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Synthesis of Epimeric 2-Deuterioestr-4-ene-3,17-diones[1]

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In order to clarify the stereochemistry of hydrogen loss from C-2 in the 19-norsteroid during the placental aromatization the stereoselective synthesis of C-2 epimeric 2-deuterioestr-4-ene-3,17-diones has been carried out. A key intermediate leading to the desired compounds, estr-2-ene-5β,17β-diol (VIII), was prepared from readily available 19-nortestosterone in several steps. Epoxidation with per-acid followed by *trans*-diaxial opening of the resulting 2β,3β-epoxide (IX) with lithium aluminum deuteride provided the 2α-deuterio-3β,5β,17β-triol (XVII). On the other hand VIII was transformed into the 2β-deuterio-3β,5β,17β-triol (XX) by treatment with deuterated diborane. Upon dehydration with thionyl chloride and subsequent oxidation with chromium trioxide-pyridine complex XVII and XX were led to 2-deuterated estr-4-ene-3,17-diones (XIX, XXII), respectively.

The biotransformation of C_{19}-steroid into the estrogen by human placenta[3] requires two general steps: (1) hydroxylation and subsequent loss of the C–19 methyl group, and (2) elimination of hydrogen atoms from both C–1 and C–2 resulting in double-bond introduction in ring A.[4] It has recently been elucidated that the hydrogen loss from C–1 and C–2 during biological aromatization is both stereospecifically β.[5] The 19-norsteroid widely used as contraceptive and anabolic drugs is also known to be aromatized in placental and ovarian tissue.[6] With regard to this aromatization mechanism it has been demonstrated that 1β-hydrogen is lost in the bioconversion to estrogen.[5b] However, the problem whether the similar mechanism is operative or not still remains undissolved. Therefore we have attempted to clarify the stereochemistry of hydrogen loss from C–2 during the placental aromatization. The design of the experiment required estr-4-ene-3,17-diones labeled with the isotope stereospecifically at 2α and 2β positions as the substrate for the enzymatic transformation. The present paper deals with the stereoselective synthesis of C–2 epimeric 2-deuterioestr-4-ene-3,17-diones starting from readily available 19-nortestosterone.

An initial project was directed to the preparation of estr-2-ene-5β,17β-diol, which would serve as a key intermediate leading to the desired compounds. First, 19-nortestosterone (I) was converted into the 4β,5β-epoxide 17-acetate (II) upon exposure to hydrogen peroxide

1) This paper constitutes Part LIII of the series entitled "Analytical Chemical Studies on Steroids"; Part LII: T. Nambara, Y.H. Bae, T. Anjyo, and S. Goya, *J. Chromatog.*, **62**, 369 (1971).
2) Location: *Aobayama, Sendai.*
3) K.J. Ryan, *J. Biol. Chem.*, **234**, 268 (1959); R.A. Meigs and K.J. Ryan, *ibid.*, **246**, 83 (1971) and references quoted therein.
4) P. Talalay, *Ann. Rev. Biochem.*, **34**, 347 (1965).
5) *a*) T. Morato, K. Raab, H.J. Brodie, M. Hayano, and R.I. Dorfman, *J. Am. Chem. Soc.*, **84**, 3764 (1962); *b*) J.D. Townsley and H.J. Brodie, *Biochemistry*, **7**, 33 (1968); *c*) H.J. Brodie, G. Possanza, and J.D. Townsley, *Biochim. Biophys. Acta*, **152**, 770 (1968); *d*) H.J. Brodie, K.J. Kripalani, and G. Possanza, *J. Am. Chem. Soc.*, **91**, 1241 (1969); *e*) J. Fishman and H. Guzik, *ibid.*, **91**, 2805 (1969); *f*) J. Fishman, H. Guzik, and D. Dixon, *Biochemistry*, **8**, 4304 (1969).
6) P.K. Besch, D.J. Watson, N. Vorys, and R.D. Barry, *Biochim. Biophys. Acta*, **69**, 417 (1963); J.D. Townsley and H.J. Brodie, *ibid.*, **144**, 440 (1967); H.J. Brodie, A.K. Pillai, and C.E. Hay, *ibid.*, **187**, 275 (1969).

in the alkaline media followed by usual acetylation.[7] It is sufficiently substantiated that the sign of Cotton effect of the 4,5-epoxy-3-ketone obeys the "reversed" octant rule and is dependent upon the sign of the octant in which the oxido oxygen is located.[8] The β-configuration of the oxido group in II was evident from the circular dichroism curve exhibiting the positive Cotton effect(for the $n\rightarrow\pi^*$ transition). Subsequent reduction with sodium borohydride under mild conditions resulted in formation of two epimeric 4β,5β-epoxy-3-ols (IIIa, IIIb). The separation of these two could not easily be attained and therefore the mixture was transformed into the 3-mesylates (IVa, IVb) by treatment with mesyl chloride and pyridine. Reaction with lithium carbonate in dimethylformamide afforded the desired 4β,5β-epoxyestr-2-en-17β-ol acetate (V) together with 4a-oxa-A-homoestra-3,5-dien-17β-ol acetate (VIb) and 17β-acetoxy-4β,5β-epoxyestran-3β-ol formate (VII), which could efficiently be separated by preparative thin-layer chromatography (TLC). The structure of VII was confirmed by leading to 4β,5β-epoxyestrane-3β,17β-diol 17-acetate (IIIa)[9] by partial hydrolysis with sodium bicarbonate. In the nuclear magnetic resonance (NMR) spectra C-4 proton signal appeared at 3.13 ppm as a doublet (J=4 cps) indicating the β-*cis* structure of the 4,5-epoxy-3-ol.[10] The structure of VIb was deduced from elemental analysis and NMR spectral data.[11] The cleavage of the 4β,5β-oxido ring in V was effected by treatment with lithium aluminum hydride yielding estr-2-ene-5β,17β-diol (VIII).

