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Studies on Metabolism of 3-Desoxyestrone. I. Synthesis of 16,17-Oxygenated Estra-1,3,5(10)-trienes[1]

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For the studies on the fate of 3-desoxyestrone in man, estra-1,3,5(10)-trienes having oxygen functions at C-16 and/or C-17, its possible metabolites, have been synthesized as shown in Chart 1.

Efficacy of 3-desoxyestrone (estra-1,3,5(10)-trien-17-one) (I) in clinical states associated with hypercholesterolemia has been observed, and it is now widely used for lipid-shifting drug with loss of the undesirable ferminizing effect.[3] However, the metabolism of this drug has not yet been clarified. Hence, the authors have attempted to isolate and characterize the metabolites for elucidation of the fate of this compound in man. The present paper deals with the synthesis of estra-1,3,5(10)-trienes having oxygen functions at C-16 and/or C-17 as the possible metabolites.

The desired compounds were prepared according to the method developed by Gallagher and his co-workers.[4] First, Δ^{16}-enol acetate[5] (II) obtained from I was oxidized with perbenzoic acid to the corresponding epoxy derivative (III), which in turn was converted to 16α-hydroxy-17-ketone (IVa) and its acetate (IVb) by treatment with sulfuric acid followed by usual acetylation. Reduction of IVa with lithium aluminum hydride furnished 16α,17β-dihydroxy derivatives (Va, Vb). When IVa was submitted to ketol rearrangement with base, 16-oxo-17β-hydroxy compounds (VIa, VIb) were afforded in satisfactory yield. Treatment of VIb with lithium aluminum hydride yielded 16β,17β-dihydroxy derivatives (VIIa, VIIb) accompanied by a small amount of by-product, whose *cis*-glycol structure was confirmed by formation of acetonide (VIII). Another 16-hydroxy-17-oxo compound (XIV) was prepared from II by lead tetraacetate oxidation.

The synthesis of the remaining *cis*-16,17-glycols (XIIIa, XIIIb) was achieved by way of Δ^{16}-compound. By the method of Caglioti, *et al.*[6] I was transformed into *p*-tosylhydrazone (XI), which was then submitted to reductive cleavage with lithium aluminum hydride. Unfortunately, the expected compound (XII) could not be isolated in the crystalline state, and therefore the crude product was subjected to further elaboration. Oxidation of XII with osmium tetroxide in the usual way resulted in formation of 16α, 17α-dihydroxy derivatives.

In addition the preparation of 16β-hydroxy derivative was undertaken. Removal of 17-acetoxyl group from VI was accomplished by refluxing with zinc dust in acetic acid providing estra-1,3,5(10)-trien-16-one (IX). Treatment of IX with sodium borohydride gave the desired 16β-hydroxy compound (Xa) as the sole product. It was transformed into the ace-

1) This paper constitutes Part XIII of the series entitled "Analytical Chemical Stuides on Steroids"; Part XII: *Chem. Pharm. Bull.* (Tokyo), **16**, 374 (1968).
2) Location: *Kita-4-bancho, Sendai.*
3) A.H. Goldkamp, W.M. Hoehn, R.A. Mikulec, E.F. Nutting, and D.L. Cook, *J. Med. Chem.*, **8**, 409 (1965).
4) N.S. Leeds, D.K. Fukushima, and T.F. Gallagher, *J. Am. Chem. Soc.*, **76**, 2943 (1954).
5) Y. Suzuki, and K. Nakama, Japan. Patent 21374 (1963); *C.A.*, **60**, 3040 (1963).
6) L. Caglioti, and M. Magi, *Tetrahedron*, **19**, 1127 (1963).

