SYNTHESIS OF 3,6α,16α-TRIHYDROXY-1,3,5(10)-ESTRATRIEN-17-ONE 6-HEMISUCCINATE AND [6,7^{3}H]-3,16α-DIHYDROXY-1,3,5(10)-ESTRATRIEN-17-ONE

Shigeo Ikegawa [1] and Jack Fishman

The Rockefeller University, New York, New York 10021

Received 3-22-82

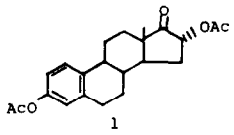
ABSTRACT

The preparation of high specific activity ^{3}H labeled 16α-hydroxyestrone and of the 6-hemisuccinate of the ketol metabolite of estradiol is described. The synthetic procedures which were required to produce these essential ingredients of a radioimmunoassay for 16α-hydroxyestrone required multistep syntheses because of the multifunctional and labile nature of the metabolite.

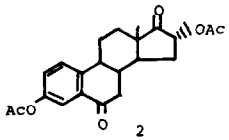
INTRODUCTION

Hydroxylation at the 16α-position of estradiol is one of the more significant transformations in the oxidative metabolism of the female sex hormone. Since the oxidation of the 17β-hydroxy group to the 17-ketone precedes this hydroxylation [2], the first product of this metabolic pathway is the ring D ketol 16α-hydroxyestrone [3] which is then, in part, converted further to estriol [4]. These two metabolites are essentially the sole products of the 16α-hydroxylative pathway because estriol undergoes little further transformation [5] and 15α-hydroxyestradiol, which is formed by an initial 15α-hydroxylation [6], is a significant metabolite only in the fetus and neonate [7]. The end product of 16α-hydroxylation, estriol, has been accorded extensive attention both as regards its content in body fluids [8] and its biological properties [9]. Its precursor, 16α-hydroxyestrone, has, however, been comparatively neglected. Its measurement in the human has been limited to urine [10] and bile [11] and it has been reported to be the dominant estrogen in the latter, albeit in a conjugated form. Recently, this ring D ketol has earned renewed interest because of the reports of

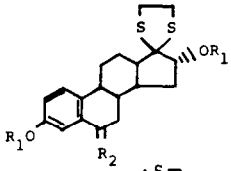
its increased formation in alcoholic cirrhosis [12] and in women and men with systemic lupus erythematosus (SLE) [13]. The possibility that this estradiol metabolite could play a role in the etiology of the SLE prompted us to examine its biological properties. The substance was found to be a potent estrogen agonist in the uterotropic assay despite an only very modest affinity for the cytosolic estrogen receptor [14]. A further distinguishing feature of 16α-hydroxyestrone was its lack of binding to the plasma carrier protein TEBG [14] for which it exhibited the lowest affinity of any sex steroid tested. The latter characteristic suggested that this estrogen might be expected to induce an estrogenic response in the human out of proportion to its plasma content, since essentially all of the material would be free or albumin bound and thus be biologically available. For these reasons the plasma content of 16α-hydroxyestrone in normals and in subjects with pathophysiological conditions such as SLE became of considerable interest. Because the levels of this material in plasma were expected to be low, a highly sensitive assay procedure such as RIA was considered to be the most feasible method of measurement. Since the substance could not be converted by an unequivocal route to any other estrogen for which a RIA was already available, an indirect procedure was not possible and a specific assay had to be developed. The two essential components of such an assay, a suitable hapten and a radiolabeled ligand of high specific activity were, therefore, required. Normally the preparation of steroid haptens and radiolabeled ligands is now a straightforward and unexceptional process but in this case the particular structure of the ketol presented problems which rendered these tasks more challenging than usual. The difficulties were associated principally with the multifunctional nature of the 16α-hydroxyestrone molecule and the



