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Studies on Estetrol. I. Synthesis of 15α,16α,17β-Trihydroxyandrost-4-en-3-one[1]

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In order to determine the precursor role of neutral steroids in the formation of estetrol in man the titled compound was prepared as a substrate by two different routes. First, 17,17-ethylenedioxy-5α-androst-15-ene-3β,5-diol (I), readily available by the known method, was employed as a starting material. Transformation into the 15α,16α-glycol structure was attained by osmium tetroxide oxidation toward the Δ^{15} double bond. Dehydration with thionyl chloride provided a mixture of the Δ^4- and Δ^5-3β,15α,16α,17β-tetraols (V), which was in turn led to the 15, 16-acetonide (VI). Oppenauer oxidation followed by removal of the dioxolane group afforded the Δ^4-3-ketosteroid (VIIIa). Alternatively, 3β,5β-dihydroxyandrost-15-en-17-one ethylene ketal (XIV), derivable from dehydroepiandrosterone in several steps, was similarly converted into androst-4-ene-3β,15α,16α,17β-tetraol (XXa). Selective oxidation of the hydroxylic group at C-3 with N-bromoacetamide provided the desired compound (VIIIa). The latter synthetic route has proved to be more favorable in respect to overall yield.

A new polar estrogen isolated from the urine of the human newborn was tentatively assigned the structure of estetrol (estra-1,3,5(10)-triene-3,15α,16α,17β-tetraol) on the basis of its chromatographic behaviors.[3] The definite evidence for the structure of this metabolite was presented from the physico-chemical data[4] and by direct comparison with the synthetic sample.[5] Recently considerable attentions have been directed to the physiological role of estetrol in the human feto-placental unit.[6] Solomon and his co-worker demonstrated that both 16α- and 15α-hydroxyandrost-4-ene-3,17-diones would serve as a precursor in the formation of estetrol, although the relative contribution of each was not known.[7] However, whether the neutral C_{19} steroid having the 15α,16α,17β-triol structure can be aromatized or not still remains unsolved, because the titled compound is hitherto unavailable as a substrate. The present paper deals with two synthetic routes leading to the desired compound from readily available dehydroepiandrosterone.

An initial project was focused to development of a method starting from 17,17-ethylenedioxy-5α-androst-15-ene-3β,5-diol (I), obtainable in five steps from dehydroepiandrosterone.[8] Removal of the protecting group at C-17 was effected by treatment with *p*-toluenesulfonic

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[1] This paper constitutes Part LXV of the series entitled "Analytical Chemical Studies on Steroids"; Part LXIV: T. Nambara, T. Anjyo, M. Ito, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 1938 (1973).

[2] Location: *Aobayama, Sendai.*

[3] A.A. Hagen, M. Barr, and E. Diczfalusy, *Acta Endocrinol.*, **49**, 207 (1965).

[4] G. Zucconi, B.P. Lisboa, E. Simonitsch, L. Roth, A.A. Hagen, and E. Diczfalusy, *Acta Endocrinol.*, **56**, 413 (1967).

[5] J. Fishman and H. Guzik, *Tetrahedron Letters*, **30**, 2929 (1967); *idem*, *J. Org. Chem.*, **33**, 3133 (1968).

[6] J. Heikkilä and H. Adlercreutz, *J. Setroid Biochem.*, **1**, 243 (1970); J. Heikkilä, *ibid.*, **2**, 83 (1971); J. Heikkilä and T. Luukkainen, *Am. J. Obst. Gyn.*, **110**, 509 (1971); M.E. Giebenhain, G.E. Tagatz, and E. Gurpide, *J. Steroid Biochem.*, **3**, 707 (1972).

[7] E.V. YoungLai and S. Solomon, *J. Clin. Endocrinol.*, **28**, 1611 (1968).

[8] R. Emiliozzi, R. Condom, M. Audinot, and L. Pichat, *Bull. Soc. Chim. France*, **1971**, 131.

