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Studies on Metabolism of 3-Desoxyestrone. III. Synthesis of 6-Oxygenated Estra-1,3,5(10)-trienes and Related Compounds[1,2]

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In the previous paper we have reported the characterization of several phenolic and neutral metabolites isolated from the rabbit urine after administration of 3-desoxyestrone.[4] Among these metabolites 6β-hydroxy-3-desoxyestrone, 3-desoxy-17α-estradiol and 17α-estradiol 17-monoacetate have not yet been reported in the literature. The present paper describes the synthesis of these compounds for the purpose of direct comparison with the urinary metabolites.

The initial project was directed to the preparation of two epimeric 6-hydroxy-3-desoxyestrone starting from 3-desoxyestrone (I). Oxidation with chromium trioxide in acetic acid[5] gave the diketone (II), whose ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectra justified the putting of the newly introduced oxo group at C–6. Catalytic reduction of the 6,17-diketone with platinum oxide afforded the 6β,17β-dihydroxy derivative (IIIa) accompanied with the 17β-hydroxy-6-ketone (IVa). On the other hand reduction with sodium borohydride gave the 6α,17β-dihydroxy derivative (VIa) as a sole product, which was evidently distinguishable from the above-mentioned reduction product. Structural assignment of the two C–6-epimers was confirmed by inspection of the nuclear magnetic resonance spectra upon leading to the diacetates. The C–6 proton signal of the 6β-acetate (IIIb) was observed as a doublet (J=4 cps) at 6.06 ppm, whereas the corresponding resonance in the C–6-epimer (VIb) as a triplet (J=6, 9 cps) at 6.09 ppm. In addition, the methyl signal of acetyl group of VIb appeared at the more downfield region than that of IIIb. These results strongly suggest that the C–6-acetoxyl group of VIb must be influenced by the deshielding effect of the benzene ring. In consequence, the β-configuration should be assigned to the hydroxyl function at C–6 in IIIa. It has already been reported that sodium borohydride reduction of the 6-oxoestrogen gave solely the 6α-hydroxy derivative, while catalytic hydrogenation with platinum oxide the 6β-epimer.[6] The present results are consistent with the previous findings in respect to the stereospecificity, although the starting material lacks the oxygen function at C–3.

Treatment of IIIa with a limited amount of benzoyl chloride in pyridine gave the 6-monobenzoate (IIIc) together with the dibenzoate (IIId), whose separation could effectively be attained by column chromatography. Oxidation of the monobenzoate with Jones reagent[7]

1) This paper constitutes Part XXV of the series entitled "Analytical Chemical Studies on Steroids"; Part XXIV: T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1200 (1969).
2) Following trivial names are used: 3-desoxyestrone, estra-1,3,5(10)-trien-17-one; 3-desoxyestradiol, estra-1,3,5(10)-trien-17β-ol; 3-desoxy-17α-estradiol, estra-1,3,5(10)-trien-17α-ol.
3) Location: *Aobayama, Sendai.*
4) *a*) T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1200 (1969); *b*) *Idem, ibid.*, **16**, 383 (1968); *c*) T. Nambara, M. Numazawa and H. Takahashi, *ibid.*, **16**, 1148 (1968).
5) B. Longwell and O. Wintersteiner, *J. Biol. Chem.*, **133**, 219 (1940).
6) O. Wintersteiner and M. Moore, *J. Am. Chem. Soc.*, **81**, 422 (1959); O. Wintersteiner, M. Moore and A.I. Cohen, *J. Org. Chem.*, **29**, 1325 (1964).
7) K. Bowden, I.M. Heilbron, E.R.H. Jones and B.C.L. Weedon, *J. Chem. Soc.*, **1946**, 39.