1



2



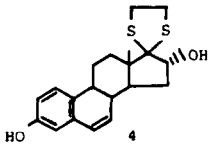
3a R_1 = Ac, R_2 = 

b R_1 = Ac, R_2 = =O

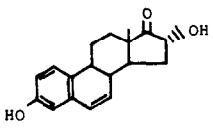
c R_1 = Ac, R_2 = 

d R_1 = H, R_2 = 

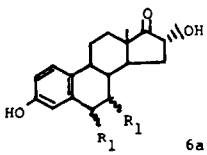
e R_1 = H, R_2 = N-O-CH_2COOH



4

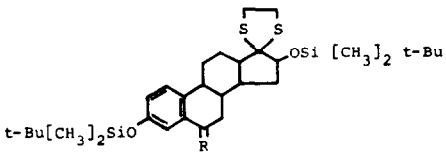


5



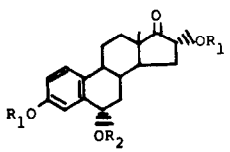
6a R_1 = H

R_1 = ^{3}H

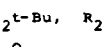


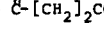
7a R = O

b R = 



8a R_1 = Si[CH_3]$_2$t-Bu, R_2 = H

b R_1 = Si[CH_3]$_2$t-Bu, R_2 = 

c R_1 = H, R_2 = 

lability of its ring D ketol structure to rearrangements [15].

High specific activity tritium labeled 16α-hydroxyestrone was required as a ligand for the radioimmunoassay procedure and also for *in vitro* and *in vivo* studies. For the latter use, it was clearly desirable to locate the isotope in a chemically and biologically stable position on the molecule. This requirement eliminated from consideration the introduction of tritium into ring D during elaboration of the α-ketol structure, and suggested the relatively stable C-6 and C-7 positions as the most suitable sites.

The use of the commercially available [6,7-^{3}H]-estrone as a starting material was impractical in view of the number of chemical steps required to achieve the transformation to 16α-hydroxyestrone [16]. Biochemical preparation by means of an *in vitro* conversion with guinea pig liver [17] had the disadvantage of poor yield and difficulty in purification from other metabolites. We therefore selected a procedure in which the radioisotope is introduced into a structure one chemical step away from 16α-hydroxyestrone and we directed our effort to the preparation of 6-dehydro-16α-hydroxyestrone.

MATERIALS AND METHODS

Catalytic reduction with tritium was done commercially by New England Nuclear Corp., Boston, MA. Radioactivity was measured in a Packard Tricarb Liquid Scintillation spectrometer model 574 using Biofluor as a scintillator. All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. IR spectra were obtained in KBr discs on a Perkins-Elmer Model 237B spectrometer. UV spectra were measured on an Aminco UV-visible spectrometer Model DW-2a. NMR spectra were recorded on a Thompson Packard TPV-60T at 60 MHz using tetramethylsilane as an internal standard. For preparative TLC silica gel GF254 (E. Merck AG, Dormstadt) was used as an adsorbent. For column chromatography silica gel (70-230 mesh) (E. Merck AG, Dormstadt) and Sephadex LH-20 (Pharmacia Fine Chemicals Corp., Piscataway, NJ) were used.

3,16α-Diacetoxy-1,3,5(10)-estratriene-6,17-dione (2). A solution of 4.87 g of 3,16α-diacetoxy-1,3,5(10)-estratrien-17-one (1) in 40 ml of glacial acetic acid was cooled at 4°C and 30 ml of an ice cold solution of CrO_3 (4 g CrO_3 in 2 ml of H_2O and 28 ml of glacial acetic acid) was added dropwise over 30 min. The reaction mixture was stirred at room temperature overnight, MeOH was added to decompose the excess reagent and the resulting solution was neutralized with 5% $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel. Elution with cyclohexane-ethyl acetate (4:1) and recrystallization of the eluate from MeOH gave 560 mg of 2 as colorless needles. m.p. 209-210°. $[\alpha]_D^{10}$+ 87.5° (C = 0.16). Anal. Calcd. for $C_{22}H_{24}O_6$: C, 68.73; H, 6.29. Found: C, 68.41; H, 6.34. NMR ($CDCl_3$)δ: 1.03 (3H, s, 18-CH_3), 2.15 (3H, s, 16α-OAc), 2.32 (3H, s, 3-OAc), 5.46 (1H, m, 16β-H), 7.28 (1H, d,d, J = 2, 8 Hz, C_2-H), 7.45 (1H, d, J = 8 Hz, C_1-H), 7.77 (1H, d, J = 2 Hz, C_4-H).