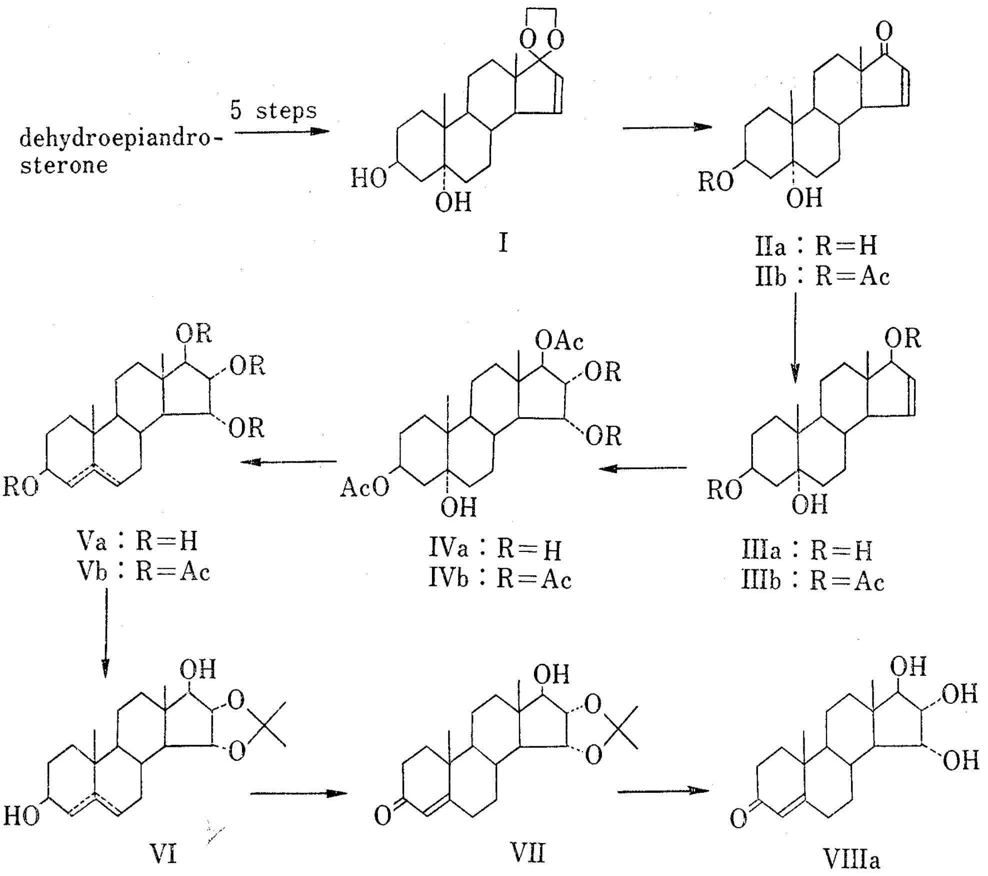

Chart 1

acid yielding the Δ^{15}-17-ketone (IIa). The circular dichroism curve of the 3-monoacetate (IIb) exhibited a negative Cotton effect indicating the lack of epimerization at C-14 during the process of deketalization.[9] Reduction of IIb with lithium aluminum hydride afforded solely the 17β-hydroxylic derivative (IIIa) without affecting any disturbance on the conjugated double bond. Subsequent treatment of the 3,17-diacetate (IIIb) with osmium tetroxide provided the 3β,5α,15α,16α,17β-pentaol 3,17-diacetate (IVa) in a reasonable yield. Configuration of the 15,16-*cis*-glycol was deduced from the result of the analogous reaction with the aromatic steroid having the Δ^{15}-17β-ol structure.[5] In addition it is sufficiently substantiated that the attack of the reagent toward the Δ^{15} double bond takes place from the less-hindered α side of the steroid molecule. Dehydration of the 5α-hydroxylic group was effected by brief treatment with thionyl chloride and pyridine. However, the reaction proceeded to both directions resulting in formation of the Δ^4 and Δ^5 compounds (Vb), whose separation could not be attained. Accordingly the mixture was submitted to further elaboration without purification. In order to utilize the steric hindrance for selective oxidation of the 3β-hydroxylic function the 3β,15α,16α,17β-tetraol (Va) was transformed into the 15,16-acetonide (VI) by treatment with acetone and phosphomolybdic acid.[10] As might be expected Oppenauer oxidation with cyclohexanone and aluminum isopropoxide occurred selectively at C-3 to furnish the Δ^4-3-ketone (VII) as a single product. Being refluxed with acid in ethanol, the 15,16-acetonide could be cleaved easily yielding the desired 15α,16α,17β-trihydroxyandrost-4-ene-3,17-dione (VIIIa).

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9) F. Sondheimer, S. Burstein, and P. Mechoulam, *J. Am. Chem. Soc.*, **82**, 3209 (1960); P. Crabbé, A. Cruz, and J. Iriarte, *Can. J. Chem.*, **46**, 349 (1968).

10) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **15**, 460 (1967).

