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Syntheses of New Haptens for Radioimmunoassay of Estradiol[1]

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In order to obtain much more specific antiserum required for radioimmunoassay of estradiol, the preparation of new promising haptens has been undertaken. First, the C-6 epimeric 6-hydroxyestradiol 3,17-bis(tetrahydropyranyl) ethers (VIII, XI) were treated with succinic anhydride and pyridine in the usual manner, respectively. Subsequent removal of the protecting groups provided the desired 6-hydroxyestradiol 6-hemisuccinates (X, XIII). Secondly, 7α-hydroxyestradiol 7-hemisuccinate (XXIII) has been also synthesized from 6-dehydroestradiol (XIV) by way of the 6α,7α-epoxide (XVIII) as a key intermediate. Reductive cleavage of the 6,7-oxido ring with metal hydride yielded solely the 7α-hydroxyl compound (XIX). Transformation into the 7-hemisuccinate (XXI) followed by elimination of the protecting group afforded XXIII in a satisfactory yield.

In recent years numerous methods based upon the principles of radioimmunoassay have been developed for the determination of estrogens and other steroid hormones in the biological fluids. With regard to estradiol several attempts have been made to obtain antiserum required for radioimmunoassay. It is well known that the immunologic specificity is less dependent on the individual steroid than on the particular functional groups occupied.[3] In most cases, however, antiserum has been prepared employing the hapten-carrier whose steroidal moiety is coupled to a protein through derivatization at one of the functional groups at position 3 or 17.[4] Accordingly such antiserum reacts with other naturally occurring estrogens, necessitating the preliminary separation. Several workers have coupled steroid to a carrier protein at site remote from the functional groups through a linkage such as 11α-hydroxyl hemisuccinate[5] and 6-O-carboxymethyloxime[6] in an attempt to obtain antibody of greater specificity. However, substitution of a bulky group at C-11 may exert the steric hindrance and alteration into trigonal carbon at C-6 may cause the distortion of the ring system reducing the specificity to a certain extent. In addition, estrogen 2- and 4-*p*-carboxyphenylazo derivatives which are coupled to a protein through the carboxyl group, have been also devised as haptens.[7] In order to obtain much more specific antiserum we have attempted to prepare the new haptens which are capable of coupling to a carrier through position 6α, 6β or 7α.[8]

An initial effort was directed to the preparation of two epimeric 6-hydroxyestradiol 6-hemisuccinates. 6-Oxoestradiol (I), readily obtainable from estradiol in three steps, was

1) This paper constitutes Part LXXI of the series entitled "Analytical Chemical Studies on Steroids"; Part LXX: T. Nambara, J. Ishiguro, Y. Kawarada, and H. Tajima, *Chem. Pharm. Bull.* (Tokyo), in **22**, 4 (1974).
2) Location: *Aobayama, Sendai.*
3) S.J. Gross, "Immunologic Methods in Steroid Determination," ed. by F.G. Péron and B.V. Caldwell, Meredith Co., New York, 1970, p. 63.
4) G. Mikhail, C.H. Wu, M. Ferin, and R.L. Vande Wiele, *Steroids*, **15**, 333 (1970).
5) F.C. Hollander and A.H.W.M. Schuurs, *Scand. J. Clin. Lab. Invest.*, Suppl. **29**, 126 (1972).
6) P.D.G. Dean, D. Exley, and M.W. Johnson, *Steroids*, **18**, 593 (1971); S.L. Jeffcoate and J.E. Searle, *ibid.*, **19**, 181 (1972); E. Kuss and R. Goebel, *ibid.*, **19**, 509 (1972); K. Wright, D.C. Collins, and J.R.K. Preedy, *ibid.*, **21**, 755 (1973).
7) S.J. Gross, D.H. Campbell, and H.H. Weetall, *Immunochem.*, **5**, 55 (1969).
8) During the course of this work the preparation of antigenic complexes of C_{19} and C_{21} steroids by coupling to a protein through position 7 has been reported.[9]

converted into the 3-benzyl ether (II), which in turn was led to the 17-tetrahydropyranyl ether (III) by treatment with dihydropyran and a catalytic amount of anhydrous *p*-toluenesulfonic acid. It is sufficiently substantiated that reduction of the 6-ketone with sodium borohydride affords the 6α-hydroxyl derivative, while with platinum oxide as a catalyst yields the 6β-epimer.[10] Treatment of III with sodium borohydride in the usual manner provided the 6α-hydroxyl derivative (IV) in a satisfactory yield. Transformation into the 6-hemisuccinate (V) was attained by refluxing with succinic anhydride in pyridine. Upon brief exposure to hydrochloric acid V was easily led to the 17β-hydroxyl derivative (VI). However, difficulties were encountered in the subsequent step, that is selective removal of the benzyl group at C-3. Catalytic hydrogenation over palladium-on-charcoal effected the simultaneous elimination of the protecting group at C-3 together with the succinoyl residue at C-6. This result prompted us to develop an alternative route.