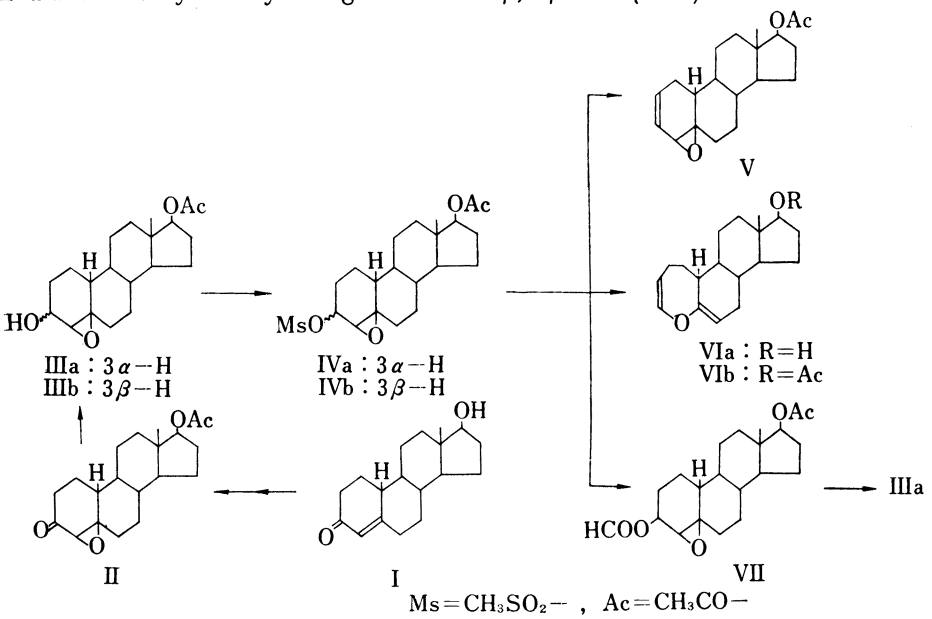

Chart 1

As a preliminary experiment toward the final goal we attempted to establish the synthetic route from VIII to estr-4-ene-3,17-dione by which the label could unambiguously be introduced at the desired position. Treatment with *m*-chloroperbenzoic acid provided two epimeric 2,3-epoxides (IX, X) in a ratio of *ca.* 3 to 1. The per-acid would attack the Δ^2-double bond

7) B. Camerino, R. Modelli, and B.Patelli, *Framaco Ed. Sci.*, **13**, 52 (1958); M.E. Wolff and C.B. Karash, *J. Org. Chem.*, **24**, 1612 (1959).
8) C. Djerassi, W. Klyne, T. Norin, G. Ohloff, and E. Klein, *Tetrahedron*, **21**, 163 (1965).
9) Upon lithium aluminum hydride reduction IIIa was transformed into estrane-3β,5β,17β-triol (XIa).
10) D.J. Collins, J.J. Hobbs, and S. Sternhell, *Tetrahedron Letters*, **1963**, 623.
11) The analogous reaction has been reported with 4β,5β-epoxycholestan-3β-ol *p*-tosylate where the diene-ether was formed in 13% yield (J.M. Coxon, R.P. Garland, M.P. Hartshorn, and G.A. Lane, *Chem. Commun.*, **1968**, 1506).

preferentially from the less-hindered β-side of the molecule, probably because A-ring is bent below to form the cage-like structure. The β-epoxide (IX) was then subjected to the reductive cleavage with lithium aluminum hydride. As was expected the *trans*-diaxial opening of the oxido ring proceeded to give the 3β,5β,17β-triol (XIa) in reasonable yield. The structural assignment was justified by direct comparison with the product derived from II. Reduction of II with metal hydride afforded two epimeric 3,5β,17β-triols (XIa, XIIa), which could readily be separated by preparative TLC. Of the two epimers the less polar one, which formed the carbonate (XIII) with phosgene indicating the 3β,5β-*cis*-diol structure, proved to be identical with the reduction product obtained from IX. Introduction of the Δ^4-double bond by dehydration was then undertaken with the 3β,5β,17β-triol 3,17-diacetate (XIb). When treated with thionyl chloride and pyridine under mild conditions XIb underwent dehydration in the expected direction to give estr-4-ene-3β,17β-diol diacetate (XIVb) in satisfactory yield. The configuration of the hydroxyl group at C-3 in the alkaline hydrolyzate (XIVa) was rationalized by inspection of NMR spectra in which an olefinic proton appeared at 5.38 ppm as a singlet.[12] Subsequent oxidation with chromium trioxide-pyridine complex gave estr-4-ene-3,17-dione (XVI) in satisfactory yield. In a similar fashion the epimeric 3α,5β,17β-triol (XIIa) was led to XVI *via* the 3,17-diacetate (XIIb) and then estr-4-ene-3α,17β-diols (XVa, XVb). The stereochemistry at C-3 was similarly verified on the basis of NMR spectral data that XVa exhibited the vinyl proton as a doublet (J=5 cps) centered at 5.55 ppm.[12]