3,16α-Diacetoxy-1,3,5(10)-estratriene-6,17-dione Bisethylenedithioketal (3a). To a solution of 220 mg of 2 in 5 ml of acetic acid were added 0.8 ml of 1,2-ethanedithiol and 0.8 ml of BF_3-etherate, and the resulting solution was allowed to stand at room temperature overnight. The reaction mixture was diluted with ether, washed with 5% NaOH and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel. Elution with benzene-ether (10:1) and recrystallization from MeOH gave 290 mg of 3a as colorless crystals. m.p. 135-138°. $[\alpha]_D^{10}$ - 14.23° (C = 0.26). Anal. Calcd. for $C_{26}H_{32}O_4S_4$: C, 58.20; H, 6.01. Found: C, 57.99; H, 5.98. NMR ($CDCl_3$)δ: 1.07 (3 H, s, 18-CH_3), 2.10 (3 H, s, 16α-OAc), 2.23 (3 H, s, 3-OAc), 3.17 (4 H, s, 17-SCH_2CH_2S-), 3.45 (4 H, m, 6-SCH_2CH_2S-), 5.1 (1 H, m, 16β-H), 6.83 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.15 (1 H, d, J = 8 Hz, C_1-H), 7.60 (1 H, d, J = 2 Hz, C_4-H).

3,16α-Diacetoxy-1,3,5(10)-estratriene-6,17-dione 17-Ethylenedithioketal (3b). To a solution of 2.2 g of 3a in 50 ml of 80% CH_3CN were added 5.4 g of $HgCl_2$ and 2.1 g of $CaCO_3$ and the reaction mixture was stirred at room temperature for 30 min. The resulting solution was diluted with AcOEt, washed with 50% ammonium acetate and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crystalline product (1.4 g) was used for further elaboration without purification. Recrystallization of a portion of the product from MeOH gave 4 as colorless leaflets. m.p. 204-205°. $[\alpha]_D^{10}$ - 45.5° (C = 0.11). Anal. Calcd. for $C_{24}H_{28}O_5S_2$: C, 62.60; H, 6.13. Found: C, 62.92; H, 6.13. NMR ($CDCl_3$)δ: 1.07 (3 H, s, 18-CH_3), 2.12 (3 H, s, 16α-OAc), 2.28 (3 H, s, 3-OAc), 3.2 (4 H, s, -SCH_2CH_2S-), 5.51 (1 H, m, 16β-H), 7.21 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.45 (1 H, d, J = 8 Hz, C_1-H), 7.72 (1 H, d, J = 2 Hz, C_4-H).

3,16α-Diacetoxy-6α-hydroxy-1,3,5(10)-estratrien-17-one Ethylenedithioketal (3c). To a solution of 200 mg of 3b in 4 ml of THF and 8 ml of MeOH were added 150 mg of $NaBH_4$ and 0.3 ml of H_2O under cooling by ice.

Abbreviations used: s = singlet, d = doublet, d,d, = doublet of doublets, m = multiplet.

The reaction mixture was stirred at 0°C for 30 min. After careful addition of AcOH to decompose the excess reagent, the resulting solution was extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by column chromatography on silica gel. Elution with benzene-ether (1:1) and recrystallization of the eluate from hexane-acetone gave 140 mg of 3c as colorless prisms. m.p. 115-116°. $[\alpha]_D^{10}$ -10° (C = 0.16). Anal. Calcd. for $C_{24}H_{30}O_5S_2$: C, 62.32; H, 6.54. Found: C, 62.62; H, 6.82. NMR ($CDCl_3$)δ: 1.00 (3 H, s, 18-CH_3), 2.13 (3 H, s, 16α-OAc), 2.25 (3 H, s, 3-OAc), 3.16 (4 H, s, -SCH_2CH_2S-), 4.7 (1 H, m, 6β-H), 5.8 (1 H, m, 16β-H), 6.9 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.16-7.28 (2 H, m, C_1- and C_4-H).