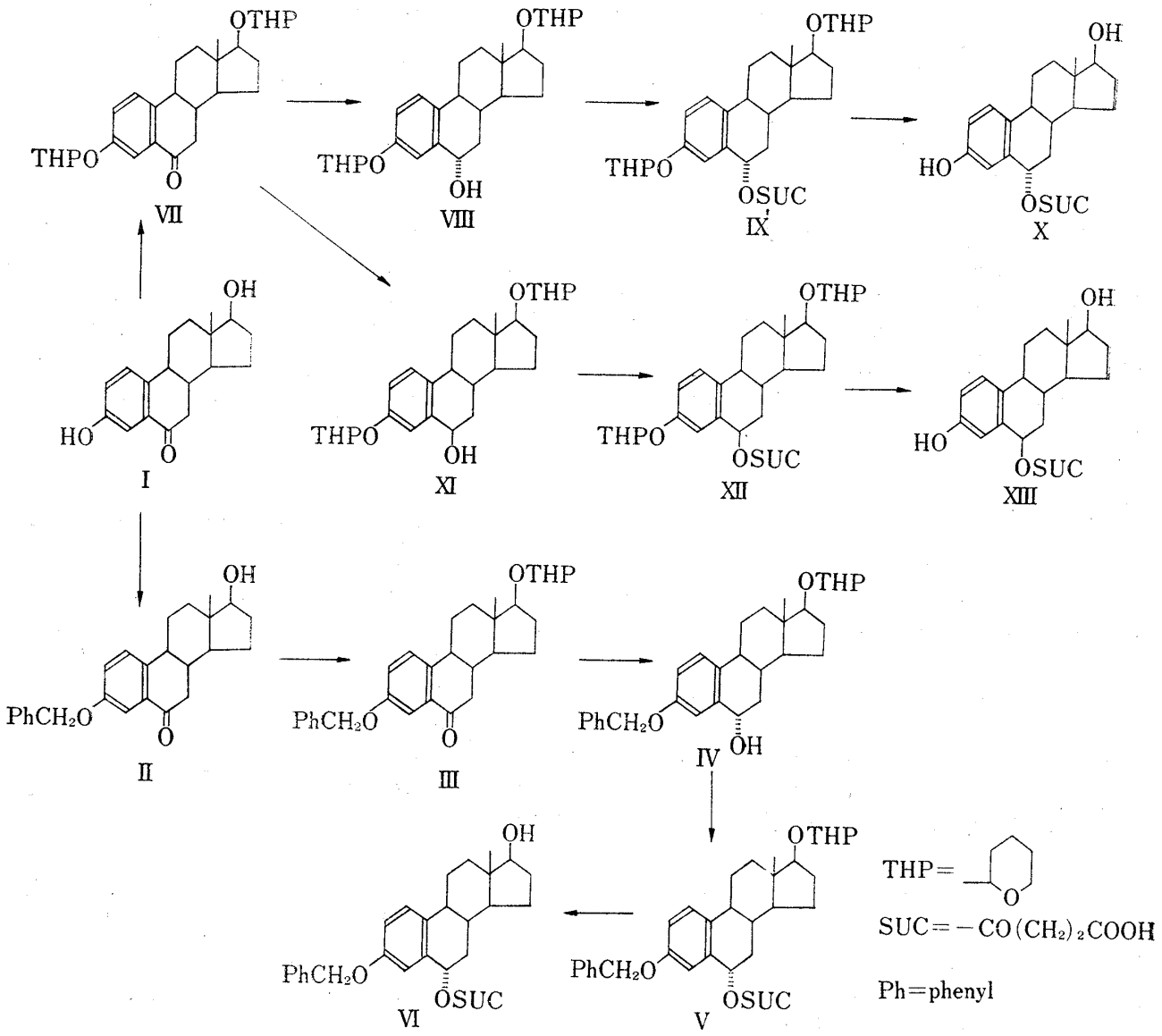

Chart 1

9) A. Weinstein, H.R. Lindner, A. Friedlander, and S. Bauminger, *Steroids*, **20**, 789 (1972); R.S. Rosenfeld, B. Rosenberg, J. Kream, and L. Hellman, *ibid.*, **21**, 723 (1973).

10) *a*) O. Wintersteiner and M. Moore, *J. Am. Chem. Soc.*, **81**, 422 (1959); *b*) O. Wintersteiner, M. Moore, and A.I. Cohen, *J. Org. Chem.*, **29**, 1325 (1964); *c*) T. Nambara, M. Numazawa, and H. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1725 (1969).