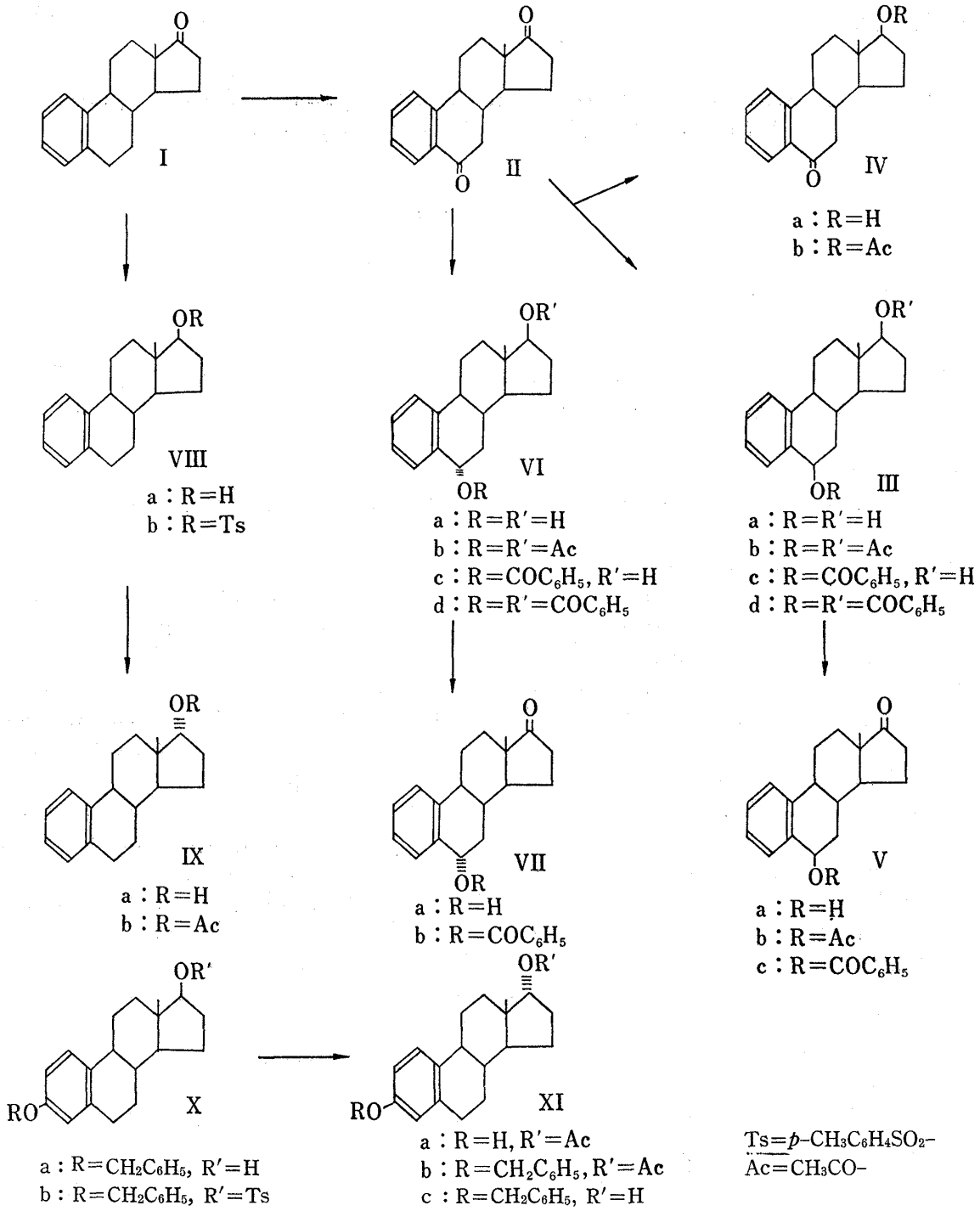

Chart 1

furnished the 6β-benzoyloxy-17-ketone (Vc), which in turn was led to the desired 6β-hydroxy-3-desoxyestrone (Va) by alkaline hydrolysis.

The preparation of the epimeric 6α-hydroxy-3-desoxyestrone (VIIa) was accomplished by the same reaction sequence. Usual benzoylation of VIa gave the 6-monobenzoate (VIc) and the dibenzoate (VId), which were effectively separated by preparative thin-layer chromatography. The monobenzoate was then oxidized with Jones reagent to give the 17-ketone (VIIb), which on hydrolysis with alkali was led to 6α-hydroxy-3-desoxyestrone (VIIa). The two epimeric 6-hydroxy-3-desoxyestrones were similar in respect to infrared spectra and chromatographic behaviors, but their trimethylsilyl derivatives could distinctly be separated

by gas–liquid chromatography. Of these two the 6β-epimer proved to be entirely identical with the metabolite in every respect.

