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Studies on Metabolism of 3-Deoxysteroids. IV.[1] Synthesis of 2,17-Oxygenated 5-Dehydro- and 5β-Androstanes[2]

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For the studies on metabolic fate of 3-deoxydehydroepiandrosterone in man, 5-dehydro- and 5β-androstanes having oxygen functions at both C-2 and C-17, the possible metabolites, have been synthesized as shown in Chart 1.

As a series of studies on metabolism of the modified steroids we have previously reported the metabolic fate of 3-deoxyestrone in rabbit.[4] A particular interest in the biotransformation of 3-deoxysteroids prompted us further to extend the investigation to C_{19} steroids. Among the possible metabolites derived from 3-deoxydehydroepiandrosterone 5-dehydro- and 5β-androstanes having oxygen function at both C-2 and C-17 have not hitherto been reported in the literatures. The present paper describes the synthesis of these 2,17-oxygenated androstanes for the purpose of direct comparison with the metabolites.

The initial project was directed to the preparation of androst-5-ene-2,17-diol starting from dehydroepiandrosterone tosylate (I). First, I was transformed into the 5α,6β-dichloro derivative (II) by treatment with sulfuryl chloride in order to protect the Δ^5-double bond.[5] When heated in dimethylsulfoxide under mild conditions,[6] II was readily converted into the Δ^2-unsaturated compound (III). Subsequent treatment with N-bromosuccinimide in acidic media[7] resulted in formation of two isomeric bromohydrins (IVa,b) together with the 2,3-dibromide (IVc) as by-product. Structural elucidation of the bromohydrins was attained by leading to the corresponding unsaturated ketones, respectively. As for one of these Jones oxidation[8] gave the α-bromoketone (VI), which exhibited an infrared absorption at 1748 cm^{-1} indicating the equatorial nature of C-bromine bond. Reductive dehalogenation of VI was accompanied with migration of the resulting double bond to give androst-4-ene-3,17-dione (VII). These results unequivocally permitted to assign the 2-bromo-3-ol structure to IVb.[9] With another bromohydrin (IVa) chromium trioxide oxidation followed by dehalogenation was performed in a similar fashion. Thus the desired androst-5-ene-2,17-dione (IX) was afforded through the 3β-bromo-2-ketone (VIII) in satisfactory yield. Hereby formation of the non-conjugated ketone system justified the putting a newly introduced oxygen function at C-2 in IVa. It is sufficiently substantiated that in the reaction of hypobromous acid with the 5α-steroidal Δ^2-olefin the initial attack of electrophilic bromine on the more exposed rear-side

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[1] The title "Studies on Metabolism of 3-Desoxyestrone" is converted to this title hereafter; Part III: T. Nambara, M. Numazawa, and H. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1725 (1969).

[2] This paper constitutes Part XXXVI of the series entitled "Analytical Chemical Studies on Steroids"; Part XXXV: T. Nambara and S. Honma, *Chem. Pharm. Bull.* (Tokyo), **18**, 1191 (1970).

[3] Location: *Aobayama, Sendai.*

[4] T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1200 (1969).

[5] T. Aoki, H. Yamamura, K. Takei, and H. Mori, *Chem. Pharm. Bull.* (Tokyo), **12**, 808 (1964).

[6] H.R. Nace, *J. Am. Chem. Soc.*, **81**, 5428 (1959); D.N. Jones and M.A. Saeed, *J. Chem. Soc.*, **1963**, 4657.

[7] G.H. Alt and D.H.R. Barton, *J. Chem. Soc.*, **1954**, 4284; T. Nakano, M. Hasegawa, and C. Djerassi, *Chem. Pharm. Bull.* (Tokyo), **11**, 465 (1963).

[8] K. Bowden, I.M. Heilbron, E.R.H. Jones, and B.C.L. Weedon, *J. Chem. Soc.*, **1946**, 39.