3,16α-Dihydroxy-1,3,5(10),6-tetraen-17-one Ethylenedithioketal (4). To a solution of 20 mg of 3c in 2 ml of MeOH was added 0.5 ml of conc HCl and the resulting solution was heated at 60°C for 2 hr. After evaporation of MeOH under reduced pressure, the solution was extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using benzene-ether (3:1) as a developing solvent. Recrystallization of the eluate from MeOH gave 11 mg of 4 as colorless leaflets. m.p. 235-238° $[\alpha]_D^{10}$ -233° (C = 0.12, MeOH). Anal. Calcd. for $C_{20}H_{24}O_2S_2$: C, 66.65; H, 6.71. Found: C, 66.83, H, 6.58. NMR ($CDCl_3$-CD_3OD = 1:1) : 0.99 (3 H, s, 18-CH_3), 3.2 (4 H, s, -SCH_2CH_2S-), 4.40 (1 H, m, 16β-H), 5.78 (1 H, d, J = 10 Hz, C_7-H), 6.37 (1 H, d, J = 10 Hz, C_6-H), 6.54 (1 H, s, C_4-H), 6.60 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.01 (1 H, d, J = 8 Hz, C_1-H).

3,16α-Dihydroxy-1,3,5(10),6-estratetraen-17-one (5). To a solution of 50 mg of 4 in 10 ml of 80% CH_3CN were added 250 mg of $HgCl_2$ and 90 mg of $CaCO_3$. The resulting mixture was refluxed with stirring overnight. The mixture was then diluted with AcOEt, washed with 50% CH_3COONH_4 and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using benzene-ether (3:1) as a developing solvent. Recrystallization of the eluate from MeOH gave 21 mg of 5 as colorless needles. m.p. 209-211°. $[\alpha]_D^{10}$ -113° (C = 0.06, MeOH). Anal. Calcd. for $C_{18}H_{22}O_3$: C, 76.03, H, 7.09, Found: C, 75.61; H, 6.97. UV λ_{max}^{MeOH}: 264 (ε 6900), 271 (ε 5800), 306 (ε 2400). NMR (d6-acetone) δ: 0.97 (3 H, s, 18-CH_3), 4.40 (1 H, m, 16β-H). 5.98 (1 H, d, J = 10 Hz, C_7-H) 6.46 (1 H, d, J = 10 Hz, C_6-H), 6.61 (1 H, d, J = 2 Hz, C_4-H), 6.68 (1 H, d,d, J = 2, 8 Hz, C_2-H). 7.05 (1 H, d, J = 8 Hz, C_1-H).

Hydrogenation of 5. A solution of 5 mg of 5 in 7 ml of AcOEt was stirred with 10% Pd/C (10 mg) at room temperature for 4 hrs under a stream of H_2 gas. After removal of the catalyst by filtration, the solvent was evaporated under reduced pressure. The product was purified by preparative TLC using benzene-ether (1:1) as a developing solvent. Recrystallization of the eluate from MeOH gave 4 mg of 16α-hydroxyestrone (6a) as colorless needles. m.p. 222-224°. Mixed melting point on admixture with an authentic sample showed no depression. IR spectra of the two samples were identical.