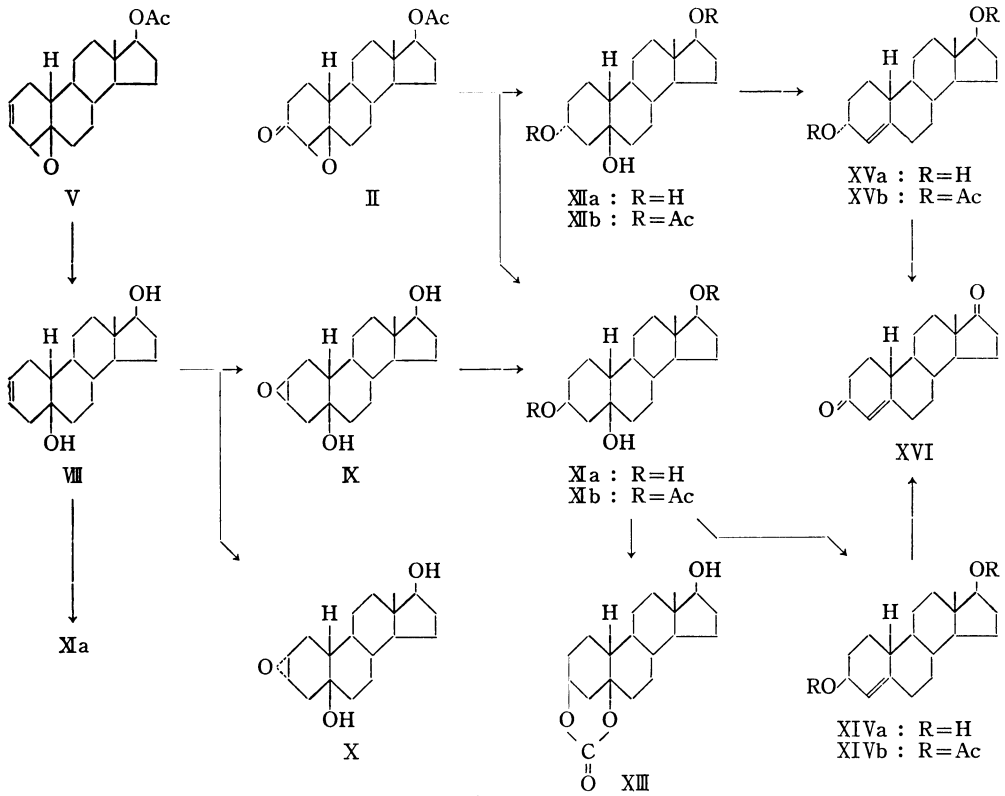

Chart 2

The next project was directed to the preliminary studies on the stereospecific labeling of the isotope at 2β-position employing the Δ^2-unsaturated 5β-steroid. Fortunately the

12) D.K. Fukushima, S. Dobriner, and H.L. Bradlow, *Biochemistry*, **5**, 1783 (1966).

reaction with diborane did take place at the β-side of the Δ^2-double bond to give the 3β-hydroxylic compound (XIa). These synthetic routes proved to be promising to introduce the isotope specifically into 2α- and 2β-positions of estr-4-ene-3,17-dione.

The synthesis of the 2α-deuterated substrate was undertaken utilizing the *trans*-diaxial opening reaction of the $2\beta,3\beta$-oxido ring. Treatment of IX with lithium aluminum deuteride furnished the 2α-deuterio-$3\beta,5\beta,17\beta$-triol (XVIIa). Usual acetylation and subsequent dehydration in the manner as described with the non-labeled compound afforded 2α-deuterioestr-4-ene-$3\beta,17\beta$-diol diacetate (XVIIIb). Hydrolysis with alkali followed by oxidation with chromium trioxide–pyridine complex gave the desired 2α-deuterioestr-4-ene-3,17-dione (XIX). The epimeric 2β-deuterio substrate was then prepared starting from VIII along the same reaction sequence as mentioned above. Treatment with deuterated diborane freshly prepared from lithium aluminum deuteride and boron trifluoride gave 2β-deuterioestrane-$3\beta,5\beta,17\beta$-triol (XXa). Usual acetylation and subsequent dehydration furnished the 2β-deuterated Δ^4-3β-ol acetate (XXIb), which on hydrolysis and chromium trioxide oxidation was led to the desired 2β-deuterioestr-4-ene-3,17-dione (XXII).