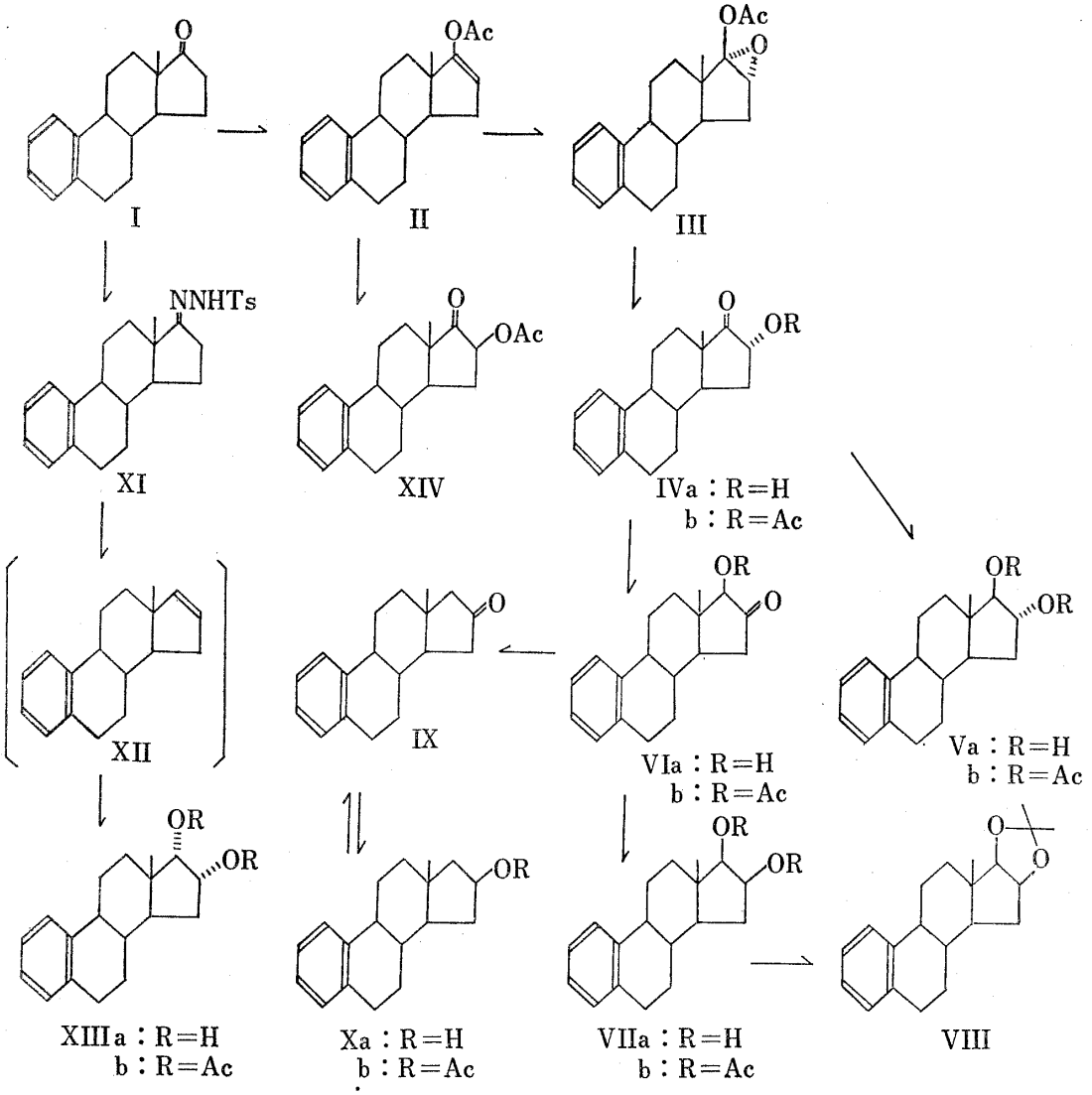

Chart 1

tate (Xb), and was backed again to IX by oxidation with Jones reagent.[7]

Since the stereochemistry of ring D in 14α-steroids is sufficiently substantiated, the assignment of structure of the above-mentioned compounds is unequivocal.

Studies on the metabolism of 3-desoxyestrone are being conducted in our laboratory and will be reported in near future.

Experimental[8]

Estra-1,3,5(10),16-tetraen-17-ol Acetate (II)——Prepared from estra-1,3,5(10)-trien-17-one (I) employing isopropenyl acetate and conc. H_2SO_4. Recrystallization from MeOH gave II as colorless plates. mp 103.5—104.5°, $[\alpha]_D^9$ +79.7° (c=2.10), (Reported mp 98.5—99.5°).[5]

16α,17α-Epoxyestra-1,3,5(10)-trien-17β-ol Acetate (III)——To a solution of II (770 mg) dissolved in $CHCl_3$ was added perbenzoic acid solution in $CHCl_3$ (0.24 M, 13 ml) and the reaction mixture was allowed to stand at room temperature for 20 hr. The solution was washed with ice-cooled 5% NaOH, H_2O and dried over

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

8) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise stated. For thin-layer chromatography (TLC) silica gel G (E. Merck Co.) was used as an adsorbent.

anhydrous Na_2SO_4. After evaporation of the solvent, the crystalline residue (770 mg) was obtained. The crude product was used for the next step without further purification. A part of this product was recrystallized from MeOH to give III as colorless plates. mp 121—123°, $[\alpha]_D^{10}$ +114.9° (c=3.66). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.84; H, 7.82.