First, I was transformed into the bis(tetrahydropyranyl) ether (VII) to protect both hydroxyl groups at C-3 and C-17. Reduction with sodium borohydride furnished solely the 6α-hydroxyl derivative (VIII), whose stereochemistry at C-6 was ascertained by leading to the known 6α-hydroxyestradiol. Subsequently, VIII was converted to the 6-hemisuccinate (IX) in the manner as described above. Upon exposure to mineral acid removal of the protecting groups was accomplished with ease to give the desired 6α-hydroxyestradiol 6-hemisuccinate (X).

The second project was focused to the synthesis of the epimeric 6β-hemisuccinate. Catalytic reduction of VII with platinum oxide provided the 6β-hydroxyl compound (XI) without affecting any disturbance on the tetrahydropyranyl ether. Configurational assignment of the C-6 hydroxyl function was justified by comparison with the authentic 6β-hydroxyestradiol upon removal of the protecting groups. Treatment with succinic anhydride and pyridine followed by acid cleavage of the ether linkage in the manner as described above afforded the desired 6β-hydroxyestradiol 6-hemisuccinate (XIII) in a reasonable yield.

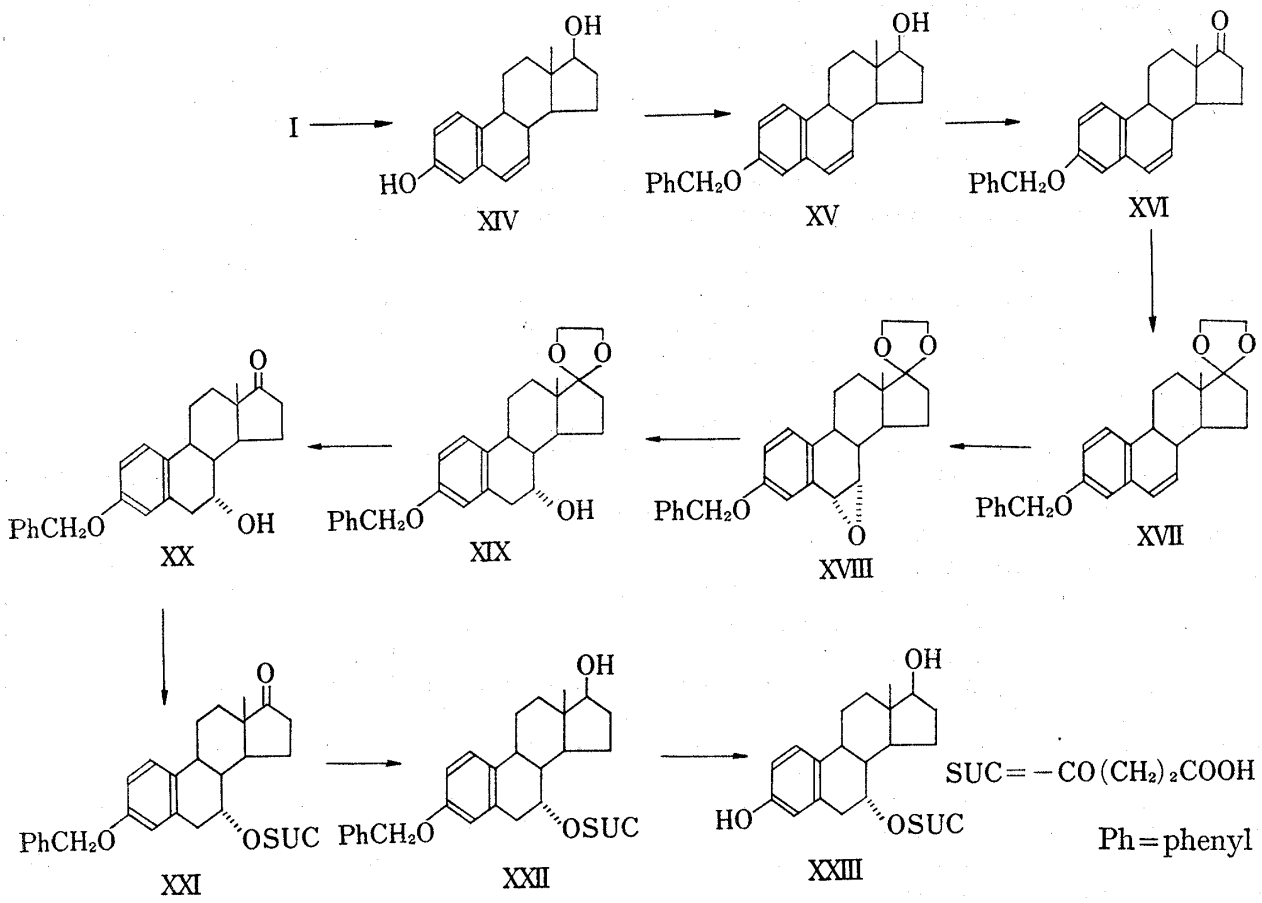

Chart 2

The preparation of 7α-hydroxyestradiol 7-hemisuccinate, a more promising hapten for obtaining the specific antibody, has been undertaken. Introduction of an oxygen function into C-7 was attempted employing the 6,7-epoxide as an key intermediate. 6-Dehydroestradiol (XIV) was transformed into the 3-benzyl ether (XV), which on chromium trioxide oxidation was led to the corresponding 17-oxo derivative (XVI). In order to protect the oxo group at C-17 XVI was converted to the ketal (XVII) by treatment with ethylene glycol and anhydrous *p*-toluenesulfonic acid. The attack of peracid toward the Δ^6 double bond did take course preferentially from the less-hindered α-side resulting in formation of the 6α,7α-epoxide

