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Synthesis of 15α-Hydroxylated Dehydroepiandrosterone and Androstenediol[1]

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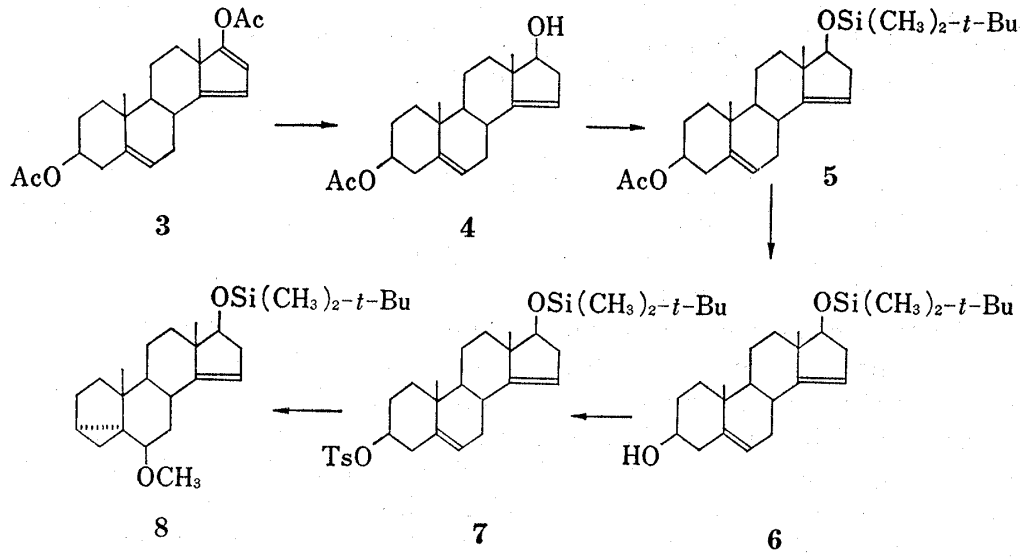
The titled compounds (**1**, **2**) have been prepared from dehydroepiandrosterone. Introduction of the hydroxyl group into the 15α-position was attained by hydroboration of the 14,15-double bond with diborane and subsequent oxidation of the organoborane with alkaline hydrogen peroxide under the protection of the 5,6-double bond as the 3,5-cyclo structure. A simple method for regeneration of the 3β-hydroxy-Δ^5-steroid from the 3,5-cyclo derivative was also developed.

15α-Hydroxydehydroepiandrosterone (**1**) and 5-androstene-3β,15α,17β-triol (**2**) are of particular interest in connection with the biosynthesis of 15α-hydroxyestriol (estetrol).[3] The occurrence of **1** in newborn infant urine as a sulfate has recently been demonstrated by means of gas chromatography–mass spectrometry.[4] During the course of our biochemical studies on the male and female hormones the availability of the 15α-hydroxylated C_{19} steroids has become an essential prerequisite. In an earlier report an attempt for the preparation of these compounds by chemical means resulted in failure,[5] and hence microbial transformation was utilized for this purpose.[4] The present paper deals with the convenient synthesis of **1** and **2** starting from dehydroepiandrosterone.

In the preceding paper of this series the new method for introducing a hydroxyl function into the 15α-position by hydration of the Δ^{14}-olefine has been established.[6] The appropriate protection of the 5,6-double bond was required, since difficulties would be anticipated for selective hydration of the 14,15-unsaturation. An initial effort was directed to the preparation of 6β-methoxy-3α,5-cyclo-5α-androst-14-en-17β-ol dimethyl-*tert*-butylsilyl ether (**8**) as a key intermediate, where the 5,6-double bond is selectively protected by leading to the *i*-steroid. For this purpose the $\Delta^{14,16}$-dien-17-ol acetate (**3**), readily obtainable from dehydroepiandrosterone by the known method,[7] was chosen as a starting material. Hydrolysis and reduction of the oxygen function at C-17 without disturbance of the 3β-acetoxyl group in **3** were simultaneously effected by treatment with sodium borohydride to yield 5,14-androstadiene-3β,17β-diol 3-acetate (**4**). Being treated with dimethyl-*tert*-butylsilyl chloride in dimethylformamide in the presence of imidazole, **4** was easily transformed into the 17-dimethyl-*tert*-butylsilyl ether (**5**). Saponification of **5** with 10% potassium hydroxide in methanol-tetrahydrofuran gave the 3β,17β-diol 17-monosilyl ether (**6**), which in turn was converted to the 3-tosylate (**7**) with *p*-toluenesulfonyl chloride and pyridine in the usual manner. When **7** was refluxed in methanol in the presence of potassium acetate, the 3,5-cyclo compound (**8**) was formed in a