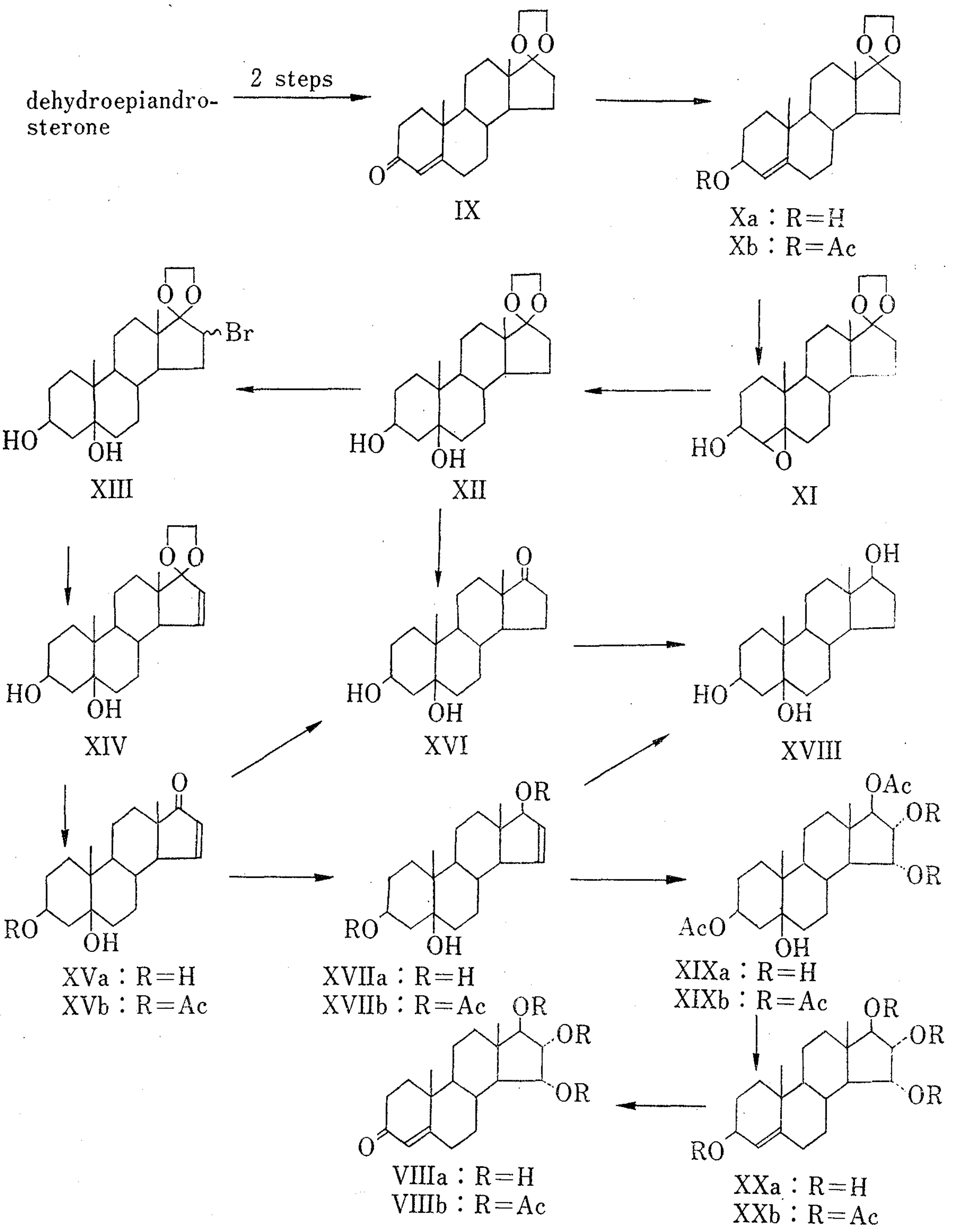

Chart 2

This synthetic route, however, proved to be unfavorable in respect of the overall yield. Therefore the next project was directed to the development of an alternative way. In order to secure the selective dehydration toward C-4 an initial effort was made on a model experiment for preparation of the $3\beta,5\beta$-dihydroxyandrostane derivative employing the method of Henbest, *et al.*[11] Treatment of androst-4-ene-$3\beta,17\beta$-diol with *m*-chloroperbenzoic acid gave the $4\beta,5\beta$-epoxide accompanied with a small amount of its epimer. The opening reaction of the oxido ring with metal hydride occurred at the expected site to furnish 5β-androstane-$3\beta,5,17\beta$-triol (XVIII), whose structure was confirmed by leading to the known 5β-hydroxy-3,17-diketone by chromium trioxide oxidation. It is to be noted that the reaction of the Δ^4-3β-ol with per acid yields the β-epoxide principally as the result of the preferential attack from the front side.

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[11] H.B. Henbest and R.A.L. Wilson, *J. Chem. Soc.*, **1957**, 1958.

On the basis of these results synthesis of the desired compound was then undertaken. First, androst-4-ene-3,17-dione ethylene ketal (IX), derivable from dehydroepiandrosterone in two steps, was reduced with lithium aluminum tri-*tert*-butoxy hydride to give the 3β-hydroxylic derivative (Xa) accompanied with a trace amount of its C-3 epimer. Treatment of Xa with *m*-chloroperbenzoic acid afforded the 4β,5β-epoxide (XI) in 60% yield. The reductive cleavage of the oxido ring with metal hydride proceeded to the expected direction yielding the 3β,5β-diol (XII) almost quantitatively. Subsequent bromination with phenyltrimethylammonium perbromide[12] in tetrahydrofuran occurred at C-16 with ease to furnish the 16ζ-bromo derivative (XIII). When heated with 1,5-diazabicyclo[5.4.0]undecene-5[8,13] in a sealed tube for a prolonged period, XIII underwent dehydrobromination to form the 15-dehydro compound (XIV) in 80% yield. Elimination of the protecting group at C-17 was attained by treatment with *p*-toluenesulfonic acid to give 3β,5β-dihydroxyandrost-15-en-17-one (XVa). The stereochemistry at C-14 was verified by leading to the saturated compound (XVI), which proved to be identical with the deketalization product derived from XII. Reduction of the Δ^{15}-17-ketone with metal hydride yielded solely the 17β-hydroxylic derivative (XVIIa) with retention of the Δ^{15} double bond. The structural assignment was rationalized by transforming into the known 3β,5β,17β-triol (XVIII) upon catalytic hydrogenation over palladium-on-charcoal. Oxidation of the 3,17-diacetate (XVIIb) with osmium tetroxide provided the 15α,16α-*cis*-glycol (XIXa) in a reasonable yield. Dehydration of the pentaol tetraacetate (XIXb) was effected by brief treatment with thionyl chloride and pyridine to yield the unsaturated compound (XXb) as a single product. On the nuclear magnetic resonance (NMR) spectrum XXb exhibited an olefinic proton signal at 5.21 ppm as a singlet indicating the preferential formation of the Δ^4 double bond. Several attempts for the selective hydrolysis of the 3-acetate under the mild conditions resulted in failure. Hence XXb was completely hydrolyzed with methanolic potassium hydroxide into the 3β,15α,16α,17β-tetraol (XXa). In the next step difficulties were encountered with the allylic oxidation with manganese dioxide, since considerable amounts of the by-products were formed. Selective oxidation of the 3-hydroxylic group was accomplished by the use of N-bromoacetamide[14] to afford the desired compound (VIIIa) in 70% yield.