(XVIII) as a single product. The cleavage of oxido ring with metal hydride proceeded in the expected direction to provide solely the 7α-hydroxyl compound (XIX). Deketalization was readily attained by brief exposure to hydrochloric acid yielding 7α-hydroxyestrone 3-benzyl ether (XX). Treatment with succinic anhydride and pyridine in the manner as described above afforded the 7-hemisuccinate (XXI), although it could not be obtained in the crystalline state. Reduction of the 17-ketone with sodium borohydride under the mild conditions and subsequent hydrogenolysis over palladium-on-charcoal provided the desired 7α-hydroxyestradiol 7-hemisuccinate (XXIII) in a satisfactory yield.

It is hoped that these new haptens may serve for preparation of the specific antiserum required for radioimmunoassay of estradiol.

Experimental[11]

3,17β-Dihydroxyestra-1,3,5(10)-trien-6-one (I)——Prepared from estradiol diacetate employing the known method. mp 279—282° (lit. mp 282—283°).[12]

3-Benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-6-one (II)——To a solution of I (600 mg) in anhydrous EtOH (50 ml) were added anhydrous K_2CO_3 (1 g) and $C_6H_5CH_2Cl$ (1 ml) and refluxed for 5 hr. The reaction mixture was poured onto ice-water and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up a crystalline product obtained was recrystallized from MeOH to give II (577 mg) as colorless needles. mp 175—177°. $[\alpha]_D^{23}$ −70.0° (c=0.10). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 80.11; H, 7.51. IR ν_{max}^{KBr} cm^{-1}: 1673 (C=O). NMR ($CDCl_3$ solution) δ: 0.78 (3H, s, 18-CH_3), 5.06 (2H, s, 3-O$\underline{H}_2C_6H_5$), 7.37 (5H, s, 3-$OCH_2C_6\underline{H}_5$).

3-Benzyloxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6-one (III)——To a solution of II (550 mg) in benzene (10 ml) were added 2,3-dihydropyran (4 ml) and anhydrous p-TsOH (13 mg) and stirred at room temperature for 1.5 hr. The resulting solution was diluted with benzene, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was submitted to preparative TLC using benzene–AcOEt (6: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.78) gave III (550 mg) as colorless oil. NMR (CCl_4 solution) δ: 0.78 (3H, s, 18-CH_3), 3.15—3.80 (3H, broad peak, 17α-H, tetrahydropyranyl-6-H), 4.35—4.75 (1H, broad s, tetrahydropyranyl-2-H), 5.02 (2H, s, 3-O$\underline{H}_2C_6H_5$), 7.30 (5H, s, 3-$OCH_2C_6\underline{H}_5$).

3-Benzyloxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6α-ol (IV)——To an ice-cooled solution of IV (600 mg) in MeOH (50 ml) was added $NaBH_4$ (110 mg) and allowed to stand for 30 min. After addition of AcOH (2 drops) to decompose the excess reagent the resulting solution was diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. Upon evaporation of solvent the crude product obtained was submitted to preparative TLC using benzene–AcOEt (6: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.45) gave IV (550 mg) as colorless oil. NMR (CCl_4 solution) δ: 0.75 (3H, s, 18-CH_3), 3.15—3.85 (3H, broad peak, 17α-H, tetrahydropyranyl-6-H), 4.40—4.75 (2H, broad, s, 6β-H, tetrahydropyranyl-2-H), 4.94 (2H, s, 3-O$\underline{H}_2C_6H_5$), 7.24 (5H, s, 3-$OCH_2C_6\underline{H}_5$).

3-Benzyloxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6α-ol Hemisuccinate (V)——To a solution of IV (250 mg) in pyridine (15 ml) was added succinic anhydride (250 mg) and refluxed for 10 hr. After evaporation of solvent the residue was diluted with ether, washed with a NaCl-saturated aq. solution, and dried over anhydrous Na_2SO_4. Upon usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using benzene–AcOEt (5: 1) as developing solvent. Elution of the adsorbent corresponding to the spot gave V (200 mg) as colorless oil. NMR (CD_3OD solution) δ: 0.78 (3H, s, 18-CH_3), 2.57 (4H, s, $-COCH_2CH_2CO-$), 3.20—3.85 (3H, broad peak, 17α-H, tetrahydropyranyl-6-H), 4.95 (2H, s, 3-O$\underline{H}_2$-C_6H_5), 7.27 (5H, s, 3-$OCH_2C_6\underline{H}_5$).