[9] Configurational assignment was tentatively deduced from the generalization that the *trans* diaxially substituted product is predominantly produced, when addition reaction involving a halonium ion is operative.

and subsequent *trans*-diaxial ring opening of the intermediate result in formation of the 3α-bromo-2β-ol as single product. In the present case it seemed therefore very likely that the β-face approach of the reagent would be favored to a certain extent by the presence of bulky halogen at C-5α and in consequence the isomeric bromohydrin (IVb) would also be produced. Upon Clemmensen reduction with zinc dust in acetic acid IVc was led to a halogen-free product formulated as androsta-3,5-dien-17-one (V). Reduction of IX with potassium borohydride gave two C-2 epimeric 2,17β-diols in a ratio of *ca.* 3 to 1, whose separation was efficiently achieved by means of alumina chromatography. Configurational assignment was deduced from the chemical shift of angular methyl group in the nuclear magnetic resonance (NMR) spectra. The C-19 proton signal of Xa appeared at the lower field than that of Xb due to 1,3-diaxial interaction. Chromatographic behaviors of two epimers also supported these assignments.

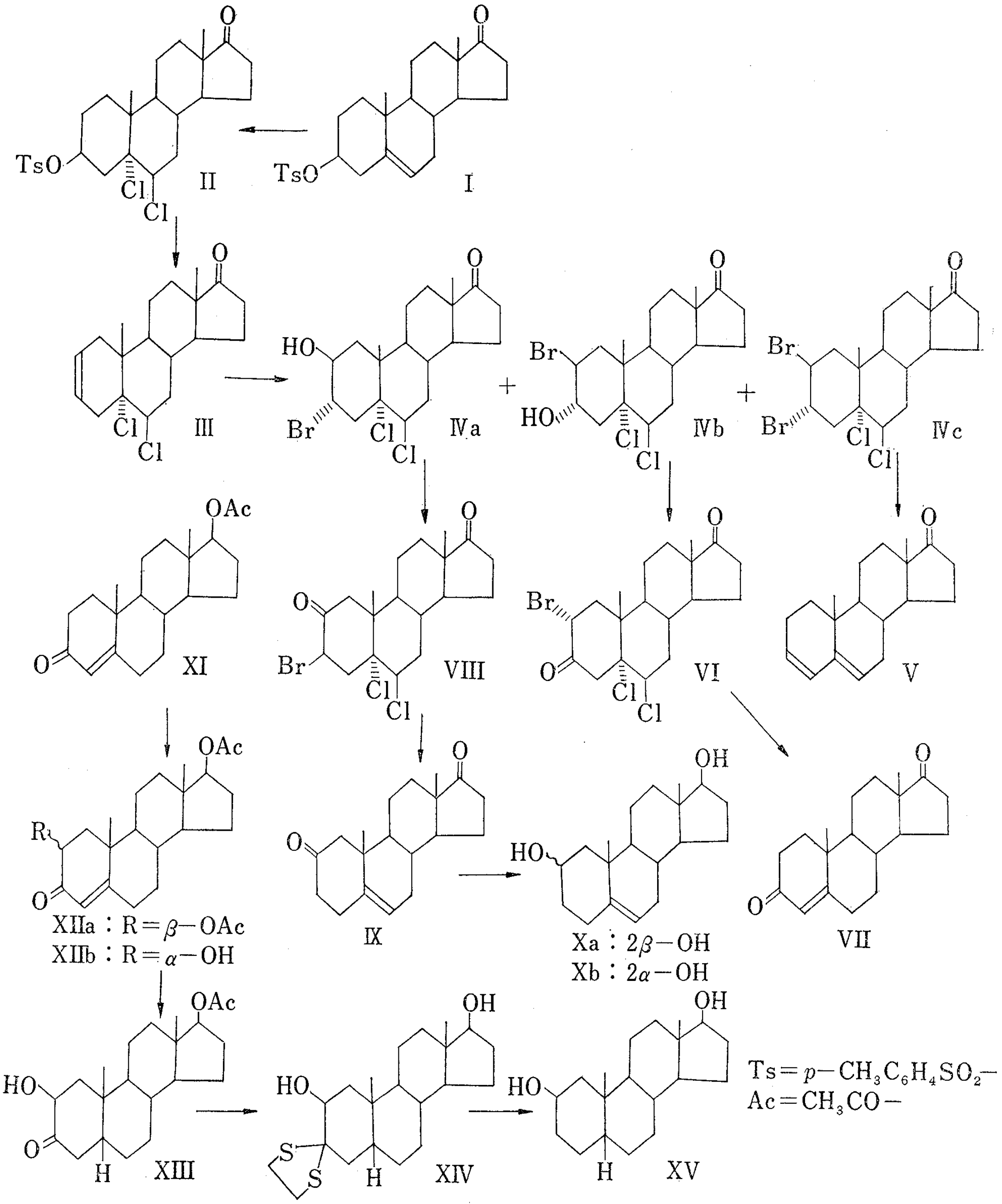

Chart 1

Next project was focused to the preparation of 5β-androstane-2,17β-diol employing the 2β-acetoxyl derivative (XIIa), derivable from testosterone acetate (XI),[10] as a starting compound. Upon treatment with potassium bicarbonate under mild conditions XIIa underwent partial hydrolysis and epimerization at C-2 to give the 2α-hydroxylic compound (XIIb). In the NMR spectra C-2 proton resonated at 4.15 ppm as a quartet (J=6, 14 cps) supporting the α-configuration of C-2 hydroxyl group. Catalytic hydrogenation of XIIb over palladium–on–calcium carbonate in N-methylpyrrolidone proceeded with success to furnish solely the saturated steroid (XIII). Stereochemistry at C-5 was rationalized by inspection of optical rotatory dispersion curve exhibiting the negative Cotton effect.