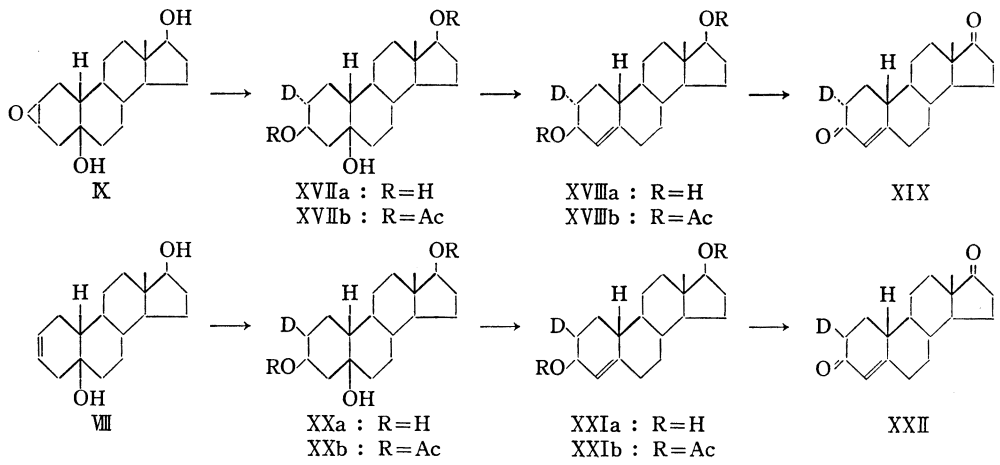

Chart 3

The infrared (IR) spectra of non-labeled estr-4-ene-3,17-dione (XVI) and two epimeric 2-deuterated steroids (XIX, XXII) were different each another in the finger print region. The locality and quantity of the isotope in these labeled steroids were determined by mass spectral technique. Inspection of the molecular ion peak, which appeared at *m/e* 273 with an increment of one mass unit, revealed that the deuterium contents of the labeled compounds were both *ca.* 98%.

It is hoped that the facile availability of the specifically labeled substrates may serve to clarify the aromatization mechanism of the 19-norsteroids.

Experimental[13]

17β-Acetoxy-4β,5β-epoxyestran-3-one (II)——To a solution of 19-nortestosterone (I) (500 mg) in MeOH (5 ml) were added 10% NaOH (1 ml) and 30% H_2O_2 (0.5 ml) at 0° and allowed to stand for 1 hr. The re-

13) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. NMR spectra were obtained on Hitachi Model R-20 spectrometer at 60 Mc; the chemical shifts are quoted as ppm downfield from tetramethylsilane used as an internal standard. IR spectra and circular dichroism curves measurements were run on JASCO Model IR-S spectrometer and Model ORD/UV-5 recorder, respectively. Mass spectra were measured by Hitachi Model RMU-7 spectrometer. For preparative TLC Silica gel H (E. Merck AG) was used as an adsorbent.

action mixture was poured onto ice-water and extracted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue obtained was treated with Ac_2O (5 ml) and pyridine (10 ml) in the usual manner. The resulting solution was diluted with ether, washed with cold 5% HCl, 5% $NaHCO_3$ and H_2O, successively and dried over anhydrous Na_2SO_4. After usual work-up the crystalline product was recrystallized from ether–hexane to give II (500 mg) as colorless needles. mp 104—105° (reported: mp 111—112°).[7] NMR (5% solution in $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 2.01 (3H, s, 17β-$OCOCH_3$), 2.98 (1H, s, 4-H), 4.58 (1H, t, J=8 cps, 17α-H). CD (c=0.12, MeOH) $[\theta]^{18}$ (mμ): 0 (350), +2800 (330), +9470 (302) (positive maximum), +2100 (270).

Reduction of II with Sodium Borohydride——To a solution of II (1.57 g) in THF (10 ml)–H_2O (3 ml) was added portionwise $NaBH_4$ (1.5 g) under ice-cooling and allowed to stand at 0° for 30 min. After decomposition of the excess reagent with AcOH the resulting solution was extracted with ether. The organic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. Evaporation of solvent gave a mixture of epimeric 17β-acetoxy-4β,5β-epoxyestran-3-ols (III) (1.74 g) as the oily product. Inspection of NMR spectra revealed that C-4 protons of 3α- and 3β-hydroxylic compounds appeared at 2.86 ppm as a singlet and 3.13 ppm as a doublet (J=4 cps), respectively indicating the composition ratio of *ca.* 1 to 2. The crude product was submitted to further step without purification.

Mesylation of III——To a solution of III (1.74 g) in pyridine (10 ml) was added dropwise $MeSO_2Cl$ (1 ml) under ice-cooling and allowed to stand at 0° for 1.5 hr. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$, 5% AcOH and H_2O, successively and dried over anhydrous Na_2SO_4. Evaporation of solvent gave 17β-acetoxy-4β,5β-epoxyestran-3-ol mesylates (IV) (*ca.* 2 g) as the semi-crystalline product. The crude product was submitted to further step without purification.

4β,5β-Epoxyestrane-3β,17β-diol 17-Acetate (IIIa)——To a solution of VII (150 mg) in MeOH (4 ml) were added 10% $NaHCO_3$ (0.7 ml) and H_2O (2 ml) and allowed to stand at room temperature for 20 min. The reaction mixture was extracted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crystalline product obtained was recrystallized from hexane–ether to give IIIa (100 mg) as colorless needles. mp 123—124°. $[\alpha]_D^{18}$ −34.6° (c=0.26). *Anal.* Calcd. for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04. Found: C, 71.98; H, 9.09. NMR (5% solution in $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 2.02 (3H, s, 17β-$OCOCH_3$), 3.13 (1H, d, J=4 cps, 4-H), 4.05 (1H, m, 3α-H), 4.61 (1H, t, J=8 cps, 17α-H).

17β-Acetoxy-4β,5β-epoxyestran-3β-ol Mesylate (IVa)——To a solution of IIIa (260 mg) in pyridine (2 ml) was added $MeSO_2Cl$ (0.15 ml) under ice-cooling and allowed to stand at 0° for 1.5 hr. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$, 5% AcOH and H_2O, successively and dried over anhydrous Na_2SO_4. After evaporation of solvent the crystalline product obtained was recrystallized from acetone–hexane to give IVa (300 mg) as colorless needles. mp 97—100° (decomp.). $[\alpha]_D^{17}$ −38.9° (c=0.45). NMR (5% solution in $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 2.03 (3H, s, 17β-$OCOCH_3$), 3.10 (3H, s, 3β-OSO_2CH_3), 3.25 (1H, d, J=4 cps, 4α-H), 4.61 (1H, t, J=8 cps, 17α-H), 5.13 (1H, m, 3α-H). This compound was unstable even in the crystalline state and therefore submitted to further step without elemental analysis.