1) Part CXII of "Studies on Steroids" by T. Nambara; Part CXI: T. Nambara, K. Sudo, and M. Sudo, *Steroids*, **27**, 111 (1976).
2) Location: *Aobayama, Sendai.*
3) E.V. YoungLai and S. Solomon, *J. Clin. Endocrinol. Metab.*, **28**, 1161 (1968).
4) R.A. Anderson, E.M. Chambaz, C. Madani, and G. Defaye, "International Symposium on Sexual Endocrinology of the Perinatal Period," Vol. 32, ed. by M.G. Forest and J. Bertrand, Inserm, Paris, 1974, p. 267.
5) R.W. Kelly and P.J. Sykes, *J. Chem. Soc.* (*C*), **1968**, 416.
6) H. Hosoda, K. Yamashita, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **23**, 3141 (1975).
7) J. Pataki and G. Siade B, *J. Org. Chem.*, **37**, 2127 (1972).

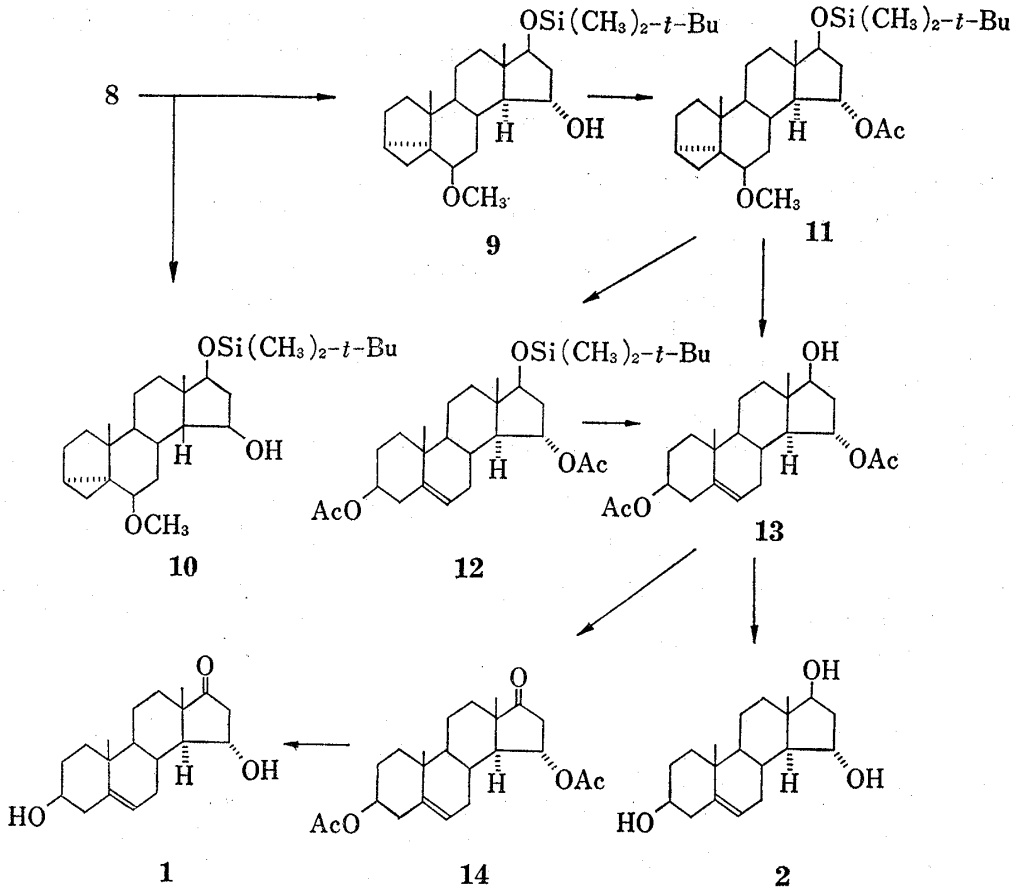


3 4 5 8 7 6

Chart 1

fairly good yield. Nuclear magnetic resonance (NMR) spectrum of **8** exhibited a three-proton multiplet at 0.25—0.70 ppm due to the cyclopropane ring besides the signals of 6β-methoxy-, 6α- and olefinic 15-protons.