16α-Hydroxyestra-1,3,5(10)-trien-17-one (IVa)——To a methanolic solution (160 ml) of III (1.8 g) was added 6 N H_2SO_4 (40 ml) and the solution was allowed to stand at room temperature for 4 days. The resulting solution was diluted with AcOEt and washed with ice-cooled 5% NaOH, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent, the crystalline residue (1.5 g) was obtained. Recrystallization from benzene gave IVa as colorless needles. mp 112—113°, $[\alpha]_D^{10}$ +101.1° (c=2.73). *Anal.* Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 79.83; H, 8.10.

16α-Hydroxyestra-1,3,5(10)-trien-17-one Acetate (IVb)——Prepared from IVa in the usual way with Ac_2O and pyridine. Recrystallization from MeOH gave IVb as colorless plates. mp 148—149°, $[\alpha]_D^{10}$ +154.9° (c=3.82). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.64; H, 7.74.

Estra-1,3,5(10)-triene-16α,17β-diol (Va)——To a solution of IVb (100 mg) in ether (10 ml) was added a suspended solution of $LiAlH_4$ (550 mg) in ether (10 ml) and refluxed for 1 hr. After decomposition of the excess reagent with moistened ether, the reaction mixture was acidified with 10% H_2SO_4 and diluted with AcOEt. The organic layer was separated and washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crude product (96 mg) was obtained. Recrystallization from acetone-hexane gave Va (80 mg) as colorless fibers. mp 143—146°, $[\alpha]_D^{10}$ +61.1°(c=2.49). *Anal.* Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 79.26; H, 8.88.

Estra-1,3,5(10)-triene-16α,17β-diol Diacetate (Vb)——Prepared from Va in the usual way with Ac_2O and pyridine. Recrystallization from MeOH gave Vb as colorless plates. mp 141°, $[\alpha]_D^{11}$ −21.5°(c=2.16). *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.12; H, 8.03.

17β-Hydroxyestra-1,3,5(10)-trien-16-one (VIa)——To a methanolic solution (180 ml) of IVb (700 mg) was added 0.1 N NaOH (130 ml) and the solution was allowed to stand at room temperature for 5 hr. The solution was neutralized with 5% HCl, concentrated and diluted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crystalline residue (700 mg) was obtaind. Recrystallization from MeOH gave VIa as colorless prisms. mp 212.5—214°, $[\alpha]_D^{11}$ −68.9° (c=1.98). *Anal.* Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 79.84; H, 8.26.

17β-Hydroxyestra-1,3,5(10)-trien-16-one Acetate (VIb)——Prepared from VIa in the usual way with Ac_2O and pyridine. Recrystallization from MeOH gave VIb as colorless plates. mp 143—144°; 166—167° (polymorphism), $[\alpha]_D^{12}$ +51.8° (c=2.55). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.92; H, 7.89

Estra-1,3,5(10)-triene-16β,17β-diol (VIIa)——To a solution of VIb (240 mg) in ether (20 ml) was added a suspended solution of $LiAlH_4$ (1.1 g) in ether (20 ml) and refluxed for 75 min. On usual work-up the crystalline product (250 mg) was obtained. Recrystallization from acetone-hexane gave VIIa (140 mg) as colorless fibers. mp 143°, $[\alpha]_D^{12}$ −25.1° (c=2.07). *Anal.* Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 79.26; H, 8.95.

Estra-1,3,5(10)-triene-16β,17β-diol Diacetate (VIIb)——Prepared from VIIa in the usual way with Ac_2O and pyridine. Recrystallization from MeOH gave VIIb as colorless plates. mp 141°, $[\alpha]_D^{12}$ −66.4° (c=3.01). *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.12; H, 7.89.

16β,17β-Isopropylidenedioxyestra-1,3,5(10)-triene (VIII)——To a solution of VIIa (110 mg) in acetone (30 ml) was added conc. H_2SO_4 (0.5 ml) under ice-cooling and the solution was allowed to stand at room temperature overnight. The resulting solution was neutralized with 5% $NaHCO_3$, concentrated and extracted with benzene. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crystalline residue was obtained. Recrystallization from MeOH gave VIII (30 mg) as colorless leaflets. mp 144—145°, $[\alpha]_D^{22}$ +56.4° (c=2.70). *Anal.* Calcd. for $C_{21}H_{28}O_2 \cdot \frac{1}{2}H_2O$: C, 78.46; H, 9.09. Found: C, 79.15; H, 8.71.