Reaction of IV with Lithium Carbonate in Dimethylformamide——To a solution of IV (503 mg) in DMF (30 ml) was added Li_2CO_3 (500 mg) and heated at 135—140° for 2 hr. The reaction mixture was diluted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue obtained was submitted to preparative TLC using benzene–ether (10: 1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.60) was eluted with ether. Recrystallization of the eluate from hexane gave 4β,5β-epoxyestr-2-en-17β-ol acetate (V) (36 mg) as colorless needles. mp 134—135°. $[\alpha]_D^{20}$ −16.7° (c=0.20). *Anal.* Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.62; H, 8.88. NMR (5% solution in $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 2.02 (3H, s, 17β-$OCOCH_3$), 2.98 (1H, d, d, J=2.5, 3.5 cps, 4α-H), 4.60 (1H, t, J=8 cps, 17α-H), 5.73 (2H, m, 2- and 3-H).

The adsorbent corresponding to the spot (*Rf* 0.87) was eluted with ether. Recrystallization of the eluate from MeOH gave 4a-oxa-A-homoestra-3,5-dien-17β-ol acetate (VIb) (68 mg) as colorless needles. mp 122—123°. $[\alpha]_D^{17}$ +30.7° (c=0.13). *Anal.* Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.88; H, 9.04. NMR (5% solution in $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 2.02 (3H, s, 17β-$OCOCH_3$), 4.60 (1H, t, J=8 cps, 17α-H), 4.65 (1H, m, 3-H), 5.24 (1H, m, 6-H), 6.20 (1H, d, d, J=7, 2 cps, 4-H).

The adsorbent corresponding to the spot (*Rf* 0.47) was eluted with ether. Recrystallization of the eluate from MeOH gave 17β-acetoxy-4β,5β-epoxyestran-3β-ol formate (VII) (126 mg) as colorless needles. mp 112—114°. $[\alpha]_D^{17}$ −52.3° (c=0.22). *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.27; H, 8.33. NMR (5% solution in $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 2.02 (3H, s, 17β-$OCOCH_3$), 3.15 (1H, d, J=4 cps, 4α-H), 4.60 (1H, t, J=8 cps, 17α-H), 5.22 (1H, m, 3α-H), 8.05 (1H, s, 3β-OCOH).

4a-Oxa-A-homoestra-3,5-dien-17β-ol (VIa)——To a solution of VIb (30 mg) in anhydrous ether (3 ml) was added $LiAlH_4$ (30 mg) portionwise and allowed to stand at room temperature for 1 hr. After decomposition of the excess reagent with moist ether, 25% Rochelle salt solution was added and extracted with ether. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crystalline product obtained was recrystallized from hexane to give VIa (20 mg) as colorless silky needles. mp 102—105°. $[\alpha]_D^{18}$ +62.5° (c=0.32). *Anal.* Calcd. for $C_{18}H_{26}O_2$: C, 78.99; H, 9.55. Found: C, 78.26; H, 9.58. NMR (5% solution in $CDCl_3$) δ: 0.78 (3H, s, 18-CH_3), 3.66 (1H, t, J=

8 cps, 17α-H), 4.65 (1H, m, 3-H), 5.25 (1H, m, 5-H), 6.13 (1H, d, d, J=7,2 cps, 4-H). IR ν_{max}^{KBr} cm^{-1}: 3270 (OH), 1653, 1670 (C=C), 1170 (C-O-C). Reacetylation of VIa with Ac_2O and pyridine in the usual manner gave VIb.

Estr-2-ene-5β,17β-diol (VIII)——To a solution of V (28 mg) in THF (3 ml) was added $LiAlH_4$ (30 mg) portionwise and refluxed for 5 hr. After decomposition of the excess reagent with moist ether, 25% Rochelle salt solution was added and extracted with AcOEt. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crystalline product obtained was recrystallized from acetone–hexane to give VIII (20 mg) as colorless needles. mp 174—176°. $[\alpha]_D^{16}$+42.9° (c=0.20). *Anal.* Calcd. for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 77.72; H, 10.27. NMR (5% solution in $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 3.61 (1H, t, J=8 cps, 17α-H), 5.60 (2H, broad s, $W1/2$=3 cps, 2- and 3-H).

Epoxidation of VIII——To a solution of VIII (200 mg) in AcOEt (20 ml) was added *m*-chloroperbenzoic acid (300 mg) and allowed to stand at room temperature for 15 hr. The resulting solution was diluted with AcOEt and washed with 10% Na_2SO_3, H_2O and dried over anhydrous Na_2SO_4. After usual work-up the oily residue obtained was submitted to preparative TLC using benzene–ether (1: 4) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.41) with $CHCl_3$ and recrystallization of the eluate from acetone–hexane gave 2β,3β-epoxyestrane-5β,17β-diol (IX) (130 mg) as colorless needles. mp 126—128°. $[\alpha]_D^{17}$+30.0° (c=0.10). *Anal.* Calcd. for $C_{18}H_{28}O_3 \cdot 1/2H_2O$: C, 71.72; H, 9.70. Found: C, 71.70; H, 9.73. NMR (5% solution in $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 3.25 (2H, m, 2α- and 3α-H), 3.60 (1H, t, J=8 cps, 17α-H).