Hydroboration of **8** and subsequent oxidation of the organoborane with alkaline hydrogen peroxide afforded two isomeric *cis*-addition products in a ratio of *ca.* 4 to 1, whose separation was readily attained by preparative thin-layer chromatography (TLC). The characteristic



9 11 10 12 13 1 14 2

Chart 2

splitting patterns of the 17α-protons in their NMR spectra permitted us to assign the structures 17β-dimethyl-*tert*-butylsilyloxy-6β-methoxy-3α,5-cyclo-5α-androstan-15α-ol (**9**) to the major product and its 14β,15β-isomer (**10**) to the minor one. It is to be noted that the presence of the bulky group at 17β would favor the attack of the reagent toward the Δ^{14}-double bond preferentially from the α-side of a molecule.

From the necessity of 15α-hydroxydehydroepiandrosterone **9** was led to the 15-acetate (**11**) by usual acetylation with acetic anhydride and pyridine. On treatment with zinc acetate in acetic acid **11** was converted to the Δ^5-compound (**12**), which was then partially hydrolyzed with hydrogen chloride in acetone to give the 3β,15α,17β-triol 3,15-diacetate (**13**). The result obtained under these conditions was found to be unsatisfactory with respect to the yield as an inevitable disadvantage of this method has previously been pointed out by Narayanan, *et al.*[8] Accordingly, development of an alternative method was then undertaken. When **11** was exposed to acetic acid in dry ether in the presence of boron trifluoride etherate, regeneration of the 5,6-double bond and simultaneous elimination of the silyl group took place to provide the desired compound (**13**) almost quantitatively. This procedure appears to be more useful for regeneration of the 3β-hydroxy-Δ^5-steroid from the 3,5-cyclo compound than the known methods employing zinc acetate-acetic acid, aluminum oxide-acetic acid[9] or acetic anhydride-boron trifluoride etherate.[8]

Hydrolysis of **13** with methanolic potassium hydroxide furnished the desired 5-androstene-3β,15α,17β-triol(**2**) in a reasonable yield. In addition, **13** was oxidized with chromic anhydride-pyridine complex to yield 15α-hydroxydehydroepiandrosterone diacetate (**14**). It is sufficiently substantiated that the β-ketol system is highly susceptible to basic conditions.[6] However, hydrolytic cleavage of the acetoxyl groups at C-15 and C-3 in **14** was accomplished with success. Upon exposure to hydrochloric acid in acetone the desired 15α-hydroxydehydroepiandrosterone (**1**) was obtained in 80% yield.

It is hoped that the availability of the titled compounds will provide the more precise knowledge on the biosynthesis of estrogens in the feto-placental unit.

Experimental[10]

5,14-Androstadiene-3β,17β-diol 3-Acetate (4)——To a solution of 5,14, 16-androstatriene-3β,17-diol diacetate (3)[7] (2.3 g) in EtOH (200 ml)–tetrahydrofuran (10 ml) was added $NaBH_4$ (3.5 g) in EtOH (20 ml)–H_2O (14 ml) at 0° and stirred at room temperature for 2 hr. After addition of 10% AcOH to decompose the excess reagent the resulting solution was concentrated to its half volume under the reduced pressure and diluted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from aq. MeOH gave **4** (1.8 g) as colorless plates. mp 157—160°. $[\alpha]_D^{21}$ −38.8° (c=0.10). NMR ($CDCl_3$) δ: 0.98 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 2.00 (3H, s, 3-$OCOCH_3$), 3.95 (1H, t, J=8 Hz, 17α-H), 4.55 (1H, m, 3α-H), 5.08 (1H, m, 15-H), 5.38 (1H, m, 6-H). *Anal.* Calcd. for $C_{21}H_{30}O_3 \cdot 1/2H_2O$: C, 74.30; H, 9.21. Found: C, 74.30; H, 9.31.