It has been reported that the 17α-hydroxylated steroids, namely 3-desoxy-17α-estradiol, 17α-estradiol and its 17-acetate, were excreted as the principal metabolites of 3-desoxyestrone in rabbit. Therefore, preparation of 3-desoxy-17α-estradiol was carried out starting from 3-desoxyestradiol (VIIIa). When the 17β-tosylate (VIIIb) was refluxed in N-methylpyrrolidone with tetrabutylammonium acetate,[8] an inversion of the C-17-substituent did take place to give the 17α-acetoxy derivative (IXb). Subsequent hydrolysis provided the desired compound, 3-desoxy-17α-estradiol (IXa).

Synthesis of 17α-estradiol 17-monoacetate was then performed. The occurrence of this novel metabolite is indicative of the existence of the steroid acetylase in rabbit. Estradiol 3-benzyl ether (Xa) was converted to the 17β-tosylate (Xb), which was then transformed into the 17α-ol acetate (XIb) in the same way as mentioned above. Hydrogenolysis with palladium-on-charcoal gave rise to debenzylation to furnish 17α-estradiol 17-monoacetate (XIa).

Thus, the proposed structures for metabolites II, VIII and VI[4a] were unequivocally established by direct comparison with these synthetic samples, respectively.

Experimental[9]

Estra-1,3,5(10)-triene-6,17-dione (II)——To a stirred solution of I (200 mg) in AcOH (1 ml) was added dropwise a solution of CrO_3 (700 mg) in 90% AcOH (7 ml) under ice-cooling and the resulting solution was allowed to stand at room temperature for 24 hr. After decomposition of excess CrO_3 by addition of EtOH (1 ml) the reaction mixture was poured into ice-water, extracted with ether, washed with $NaHCO_3$ solution and H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent repeated recrystallization of the product from MeOH gave II (29 mg) as colorless prisms. mp 181.5—182°. $[\alpha]_D^{16}$ +57.0° (c=0.35). *Anal.* Calcd. for $C_{18}H_{20}O_2$: C, 80.56; H, 7.51. Found: C, 80.78; H, 7.64. IR ν_{max}^{KBr} cm^{-1}: 1675 (conjugated C=O), 1730 (5-membered ring C=O). UV λ_{max}^{EtOH} mμ (ε): 252 (17000), 294 (2200). NMR ($CDCl_3$ solution) δ: 0.92 (3H, s, 18-CH_3), 8.08 (1H, d, J=7 cps, 4-H).

Catalytic Reduction of II with Platinum Oxide——A solution of II (300 mg) in EtOH (25 ml) was shaken with PtO_2 (240 mg) under a stream of H_2 for 5 hr. After usual work-up the crystalline product was chromatographed on Al_2O_3. Recrystallization of the 1st eluate from acetone gave 17β-hydroxyestra-1,3,5(10)-trien-6-one (IVa) (100 mg) as colorless plates. mp 143—144°. $[\alpha]_D^{25}$ −4.5° (c=0.22). *Anal.* Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 80.38; H, 8.42. NMR ($CDCl_3$ solution) δ: 0.80 (3H, s, 18-CH_3), 3.74 (1H, t, J=9, 11 cps, 17α-H), 8.05 (1H, d, J=7 cps, 4-H). Recrystallization of the 2nd eluate from ether gave estra-1,3,5(10)-triene-6β,17β-diol (IIIa) (180 mg) as colorless needles. mp 111—113°/157—158°. $[\alpha]_D^{25}$ +98.3° (c=0.14). *Anal.* Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 80.07; H, 8.06.

Estra-1,3,5(10)-triene-6β,17β-diol Diacetate (IIIb)——Treatment of IIIa with Ac_2O and pyridine followed by recrystallization from MeOH gave IIIb as colorless prisms. mp 149—151°. $[\alpha]_D^{26}$ +60.9° (c=0.16). *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.12; H, 7.67. NMR ($CDCl_3$ solution) δ: 0.85 (3H, s, 18-CH_3), 2.04 (6H, s, 6β-$OCOCH_3$, 17β-$OCOCH_3$), 4.70 (1H, t, J=7, 8 cps, 17α-H), 6.06 (1H, d, J=4 cps, 6α-H), 7.28 (4H, aromatic ring).