[6,7-^{3}H]-3,16α-Dihydroxy-1,3,5(10)-estratrien-]7-one (6b). A solution of 20 mg of 6a in 2 ml of AcOEt containing 10 mg of 10% palladium on charcoal was reduced with 25 curies of tritium. The catalyst was removed by filtration, the solvent was evaporated, and the residue was treated twice with MeOH to remove labile tritium. The crude product contained 3.909 Ci. 0.1 curies of the product was submitted to preparative TLC impregnated with 5% $AgNO_3$ using $CHCl_3$-EtOH (96:4) as a developing solvent. The eluate was further purified by column (1 x 18 cm) chromatography on 1 g of Sephadex LH-20. Elution with 10-16 ml of isooctane-benzene-methanol (62:20:18) gave 30 mCi of 6b. An aliquot containing 177,760 cpm was diluted with 24 mg of inert 16α-hydroxyestrone, and recrystallized from MeOH. The successive specific activities were 7256, 7259, and 7321 cpm/mg indicating a radiohomogeneity of 100%. Another aliquot of 6b was scanned following the above thin layer chromatography without $AgNO_3$ and showed a single radioactive peak coincident with the unlabeled 16α-hydroxyestrone. The specific activity of the synthesized material was approximately 56 curies per millimole.

3,16α-Dihydroxy-1,3,5(10)-estratriene-6,17-dione 17-Ethylenedithioketal (3d). To a solution of 10 mg of 3,16α-diacetoxy-1,3,5(10)-estratriene-6,17-dione 17-ethylenedithioketal 3b in methanol (1 ml) and dioxane (0.5 ml) was added 30% KOH (0.1 ml), and the solution was stirred at room temperature overnight. The reaction mixture was neutralized with 10% acetic acid and was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the product from methanol gave 3d (7 mg) as colorless prisms: m.p. 240-243°. $[\alpha]_D^{10}$ -15.4° (C = 0.1). NMR (4% solution in $CDCl_3$-$(CD_3)_2CO$ = 1:1)δ: 1.07 (3 H, s, 18-CH_3), 3.23 (4 H, s, -SCH_2CH_2S-), 4.5 (1 H, m, 16β-H), 6.98 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.25 (1 H, d, J = 8 Hz, C_1-H), 7.37 (1 H, d, J = 2 Hz, C_4-H). Anal. Calcd. for $C_{20}H_{24}O_3S_2$: C, 63.82; H, 6.43. Found: C, 63.94; H, 6.36.

3,16α-Dihydroxy-1,3,5(10)-estratriene-6,17-dione 6-(O-Carboxymethyl) oxime 17-Ethylenedithioketal (3e). To a solution of 3d (80 mg) in methanol (10 ml) was added carboxymethoxylamine-HCl (200 mg) in 2N NaOH (0.9 ml), and the solution was refluxed for 2 hr. After addition of water and 2N NaOH to adjust to pH 11, the resulting solution was extracted with ethyl acetate. The organic layer was acidified to pH 1 with conc hydrochloric acid and was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The crude product was purified by preparative TLC using ethyl acetate-methanol-acetic acid (50:10:0.1) as a solvent. Recrystallization of the eluate from methanol gave 3e (87 mg) as colorless crystals. m.p. 300° (decomp). NMR (4% in d6-DMSO): 0.9 (3 H, s, 18-CH_3), 3.2 (4 H, s, -SCH_2CH_2S-), 4.5 (3 H, m, 16β-H and -$NOCH_2COOH$), 6.6-7.2 (3H, unidentified aromatic proton).

3,16α-Bis(tert-butyldimethylsilyloxy)-1,3,5(10)-estratriene-6,17-dione 17-Ethylenedithioketal (7a). To a solution of 3d (150 mg) in dimethylformamide (0.6 ml) and pyridine (0.3 ml) were added imidazole (1 g) and t-butyldimethylsilyl chloride (500 mg), and the solution was stirred at room temperature overnight. The resulting mixture was diluted with ether, washed with water, dried over anhydrous Na_2SO_4, and evaporated

in vacuo. The product was subjected to column chromatography on silica gel. Elution with cyclohexane-ethyl acetate (20:1 v/v) gave 7a (300 mg) as colorless granules. NMR (4% in $CDCl_3$)δ: 0.1 (6 H, s, 16-OSi$(CH_3)_2$), 0.2 (6 H, s, 3-OSi$(CH_3)_2$), 0.93 (3 H, s, 18-CH_3), 0.97 (12 H, s, 16-OSi-t-Bu), 1.4 (12 H, s, 3-OSi-t-Bu), 3.13 (4 H, m, -SCH_2CH_2S-), 4.57 (1 H, m, 16β-H), 6.9 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.27 (1 H, d, J = 8 Hz, C_1-H), 7.47 (1 H, d, J = 2 Hz, C_4-H).