The identity of the final products obtained by two different routes was justified by the usual criteria. The latter synthetic method has proved to be of more advantage for facile arrival at the goal. It is hoped that the availability of 15α,16α,17β-trihydroxyandrost-4-en-3-one as a substrate will provide the more precise knowledge on the biosynthesis of estetrol in the feto-placental unit.

Experimental[15]

3β,5α-Dihydroxyandrost-15-en-17-one (IIa)——To a solution of 17,17-ethylenedioxy-5α-androst-15-ene-3β,5-diol (I)[8] (1.3 g) in 85% aq. acetone (100 ml) was added *p*-TsOH·H_2O (80 mg) and stirred at room temperature for 1 hr. The resulting solution was extracted with AcOEt. The organic layer was separated,

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[12] L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley and Sons, Inc., New York, 1967, p. 855.

[13] M. Fieser and L.F. Fieser, "Reagents for Organic Synthesis," Vol. 2, Wiley-Interscience, New York, 1969, p. 101.

[14] K. Morita, *Bull. Chem. Soc.* (Japan), 32, 227 (1959).

[15] All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Ultraviolet (UV) and infrared (IR) spectra were run on Hitachi Model EPS-3 and JASCO Model IR-S spectrophotometers, respectively. Nuclear magnetic resonance (NMR) spectra were recorded on Hitachi Model R-20A spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet. For thin-layer chromatography (TLC) silica gel H (E. Merck AG, Darmstadt) was used as an adsorbent.

washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from acetone–hexane to give IIa (1.1 g) as colorless needles. mp 253.5—254.5°. $[\alpha]_D^{20}$ −31.6° (c=0.03, MeOH). *Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.06; H, 9.49.

3β,5α-Dihydroxyandrost-15-en-17-one 3-Acetate (IIb)——Treatment of IIa with Ac_2O and pyridine in the usual manner followed by recrystallization from acetone–hexane gave IIb as colorless needles. mp 209—210°. $[\alpha]_D^{20}$ −215.3° (c=0.04). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.88; H, 8.70. NMR (5% solution in $CDCl_3$) δ: 1.08 (6H, s, 18- and 19-CH_3), 2.01 (3H, s, 3β-$OCOCH_3$), 5.20 (1H, m, 3α-H), 6.00 (1H, q, J=3.6 Hz, 16-H), 7.50 (1H, q, J=1.5, 6 Hz, 15-H). CD (c=0.17, dioxane) $[\theta]^{28}$ (nm): 0 (400), −1452 (360), −1386 (357), 0 (300).

5α-Androst-15-ene-3β,5,17β-triol (IIIa)——To an ice-cooled solution of IIb (2 g) in ether (150 ml)-tetrahydrofuran (THF) (50 ml) was added $LiAlH_4$ (400 mg) portionwise and stirred at 0° for 1 hr. The excess reagent was then decomposed by addition of moist AcOEt. The organic layer was separated, washed with 20% Rochelle salt solution and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from acetone–hexane to give IIIa (1.5 g) as colorless needles. mp 197—198°. $[\alpha]_D^{18}$ −25.9° (c=0.11, MeOH). *Anal.* Calcd. for $C_{19}H_{30}O_3 \cdot 3/4H_2O$: C, 71.32; H, 9.92. Found: C, 71.48; H, 9.92.

5α-Androst-15-ene-3β,5,17β-triol 3,17-Diacetate (IIIb)——Treatment of IIIa with Ac_2O and pyridine in the usual manner followed by recrystallization from acetone–hexane gave IIIb as colorless needles. mp 163—164°. $[\alpha]_D^{18}$ +18.8° (c=0.16), *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.81; H, 9.04. NMR (5% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 2.01 (3H, s, –$OCOCH_3$), 2.08 (3H, s, –$OCOCH_3$), 5.05 (1H, m, 3α-H), 5.28 (1H, d, J=2 Hz, 17α-H), 5.62 (1H, q, J=2, 6 Hz, 16-H), 5.96 (1H, d, J=6 Hz, 15-H).