3-Benzyloxyestra-1,3,5(10)-triene-6α,17β-diol 6-Hemisuccinate (VI)——To a solution of V (350 mg) in acetone (30 ml) was added 5% HCl (1 ml) and stirred at room temperature for 1 hr. The resulting solution was neutralized with 5% $NaHCO_3$ and concentrated. An oily residue was extracted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was submitted to preparative TLC by multiple runs using benzene–AcOEt (5: 1) as developing solvent. Elution

11) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Infrared (IR) spectra were run on a JASCO Model IR-S spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Hitachi Model R-20A spectrometer at 60MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, and m=multiplet. For preparative thin-layer chromatography (TLC) Silica gel HF_{254} (E. Merck AG) was used as adsorbent.

12) B. Longwell and O. Wintersteiner, *J. Biol. Chem.*, **133**, 219 (1940).

of the adsorbent corresponding to the spot and recrystallization of the eluate from MeOH gave VI (300 mg) as colorless needles. mp 118—119°. $[\alpha]_D^{23}$+60.0° (c=0.05, MeOH). *Anal.* Calcd. for $C_{29}H_{34}O_6 \cdot 1\frac{1}{2}H_2O$: C, 68.87; H, 7.38. Found: C, 69.37; H, 7.30. IR ν_{max}^{KBr} cm^{-1}: 1727, 1756 (C=O). NMR ($CDCl_3$ solution) δ: 0.72 (3H, s, 18-CH_3), 2.59 (4H, s, $-COCH_2CH_2CO-$), 3.40—3.80 (1H, m, 17α-H), 4.98 (2H, s, 3-$OCH_2C_6H_5$), 5.83—6.18 (1H, broad, 6β-H), 7.32 (5H, s, 3-$OCH_2C_6H_5$).

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6-one (VII)——To a solution of I (200 mg) in benzene (120 ml) were added 2,3-dihydropyran (0.8 ml) and anhydrous p-TsOH (8 ml) and refluxed for 5 hr. The resulting solution was diluted with benzene, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was recrystallized from MeOH to give VII (130 mg) as colorless needles. mp 147—149°. $[\alpha]_D^{25}$+14.3° (c=0.10). *Anal.* Calcd. for $C_{28}H_{38}O_5$: C, 73.98; H. 8.43. Found: C, 73.39; H, 8.05. NMR (CCl_4 solution) δ: 0.81 (3H, s, 18-CH_3), 3.30—3.90 (5H, broad peak, 17α-H, tetrahydropyranyl-6-H), 4.42—4.75 (1H, broad s, tetrahydropyranyl-2-H), 5.39 (1H, broad s, tetrahydropyranyl-2-H).

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6α-ol (VIII)——To an ice-cooled solution of VII (40 mg) in MeOH (50 ml) was added $NaBH_4$ (8 mg) and allowed to stand for 1 hr. After addition of AcOH (2 drops) to decompose the excess reagent the resulting solution was diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was recrystallized from MeOH to give VIII (30 mg) as colorless needles. mp 100—101°. $[\alpha]_D^{25}$+199.0° (c=0.10). *Anal.* Calcd. for $C_{28}H_{40}O_5 \cdot \frac{1}{2}H_2O$: C, 72.10; H, 8.79. Found: C, 72.30; H, 8.56. NMR (CCl_4 solution) δ: 0.82 (3H, s, 18-CH_3), 3.38—3.85 (5H, broad peak, 17α-H, tetrahydropyranyl-6-H), 4.60 (2H, broad s, 6β-H, tetrahydropyranyl-2-H), 5.38 (1H, broad s, tetrahydropyranyl-2-H).

Transformation of VIII into 6α-Hydroxyestradiol——To a solution of VIII (20 mg) in MeOH (5 ml) was added 1N HCl (0.3 ml) and stirred at room temperature for 1 hr. The resulting solution was extracted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of solvent the crude product obtained was recrystallized from aq. MeOH to give 6α-hydroxyestradiol (8 mg) as colorless needles. mp 247—250°. Mixed melting point on admixture with the authentic sample showed no depression. 3,6,17-Triacetate: Treatment with Ac_2O and pyridine in the usual manner followed by recrystallization from aq. acetone gave the triacetate as colorless needles. mp 143—145° (lit. mp 144—145°).[10a]

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6α-ol Hemisuccinate (IX)——To a solution of VIII (53 mg) in pyridine (15 ml) was added succinic anhydride (45 mg) and refluxed for 17 hr. After evaporation of solvent the residue was diluted with ether, washed with a NaCl-saturated aq. solution, and dried over anhydrous Na_2SO_4. Upon usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using benzene–AcOEt (4: 1) as developing solvent. Elution of the adsorbent corresponding to the spot gave IX (60 mg) as colorless oil. NMR (CCl_4 solution) δ: 0.80 (3H, s, 18-CH_3), 2.61 (4H, s, $-COCH_2CH_2CO-$), 3.15—3.85 (5H, broad peak, 17α-H, tetrahydropyranyl-6-H).