17β-Dimethyl-*tert*-butylsilyloxy-5,14-androstadien-3β-ol Acetate (5)——To a solution of **4** (2.3 g) in dimethylformamide (25 ml) were added imidazole (10 g) and dimethyl-*tert*-butylsilyl chloride (4.3 g) and allowed to stand at room temperature overnight. 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 **5** (2.4 g) as colorless leaflets. mp 118—120°. $[\alpha]_D^{21}$ −33.3° (c=0.10). NMR ($CDCl_3$) δ: 0.04 (6H, s, 17-OSi-$(CH_3)_2$), 0.89 (9H, s, 17-OSi-*t*-Bu), 0.93 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.00 (3H, s, 3-$OCOCH_3$), 3.90 (1H, t, J=8 Hz, 17α-H), 4.55 (1H, m, 3α-H), 5.08 (1H, m, 15-H), 5.38 (1H, m, 6-H). *Anal.* Calcd. for $C_{27}H_{44}O_3Si$: C, 72.92; H, 9.97. Found: C, 72.97; H, 10.13.

8) C.R. Narayanan, S.R. Prakash, and B.A. Nagasampagi, *Chem. Ind.* (London), **1974**, 966.
9) A. Metayer and M. Barbier, *Bull. Soc. Chim. Fr.*, **1972**, 3625.
10) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Infrared (IR) spectra were run on a JASCO Model IRA-1 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, sx=sextet, and m=multiplet. For preparative TLC silica gel H (E. Merck AG, Darmstadt) was used as an adsorbent.

5,14-Androstadiene-3β,17β-diol 17-Dimethyl-*tert*-butylsilyl Ether (6)——To a solution of **5** (1.2 g) in MeOH (4 ml)-tetrahydrofuran (6 ml) was added 10% KOH (4 ml) and allowed to stand at room temperature for 1 hr. The resulting solution was concentrated under reduced pressure and diluted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **6** (1.1 g) as colorless needles. mp 150—151°. $[\alpha]_D^{21}$ −28.6° (c=0.10). NMR (CCl_4) δ: 0.02 (6H, s, 17-OSi$(CH_3)_2$), 0.88 (12H, s, 18-CH_3 and 17-OSi-*t*-Bu), 1.00 (3H, s, 19-CH_3), 3.36 (1H, m, 3α-H), 3.84 (1H, t, J=8 Hz, 17α-H), 5.00 (1H, m, 15-H), 5.28 (1H, m, 6-H). *Anal.* Calcd. for $C_{25}H_{42}O_2Si$: C, 74.57; H, 10.51. Found: C, 74.35; H, 10.75.

3β-*p*-Toluenesulfonyloxy-5,14-androstadien-17β-ol Dimethyl-*tert*-butylsilyl Ether (7)——To a solution of **6** (1 g) in pyridine (10 ml) was added *p*-toluenesulfonyl chloride (2.5 g) and stirred at 0° for 2 days. The resulting solution was poured onto ice-water and the precipitate was collected by filtration, washed with H_2O, and dried. The crude product (1.3 g) was submitted to further elaboration without purification. A portion of the product was recrystallized from acetone to give **7** as colorless needles. mp 129.5—130.5°. $[\alpha]_D^{26}$ −16.1° (c=0.10). NMR (CCl_4) δ: 0.03 (6H, s, 17-OSi$(CH_3)_2$), 0.88 (12H, s, 18-CH_3 and 17-OSi-*t*-Bu), 0.99 (3H, s, 19-CH_3), 2.43 (3H, s, 3-$OSO_2C_6H_4$-CH_3), 3.83 (1H, t, J=8 Hz, 17α-H), 4.15 (1H, m, 3α-H), 5.00 (1H, m, 6-H), 7.22, 7.69 (each 2H, d, J=8 Hz, aromatic proton). *Anal.* Calcd. for $C_{32}H_{48}O_4SSi$: C, 69.01; H, 8.67. Found: C, 69.03; H, 8.78.

6β-Methoxy-3α,5-cyclo-5α-androst-14-en-17β-ol Dimethyl-*tert*-butylsilyl Ether (8)——To a solution of **7** (1.3 g) in MeOH (70 ml) was added fused AcOK (3 g) and refluxed for 40 min. The resulting solution was concentrated under reduced pressure and diluted with ether. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Purification of the crude product by preparative TLC using hexane–AcOEt (30: 1) as developing solvent gave **8** (990 mg) as colorless oil. NMR (CCl_4) δ: 0.04 (6H, s, 17-OSi$(CH_3)_2$) 0.25—0.70 (3H, m, cyclopropane), 0.89 (9H, s, 17-OSi-*t*-Bu), 0.94 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.71 (1H, broad s, 6α-H), 3.24 (3H, s, 6β-OCH_3), 3.85 (1H, t, J=8 Hz, 17α-H), 4.98 (1H, m, 15-H).