3-16α-Bis(tert-butyldimethylsilyloxy)-6α-hydroxy-1,3,5(10)-estratrien-17-one Ethylenedithioketal (7b). To a solution of 7a (300 mg) in methanol (20 ml) was added sodium borohydride (100 mg), and the reaction mixture was stirred at 0°C for 5 min. After careful decomposition of the excess reagent with acetic acid, the resulting solution was extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The product was subjected to preparative TLC using benzene-ether (20:1) as a solvent. Recrystallization of the eluate from methanol gave 7b (245 mg) as colorless needles. m.p. 103-105°. $[\alpha]_D^{10}$ -46.7° (C = 0.15). NMR (4% in $CDCl_3$)δ: 0.09 (6 H, s, 16-OSi$(CH_3)_2$), 0.17 (6 H, s, 3-OSi$(CH_3)_2$), 0.9 (3 H, s, 18-CH_3), 0.91 (12 H, s, 16-OSi-t-Bu), 0.96 (12 H, s, 3-OSi-t-Bu), 3.1 (4 H, m, -SCH_2CH_2S-), 4.3-4.9 (2 H, m, 16β- and 6β-H), 6.63 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.03 (1 H, d, J = 2 Hz, C_4-H), 7.07 (1 H, d, J = 8 Hz, C_1-H). Anal. Calcd. for $C_{32}H_{54}O_3S_2Si_2$: C, 63.30; H, 8.98. Found: C, 63.52; H, 8.89.

3,16α-Bis(tert-butyldimethylsilyloxy)-6α-hydroxy-1,3,5(10)-estratrien-17-one (8a). To a solution of 7b (400 mg) in 80% acetonitrile (40 ml) were added mercuric chloride (1.8 g) and calcium carbonate (0.75 g) and the solution was refluxed with continuous stirring overnight. The resulting solution was diluted with ethyl acetate, washed with 50% CH_3COONH_4 and water, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The product was subjected to column chromatography on silica gel. Elution with benzene-ether (50:1) and recrystallization of the eluate from methanol gave 8a (280 mg) as colorless plates. m.p. 108-111°. $[\alpha]_D^{10}$ +120.8° (C = 0.24). NMR (4% in $CDCl_3$)δ: 0.17 (6 H, s, 16-OSi$(CH_3)_2$), 0.23 (6 H, s, 3-OSi$(CH_3)_2$), 0.91 (15 H, s, 16-OSi-t-Bu and 18-CH_3), 1.00 (12 H, s, 3-OSi-t-Bu), 4.33 (1 H, m, 16β-H), 4.73 (1 H, m, 6β-H), 6.70 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.08 (1 H, d, J = 2 Hz, C_4-H), 7.13 (1 H, d, J = 8 Hz, C_1-H). Anal. Calcd. for $C_{30}H_{50}O_4Si_2$: C, 67.86; H, 9.51, Found: C, 67.81; H, 9.39.

