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The Synthesis and Nuclear Magnetic Resonance Spectra of Epimeric 16-Deuterio-13*α*-androstan-17-ols[1]

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Four epimeric 16-deuterio-5*α*,13*α*-androstane-3*β*,17-diols were prepared starting from 3*β*-hydroxy-5*α*,13*α*-androstan-17-one, and the conformation of ring D in 13*α*-steroids is discussed based upon the correlation of dihedral angles with the observed coupling constants of H16,17. The chemistry of the 16*β*,17*β*-epoxy and Δ^{16} compounds is also described.

In a previous publication the authors reported the synthesis and conformational analysis of the two epimeric 16-bromo-5*α*,13*α*-androstan-17-ones.[3)] The conformation of the fused cyclopentane ring in 13*α*-testosterone has also been studied by Chinn, where the correlation of dihedral angles with the coupling constants as defined by the Karplus equation was used.[4)] Recently, it has been demonstrated that C-16-deuterated compounds are much more suitable for the study of 17-proton resonance, since the deuterium substitution simplifies the ABX pattern to the AX without electronic influence or steric distortion of ring D.[5)] Therefore, the authors have attempted to employ the pertinent deuterated compounds for the conformational studies of 13*α*-steroids. The present paper deals with the synthesis of the four epimeric 16-deuterio-5*α*,13*α*-androstane-3*β*,17-diols (IXb, c, Xb, c) and conformation of ring D on the basis of their nuclear magnetic resonance spectra.

In order to ensure the stereoselectivity the *trans*-16-deuterio-17-hydroxy compounds were synthesized starting from the 16,17-epoxides by reduction with lithium aluminum deuteride. The course of the reductive cleavage of the 16*α*,17*α*-epoxide with metal hydride was previously reported,[3)] but the stereochemistry of the opening of its epimer has not yet been elucidated. Therefore, it was initially necessary to prepare 16*β*,17*β*-epoxy-5*α*,13*α*-androstan-3*β*-ol (Va).

Treatment of the 16*α*,17*α*-epoxide (IIb) with acetic acid resulted in formation of 5*α*,13*α*-androstane-3*β*,16*β*,17*α*-triol 3,16-diacetate (III), where the stereochemistry at C-16 and C-17 was tentatively assigned based upon the analogous reactions. The resulting triol diacetate was further converted to the 17-mesylate (IV) with methanesulfonyl chloride and pyridine. When the mesylate was refluxed with alkali, the desired 16*β*,17*β*-epoxide (Va), readily distinguished from its epimer (IIa), was formed.

Reduction of this new epoxide with lithium aluminum hydride yielded the 17*β*-hydroxy derivative (Xa) as the sole product, whose structure was confirmed by comparison with an authentic sample. The mode of the epoxide opening suggests that the steric effect of the C-13-methyl group on the *α*-side of C-17 favors the preferential attack of the reagent at C-16 to give the axial alcohol.