Estra-1,3,5(10)-trien-16-one (IX)——i) To a boiled solution of VIb (245 mg) dissovled in AcOH (30 ml) and Ac_2O (3 ml) was added Zn dust (7 g) portionwise during 30 min, and the resulting solution was refluxed under stirring for 14 hr. The reaction mixture was filtered and the cake separated was washed with EtOH. The combined filtrate was concentrated *in vacuo*, acidified with 5% HCl and extracted with ether. The organic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crude product obtained was chromatographed on Al_2O_3 (5 g). Elution with hexane-benzene (9:1) and recrystallization of the eluate from MeOH gave IX (61 mg) as colorless prisms. mp 143—145.5°, $[\alpha]_D^{25.5}$ −107.6° (c=2.82). *Anal.* Calcd. for $C_{18}H_{22}O$: C, 84.99; H, 8.72. Found: C, 84.44; H, 8.63.

ii) To a solution of Xa (15 mg) in acetone (2 ml) was added 8 N CrO_3 solution[7] (0.015 ml) under ice-cooling 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. On usual work-up the crystalline residue (10 mg) was obtained. Recrystallization from MeOH gave IX as colorless plates, identical with the sample obtained in i).

Estra-1,3,5(10)-trien-16β-ol (Xa)——To a methanolic solution (20 ml) of IX (180 mg) was added $NaBH_4$ (65 mg) under ice-cooling and kept at 0° for 30 min, and then at room temperature for 30 min. After

decomposition of the excess reagent with AcOH, the resulting solution was extracted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up, the crystalline residue (170 mg) was obtained. Recrystallization from acetone–hexane gave Xa as colorless leaflets. mp 138—139°, $[\alpha]_D^{22}$ +94.5° (c=2.16). *Anal.* Calcd. for $C_{18}H_{24}O$: C, 84.32; H, 9.44. Found: C, 84.42; H, 9.25.

Estra-1,3,5(10)-trien-16β-ol Acetate (Xb)——Prepared from Xa in the usual way with Ac_2O and pyridine. Recrystallization from MeOH gave Xb as colorless leaflets. mp 115—116°, $[\alpha]_D^{22}$ +77.1° (c=2.91). *Anal.* Calcd. for $C_{20}H_{26}O_2$: C, 80.49; H, 8.78. Found: C, 81.06; H, 8.80.

Estra-1,3,5(10) trien-17-one *p*-Tosylhydrazone (XI)——A methanolic solution (55 ml) of I (1 g) and *p*-tosylhydrazide (870 mg) containing AcOH (0.5 ml) was refluxed for 20 hr. On usual work-up the crystalline residue was obtained. Recrystallization from MeOH gave XI as colorless plates. mp 182—183°, $[\alpha]_D^{12}$ +66.0° (c=2.76). *Anal.* Calcd. for $C_{25}H_{30}O_2N_2S$: C, 71.06; H, 7.16; N, 6.63. Found: C, 71.31; H, 7.21; N, 6.26.

Estra-1,3,5(10),16-tetraene (XII)——To a solution of XI (1.1 g) in THF (50 ml) was added $LiAlH_4$ (2.2 g) and refluxed for 10 hr. On work-up in the same manner as described in Va, the crude product obtained was chromatographed on Al_2O_3 (15 g). Elution with hexane gave XII (340 mg) as oily product. TLC: *Rf* 0.50 (hexane); NMR (τ): 4.15 (2H, multiplet –CH=CH–).

Estra-1,3,5(10)-triene-16α,17α-diol (XIIIa)——To a solution of XII (320 mg) in benzene (6 ml)–pyridine (0.6 ml) was added OsO_4 (390 mg) and allowed to stand at room temperature for 24 hr. To the resulting solution were added H_2O (20 ml), MeOH (10 ml), Na_2SO_3 (2.2 g) and $KHCO_3$ (2.4 g) successively and the mixed solution was stirred for 5 hr. The reaction mixture was shaken with $CHCl_3$ (40 ml) for 1.5 hr and then filtered. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was chromatographed on Al_2O_3 (10 g). Elution with benzene–ether (7:3) and ether and recrystallization of the eluate (130 mg) from acetone–hexane gave XIIIa as colorless fibers. mp 75—78°; 112° (polymorphism), $[\alpha]_D^{12}$ +45.8° (c=2.01). *Anal.* Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 78.57; H, 8.74.

Estra-1,3,5(10)-triene-16α,17α-diol Diacetate (XIIIb)——Prepared from XIIIa in the usual way with Ac_2O and pyridine. Recrystallization from MeOH gave XIIIb as colorless needles. mp 94°, $[\alpha]_D^{12}$ +30.0° (c=1.87). *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.01; H, 7.89.

16β-Hydroxyestra-1,3,5(10)-trien-17-one Acetate (XIV)——To a solution of II (100 mg) in AcOH (1.6 ml)–Ac_2O (0.16 ml) was added $Pb(AcO)_4$ (120 mg) and allowed to stand at room temperature for 3 days. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crystalline residue (110 mg) was obtained. Recrystallization from MeOH gave XIV as colorless needles. mp 190—192°, $[\alpha]_D^{13}$ +125.9° (c=2.47). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.62; H, 7.61.

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