Estra-1,3,5(10)-triene-3,6α,17β-triol 6-Hemisuccinate (X)——To a solution of IX (75 mg) in MeOH (20 ml) was added 1N HCl (5 ml) and stirred at room temperature for 1 hr. The resulting solution was neutralized with 5% $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product was purified by preparative TLC and recrystallized from aq. MeOH to give X (65 mg) as colorless needles. mp 164—166°. $[\alpha]_D^{25}$+302.0° (c=0.04, MeOH). *Anal.* Calcd. for $C_{22}H_{28}O_6 \cdot \frac{1}{2}H_2O$: C, 66.48; H, 7.10. Found: C, 66.40; H, 7.10. IR ν_{max}^{KBr} cm^{-1}: 1720, 1740 (C=O). NMR (CD_3OD solution) δ: 0.82 (3H, s, 18-CH_3), 2.69 (4H, s, $-COCH_2CH_2CO-$), 5.98 (1H, m, 6β-H).

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6β-ol (XI)——A solution of VII (200 mg) in EtOH (15 ml) was shaken with PtO_2 (150 mg) under a stream of H_2 gas at room temperature for 5 hr. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. The crude product obtained was submitted to preparative TLC by multiple runs using benzene–ether (5: 1) as developing solvent. Elution of the adsorbent corresponding to the spot gave XI (150 mg) as colorless oil. NMR (CCl_4 solution) δ: 0.80 (3H, s, 18-CH_3), 3.40—3.90 (5H, broad peak, 17α-H, tetrahydropyranyl-6-H), 4.60 (2H, broad s, 6α-H, tetrahydropyranyl-2-H), 5.40 (1H, broad s, tetrahydropyranyl-2-H).

Transformation of XI into 6β-Hydroxyestradiol——Treatment of XI (20 mg) with 1N HCl in the manner as described in VIII followed by recrystallization from aq. MeOH gave 6β-hydroxyestradiol (5 mg) as colorless needles. mp 191—194°. Mixed melting point on admixture with the authentic sample showed no depression. 3,6,17-Triacetate: Usual acetylation with Ac_2O and pyridine followed by recrystallization from aq. acetone gave the triacetate. mp 175—178° (lit. mp 176—178°).[10a]

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-trien-6β-ol Hemisuccinate (XII)——To a solution of XI (150 mg) in pyridine (15 ml) was added succinic anhydride (150 mg) and refluxed for 10 hr. After evaporation of solvent the residue was diluted with ether, washed with a NaCl-saturated aq. solution, and dried over anhydrous Na_2SO_4. Upon usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using benzene–AcOEt (4: 1) as developing solvent. Elution of the adsorbent corresponding to the spot gave XII (120 mg) as colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1740 (C=O). NMR (CCl_4 solution) δ: 0.80 (3H, s, 18-CH_3), 2.61 (4H, s, $-COCH_2CH_2CO-$), 3.30—3.85 (5H, broad peak, 17α-H, tetra-

hydropyranyl-6-H), 4.40—4.65 (2H, broad s, 6α-H, tetrahydropyranyl-2-H), 5.32 (1H, broad s, tetrahydropyranyl-2-H).

Estra-1,3,5(10)-triene-3,6β,17β-triol 6-Hemisuccinate (XIII)——To a solution of XII (120 mg) in MeOH (30 ml) was added 1N HCl (5 ml) and stirred at room temperature for 1 hr. The resulting solution was neutralized with 5% $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was purified by preparative TLC and recrystallized from aq. MeOH to give XIII (60 mg) as colorless needles. mp 164—166°. $[\alpha]_D^{20}$+25.0° (c=0.10, MeOH). *Anal.* Calcd. for $C_{22}H_{28}O_6 \cdot H_2O$: C, 65.01; H, 7.44. Found: C, 65.12; H, 6.99. IR ν_{max}^{KBr} cm^{-1}: 1720, 1740 (C=O). NMR ($CDCl_3$–CD_3OD) δ: 0.78 (3H, s, 18-CH_3), 2.62 (4H, s, –$COCH_2CH_2CO$–), 6.00 (1H, m, 6α-H).