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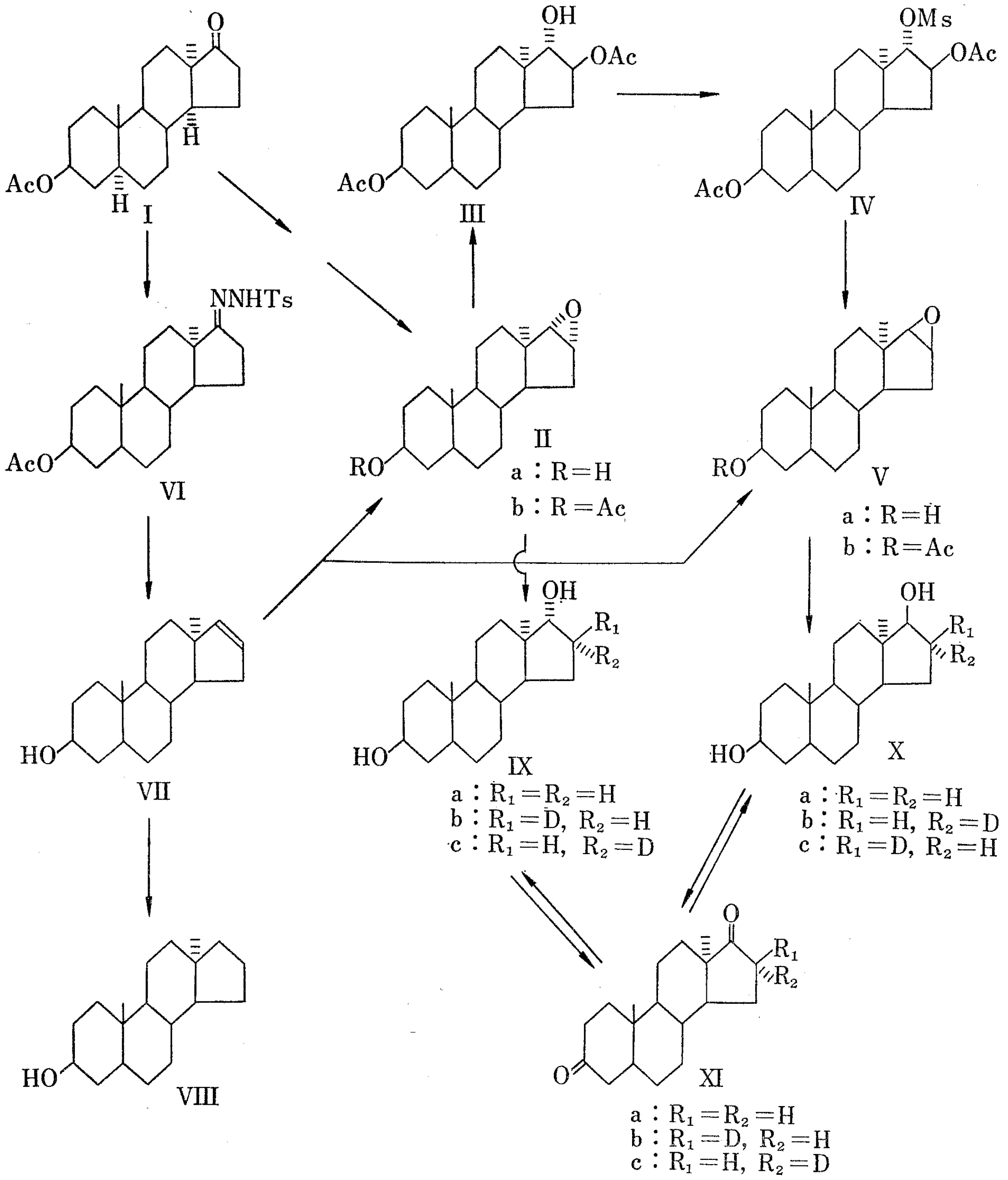
1) This paper constitutes Part XVIII of the series entitled "Analytical Chemical Studies on Steroids"; Part XVII: T. Nambara and Y. Matsuki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1394 (1968).

2) Location: *a) Kita-4-bancho, Sendai*; *b) Bronx, New York.*

3) T. Nambara, H. Hosoda, and S. Goya, *Chem. Ind.*, **1967**, 1703; *Chem. Pharm. Bull.* (Tokyo), **16**, 1266 (1968).

4) L.J. Chinn, *J. Org. Chem.*, **30**, 4165 (1965).

5) J. Fishman, *J. Am. Chem. Soc.*, **87**, 3455 (1965).



$Ms=CH_3SO_2-$, $Ts=p-CH_3C_6H_4SO_2-$, $Ac=CH_3CO-$

Chart 1

The synthetic route to the 16β,17β-oxido compound mentioned above was somewhat tedious, and a more accessible way leading to the 16,17-epoxide was desired. The starting material, 3β-acetoxy-5α,13α-androstan-17-one (I),[3,6] was transformed into the *p*-tosylhydrazone (VI) in the usual way. When the *p*-tosylhydrazone was reduced with lithium aluminum hydride in tetrahydrofuran,[7] 5α,13α-androst-16-en-3β-ol (VII) was produced in satisfactory yield. On catalytic hydrogenation over palladium-on-charcoal this unsaturated product was transformed into 5α,13α-androstan-3β-ol (VIII). Treatment of the Δ^{16} compound (VII) with perbenzoic acid gave a mixture of two epimeric 16,17-epoxy-5α,13α-androstan-3β-ols (IIa, Va), which could not be separated. Upon usual acetylation, however, the mixture could be separated with relative ease by means of column chromatography on silica