5α-Androstane-3β,5,15α,16α,17β-pentaol 3,15,16,17-Tetraacetate (IVb)——To a solution of IIIb (450 mg) in benzene (15 ml)–pyridine (2 ml) was added OsO_4 (500 mg) and stirred at room temperature for 45 hr. Then a solution of Na_2SO_3 (5 g) and $KHCO_3$ (3 g) in MeOH (50 ml)–H_2O (20 ml) was added and stirred at room temperature for 4 hr. The resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave an oily residue, which in turn was treated with Ac_2O (5 ml) and pyridine (2.5 ml). After usual workup the crude product was recrystallized from acetone–hexane to give IVb (150 mg) as colorless needles. mp 260—262°. $[\alpha]_D^{20}$ +40.7° (c=0.07). *Anal.* Calcd. for $C_{27}H_{40}O_9$: C, 63.76; H, 7.93. Found: C, 63.31; H, 8.10. NMR (5% solution in $CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.00 (12H, s, 3β-, 15α-, 16α-, and 17β –$OCOCH_3$), 5.10 (4H, m, 3α-, 15β-, 16β-, and 17α-H).

15α,16α,17β-Trihydroxyandrost-4-en-3-one 15,16-Acetonide (VII)——To a solution of IVb (70 mg) in pyridine (1.5 ml) was added $SOCl_2$ (0.5 ml) at 0° and allowed to stand at room temperature for 5 min. After careful addition of H_2O to decompose the excess reagent the resulting solution was extracted with AcOEt. The organic layer was separated, washed with 5% $NaHCO_3$, 5% HCl and H_2O, successively, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave androst-4- and -5-ene-3β,15α,16α,17β-tetraol tetraacetate (Vb) (60 mg) as colorless oil. NMR (3% solution in $CDCl_3$) δ: 0.91 (3H, s, 18-CH_3), 1.04 (1.8H, s, 19-CH_3), 1.08 (1.2H, s, 19-CH_3), 2.02, 2.06 (12H, s, 3β-, 15α-, 16α-, and 17β-$OCOCH_3$), 4.80—5.10, 5.18—5.46 (5H, m, 3α-, 4-, 6-, 15β-, 16β-, and 17α-H). Then Vb was dissolved in 50% aq. MeOH (20 ml) containing KOH (1 g) and stirred at 45° overnight. The resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. On usual work-up androst-4- and -5-ene-3β,15α,16α,17β-tetraol (Va) (35 mg) was obtained as colorless oil. To a solution of Va in acetone (10 ml) was added 5% phosphomolybdic acid solution in aq. acetone (0.5 ml) and allowed to stand at room temperature for 30 min. After addition of 30% NH_4OH (1 ml) the resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave androst-4- and -5-ene-3β,15α,16α,17β-tetraol 15,16-acetonide (VI) (35 mg) as colorless oil. To a solution of VI in toluene (10 ml)–cyclohexanone (0.5 ml) was added $Al(iso\text{-}PrO)_3$ (20 mg) and refluxed for 18 hr. The resulting solution was diluted with AcOEt and washed with 20% Rochelle salt solution and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC using hexane–AcOEt (1:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.45) was eluted with AcOEt and the eluate was recrystallized from acetone to give VII (25 mg) as colorless needles. mp 180—182°. $[\alpha]_D^{18}$ −23.8° (c=0.04). *Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.00; H, 8.83. NMR (5% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 1.19 (3H, s, 19-CH_3), 1.28 (3H, s, $\rangle C(CH_3)_2$), 1.48 (3H, s, $\rangle C(CH_3)_2$), 3.65 (1H, d, J=5 Hz, 17α-H), 4.45 (2H, m, 15β- and 16β-H), 5.72 (1H, s, 4-H).

17,17-Ethylenedioxyandrost-4-en-3β-ol (Xa)——To a stirred solution of $LiAlH_4$ (6 g) in anhydrous ether (350 ml) was added dropwise an ethereal solution of *tert*-BuOH (53 ml in 30 ml) under ice-cooling. To this solution was added a solution of 17,17-ethylenedioxyandrost-4-en-3-one (IX) (6 g) in anhydrous ether (200 ml) and stirred at room temperature for 1 hr. After addition of moist AcOEt to decompose the excess reagent the organic layer was separated, washed with 20% Rochelle salt solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product obtained from MeOH gave Xa (4.53 g) as colorless plates. mp 205—207°. $[\alpha]_D^{18}$ +37.3° (c=0.12). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70.

Found: C, 76.14; H, 9.71. Julia, *et al.* prepared this compound by the different method (reported: mp 205—206°).[16]

17,17-Ethylenedioxyandrost-4-en-3β-ol Acetate (Xb)——Treatment of Xa with Ac_2O and pyridine in the usual manner followed by recrystallization from acetone–hexane gave Xb as colorless plates. mp 172—174°. $[\alpha]_D^{20}$ +58.8° (c=0.09). *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.81; H, 9.15. Loken, *et al.* prepared this compound by the different method (reported: mp 173—176°).[17]

4β,5β-Epoxy-17,17-ethylenedioxyandrostan-3β-ol (XI)——To a solution of Xa (1 g) in benzene (50 ml) was added *m*-chloroperbenzoic acid (600 mg) and stirred at room temperature for 3 hr. The resulting solution was diluted with AcOEt, washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product obtained from acetone–hexane gave XI (625 mg) as colorless plates. mp 150—152°. $[\alpha]_D^{19}$ −10.5° (c=0.10). *Anal.* Calcd. for $C_{21}H_{32}O_4$; C, 72.38: H, 9.26. Found: C, 72.47; H, 9.34. Camerino, *et al.* prepared this compound by the different method (reported :mp 154—155°).[18]