17β-Hydroxyestra-1,3,5(10)-trien-6-one Acetate (IVb)——Treatment of IVa with Ac_2O and pyridine followed by recrystallization from aq. acetone gave IVb as colorless prisms. mp 222—223°. $[\alpha]_D^{26}$ −28.1° (c=0.14). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.75; H, 7.58.

Benzoylation of IIIa——To a solution of IIIa (100 mg) in pyridine (1.5 ml) was added C_6H_5COCl (0.2 ml) dropwise under cooling in ice-water and allowed to stand at room temperature for 50 min. The reaction mixture was diluted with ether, washed with 5% $NaHCO_3$, 5% HCl and H_2O, successively and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily product was chromatographed on Al_2O_3. Elution with benzene gave estra-1,3,5(10)-triene-6β,17β-diol dibenzoate (IIId) (8 mg) as colorless oil. Further elution with benzene and recrystallization of the eluate from acetone gave estra-1,3,5(10)-triene-6β,17β-diol 6-

8) H.B. Henbest and W.R. Jackson, *J. Chem. Soc.*, **1962**, 954.

9) All melting points were taken on a 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 measured on Hitachi Model H-60 spectrometer at 60 Mc in $CDCl_3$; the chemical shifts are quoted as ppm downfield from $(CH_3)_4Si$, an internal standard. For thin-layer chromatography (TLC) silica gel H (E. Merck AG) was used as adsorbent.

benzoate (IIIc) (35 mg) as colorless prisms. mp 140—142°. $[\alpha]_D^{23}$ +103.8° (c=0.14). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 80.13; H, 7.39.

6β-Hydroxy-1,3,5(10)-trien-17-one (Va)——To a stirred solution of IIIc (30 mg) in acetone (2 ml) was added Jones reagent (0.1 ml) under ice-cooling and allowed to stand at 0° for 10 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. On evaporation of solvent the oily residue was obtained. Unfortunately 6β-hydroxyestra-1,3,5(10)-trien-17-one benzoate (Vc) could not be crystallized and therefore submitted to further elaboration without purification. A solution of Vc (30 mg) dissolved in 2.5% methanolic KOH (5 ml) was allowed to stand at room temperature for 5 hr. After usual work-up the crude product was recrystallized from acetone to give Va (21 mg) as colorless needles. mp 220—223°. $[\alpha]_D^{24}$ +174.3° (c=0.11). *Anal.* Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 80.51; H, 8.05. IR ν_{max}^{KBr} cm^{-1}: 1723 (5-membered ring C=O), 1403 (active CH_2), 3500 (OH).

6β-Hydroxyestra-1,3,5(10)-trien-17-one Acetate (Vb)——Treatment of Va with Ac_2O and pyridine followed by recrystallization from aq. acetone gave Vb as colorless plates. mp 176—179°. $[\alpha]_D^{23}$ +159.0° (c=0.15). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.91; H, 7.65. NMR ($CDCl_3$ solution) δ: 0.94 (3H, s, 18-CH_3), 2.06 (3H, s, 6β-$OCOCH_3$), 6.10 (1H, d, J=4 cps), 7.33 (4H, aromatic ring).

Estra-1,3,5(10)-triene-6α,17β-diol (VIa)——To a solution of II (45 mg) in MeOH (5 ml) was added $NaBH_4$ (35 mg) portionwise under ice-cooling and allowed to stand at room temperature for 30 min. After addition of a few drops of AcOH the resulting solution was extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product (47 mg) was recrystallized from acetone to give VIa (29 mg) as colorless needles. mp 148—150°. $[\alpha]_D^{15}$ +83.4° (c=0.37). *Anal.* Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 79.69; H, 8.80.