Elution of the adsorbent corresponding to the spot (Rf 0.27) with $CHCl_3$ and recrystallization of the eluate from acetone–hexane gave 2α,3α-epoxyestrane-5β,17β-diol (X) (50 mg) as colorless needles. mp 206—207°. $[\alpha]_D^{17}$+30.6° (c=0.20). *Anal.* Calcd. for $C_{18}H_{28}O_3$: C, 73.93; H, 9.65. Found: C, 73.57; H, 9.80. NMR (5% solution in $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 3.20 (2H, m, 2β- and 3β-H), 3.61 (1H, t, J=8 cps, 17α-H).

Reduction of II with Lithium Aluminum Hydride——To a solution of II (200 mg) in anhydrous THF (20 ml) was added $LiAlH_4$ (200 mg) portionwise and refluxed for 5 hr. After decomposition of the excess reagent with moist ether, 25% Rochelle salt solution was added and extracted with AcOEt. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC using ether as developing solvent. The adsorbent corresponding to the spots (Rf 0.59 and 0.32) was eluted with MeOH–AcOEt and the eluate was treated with Ac_2O and pyridine in the usual manner, respectively. The crude acetate derived from the less polar compound was submitted to preparative TLC using benzene–ether (10: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.17) with ether and recrystallization of the eluate from hexane gave estrane-3β,5β,17β-triol 3,17-diacetate (XIb) (60 mg) as colorless leaflets. mp 141—142°. $[\alpha]_D^{18}$+37.5° (c=0.20). *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.81; H, 9.02. NMR (5% solution in $CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 2.03 (3H, s, 17β-$OCOCH_3$), 2.08 (3H, s, 3β-$OCOCH_3$), 3.00 (1H, broad s, 5β-OH), 4.61 (1H, t, J=8 cps, 17α-H), 5.22 (1H, m, $W1/2$=7 cps, 3α-H).

The crude acetate derived from the more polar compound was submitted to preparative TLC using benzene–ether (10: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.10) with ether and recrystallization of the eluate from acetone–hexane gave estrane-3α,5β,17β-triol 3,17-diacetate (XIIb) (50 mg) as colorless leaflets. mp 176—178°. $[\alpha]_D^{16}$+28.0° (c=0.25). *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.58; H, 8.97. NMR (5% solution in $CDCl_3$) δ: 0.79 (3H, s, 18-CH_3), 2.02 (6H, s, 3α- and 17β-$OCOCH_3$), 4.60 (1H, t, J=8 cps, 17α-H), 5.10 (1H, m, $W1/2$=20 cps, 3β-H).

Estrane-3β,5β,17β-triol (XIa)——i) XIb (25 mg) was dissolved in 5% methanolic KOH (7 ml) and allowed to stand at room temperature for 16 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crystalline product obtained was recrystallized from MeOH–benzene to give XIa (15 mg) as colorless leaflets. mp 230—232°. $[\alpha]_D^{23}$+22.5° (c=0.10, MeOH). *Anal.* Calcd. for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27. Found: C, 73.45; H, 10.29.

ii) To a solution of IX (6 mg) in anhydrous THF (2 ml) was added $LiAlH_4$ (30 mg) portionwise and refluxed for 4 hr. After decomposition of the excess reagent with moist ether, 25% Rochelle salt solution was added and extracted with AcOEt. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crystalline product obtained was recrystallized from MeOH–benzene to give XIa (4 mg) as colorless leaflets. mp 229—231°. Mixed mp on admixture with the sample obtained in i) showed no depression and IR spectra of two samples were entirely identical in every respect.

iii) To a solution of IIIa (17 mg) in THF (5 ml) was added $LiAlH_4$ (50 mg) portionwise and refluxed for 4 hr. The reaction mixture was treated in the manner as described in ii). The crystalline product thus obtained was recrystallized from MeOH–benzene to give XIa (13 mg) as colorless leaflets. mp 226—228°. Mixed mp on admixture with the sample obtained in i) showed no depression and IR spectra of two samples were entirely identical in every respect.

iv) To a stirred solution of $LiAlH_4$ (20 mg) in anhydrous ether (2 ml) were added the solutions of VIII (10 mg) in anhydrous THF (1 ml) and of BF_3-etherate (170 mg) in ether (2 ml) at 0° over a period of 30 min under a stream of N_2 gas. After stirring at room temperature for 1 hr the excess reagent was decomposed

by careful ad .ition of moist ether. The organic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. Evaporation of solvent gave the oily residue. To a stirred solution of this product dissolved in THF (4 ml) were added 10% NaOH (1 ml) and 30% H_2O_2 (1 ml) and allowed to stand at 0° for 1 hr. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was separated, washed with 10% $NaHSO_3$, H_2O and dried over anhydrous Na_2SO_4. After usual work-up the semi-crystalline product obtained was submitted to preparative TLC using ether as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.59) with AcOEt and recrystallization of the eluate from MeOH–benzene gave XIa (4 mg) as colorless leaflets. mp 234—236°. Mixed mp on admixture with the sample obtained in i) showed no depression and IR spectra of two samples were entirely identical in every respect.