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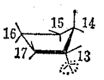
6) T. Nambara, T. Kudo, H. Hosoda, and S. Goya, *J. Chromatog.*, **31**, 210 (1967).
7) L. Caglioti and M. Magi, *Tetrahedron*, **19**, 1127 (1963).

gel to furnish α– and β–epoxides (IIb, Vb) in the ratio of 1 to 2. It is interesting that epoxidation of the Δ^{16}–double bond with peracid proceeds from both sides of the molecule to give α– and β–epoxy derivatives in the 13α series. This may be due to the C/D *cis* 13α–linkage, where the β–side is crowded due to the cage–like structure, while the α–side is sterically hindered by the presence of the angular methyl group. For the preparation of the epimeric 17–hydroxy derivatives the mixture of the 16,17–epoxides was further reduced with lithium aluminum hydride. The two epimeric 5α,13α–androstane–3β,17–diols (IXa, Xa) could then be separated by means of thin–layer chromatography. The epimeric *trans*–16–deuterio–17–hydroxy compounds (IXb, Xb) were similarly prepared from the mixture of the epimeric 16,17–epoxides by reduction with lithium aluminum deuteride and subsequent chromatographic purification.

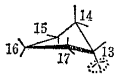
Table I. Coupling Constants of H16, 17 of Epimeric 16–Deuterio–5α,13α–androstane–3β,17–diols[a]

Configuration of H–16 and –17	Conformation A	Conformation B	Conformation C	Found[b]
16β, 17α (Xb)	2.2	0	0	0
16α, 17α (Xc)	8.2	7.4	5.2	5.1
16α, 17β (IXb)	2.2	5.1	7.7	7.1
16β, 17β (IXc)	8.2	7.4	5.2	8.6

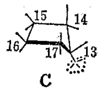
a) All J values are in cycles per second.
b) These bands appeared somewhat broadened. It is probably due to H–D coupling and H17α, 14 through 4σ bonds or H17β, 18 coupling. Upon irradiation at the frequency of 18–CH_3 resonance, H17β signal was observed sharpened. The H17α and H17β signals appeared at 6.28 and 5.85τ, respectively.



A



B



C

The preparation of *cis*–16–deuterio–17–ols (IXc, Xc) was somewhat more involved. The 3β,17α–dihydroxy compound (IXa) was oxidized with chromium trioxide in acetic acid providing 5α,13α–androstane–3,17–dione (XIa). The 16β–deuterio–3,17–dione (XIb) prepared from 16β–deuterio–3β,17α–diol (IXb) in the same manner,[8] upon lithium aluminum hydride reduction followed by chromatographic purification, gave the desired 16β–deuterio–5α, 13α–androstane–3β,17β–diol (Xc) together with its 17–epimer. The preparation of the remaining 16α–deuterio–5α,13α–androstane–3β,17α–diol (IXc) followed the same reaction sequence, namely chromium trioxide oxidation and subsequent reduction with metal hydride starting from 16α–deuterio–5α,13α–androstane–3β,17β–diol (Xb) through the 16α–deuterio–3,17–dione (XIc). The infrared spectra of these 16–deuterated compounds were characterized by the presence of C–D stretching band at 2144—2174 cm^{-1}.

The coupling constants of the C–17 proton were measured in the four epimeric 16–deuterio–5α,13α–androstane–3β,17–diols obtained above. The coupling of H16β,17β was found to be larger than that of H16α,17α despite the similarity of their dihedral angles. Williams and Bhacca[9] reported that in the cyclohexane ring the coupling constant of a proton attached to a carbon bearing an equatorial hydroxyl group is 2—3 cps larger than that of a proton on a carbon having an axial hydroxyl group. This finding, if extended to the five–membered ring, would account for the observed discrepancy. The observed coupling constants for

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8) It has already been confirmed that oxidation of 17–hydroxy compound to the 17–ketone with chromium trioxide and subsequent work–up proceed without loss of isotope at C–16 (G.K. Helkamp and B.F. Rickborn, *J. Org. Chem.*, **22**, 479 (1957)).