Estra-1,3,5(10)-triene-6α,17β-diol Diacetate (VIb)——Treatment of VIa with Ac_2O and pyridine followed by recrystallization from MeOH gave VIb as colorless powders. mp 114—115°. $[\alpha]_D^{25}$ +38.7° (c=0.17). *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.38; H, 8.25. NMR ($CDCl_3$ solution) δ: 0.81 (3H, s, 18-CH_3), 2.04 (3H, s, 17β-$OCOCH_3$), 2.11 (3H, s, 6α-$OCOCH_3$), 4.69 (1H, t, J=7, 8 cps, 17α-H), 6.09 (1H, t, J=6, 9 cps, 6β-H), 7.28 (4H, aromatic ring).

Benzoylation of VIa——To a solution of VIa (100 mg) in pyridine (2 ml) was added C_6H_5COCl (0.2 ml) dropwise under ice-cooling and allowed to stand at room temperature for several min. The reaction mixture was extracted with ether, washed with 5% HCl, H_2O and 5% $NaHCO_3$, successively and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue was submitted to preparative TLC using benzene as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.14) and recrystallization of the eluate from MeOH gave estra-1,3,5(10)-triene-6α,17β-diol 6-benzoate (VIc) (11 mg) as colorless needles. mp 178—180°. $[\alpha]_D^{7}$ +75.5° (c=0.15). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.58; H, 7.45. Elution of the adsorbent corresponding to the spot (*Rf* 0.62) and recrystallization of the eluate from benzene gave estra-1,3,5(10)-triene-6α,17β-diol dibenzoate (VId) as colorless needles. mp 157.5—159.5°. $[\alpha]_D^{18}$ +49.9° (c=0.10). *Anal.* Calcd. for $C_{32}H_{32}O_4$: C, 79.97; H, 6.71. Found: C, 80.14; H, 6.99.

6α-Hydroxyestra-1,3,5(10)-trien-17-one Benzoate (VIIb)——To a solution of VIc (13 mg) in acetone (2 ml) was added Jones reagent (3 drops) 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 was recrystallized from MeOH to give VIIb (7 mg) as colorless needles. mp 200—204°. $[\alpha]_D^{18}$ +141.6° (c=0.12). *Anal.* Calcd. for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00. Found: C, 80.38; H, 6.92.

6α-Hydroxyestra-1,3,5(10)-trien-17-one (VIIa)——Hydrolysis of VIIb with 2.5% methanolic KOH in the usual manner and recrystallization of the hydrolyzate from acetone gave VIIa as colorless plates. mp 214—216°. $[\alpha]_D^{25}$ +149.5° (c=0.04). *Anal.* Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 79.93; H, 8.05. IR ν_{max}^{KBr} cm^{-1}: 1725 (5-membered ring C=O), 1404 (active CH_2), 3585 (OH).

Estra-1,3,5(10)-trien-17β-ol *p*-Toluenesulfonate (VIIIb)——To a solution of VIIIa (1 g) in pyridine (15 ml) was added *p*-TsCl (1.8 g) and stirred at 0° for 5 hr and then at room temperature for 3 days. The reaction mixture was poured into ice-water and the precipitate was filtered and washed with H_2O. Recrystallization from AcOEt gave VIIIb (1.17 g) as colorless needles. mp 177—178°. $[\alpha]_D^{14}$ +5.0° (c=0.42). *Anal.* Calcd. for $C_{25}H_{30}O_3S$: C, 73.15; H, 7.37. Found: C, 73.39; H, 7.43.

Estra-1,3,5(10)-trien-17α-ol Acetate (IXb)——A solution of VIIIb (200 mg) in N-methylpyrrolidone (4 ml) was heated at 160° with tetrabutylammonium acetate (1.2 g) for 2.5 hr. The reaction mixture was poured into ice-water and extracted with ether. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent the oily residue was submitted to preparative TLC using benzene as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.64) and recrystallization of the eluate from MeOH gave IXb (60 mg) as colorless needles. mp 158—161°. $[\alpha]_D^{14}$ +35.5° (c=0.51). *Anal.* Calcd. for $C_{20}H_{26}O_2$: C, 80.49; H, 8.78. Found: C, 80.46; H, 8.82. NMR ($CDCl_3$ solution) δ: 0.80 (3H, s, 18-CH_3), 2.05 (3H, s, 17α-$OCOCH_3$), 4.88 (1H, d, J=6 cps, 17β-H).