Estrane-3α,5β,17β-triol (XIIa)——XIIb (50 mg) was dissolved in 5% methanolic KOH (14 ml) and allowed to stand at room temperature for 16 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crystalline product obtained was recrystallized from MeOH–benzene to give XIIa (30 mg) as colorless needles. mp 212—214°. $[\alpha]_D^{16}$ +20.0° (c=0.10, MeOH). *Anal.* Calcd. for $C_{18}H_{30}O_3 \cdot \frac{1}{2}H_2O$: C, 71.25; H, 10.30. Found: C, 71.78; H, 10.47.

Estrane-3β,5β,17β-triol 3,5-Carbonate (XIII)——To a solution of XIa (50 mg) in pyridine (3 ml) and $CHCl_3$ (7 ml) was added 30% $COCl_2$ solution in toluene (2 ml) at −20° and allowed to stand at room temperature for 2 hr. The reaction mixture was poured onto ice–water and extracted with $CHCl_3$. The organic layer was washed with cold 5% HCl, 5% $NaHCO_3$ and H_2O, successively and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue was submitted to preparative TLC using ether as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.41) with $CHCl_3$ and recrystallization of the eluate from $CHCl_3$–hexane gave XIII (20 mg) as colorless needles. mp 283—285°. $[\alpha]_D^{22}$ +50.0° (c=0.13). *Anal.* Calcd. for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 70.85; H, 8.87. NMR (5% solution in $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 3.62 (1H, t, J=8 cps, 17α-H), 4.69 (1H, m, $W\frac{1}{2}$=9 cps, 3α-H). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1733 (C=O).

Estr-4-ene-3β,17β-diol Diacetate (XIVb)——To a solution of XIb (50 mg) in pyridine (2 ml) was added $SOCl_2$ (0.7 ml) at 0° and allowed to stand for 5 min. After decomposition of the excess reagent with ice–water the reaction mixture was extracted with $CHCl_3$, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue obtained was submitted to preparative TLC using benzene as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.10) was eluted with ether. Recrystallization of the eluate from hexane gave XIVb (35 mg) as colorless needles. mp 134—136°. $[\alpha]_D^{21}$ −20.0° (c=0.20). *Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.45; H, 9.10. NMR (5% solution in $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 2.02 (6H, s, 3β- and 17β-$OCOCH_3$), 4.59 (1H, t, J= 8 cps, 17α-H), 5.20 (1H, m, 3α-H), 5.30 (1H, s, 4-H). Hartman prepared this compound by the different method (reported: mp 139.2—140.2°).[14]

Estr-4-ene-3β,17β-diol (XIVa)——XIVb (35 mg) was dissolved in 5% methanolic KOH (5 ml) and allowed to stand at room temperature for 3 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the semi-crystalline product obtained was submitted to preparative TLC using hexane–AcOEt (5: 3) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.23) was eluted with $CHCl_3$. Recrystallization of the eluate from acetone–hexane gave XIVa (20 mg) as colorless needles. mp 167—169°. NMR (5% solution in $CDCl_3$) δ: 0.76 (3H, s, 18-CH_3), 3.61 (1H, t, J=8 cps, 17α-H), 4.15 (1H, m, 3α-H), 5.38 (1H, s, 4-H). Hartman prepared this compound by the different method (reported: mp 169.4—170.6°).[14]

Estr-4-ene-3α,17β-diol Diacetate (XVb)——To a solution of XIIb (30 mg) in pyridine (2 ml) was added $SOCl_2$ (0.7 ml) at 0° and allowed to stand for 5 min. After decomposition of the excess reagent with ice–water the reaction mixture was extracted with $CHCl_3$, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue obtained was submitted to preparative TLC using benzene as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.11) was eluted with ether. Recrystallization of the eluate from hexane gave XVb (20 mg) as colorless needles. mp 111—112°. $[\alpha]_D^{23}$ +155.0° (c=0.10). *Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.57; H, 9.26. NMR (5% solution in $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 2.02 (6H, s, 3α- and 17β-$OCOCH_3$), 4.59 (1H, t, J= 8 cps, 17α-H), 5.13 (1H, m, 3β-H), 5.50 (1H, d, J=5 cps, 4-H). Hartman prepared this compound by the different method (reported: mp 112—113.5°).[14]

Estr-4-ene-3α,17β-diol (XVa)——XVb (37 mg) was dissolved in 5% methanolic KOH (5ml) and allowed to stand at room temperature for 3 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the semi-crystalline product obtained was submitted to preparative TLC using hexane–AcOEt (5: 3) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.15) was eluted with $CHCl_3$. Recrystallization of the eluate from acetone–hexane gave XVa (20 mg) as colorless needles. mp 205—206°. NMR (5% solution in $CDCl_3$) δ: 0.77 (3H,

14) J.A. Hartman, *J. Am. Chem. Soc.*, **77**, 5151 (1955).

s, 18-CH_3), 3.65 (1H, t, J=8 cps, 17α-H), 4.14 (1H, m, 3β-H), 5.55 (1H, d, J=5 cps, 4-H). Hartman prepared this compound by the different method (reported: mp 206—207.8°).[14]

Oxidation of XIVa and XVa with Chromium Trioxide-Pyridine Complex——i) To a solution of XIVa (15 mg) in pyridine (1 ml) was added CrO_3–pyridine complex (0.5 ml) and allowed to stand at room temperature for 5 hr. The reaction mixture was extracted with AcOEt, washed with 10% AcOH, 5% $NaHCO_3$ and H_2O, successively and dried over anhydrous Na_2SO_4. After evaporation of solvent the crystalline product obtained was submitted to preparative TLC using hexane–AcOEt (5: 3) as developing solvent. The adsorbent corresponding to the spot (Rf 0.26) was eluted with $CHCl_3$. Recrystallization of the eluate from acetone–hexane gave estr-4-ene-3,17-dione (XVI) (8 mg) as colorless leaflets. mp 168—169°. Mixed mp on admixture with the authentic sample showed no depression and IR spectra of two samples were entirely identical in every respect.

ii) Treatment of XVa (15 mg) with CrO_3–pyridine complex (0.5 ml) in pyridine (1 ml) in the manner as described in i). Recrystallization from acetone–hexane gave XVI (8 mg) as colorless leaflets. mp 168—169°. Mixed mp on admixture with the authentic sample showed no depression and IR spectra of two samples were entirely identical in every respect.