9) D.H. Williams and N.S. Bhacca, *J. Am. Chem. Soc.*, **86**, 2742 (1964).

17β-proton should be reduced by an intermediate value up to 2 cps for comparison with the calculated values derived from the Karplus equation.[10]

The torsional angles determined by Brutcher, *et al.*[11] for cyclopentane permit the calculation of H16,17 dihedral angles for the three possible conformations A, B and C. In Table I the coupling constants calculated from the angles by the Karplus equation are listed and compared with the experimental values obtained in the present study. It is evident that in both sets of the 17-hydroxy series the observed values are in qualitative agreement with those calculated for conformations B or C, but not A. A definite choice between these two conformations is not possible due to the limitations of the Karplus equation. Chinn also compared the sum of the coupling constants of 17-proton with both H16α and H16β of the 17-epimeric 13α-testosterones, which appeared as X portion of the ABX system, with the calculated values for the possible ring D conformations. He also reached the same conclusion that ring D could exist in conformations B or C, but not A, if ring C is assumed to be in the chair form.[4] However, it should be now emphasized that the present work is certainly more definitive. The small differences in coupling constants observed in the 13α series with the corresponding values in the 13β series[5] may very well be due to distorsion of ring C from the chair form in the *cis* structure.

Further studies on the conformational analysis of ring D with deuterated compounds are being extended to the 14β-ring system and will be reported in near future.

Experimental[12]

16α,17α-Epoxy-5α,13α-androstan-3β-ol Acetate (IIb)——16α,17α-Epoxy-5α,13α-androstan-3β-ol[3] (IIa) (30 mg) was treated with pyridine (0.4 ml) and Ac_2O (0.2 ml) in the usual manner. Recrystallization from aq. MeOH gave IIb (30 mg) as colorless plates. mp 123—124°, $[\alpha]_D^{17}$ −20.0° (c=0.15). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.15; H, 9.73.

5α,13α-Androstane-3β,16β,17α-triol 3,16-Diacetate (III)——A solution of IIb (24 mg) in glacial AcOH (3 ml) was boiled under reflux for 2 hr. The resulting solution was concentrated under reduced pressure, and the residue obtained was diluted with ether. The organic extract was washed with cold 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the residue was taken up in benzene and chromatographed on silica gel (3 g). Elution with benzene-ether (10:1) and recrystallization of the eluate from acetone-hexane gave III (10 mg) as colorless needles. mp 175—177°, $[\alpha]_D^{20}$ −67.9° (c=0.14). *Anal.* Calcd. for $C_{23}H_{36}O_5 \cdot \frac{1}{2}H_2O$: C, 68.80; H, 9.29. Found: C, 69.06; H, 9.24.

3β,16β-Diacetoxy-5α,13α-androstan-17α-ol Methanesulfonate (IV)——To a solution of III (26 mg) in pyridine (0.2 ml) was added $MeSO_2Cl$ (0.03 ml), and the resulting solution was allowed to stand at 0° overnight. The reaction mixture was diluted with ether and washed with 5% $NaHCO_3$, 5% HCl and H_2O successively, and dried over anhydrous Na_2SO_4. Upon evaporation of solvent the crystalline product was obtained. Recrystallization from aq. MeOH gave IV (22 mg) as colorless plates. mp 172—173°, $[\alpha]_D^{18}$ +9.1° (c=0.11). *Anal.* Calcd. for $C_{24}H_{38}O_7S$: C, 61.26; H, 8.14. Found: C, 61.44; H, 7.87.

16β,17β-Epoxy-5α,13α-androstan-3β-ol (Va)——i) A solution of IV (29 mg) in 5% methanolic KOH (2 ml) was boiled under reflux for 2 hr. After evaporation of solvent under reduced pressure the residue was taken up in AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was dissolved in benzene and chromatographed on silica gel (3 g). Elution with benzene-ether (9:1) and recrystallization of the eluate from ether-hexane gave Va (8 mg) as colorless prisms. mp 116—117°, $[\alpha]_D^{20}$ −87.5° (c=0.08). *Anal.* Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.53; H, 10.38.

ii) A solution of Vb (10mg) dissolved in 5% methanolic KOH (10 ml) was boiled under reflux for 30 min. On usual work-up the crude product was obtained. Recrystallization from ether-hexane gave Va (6 mg) as colorless prisms. mp 113-114°. Mixed mp on admixture with the sample obtained in i) showed no depression.