17,17-Ethylenedioxy-5β-androstane-3β,5-diol (XII)——To a solution of XI (11 g) in anhydrous THF (250 ml) was added $LiAlH_4$ (25 g) portionwise and refluxed for 1 hr. The excess reagent was decomposed by careful addition of moist AcOEt. The organic layer was separated, washed with 20% Rochelle salt solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was submitted to chromatography on Al_2O_3 (150 g). Elution with benzene and recrystallization of the eluate from MeOH gave XII (10.4 g) as colorless plates. mp 212—213°. $[\alpha]_D^{14}$ −22.0° (c=0.09). *Anal.* Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.82; H, 9.79. NMR (5% solution in $CDCl_3$) δ: 0.81 (3H, s, 18- or 19-CH_3), 0.91 (3H, s, 19- or 18-CH_3), 3.84 (4H, s, -OCH_2CH_2O–), 4.00—4.20 (1H, m, 3α-H).

16ζ-Bromo-17,17-ethylenedioxy-5β-androstane-3β,5-diol (XIII)——To a solution of XII (9.3 g) in anhydrous THF (190 ml) was added phenyltrimethylammonium perbromide[12] (9.7 g) under ice-cooling and allowed to stand at 4° overnight. The resulting solution was diluted with AcOEt, washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and H_2O, successively, and dried over anhydrous Na_2SO_4. After evaporation of solvent the residue obtained was recrystallized from acetone to give XIII (6.74 g) as colorless needles. mp 224—226°. $[\alpha]_D^{16}$ +24.4° (c=0.12). *Anal.* Calcd. for $C_{21}H_{33}O_4Br$: C, 58.73; H, 7.80; Br, 18.61. Found: C, 58.89; H, 7.84; Br, 18.52. NMR (5% solution in $CDCl_3$) δ: 0.86 (3H, s, 18- or 19-CH_3), 0.92 (3H, s, 19- or 18-CH_3), 3.72—4.28 (5H, m, 3α-H and –OCH_2CH_2O–), 4.29—4.65 (1H, m, 16-H).

17,17-Ethylenedioxy-5β-androst-15-ene-3β,5-diol (XIV)——A solution of XIII (6.7 g) in 1,5-diazabicyclo[5.4.0]undecene-5[13] (15 ml) was heated at 140° in a sealed tube for a week. After addition of the reagent (5 ml) the reaction was further carried out for 2 days. The reaction mixture was extracted with $CHCl_3$ and the extract was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was chromatographed on Al_2O_3 (80 g). Elution with benzene–ether (5: 1 to 1: 1) and recrystallization of the eluate from acetone gave XIV (4.4 g) as colorless plates. mp 191—192°. $[\alpha]_D^{17}$ −53.1° (c=0.08). *Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.28; H, 9.33. NMR (5% solution in $CDCl_3$) δ: 0.90 (3H, s, 18- or 19-CH_3), 0.97 (3H, s, 19- or 18-CH_3), 3.91 (4H, d, J=1.8 Hz, –OCH_2CH_2O–), 4.00—4.20 (1H, m, 3α-H), 5.69 (1H, q, J=3, 6 Hz, 15-H), 6.13 (1H, q, J=1.8, 6 Hz, 16-H).

3β,5β-Dihydroxyandrost-15-en-17-one (XVa)——To a solution of XIV (2 g) in acetone (100 ml) was added aq. solution of *p*-TsOH·H_2O (100 mg in 17 ml) and stirred at room temperature for 1.5 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 crude product from acetone–hexane gave XVa (1.65 g) as colorless plates. mp 201.5—203°. $[\alpha]_D^{15}$ −57.0° (c=0.03). *Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.85; H, 9.22. NMR (5% solution in $CDCl_3$) δ: 1.03 (3H, s, 18- or 19-CH_3), 1.07 (3H, s, 19- or 18-CH_3), 4.05—4.30 (1H, m, 3α-H), 6.01 (1H, q, J=3, 6 Hz, 15-H), 7.50 (1H, q, J=1.8, 6 Hz, 16-H). IR ν_{max}^{KBr} cm^{-1}: 1715, 1630. UV λ_{max}^{EtOH} nm (ε): 237 (8300).

3β,5β-Dihydroxyandrost-15-en-17-one 3-Acetate (XVb)——Treatment of XVa with Ac_2O and pyridine in the usual manner followed by recrystallization from acetone–hexane gave XVb as colorless needles. mp 167.5—169°. $[\alpha]_D^{16}$ +8.8° (c=0.11). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 73.08; H, 8.67. NMR (5% solution in $CDCl_3$) δ: 1.04 (3H, s, 18- or 19-CH_3), 1.06 (3H, s, 19- or 18-CH_3), 2.08 (3H, s, 3β-$OCOCH_3$), 5.10—5.32 (1H, m, 3α-H), 6.01 (1H, q, J=3, 6 Hz, 15-H), 7.50 (1H, q, J=1.8, 6 Hz, 16-H).