Hydration of 8——To a solution of **8** (1.1 g) in anhydrous tetrahydrofuran (15 ml) was introduced B_2H_6 gas generated by adding a solution of BF_3–etherate (22 ml) in diglyme (33 ml) to a solution of $NaBH_4$ (11 g) in diglyme (50 ml). The gas was passed at 0° for 1 hr and then at room temperature for 1.5 hr by means of a slow stream of N_2. To the reaction mixture was carefully added a solution of 30% H_2O_2 (5.5 ml) in 10% NaOH (7 ml) and allowed to stand at 0° for 1 hr. The resulting solution was diluted with ether, washed with 5% $NaHSO_3$, 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 (8: 1) as developing solvent. Recrystallization of the major product from aq. MeOH gave **9** (770 mg) as colorless needles. mp 60—64°. $[\alpha]_D^{26}$ +55.8° (c=0.21). NMR (CCl_4) δ: 0.02 (6H, s, 17-OSi$(CH_3)_2$), 0.25—0.70 (3H, m, cyclopropane), 0.77 (3H, s, 18-CH_3), 0.89 (9H, s, 17-OSi-*t*-Bu), 1.00 (3H, s, 19-CH_3), 2.70 (1H, broad s, 6α-H), 3.29 (3H, s, 6β-OCH_3), 3.76 (1H, t, J=8 Hz, 17α-H), 4.02 (1H, m, 15β-H). *Anal.* Calcd. for $C_{26}H_{46}O_3Si$: C, 71.84; H, 10.67. Found: C, 71.54; H, 10.70. The minor product obtained from the less polar fraction was recrystallized from MeOH to give **10** (190 mg) as colorless prisms. mp 126—127.5°. $[\alpha]_D^{22}$ +32.7° (c=0.20). NMR (CCl_4) δ: 0.04 (6H, s, 17-OSi$(CH_3)_2$), 0.25—0.70 (3H, m, cyclopropane), 0.90 (9H, s, 17-OSi-*t*-Bu) 0.95 (3H, s, 19-CH_3), 1.03 (3H, s, 18-CH_3), 2.73 (1H, broad s, 6α-H), 3.27 (3H, s, 6β-OCH_3), 3.53 (1H, d, J=5 Hz, 17α-H), 4.06 (1H, m, 15α-H). *Anal.* Calcd. for $C_{26}H_{46}O_3Si$: C, 71.84; H, 10.67. Found: C, 71.95; H, 10.93.

17β-Dimethyl-*tert*-butylsilyloxy-6β-methoxy-3α,5-cyclo-5α-androstan-15α-ol Acetate (11)——A solution of **9** (350 mg) in pyridine (3 ml) and Ac_2O (1.5 ml) was allowed to stand at room temperature overnight. The resulting solution was poured onto ice-water and extracted with ether. The organic layer was washed with 10% AcOH, ice-cooled 5% $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **11** (340 mg) as colorless needles. mp 128.5—129.5°. $[\alpha]_D^{25}$ +68.8° (c=0.10). NMR (CCl_4) δ: 0.04 (6H, s, 17-OSi$(CH_3)_2$), 0.25—0.70 (3H, m, cyclopropane), 0.82 (3H, s, 18-CH_3), 0.88 (9H, s, 17-OSi-*t*-Bu), 1.00 (3H, s, 19-CH_3), 1.93 (3H, s, 15-$OCOCH_3$), 2.63 (1H, m, 6α-H), 3.24 (3H, s, 6β-OCH_3), 3.70 (1H, t, J=8 Hz, 17α-H), 4.82 (1H, sx, J=9, 9, 5 Hz, 15β-H). *Anal.* Calcd. for $C_{28}H_{48}O_4Si$: C, 70.54; H, 10.15. Found: C, 70.32; H, 10.11.