[11] It is of interest that stereospecific hydrogenation did take place from the β-side of the molecule probably due to the presence of a hydroxyl group at C-2. In addition the splitting pattern (J_{AX}=6 cps, J_{BX}=14 cps) of the proton attached to the carbon bearing free hydroxyl group justified to assign the β-configuration to the oxygen function at C-2. It is to be noted that saturation of the Δ^4-double bond was accompanied with spontaneous epimerization at C-2 yielding the thermodynamically more stable 2β-hydroxylic derivative (XIII). Removal of the oxo group was then carried out by reductive means as usual. The 3-oxo-2β,17β-diol monoacetate was transformed into the corresponding thioketal by the boron trifluoride procedure of Fieser[12] and then to the 2,17-dihydroxylic compound (XIV) by alkaline hydrolysis. Raney nickel desulfurization proceeded without any evident hydrogenolysis at C-2 to give the desired 5β-androstane-2β,17β-diol (XV) in satisfactory yield.

It is hoped that the authentic specimens thus obtained may be helpful for characterization of the metabolites derived from 3-deoxydehydroepiandrosterone.

Experimental[13]

3β-Hydroxy-5α,6β-dichloroandrostan-17-one p-Toluenesulfonate (II)——To a solution of dehydroepiandrosterone p-toluenesulfonate (I) (1 g) in pyridine (6 ml) was added SO_2Cl_2 (0.2 ml) dropwise under ice–cooling and stirred at room temperature for 5 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was recrystallized from acetone to give II (380 mg) as colorless prisms. mp 174—177°. $[\alpha]_D^{14}$ $-13.7°$ (c=0.22). *Anal.* Calcd. for $C_{24}H_{34}O_4SCl_2$: C, 60.82; H, 6.67. Found: C, 60.70; H, 6.66.