3β,5β-Dihydroxyandrostan-17-one (XVI)——i) To a solution of XII (50 mg) in acetone (4 ml) was added an aq. solution of *p*-TsOH·H_2O (5 mg in 1.25 ml) and stirred at room temperature for 1 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 from acetone–hexane gave XVI (40 mg) as colorless plates. mp 201—203°. $[\alpha]_D^{18}$ −48.4° (c=0.03). *Anal.* Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.56; H, 10.08. NMR (5% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 4.05—4.25 (1H, m, 3α-H).

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16) S. Julia and J.P. Lavaux, *Bull. Soc. Chim. France*, **1963**, 1223.
17) B. Loken and M. Gut, *Steroids*, **1**, 39 (1965).
18) B. Camerino, R. De Castiglione, and G. Bosisio, *Farmaco Ed. Sci.*, **19**, 312 (1964).

ii) A solution of XVa (20 mg) in EtOH (5 ml) was shaken with 10% Pd/C (10 mg) under a stream of H_2 gas at room temperature for 1 hr. After removal of the catalyst by filtration the filtrate was evaporated. Recrystallization from acetone–hexane gave XVI (12 mg) as colorless plates. mp 202—204°. Mixed melting point on admixture with the sample obtained in i) showed no depression and IR spectra of two samples were entirely identical.

5β-Androst-15-ene-3β,5,17β-triol (XVIIa)——To a solution of XVb (200 mg) in anhydrous ether (55 ml) was added $LiAlH_4$ (50 mg) portionwise and stirred at 0° for 2 hr. The excess reagent was decomposed by careful addition of moist AcOEt. The organic layer was separated, washed with 20% Rochelle salt solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from acetone–hexane gave XVIIa (160 mg) as colorless plates. mp 206—207°. $[\alpha]_D^{16}$ −21.4° (c=0.09, MeOH). *Anal.* Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.27; H, 9.80.

5β-Androst-15-ene-3β,5,17β-triol 3,17-Diacetate (XVIIb)——Treatment of XVIIa with Ac_2O and pyridine in the usual manner followed by recrystallization from acetone–hexane gave XVIIb as colorless plates. mp 135—137°. $[\alpha]_D^{19}$ −38.3° (c=0.12). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.59; H, 9.09. NMR (5% solution in $CDCl_3$), δ: 0.82 (3H, s, 18- or 19-CH_3), 1.00 (3H, s, 19- or 18-CH_3), 2.08 (6H, s, 3β- and 17β-$OCOCH_3$), 5.12—5.36 (2H, m, 3α- and 17α-H), 5.62 (1H, m, 15- or 16-H), 5.95 (1H, m, 16- or 15-H).

5β-Androstane-3β,5,17β-triol (XVIII)——i) Treatment of XVI (10 mg) with $LiAlH_4$ in the manner as described in XVIIa followed by recrystallization from acetone–hexane gave XVIII (7 mg) as colorless needles. mp 196—198°. $[\alpha]_D^{19}$ +92.1° (c=0.04, MeOH). *Anal.* Calcd. for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 74.18; H, 10.65. NMR (2% solution in $CDCl_3$) δ: 0.73 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.60 (1H, t, J=6, 8 Hz, 17α-H), 4.05—4.25 (1H, m, 3α-H).

ii) A solution of XVIIa (10 mg) in EtOH (3 ml) was shaken with 10% Pd/C (10 mg) under a stream of H_2 gas at room temperature overnight. After usual work-up the crude product was recrystallized from acetone–hexane to give XVIII (6 mg) as colorless needles. mp 196—200.° Mixed melting point on admixture with the sample obtained in i) showed no depression and IR spectra of two samples were entirely identical.

iii) To a stirred solution of $LiAlH_4$ (400 mg) in anhydrous ether (25 ml) was added dropwise an ethereal solution of *tert*-BuOH (3.5 ml in 10 ml) under ice-cooling. To this solution was added a solution of testosterone (300 mg) in anhydrous ether (10 ml) and stirred at room temperature for 50 min. After addition of moist AcOEt to decompose the excess reagent, the organic layer was separated, washed with 20% Rochelle salt solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from aq. MeOH gave androst-4-ene-3β,17β-diol (290 mg) as colorless leaflets. mp 158—160°. Julia, *et al.* prepared this compound by the different method (reported: mp 156°).[19] To a solution of the 3β,17β-diol (250 mg) in MeOH (10 ml) was added *m*-chloroperbenzoic acid (140 mg) and allowed to stand at room temperature for 3 hr. The resulting solution was diluted with AcOEt, washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was submitted to preparative TLC using benzene–AcOEt (2:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.50) gave 4β,5β-epoxyandrostane-3β,17β-diol (226 mg) as colorless oil. To a solution of the epoxide (220 mg) in anhydrous THF was added $LiAlH_4$ (50 mg) and refluxed for 3 hr. After addition of moist AcOEt to decompose the excess reagent, the organic layer was separated, washed with 20% Rochelle salt solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from acetone–hexane gave XVIII (150 mg) as colorless needles. mp 196—198°. Mixed melting point on admixture with the sample obtained in i) showed no depression and IR spectra of two samples were entirely identical.