Estra-1,3,5(10),6-tetraene-3,17β-diol (XIV)——Prepared from I employing the known method. mp 224.5—225.5°. (lit. mp 215—220°).[13,14]

3-Benzyloxyestra-1,3,5(10),6-tetraen-17β-ol (XV)——To a solution of XIV (520 mg) in anhydrous EtOH (50 ml) were added anhydrous K_2CO_3 (1.2 g) and $C_6H_5CH_2Cl$ (2 ml) and refluxed for 5 hr. The reaction mixture was poured onto ice-water and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up a crystalline product obtained was recrystallized from MeOH to give XV (600 mg) as colorless needles. mp 101—103°. $[\alpha]_D^{23}$−160.0° (c=0.10). *Anal.* Calcd. for $C_{25}H_{28}O_2$: C, 83.29; H, 7.83. Found: C, 83.81; H, 8.02. NMR ($CDCl_3$ solution) δ: 0.78 (3H, s, 18-CH_3), 3.75 (1H, m, 17α-H), 5.03 (2H, s, 3-$OCH_2C_6H_5$), 7.37 (5H, s, 3-$OCH_2C_6H_5$).

3-Benzyloxyestra-1,3,5(10),6-tetraen-17-one (XVI)——To a solution of XV (600 mg) in acetone (30 ml) was added 8N CrO_3 (1 ml) dropwise under ice-cooling and stirred for 8 min. The resulting solution was poured onto ice-water and extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was submitted to column chromatography on silica gel. Elution with benzene–ether (95: 5) and recrystallization of the eluate from MeOH gave XVI (500 mg) as colorless leaflets. mp 151—152°. $[\alpha]_D^{25}$−150.0° (c=0.10). *Anal.* Calcd. for $C_{25}H_{26}O_2$: C, 83.76; H, 7.31. Found: C, 83.37; H, 7.53. NMR ($CDCl_3$ solution) δ: 0.90 (3H, s, 18-CH_3), 5.04 (2H, s, 3-$OCH_2C_6H_5$), 7.37 (5H, s, 3-$OCH_2C_6H_5$).

3-Benzyloxy-17,17-ethylenedioxyestra-1,3,5(10),6-tetraene (XVII)——To a solution of XVI (600 mg) in benzene (50 ml) were added ethylene glycol (4.5 ml) and anhydrous p-TsOH (20 mg) and the moisture was azeotropically removed by slow distillation over a period of 8 hr. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from MeOH gave XVII (560 mg) as colorless prisms. mp 128—130°. $[\alpha]_D^{25}$−65.0° (c=0.10). *Anal.* Calcd. for $C_{27}H_{30}O_3$: C, 80.56; H, 7.51. Found: C, 81.10; H, 7.47. NMR (CCl_4 solution) δ: 0.83 (3H, s, 18-CH_3), 3.79 (4H, s, –OCH_2CH_2O–), 4.96 (2H, s, 3-$OCH_2C_6H_5$), 7.26 (5H, s, 3-$OCH_2C_6H_5$).

3-Benzyloxy-6α,7α-epoxy-17,17-ethylenedioxyestra-1,3,5(10)-triene (XVIII)——To a solution of XVII (340 mg) in CH_2Cl_2 (15 ml) was added m-chloroperbenzoic acid (210 mg) and allowed to stand at room temperature for 15 hr. The resulting solution was diluted with ether, washed with 5% $Na_2S_2O_3$, 5% $NaHCO_3$, and H_2O, successively, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was submitted to column chromatography on Al_2O_3. Elution with benzene and recrystallization of the eluate from benzene–ether gave XVIII (260 mg) as colorless prisms. mp 142—145°. $[\alpha]_D^{23}$−50.0° (c=0.10). *Anal.* Calcd. for $C_{27}H_{30}O_4$: C, 77.48; H, 7.23. Found: C, 77.82; H, 7.17. NMR (CCl_4 solution) δ: 0.83 (3H, s, 18-CH_3), 3.43 (1H, d, J=2.5 Hz, 7β-H), 3.51 (1H, d, J=2.5 Hz, 6β-H), 3.79 (4H, s, –OCH_2CH_2O–), 4.98 (2H, s, 3-$OCH_2C_6H_5$), 7.28 (5H, s, 3-$OCH_2C_6H_5$).

3-Benzyloxy-17,17-ethylenedioxyestra-1,3,5(10)-trien-7α-ol (XIX)——To a solution of XVIII (260 mg) in anhydrous tetrahydrofuran (THF) (10 ml) was added a solution of $LiAlH_4$ (740 mg) in THF (5 ml) under ice-cooling and refluxed for 9 hr. After careful addition of moist ether to decompose the excess reagent the resulting solution was acidified with HCl and extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was submitted to preparative TLC by multiple runs using hexane–AcOEt (5: 1) as developing solvent. Elution of the adsorbent corresponding to the spot and recrystallization of the eluate from EtOH gave XIX (166 mg) as colorless leaflets. mp 148—150°. $[\alpha]_D^{23}$+30.0° (c=0.10). *Anal.* Calcd. for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 76.72; H, 7.74. NMR ($CDCl_3$ solution) δ: 0.87 (3H, s, 18-CH_3), 2.86 (4H, s, –OCH_2CH_2O–), 4.12 (1H, t, J=4 Hz, 7β-H), 4.99 (2H, s, 3-$OCH_2C_6H_5$), 7.34 (5H, s, 3-$OCH_2C_6H_5$).