Estra-1,3,5(10)-trien-17α-ol(IXa)——Hydrolysis of IXb with 2.5% methanolic KOH and recrystallization of the hydrolyzate from acetone–hexane gave IXa as colorless needles. mp 158—161°. $[\alpha]_D^{20}$ +44.8° (c=0.17). *Anal.* Calcd. for $C_{18}H_{24}O$: C, 84.32; H, 9.44. Found: C, 84.41; H, 9.38.

3-Benzyloxyestra-1,3,5 (10)-trien-17β-ol *p*-Toluenesulfonate (Xb)——To a solution of Xa (640 mg) in pyridine (18 ml) was added *p*-TsCl (1.94 g) and the reaction mixture was treated in the same manner as described in VIIIb. Recrystallization from ether gave Xb (578 mg) as colorless plates. mp 116—117°. $[\alpha]_D^{19}$+10.2° (c=0.78). *Anal.* Calcd. for $C_{32}H_{36}O_4S$: C, 74.39; H, 7.02. Found: C, 74.12; H, 7.10.

3-Benzyloxyestra-1,3,5(10)-trien-17α-ol Acetate (XIb)——A solution of Xb (300 mg) in N-methylpyrrolidone (5 ml) was heated at 160° with tetrabutylammonium acetate (1.5 g) for 7 hr. The reaction mixture was treated in the same manner as described in IXb. The crude product was submitted to preparative TLC using benzene as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.70) and recrystallization of the eluate from EtOH gave XIb (93 mg) as colorless leaflets. mp 99—100°. $[\alpha]_D^{18}$ +30.4° (c=0.39). *Anal.* Calcd. for $C_{27}H_{32}O_3$: C, 80.16; H, 7.97. Found: C, 80.19; H, 7.87. Elution of the adsorbent corresponding to the spot (*Rf* 0.92) and recrystallization of the eluate from EtOH gave 3-benzyloxyestra-1,3,5(10),16-tetraene (27 mg) as colorless needles. mp 74—77°. $[\alpha]_D^{14}$ +57.1° (c=0.21). *Anal.* Calcd. for $C_{25}H_{28}O$: C, 87.16; H, 8.19. Found: C, 86.91; H, 7.97.

Estra-1,3,5(10)-triene-3,17α-diol 17-Acetate (XIa)——A solution of XIb (85 mg) in EtOH (30 ml) was shaken with 5% Pd/C (60 mg) under a stream of H_2 overnight. After removal of the catalyst by filtration the filtrate was concentrated to give the crystalline product. Recrystallization from aq. MeOH gave XIa (38 mg) as colorless prisms. mp 188.5—190°. $[\alpha]_D^{19}$ +38.5° (c=0.44). *Anal.* Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.10; H, 8.27.

3-Benzyloxyestra-1,3,5(10)-trien-17α-ol (XIc)——Hydrolysis of XIb with 2.5% methanolic KOH and recrystallization of the hydrolyzate from acetone–hexane gave XIc as colorless prisms. mp 99—100°. $[\alpha]_D^{18.5}$ +33.3° (c=0.21). *Anal.* Calcd. for $C_{25}H_{30}O_2$: C, 82.83; H, 8.34. Found: C, 82.90; H, 8.30.

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Studies on the Syntheses of Heterocyclic Compounds. CCCXXI.[1] Synthesis of Isoquinoline Derivatives having Twenty-four Membered Ring System by Ullmann Reaction

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In a previous paper[3] we have reported that Ullmann reaction of 1-phenylpropylisoquinoline derivative (I) gave only twenty-two membered ring compounds (Va—b) as an intermolecular condensation product, but no intramolecular condensation product (III) was obtained. Therefore, Ullmann reaction of 1-[4-(3-bromo-4-methoxyphenyl)butyl]-1,2,3,4-tetrahydro-7-hydroxy-6-methoxy-2-methylisoquinoline (II) having one more methylene group than the compound (I) was carried out, in order to examine whether both inter- and intramolecular

1) Part CCCXX: T. Kametani, K. Ohkubo, and S. Takano, *Yakugaku Zasshi*, **89**, 1048 (1969).
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3) T. Kametani, H. Iida, and S. Tanaka, *Yakugaku Zasshi*, **89**, 230 (1969).