5α,6β-Dichloroandrost-2-en-17-one (III)——A solution of II (380 mg) in dimethylsulfoxide (5 ml) was heated at 100° for 21 hr. The resulting solution was poured into ice–water, and the precipitate was collected by filtration, washed with H_2O and dissolved in ether. The ethereal solution was dried over anhydrous Na_2SO_4 and then evaporated to dryness. Recrystallization of the crude product from MeOH gave III (65 mg) as colorless needles. mp 154—156°. $[\alpha]_D^{14}$ $+7.5°$ (c=0.13). *Anal.* Calcd. for $C_{19}H_{26}OCl_2$: C, 66.74; H, 7.66. Found: C, 66.75; H, 7.34. NMR (5% solution in $CDCl_3$) δ: 4.50 (1H, m, 6-H), 5.66 (2H, m, 2-H, 3-H).

2β-Hydroxy-3α-bromo-5α,6β-dichloroandrostan-17-one (IVa), 2β-Bromo-3α-hydroxy-5α,6β-dichloroandrostan-17-one (IVb), 2β,3α-Dibromo-5α,6β-dichloroandrostan-17-one (IVc)——To a solution of III (500 mg) in 5% aq. t-BuOH (3 ml)-dioxane (5 ml) were added N-bromosuccinimide (1 g) dissolved in dioxane (16 ml) and 1N $HClO_4$ (1 ml) and allowed to stand at room temperature for 20 min. The resulting solution was concentrated *in vacuo* below 45°, diluted with H_2O and then extracted with ether. After usual work–up the sticky residue obtained was submitted to preparative TLC using hexane–AcOEt (7:3) as developing solvent. Elution of the adsorbent corresponding to the most polar spot (Rf: 0.46) and recrystallization

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10) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 4712 (1953); R.L. Clarke, K. Dobriner, A. Mooadian, and C.M. Martini, *ibid.*, **77**, 661 (1955).

11) C. Djerassi, "Optical Rotatory Dispersion," McGraw–Hill Book Co., New York, 1960, p. 49.

12) L.F. Fieser, *J. Am. Chem. Soc.*, **76**, 1945 (1954).

13) All melting points were taken on a micro hot–stage apparatus and are uncorrected. The optical rotations were measured in $CHCl_3$ unless otherwise stated. The rotatory dispersion curves were obtained on JASCO Model ORD/UV-5 recorder. The infrared (IR) spectral measurements were performed on JASCO Model IR-S spectrophotometer. The NMR spectra were run on Hitachi Model H-60 spectrometer at 60 Mc: the chemical shifts are quoted as ppm downfield from $(CH_3)_4Si$ as an internal reference. Abbreviations used s=singlet, t=triplet, q=quartet and m=multiplet. For preparative thin–layer chromatography (TLC) Silica gel H (E. Merck AG) was used as an adsorbent.

of the eluate from acetone gave IVa (90 mg) as colorless needles. mp 162—164°. $[\alpha]_D^{20}$ $+41.0°$ ($c=0.10$). *Anal.* Calcd. for $C_{19}H_{27}O_2BrCl_2$: C, 52.07; H, 6.21. Found: C, 51.81; H, 6.13. Elution of the adsorbent corresponding to the second spot (*Rf*: 0.53) and recrystallization of the eluate from $CHCl_3$–hexane gave IVb (68 mg) as colorless needles. mp 173—177°. $[\alpha]_D^{20}$ $+66.5°$ ($c=0.08$). *Anal.* Calcd. for $C_{19}H_{27}O_2BrCl_2$: C, 52.07; H, 6.21. Found: C, 52.01; H, 6.44. Elution of the adsorbent corresponding to the remaining spot (*Rf*: 0.76) and recrystallization of the eluate from AcOEt gave IVc (105 mg) as cololress prisms. mp 192.5—194.5°. $[\alpha]_D^{20}$ $+77.5°$ ($c=0.26$). *Anal.* Calcd. for $C_{19}H_{26}OBr_2Cl_2$: C, 45.54; H, 5.23. Found: C, 45.95; H, 5.30.