3-Benzyloxy-7α-hydroxyestra-1,3,5(10)-trien-17-one (XX)——To a solution of XIX (166 mg) in MeOH (15 ml) was added 5% HCl (5 ml) and stirred at room temperature for 1 hr. The resulting solution was neutralized with 5% $NaHCO_3$ and then extracted with ether. After usual work-up the crude product obtained was recrystallized from MeOH to give XX (130 mg) as colorless prisms. mp 154—155°. $[\alpha]_D^{23}$+120.0° (c=0.10). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.85; H, 7.52. NMR ($CDCl_3$ solution) δ: 0.89 (3H, s, 18-CH_3), 4.18 (1H, t, J=4 Hz, 7β-H), 5.02 (2H, s, 3-$OCH_2C_6H_5$), 7.36 (5H, s, 3-$OCH_2C_6H_5$).

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3-Benzyloxy-7α-hydroxyestra-1,3,5(10)-trien-17-one Hemisuccinate (XXI)——To a solution of XX (130 mg) in pyridine (15 ml) was added succinic anhydride (130 mg) and refluxed for 50 hr. The resulting solution was concentrated and then extracted with AcOEt. The organic layer was washed with a NaCl-saturated aq. solution and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using benzene–AcOEt (4: 1) as developing solvent. Elution of the adsorbent corresponding to the spot gave XXI (120 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.86 (3H, s, 18-CH_3), 2.56 (4H, s, $-COCH_2CH_2CO-$), 5.01 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 5.40 (1H, t, $J=4$ Hz, 7β-H), 7.35 (5H, s, 3-$OCH_2C_6\underline{H}_5$). Methyl ester: To a solution of XXI (20 mg) in MeOH (10 ml) was added an ethereal solution of CH_2N_2 and allowed to stand at room temperature for 10 min. After addition of AcOH (1 drop) to decompose the excess reagent the resulting solution was concentrated. An oily residue obtained was taken up with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. Usual work-up gave the methyl ester (20 mg) as colorless oil. NMR (CCl_4 solution) δ: 0.82 (3H, s, 18-CH_3), 2.45 (4H, s, $-COCH_2CH_2CO-$), 3.49 (3H, s, $-COOCH_3$), 4.89 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 5.28 (1H, t, $J=4$ Hz, 7β-H), 7.21 (5H, s, 3-$OCH_2C_6\underline{H}_5$).

3-Benzyloxyestra-1,3,5(10)-triene-7α,17β-diol 7-Hemisuccinate (XXII)——To a solution of XXI (120 mg) in MeOH (20 ml) was added a methanolic solution (1.5 ml) of $NaBH_4$ (200 mg) at -10——$13°$ and allowed to stand for 5 hr. After addition of AcOH (2 drops) to decompose the excess reagent the resulting solution was extracted with AcOEt. The organic layer was washed with a NaCl-saturated aq. solution and dried over anhydrous Na_2SO_4. After evaporation of solvent a crystalline product obtained was recrystallized from aq. MeOH to give XXII (100 mg) as colorless needles. mp 120—122°. $[\alpha]_D^{24}+2.6°$ ($c=0.38$, MeOH). *Anal.* Calcd. for $C_{29}H_{34}O_6\cdot\frac{1}{2}H_2O$: C, 71.43; H, 7.24. Found: C, 71.63; H, 7.09. NMR ($CDCl_3$ solution) δ: 0.75 (3H, s, 18-CH_3), 2.58 (4H, s, $-COCH_2CH_2CO-$), 3.67 (1H, m, 17α-H), 4.98 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 5.23 (1H, t, $J=3$ Hz, 7β-H), 7.34 (5H, s, 3-$OCH_2C_6\underline{H}_5$).

Estra-1,3,5(10)-triene-3,7α,17β-triol 7-Hemisuccinate (XXIII)——A solution of XXII (100 mg) in AcOEt (20 ml) was shaken with 5% Pd/C (100 mg) under a stream of H_2 gas at room temperature for 24 hr. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. The crude product obtained was purified by preparative TLC by multiple runs using benzene–AcOEt (3: 1) as developing solvent to give XXIII (60 mg) as colorless amorphous substance. mp 116—119°. $[\alpha]_D^{24}+31.3°$ ($c=0.08$, MeOH). *Anal.* Calcd. for $C_{22}H_{28}O_6\cdot\frac{1}{4}H_2O$: C, 67.24; H, 7.31. Found: C, 67.25; H, 7.13. IR ν_{max}^{KBr} cm^{-1}: 1717, 1733 (C=O). NMR (CD_3OD solution) δ: 0.75 (3H, s, 18-CH_3), 2.51 (4H, s, $-COCH_2CH_2CO-$), 3.63 (1H, m, 17α-H), 5.22 (1H, t, $J=4$ Hz, 7β-H).

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