2α-Deuterioestrane-3β,5β,17β-triol (XVIIa)——To a solution of IX (130 mg) in anhydrous THF (40 ml) was added $LiAlD_4$ (130 mg) portionwise and refluxed for 3 hr. After decomposition of the excess reagent with moist ether, 25% Rochelle salt solution was added and extracted with AcOEt. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crystalline product obtained was recrystallized from MeOH–benzene to give XVIIa (80 mg) as colorless needles. mp 227—228°. Mixed mp on admixture with XIa showed no depression.

2α-Deuterioestrane-3β,5β,17β-triol 3,17-Diacetate (XVIIb)——Treatment of XVIIa (80 mg) with Ac_2O (1 ml) and pyridine (2 ml) in the usual manner followed by recrystallization from hexane gave XVIIb (60 mg) as colorless needles. mp 141—142°. Mixed mp on admixture with XIb showed no depression.

2α-Deuterioestr-4-ene-3β,17β-diol Diacetate (XVIIIb)——XVIIb (80 mg) was treated with $SOCl_2$ (1.3 ml) and pyridine (3 ml) in the same manner as described in XIVb. The crude product was purified by preparative TLC in the system hexane–benzene (1: 10) to give XVIIIb (50 mg).

2α-Deuterioestr-4-ene-3β,17β-diol (XVIIIa)——XVIIIb (50 mg) was treated with 5% methanolic KOH (4 ml) in the same manner as described in XIVa to give XVIIIa (38 mg).

2α-Deuterioestr-4-ene-3,17-dione (XIX)——XVIIIa (38 mg) was treated with CrO_3–pyridine (0.5 ml) complex in the same manner as described in XVI. Recrystallization from acetone–hexane gave XIX (14 mg) as colorless leaflets. mp 167—168°. Mixed mp on admixture with XVI showed no depression. IR ν_{max}^{KBr} cm^{-1}: 1670, 1738 (C=O). NMR ($CDCl_3$ solution) δ: 0.93 (3H, s, 18-CH_3), 5.82, (1H, broad s, 4-H). Mass Spectrum m/e: 273 (M^+).

2β-Deuterioestrane-3β,5β,17β-triol (XXa)——To a stirred solution of $LiAlD_4$ (110 mg) in anhydrous ether (5 ml) were added the solutions of VIII (50 mg) in anhydrous THF (6 ml) and of BF_3-etherate (1.3 g) in ether (8 ml) at 0° over a period of 30 min under a stream of N_2 gas. After stirring at room temperature for 1 hr the excess reagent was decomposed by careful addition of moist ether. The orgaic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. Evaporation of solvent gave the oily residue. To a stirred solution of this product dissolved in THF (10 ml) were added 10% NaOH (6 ml) and 30% H_2O_2 (6 ml) and allowed to stand at 0° for 1 hr. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was separated, washed with 10% $NaHSO_3$, H_2O and dried over anhydrous Na_2SO_4. After usual work-up the semi-crystalline product obtained was submitted to preparative TLC using ether as developing solvent. The adsorbent corresponding to the spot (Rf 0.59) was eluted with AcOEt. The eluate was homogeneous and therefore subjected to further step without recrystallization.

2β-Deuterioestrane-3β,5β,17β-triol 3,17-Diacetate (XXb)——Treatment of XXa (20 mg) with Ac_2O (0.5 ml) and pyridine (1 ml) followed by recrystallization from hexane gave XXb (16 mg) as colorless leaflets. mp 140—142°. Mixed mp on admixture with XIb showed no depression.

2β-Deuterioestr-4-ene-3β,17β-diol Diacetate (XXIb)——XXb (15 mg) was treated with $SOCl_2$ (0.2 ml) and pyridine (0.5 ml) in the same manner as described in XIVb. The crude product was purified by preparative TLC in the system hexane–benzene (1: 10) to give XXIb (10 mg).

2β-Deuterioestr-4-ene-3β,17β-diol (XXIa)——XXIb (10 mg) was treated with 5% methanolic KOH (2 ml) in the same manner as described in XIVa to give XXIa (8 mg).

2β-Deuterioestr-4-ene-3,17-dione (XXII)——XXIa (8 mg) was treated with CrO_3–pyridine complex in the same manner as described in XVI. Recrystallization from acetone–hexane gave XXII (3 mg) as colorless leaflets. mp 170—171°. Mixed mp on admixture with XVI showed no depression. IR ν_{max}^{KBr} cm^{-1}: 1670, 1738 (C=O). NMR ($CDCl_3$ solution) δ: 0.93 (3H, s, 18-CH_3), 5.82 (1H, broad s, 4-H). Mass Spectrum m/e: 273 (M^+).

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