOEt (100: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XVIIb (140 mg) as colorless needles. mp 97—99°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass Spectrum *m/e* 519 (M^+) (98% d_1). In the NMR spectrum the C-4 proton signal disappeared completely.

4-d_1-Androst-4-ene-3β,17β-diol (XVIIa)——To a solution of XVIIb (80 mg) in THF (0.5 ml) was added (n-C_4H_9)$_4$NF (2 ml) and stirred at room temperature for 20 hr. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using benzene–EtOH (10: 1) as developing solvent. Recrystallization of the eluate from MeOH gave XVIIa (36 mg) as colorless plates. mp 154—155°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass Spectrum *m/e*: 291 (M^+) (98% d_1). In the NMR spectrum the C-4 proton signal disappeared completely.

Oxidation of XVIIa with CrO_3-Pyridine Complex——To a solution of XVIIa (28 mg) in pyridine (0.5 ml) was added CrO_3-pyridine complex (1: 20 w/v) (1 ml) and allowed to stand at room temperature for 30 min. The resulting solution was diluted with AcOEt, washed with 10% Na_2CO_3 and H_2O, dried over anhydrous Na_2SO_4,

and evaporated. The crude product was submitted to preparative TLC using hexane–AcOEt (1 : 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.60) and recrystallization of the eluate from acetone–hexane gave 4-d_1-androst-4-ene-3,17-dione (XIX) (6 mg) as colorless plates. mp 170.5—172°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass Spectrum *m/e*: 287 (M^+) (98% d_1). Elution of the adsorbent corresponding to the spot (*Rf* 0.47) and recrystallization of the eluate from acetone–hexane gave 4-d_1-testosterone (XVIII) (4 mg) as colorless plates. mp 143.5—144°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass Spectrum *m/e*: 289 (M^+) (98% d_1). In the NMR spectra of XIX and XVIII the C-4 proton signal disappeared completely.

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