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[10] M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); *J. Am. Chem. Soc.*, **85**, 2870 (1963).

[11] F.V. Brutcher, Jr. and W. Bauer, Jr. *J. Am. Chem. Soc.*, **84**, 2233, 2236 (1962).

[12] All melting points were taken on micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise stated. Infrared spectra measurements were run on Hitachi Model EPI-2 spectrophotometer. Nuclear magnetic resonance spectra were obtained on Hitachi Model H-60 spectrometer operated at 60 Mcps in $CDCl_3$ containing tetramethylsilane (internal standard) and a trace of D_2O.

Reduction of 16β,17β-Epoxy-5α,13α-androstan-3β-ol (Va) with Lithium Aluminum Hydride——To a solution of Va (15 mg) in ether (3 ml) was added $LiAlH_4$ (40 mg) portionwise under cooling in ice–bath, and the resulting solution was boiled under reflux for 6 hr. After usual work–up the crude product obtained was recrystallized from acetone–hexane to give 5α,13α–androstane–3β,17β–diol (Xa) (10 mg) as colorless plates. mp 167—169°. Mixed mp on admixture with the authentic sample³⁾ showed no depression.

16β,17β-Epoxy-5α,13α-androstan-3β-ol Acetate (Vb)——A 70 mg portion of the crude product, obtained from VII by epoxidation with perbenzoic acid, was dissolved in Ac_2O (0.5 ml) and pyridine (1 ml) and allowed to stand at room temperature overnight. After usual work–up an oily residue obtained was dissolved in hexane and chromatographed on silica gel (3 g). Elution with benzene and recrystallization of the eluate from aq. MeOH gave Vb (35 mg) as colorless plates. mp 112—113°, $[\alpha]_D^{18}$ −63.9° (c=0.18). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.01; H, 9.63. Further elution with benzene–ether (15:1) and recrystallization of the eluate from aq. MeOH gave Vb (18 mg) as colorless plates. mp 122—123°. Mixed mp on admixture with the sample obtained from IIa by usual acetylation showed no depression.

3β-Acetoxy-5α,13α-androstan-17-one *p*-Tosylhydrazone (VI)——To a solution of 3β–hydroxy–5α,13α–androstan–17–one acetate (I) (1 g) in MeOH (50 ml) were added *p*–tosylhydrazine (1.5 g) and AcOH (5 ml), and the resulting solution was boiled under reflux for 4 days. On cooling the crystalline product separated was filtered and recrystallized from MeOH to give VI (1.1 g) as colorless plates. mp 228—230°, $[\alpha]_D^{20}$ −106.7° (c=0.15). *Anal.* Calcd. for $C_{28}H_{40}O_4N_2S$: C, 67.17; H, 8.05; N, 5.60. Found: C, 67.04; H, 8.07; N, 5.71.

5α,13α-Androst-16-en-3β-ol (VII)——To a solution of VI (1.08 g) in THF (50 ml) was added $LiAlH_4$ (2.6 g) and the reaction mixture was boiled under reflux for 15 hr. After usual work–up the crude product obtained was chromatographed on Al_2O_3 (10 g). Elution with benzene–ether (2:1) and recrystallization of the eluate from aq. MeOH gave VII (300 mg) as colorless needles. mp 99—100°, $[\alpha]_D^{21}$ −109.4° (c=0.16). *Anal.* Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 83.03; H, 10.93.

5α,13α-Androstan-3β-ol (VIII)——A solution of VII (20 mg) dissolved in AcOEt (5 ml) was shaken with 5% Pd/C (20 mg) in the stream of H_2 at room temperature for 5 days. After removal of catalyst by filtration the filtrate was concentrated to give the crystalline product. Recrystallization from aq. MeOH gave VIII (18 mg) as colorless leaflets. mp 109—111°, $[\alpha]_D^{21}$ −61.5° (c=0.13). *Anal.* Calcd. for $C_{19}H_{32}O$: C, 82.54; H, 11.66. Found: C, 82.13; H, 11.68.