5β-Androstane-3β,5,15α,16α,17β-pentaol 3,15,16,17-Tetraacetate (XIXb)——To a solution of XVIIb (1.8 g) in benzene (60 ml)-pyridine (5 ml) was added dropwise a solution of OsO_4 (3.5 g) in benzene (7 ml) and stirred at room temperature for 22 hr. Then a solution of Na_2SO_3 (6 g) and $KHCO_3$ (6 g) in MeOH (200 ml)-H_2O (90 ml) was added and stirred for 2 hr. The resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave 5β-androstane-3β,5,15α,16α,17β-pentaol 3,17-diacetate (XIXa) (900 mg) as brownish yellow oil. The crude product was further treated with Ac_2O (7 ml) and pyridine (7 ml) overnight. After usual work-up the residue obtained was submitted to chromatography on Al_2O_3 (30 g). Elution with benzene gave XIXb (660 mg) as colorless oil. NMR (5% solution in $CDCl_3$) δ: 0.89 (3H, s, 18- or 19-CH_3), 0.98 (3H, s, 19- or 18-CH_3), 2.01, 2.05, 2.07 (12H, s, 3β-, 15α-, 16α-, and 17β-$OCOCH_3$), 4.82—5.07, 5.10—5.40 (4H, m, 3α-, 15β-, 16β-, and 17α-H).

Androst-4-ene-3β,15α,16α,17β-tetraol Tetraacetate (XXb)——To a solution of XIXb (40 mg) in pyridine (1 ml) was added $SOCl_2$ (0.1 ml) at 0° and stirred at room temperature for 5 min. After careful addition of H_2O the resulting solution was extracted with AcOEt. The organic layer was separated, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was recrystallized from acetone–hexane to give XXb (24 mg) as colorless needles. mp 163—164.5°. $[\alpha]_D^{14}$ −80.2° (c=0.10). *Anal.* Calcd. for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 65.87; H, 7.57. NMR (5% solution in $CDCl_3$) δ:

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[19] S. Julia and C. Moutonnier, *Bull. Soc. Chim. France*, **1964**, 321.

0.90 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.00, 2.03 (12H, s, 3β-, 15α-, 16α-, and 17β-$OCOCH_3$), 4.80—5.08, 5.15—5.40 (4H, m, 3α-, 15β-, 16β-, and 17α-H), 5.21 (1H, broad s, 4-H).

15α,16α,17β-Trihydroxyandrost-4-en-3-one (VIIIa)——i) A solution of XXb (85 mg) in 5% methanolic KOH (10 ml) was stirred at 35° overnight. The resulting solution was extracted with AcOEt–*n*-BuOH (1: 1). The organic layer was separated, washed with H_2O, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave androst-4-ene-3β,15α,16α,17β-tetraol (XXa) as colorless amorphous substance. Re-acetylation with Ac_2O and pyridine in the usual manner gave XXb. To a solution of XXa in pyridine–benzene (2: 1) (2.5 ml) was added N-bromoacetamide (30 mg) and allowed to stand at room temperature for 1.5 hr. The resulting solution was extracted with AcOEt–*n*-BuOH (1: 1). The organic layer was washed with 10% $NaHSO_3$, 10% NaOH, 5% H_2SO_4 and H_2O, successively, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from MeOH gave VIIIa (45 mg) as colorless needles. mp 253—255°. $[\alpha]_D^{17}$ +45.7° (c=0.04, MeOH). *Anal.* Calcd. for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 71.01; H, 8.83. NMR (3% solution in CD_3OD) δ: 0.82 (3H, s, 18-CH_3), 1.26 (3H, s, 19-CH_3), 3.70—4.10 (3H, m, 15β-, 16β- and 17α-H), 5.73 (1H, s, 4-H). IR ν_{max}^{KBr} cm^{-1}: 1657 (C=O), 1619 (C=C).

ii) To a solution of VII (15 mg) in EtOH (1.5 ml) was added 10% H_3PO_4 (0.5 ml) and refluxed for 30 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 from AcOEt–hexane gave VIIIa (5 mg) as colorless amorphous substance. mp 242—245°. Mixed melting point on admixture with the sample obtained in i) showed no depression. The thin-layer chromatographic behaviors of two samples were entirely identical.

15α,16α,17β-Trihydroxyandrost-4-en-3-one Triacetate (VIIIb)——Treatment of VIIIa with Ac_2O and pyridine in the usual manner followed by recrystallization from acetone–hexane gave VIIIb as colorless needles. mp 221—222°. $[\alpha]_D^{15}$ −48.4° (c=0.02). *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 66.96; H, 7.69. NMR (5% solution in $CDCl_3$) δ: 0.93 (3H, s, 18-CH_3), 1.19 (3H, s, 19-CH_3), 2.01, 2.04 (9H, s, 15α-, 16α-, and 17β-$OCOCH_3$), 4.80—5.10, 5.20—5.40 (3H, m, 15β-, 16β-, and 17α-H), 5.71 (1H, s, 4-H).

5β-Hydroxyandrostane-3,17-dione——XVIII (15 mg) was treated with CrO_3–pyridine complex (100 mg in 1 ml) at room temperature overnight. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was separated, washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, successively, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from acetone–hexane gave 5β-hydroxyandrostane-3,17-dione (10 mg) as colorless plates. mp 182—185°. Mixed melting point on admixture with the authentic sample[20] showed no depression and IR spectra of two samples were entirely identical.

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20) B. Camerino and D. Cattapan, *Farmaco Ed. Sci.*, 13, 39 (1958).