Androsta-3,5-dien-17-one (V)——A solution of IVc (30 mg) in AcOH (1.5 ml) was refluxed with Zn dust (0.45 g) for 7 hr. Similar treatment as described in IX followed by recrystallization from hexane–ether gave V (3 mg) as pale yellow leaflets. mp 87—92°. Mixed melting point on admixture with the authentic sample showed no depression and IR spectra of two samples were entirely identical. TLC: *Rf* 0.61 (hexane–AcOEt (4:1)).

2*α*-Bromo-5*α*,6*β*-dichloroandrostane-3,17-dione (VI)——To a solution of IVb (50 mg) in acetone (2 ml) was added a few drops of Jones reagent at 0° and allowed to stand for 5 min. The reaction mixture was treated in a similar manner as described in VIII. The crude product obtained was purified by preparative TLC using benzene–ether (6:1) as developing solvent. Recrystallization from ether gave VI (28 mg) as colorless needles. mp 159—163° (decomp.). $[\alpha]_D^{26}$ $+9.4°$ ($c=0.32$). *Anal.* Calcd. for $C_{19}H_{25}O_2BrCl_2$: C, 52.31; H, 5.77. Found: C, 51.95; H, 5.59. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1748 (C=O).

Androst-4-ene-3,17-dione (VII)——A solution of VI (56 mg) in AcOH (3 ml) was refluxed with Zn dust (1.2 g) for 5 hr. Similar treatment as described in IX followed by recrystallization from MeOH gave VII (7 mg) as colorless needles. mp 170—173°. Mixed melting point on admixture with the authentic sample showed no depression and IR spectra of two samples were entirely identical.

3*β*-Bromo-5*α*,6*β*-dichloroandrostane-2,17-dione (VIII)——To a solution of IVa (100 mg) in acetone (5 ml) was added a few drops of Jones reagent at 0° and allowed to stand for 5 min. The reaction mixture was poured into ice–water and extracted with ether. The organic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was recrystallized from AcOEt to give VIII (56 mg) as colorless needles. mp 203—206° (decomp.). $[\alpha]_D^{20}$ $+7.0°$ ($c=0.14$). *Anal.* Calcd. for $C_{19}H_{25}O_2BrCl_2$: C, 52.31; H, 5.77. Found: C, 52.49; H, 5.78. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740 (C=O).

Androst-5-ene-2,17-dione (IX)——To a solution of VIII (250 mg) in AcOH (10 ml) was added Zn dust (5 g) and the suspended solution was refluxed for 3 hr. The Zn cake was filtered off and washed with MeOH. The combined filtrates were concentrated and then extracted with ether. After usual work–up the crude product obtained was recrystallized from aq. acetone to give IX (110 mg) as colorless needles. mp 139—140°. $[\alpha]_D^{20}$ $-34.5°$ ($c=0.13$). *Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.82; H, 9.05.

Androst-5-ene-2*β*,17*β*-diol (Xa), Androst-5-ene-2*α*,17*β*-diol (Xb)——To a solution of IX (250 mg) in MeOH (5 ml) was added KBH_4 (200 mg) portionwise at 0° and the resulting solution was allowed to stand at room temperature overnight. After evaporation of solvent the residue obtained was extracted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. According to TLC the product appeared to consist of two isomeric 2,17β-diols (Xa, b) in a ratio of *ca.* 3 to 1. The crude product was adsorbed on Al_2O_3 and then eluted with increasing concentration of MeOH in benzene. Recrystallization of the first eluate from MeOH gave Xa (45 mg) as colorless fibers. mp 201—204°. $[\alpha]_D^{20}$ $-42.3°$ ($c=0.15$, MeOH). *Anal.* Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 77.89; H, 10.41. NMR (2.5% solution in $CDCl_3$) δ: 0.76 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3). Recrystallization of the second eluate from acetone gave Xb (22 mg) as colorless needles. mp 218—220°. $[\alpha]_D^{20}$ $-55.7°$ ($c=0.13$, MeOH). *Anal.* Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 79.08; H, 10.57. NMR (2.5% solution in $CDCl_3$) δ: 0.75 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3).