17β-Dimethyl-*tert*-butylsilyloxy-5-androstene-3β,15α-diol Diacetate (12)——To a solution of **11** (305 mg) in AcOH (9.5 ml) was added freshly fused $Zn(OAc)_2$ (1.5 g) and refluxed for 20 min with stirring. The resulting solution was cooled and extracted with ether. The organic layer 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 benzene–ether (50: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **12** (98 mg) as colorless needles. mp 100—102°. $[\alpha]_D^{18}$ −27.3° (c=0.11). NMR (CCl_4) δ: 0.04 (6H, s, 17-OSi$(CH_3)_2$), 0.77 (3H, s, 18-CH_3), 0.88 (9H, s, 17-OSi-*t*-Bu), 1.03 (3H, s, 19-CH_3), 1.93 (6H, s, 3- and 15-$OCOCH_3$), 3.71 (1H, t, J=8 Hz, 17α-H), 4.50 (1H, m, 3α-H), 4.83 (1H, m, 15β-H), 5.30 (1H, m, 6-H). *Anal.* Calcd. for $C_{29}H_{48}O_5Si$: C, 69.00; H, 9.59. Found: C, 69.31; H, 9.59.

5-Androstene-3β,15α,17β-triol 3,15-Diacetate (13)——i) To a solution of **12** (97 mg) in acetone (1 ml) was added a solution of HCl (220 mg) in acetone (2.5 ml) and allowed to stand at room temperature for 20 min. 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 from acetone–hexane gave **13** (68 mg) as colorless needles. mp 162—164°. $[\alpha]_D^{25}$ −25.0° (c=0.10). NMR ($CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3),

2.02 (6H, s, 3- and 15-$OCOCH_3$), 3.81 (1H, t, J=8 Hz, 17α-H), 4.55 (1H, m, 3α-H), 4.90 (1H, sx, J=9, 9, 5 Hz), 5.30 (1H, m, 6-H). *Anal.* Calcd. for $C_{22}H_{34}O_5 \cdot 1/2H_2O$: C, 69.14; H, 8.83. Found: C, 69.25; H, 8.88.

ii) To a solution of **11** (47 mg) in anhydrous ether (2 ml) were added AcOH (1 ml) and BF_3–etherate (1 ml) and allowed to stand at room temperature for 2 hr. 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 from acetone–hexane gave **13** (38 mg) as colorless needles. mp 160—162°. IR spectra of the two samples obtained in i) and ii) were entirely identical in every respect.

3β,15α-Diacetoxy-5-androsten-17-one (14)——To a solution of **13** (50 mg) in pyridine (1 ml) was added CrO_3–pyridine complex (10% w/v) (2 ml) and allowed to stand at room temperature for 3 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 residue from MeOH gave **14** (42 mg) as colorless needles. mp 187—188°. $[\alpha]_D^{23}$ +32.6° (c=0.11). NMR (CCl_4) δ: 0.95 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 1.95, 1.99 (each 3H, s, 3- and 15-$OCOCH_3$), 3.03 (1H, dd, J=8, 18 Hz, 16ζ-H), 4.50 (1H, m, 3α-H), 5.05 (1H, m, 15β-H), 5.30 (1H, m, 6-H). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.87; H, 8.36.

5-Androstene-3β,15α,17β-triol (2)——To a solution of **13** (36 mg) in MeOH (2 ml) was added 10% KOH (0.5 ml) and allowed to stand at room temperature for 4 hr. 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–benzene gave **2** (25 mg) as colorless prisms. mp 262—265.5°/273.5—275°. $[\alpha]_D^{20}$ −24.0° (c=0.10, EtOH). *Anal.* Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.39; H, 9.96.

3β,15α-Dihydroxy-5-androsten-17-one (15α-Hydroxydehydroepiandrosterone) (1)——To a solution of **14** (35 mg) in acetone (6 ml) was added 5 N HCl (3 ml) and allowed to stand at room temperature for 7 hr. The resulting solution was neutralized with 5% $NaHCO_3$ and concentrated under reduced pressure. The residue 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 (1 : 3) as developing solvent. Recrystallization of the eluate from aq. acetone gave **1** (22 mg) as colorless needles. mp 219.5—221°. $[\alpha]_D^{22}$ +29.8° (c=0.11). NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.07 (3H, s, 19-CH_3), 3.01 (1H, dd, J=8, 18 Hz, 16ζ-H), 3.55 (1H, m, 3α-H), 4.36 (1H, sx, J=9, 9, 5 Hz), 5.40 (1H, m, 6-H). *Anal.* Calcd. for $C_{19}H_{28}O_3 \cdot 1/4H_2O$: C, 73.87; H, 9.30. Found: C, 73.75; H, 9.45.

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