Epoxidation of 5α,13α-Androst-16-en-3β-ol with Perbenzoic Acid——To a solution of VII (215 mg) in $CHCl_3$ (6 ml) was added $C_6H_5CO_3H$–$CHCl_3$ solution (0.37 M, 2.7 ml), and the resulting solutin was allowed to stand at room temperature for 5 hr. The reaction mixture was diluted with ether, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily residue obtained was taken up in hexane–benzene (1:1) and chromatographed on silica gel (3 g). Elution with benzene to benzene–ether (5:1) gave a mixture of the epimeric 16,17–epoxy–5α,13α–androstan–3β–ols (200 mg). Separation of these epimers could not be achieved and hence the crude product was submitted to further elaboration without purification.

Reduction of Epimeric 16,17-Epoxy-5α,13α-androstan-3β-ols with Lithium Aluminum Deuteride——To a solution of the mixture of IIa and Va (50 mg) in ether (5 ml) was added $LiAlD_4$ (100 mg) portionwise under cooling in ice–bath. The resulting solution was boiled under reflux for 5 hr. After usual work–up the crude product obtained was submitted to the preparative TLC on silica gel H using hexane–AcOEt (5:3) as solvent. The adsorbent of the zones corresponding to each spot (*Rf* 0.33, 0.42) was eluted with acetone. Recrystallization of the eluates from hexane–acetone gave 16β–deuterio–5α,13α–androstane–3β,17α–diol (IXb) (10 mg), mp 199—200°, and 16α–deuterio–5α,13α–androstane–3β,17β–diol (Xb) (20 mg), mp 170—171°, respectively.

5α,13α-Androstane-3,17-dione (XIa)——To a solution of IXa (10 mg) in glacial AcOH (1 ml) was added 2% CrO_3 solution in 98% AcOH (0.2 ml), and the resulting solution was allowed to stand at room temperature for 5 hr. After addition of MeOH to decompose the excess CrO_3, the reaction mixture was diluted with H_2O and extracted with ether. On usual work–up the crystalline product was obtained. Recrystallization from acetone–hexane gave XIa (10 mg) as colorless prisms. mp 165—167°, $[\alpha]_D^{19}$ −75.0° (c=0.12). *Anal.* Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 79.22; H, 10.10.

16α-Deuterio-5α,13α-androstane-3,17-dione (XIc)——Xb (40 mg) was oxidized with 2% CrO_3 in AcOH (1 ml) in the same manner as XIa. After addition of MeOH to decompose the excess CrO_3 the reaciton mixture was diluted with H_2O and extracted with ether. Organic extract was washed with cold 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work–up the crystalline product was obtained. Recrystallization from acetone–hexane gave XIc (36 mg) as colorless leaflets. mp 165—167°.

16β-Deuterio-5α,13α-androstane-3,17-dione (XIb)——IXb (35 mg) was oxidized with 2% CrO_3 in AcOH (1 ml) in the same manner as XIa. The crude product (32 mg) obtained was homogeneous according to TLC and therefore submitted to further elaboration without purification.

16α-Deuterio-5α,13α-androstane-3β,17α-diol (IXc)——To a solution of XIc (36 mg) in ether (20 ml) was added ethereal solution of $LiAlH_4$ (40 mg) under cooling in ice–bath. After allowing to stand at room temperature the moist ether was added to decompose the excess reagent. After usual work–up the crude product obtained was submitted to the preparative TLC on silica gel H using hexane–AcOEt (5:3) as solvent. The adsorbent of the zone corresponding to the spot (*Rf* 0.33)was eluted with acetone and the eluate was recrystallized from acetone–hexane to give IXc (20 mg) as colorless prisms. mp 196°. Xb (5 mg)was also obtained from the eluate of the corresponding zone (*Rf* 0.42).

16β-Deuterio-5α,13α-androstane-3β,17β-diol (Xc)——XIb (32 mg) was submitted to reduction with $LiAlH_4$ (40 mg) followed by the preparative TLC. The eluate of the corresponding zone (*Rf* 0.42) was recrystallized from acetone–hexane to give Xc (5 mg) as colorless needles. mp 171—172°. IXb (20 mg) was also obtained from the eluate of the zone of *Rf* 0.33.

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