2*α*,17*β*-Dihydroxyandrost-4-en-3-one 17-Acetate (XIIb)——To a solution of XIIa (1 g) in MeOH (50 ml) was added 10% $KHCO_3$ (10 ml) and heated at 50° for 5 hr. Then, 10% $KHCO_3$ (10 ml) was added and heated again for 7 hr. After evaporation of solvent an oily residue obtained was extracted with AcOEt, washed with 5% HCl and H_2O, and dried over anhydrous Na_2SO_4. On usual work–up a pale yellow product was obtained. Recrystallization from acetone gave XIIb (0.32 g) as colorless needles. mp 223—224°. $[\alpha]_D^{24}$ $+75.0°$ ($c=0.21$). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.78; H, 8.69. NMR (5% solution in $CDCl_3$) δ: 4.15 (1H, q, $J=14$, 6 cps, 2β-H), 4.57 (1H, t, $J=7.5$ cps, 17α-H). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1228, 1730 (–$OCOCH_3$), 1684 (conjugated C=O), 3400 (–OH).

2*β*,17*β*-Dihydroxy-5*β*-androstan-3-one 17-Acetate (XIII)——A solution of XIIb (190 mg) in N-methylpyrrolidone (10 ml) was shaken with 5% $Pd/CaCO_3$ (190 mg) under a current of H_2 at room temperature for 3 days. After removal of catalyst by filtration the filtrate was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the pale yellow product obtained was submitted to preparative TLC using benzene–ether (6:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf*: 0.47) and recrystallization of the eluate from MeOH gave XIII (82 mg) as colorless needles. mp 163.5—164°. $[\alpha]_D^{21}$ $-8.1°$ ($c=0.19$). *Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.10; H, 9.42. NMR (5% solution in $CDCl_3$) δ: 0.79 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 4.16 (1H, q, $J=14$, 6 cps, 2α-H). ORD ($c=0.11$, MeOH) $[\phi]^{17}$ (mμ): −159° (390), −223° (350), −1178° (300) (trough), 0° (284), +1814° (254) (peak), +1655° (230).

3,3-Ethylenedithio-5β-androstane-2β,17β-diol (XIV)——To a solution of XIII (95 mg) in AcOH (5 ml) were added ethanedithiol (0.38 ml) and BF_3–etherate (0.38 ml), successively and allowed to stand at room temperature for 3 days. The solution was diluted with AcOEt, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work-up a crystalline product (100 mg) was obtained. To a solution of this crude product dissolved in dioxane (5 ml) was added methanolic 10% KOH (6 ml) and refluxed for 5 hr. The resulting solution was diluted with AcOEt, washed with 5% HCl and H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the residue was submitted to preparative TLC using benzene–ether (2:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf*: 0.17) and recrystallization of the eluate from acetone gave XIV (40 mg) as colorless needles. mp 103—104°. $[\alpha]_D^{17.5}$ +5.4° ($c=0.19$). *Anal.* Calcd. for $C_{21}H_{34}O_2S_2$: C, 65.92; H, 8.96. Found: C, 65.40; H, 9.10. NMR (5% solution in $CDCl_3$) δ: 3.75 (1H, q, $J=12.6$, 4.2 cps, 2α-H), 3.16 (1H, t, $J=3.6$ cps, 17α-H).

5β-Androstane-2β,17β-diol (XV)——To a solution of XIV (40 mg) in EtOH (4 ml) was added Raney-Ni (W_2) (*ca.* 1.2 g) and refluxed for 6 hr. After removal of the metal by filtration the filtrate was concentrated to give a crystalline product. 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*: 0.08) and recrystallization of the eluate from acetone gave XV (11.1 mg) as colorless needles. mp 179—180°. $[\alpha]_D^{20}$ −4.4° ($c=0.07$). *Anal.* Calcd. for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found: C, 77.72; H, 10.98. NMR (3.8% solution in $CDCl_3$) δ: 0.72 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3).

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