3,16α-Bis(tert-butyldimethylsilyloxy)-6α-hydroxy-1,3,5(10)-estratrien-17-one Hemisuccinate (8b). To a solution of 8a (100 mg) in pyridine (3 ml) was added succinic anhydride (200 mg), and the resulting solution was refluxed for 2.5 hr. After evaporation of pyridine in vacuo, the residue was diluted with ether, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The product was subjected to column chromatography on silica gel. Elution with cyclohexane-ethyl acetate (1:2) gave 8b (108 mg) as colorless amorphous powder. NMR (4% in $CDCl_3$) : 0.17 (6 H, s, 16-OSi$(CH_3)_2$), 0.23 (6 H, s, 3-OSi$(CH_3)_2$), 0.91 (15 H, s, 16-OSi-t-Bu and 18-CH_3), 1.00 (12 H, s, 3-OSi-t-Bu), 2.6 (4 H, m, -$OCOCH_2CH_2COOH$), 4.37 (1 H, m, 16β-H), 6.07 (1 H, m, 6β-H), 6.71 (1 H, d, J = 2 Hz, C_4-H), 6.77 (1 H, d,d, J = 2, 8 Hz, C_2-H), 7.17 (1 H, d, J = 8 Hz, C_1-H).

3,6α,16α-Trihydroxy-1,3,5(10)-estratrien-17-one 6-Hemisuccinate (8c). To a solution of 8b (25 mg) in acetone (1 ml) was added 1N HCl (0.2 ml) and the solution was allowed to stand at room temperature overnight. The resulting solution was diluted with H_2O and was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The product was subjected to column chromatography on silica gel. Elution with ethyl acetate-methanol (3:1) gave 8c (14 mg) as colorless granules. m.p. 170-173°. NMR ($CDCl_3$-CD_3OD = 1:4)δ: 0.93 (3 H, s, 18-CH_3), 2.67 (4 H, m, -$OCOCH_2CH_2COOH$), 4.37 (1 H, m, 16β-H), 6.03 (1 H, m, 6β-H), 6.3-6.8 (2 H, unresolved m, C_2- and C_4-H), 7.11 (1 H, d, J = 8 Hz, C_1-H). Anal. Calcd. for $C_{22}H_{26}C_7.2½\ H_2O$: C, 59.04: H. 6.43. Found. C, 59.05; H, 6.21.

RESULTS AND DISCUSSION

Benzylic oxidation of 3,16α-diacetoxyestrone (1), which can be obtained from estrone in three steps, with chromic acid in acetic acid gave the 6-keto derivative 2 in reasonable yield. Difficulties were encountered in selective reduction of the carbonyl function at C-6 and an alternative route was therefore required to generate the 6-hydroxyl without reduction. of the 17-ketone. Treatment of 2 with 1,2-ethanedithiol in acetic acid in the presence of boron trifluoride etherate gave the 6,17-bisethylene-dithioketal 3a in quantitative yield. Selective desulfurization of the benzylic C-6 ketone was accomplished quantitatively by brief treatment with mercuric chloride and calcium carbonate in 80% acetonitrile to yield 3b. The structure of 3b was confirmed by nuclear magnetic resonance (NMR) spectrum with the singlet signal due to the ethylenedithioketal function at C-17 appearing at 3.2 ppm, while the multiplet signal due to the same function at C-6 (3.45 ppm in 3a) was absent. Reduction of 3b with sodium borohydride ($NaBH_4$) afforded the 6α-hydroxy derivative 3c. The α configuration of the hydroxyl group was assigned by analogy to other reductions at this position [18]. Dehydration of the hydroxyl group at C-6 and hydrolysis of the acetates occurred simultaneously when 5 was heated with hydrochloric acid in methanol and gave the 3,16α-dihydroxy-6-dehydro derivative 4 in satisfactory yield. Upon refluxing with mercuric chloride and calcium carbonate in 80% acetonitrile for

an extended period, 4 was converted to the desired 3,16α-dihydroxy-1,3,5(10),6-estratetraen-17-one (5). The Δ^6 structure was confirmed by spectral data. The NMR spectrum exhibited a doublet signal due to the C-6 and C-7 protons which appeared at 6.46 ppm and at 5.98 ppm. Absorption at 264 nm and at 306 nm in the ultraviolet (UV) spectrum also identified the presence and location of the double bond. Catalytic hydrogenation of 5 yielded 16α-hydroxyestrone 6a which was identical with the authentic material.

Reduction of the benzylic double bond in 5 with 25 Curies of tritium provided [6,7-^{3}H]-16α-hydroxyestrone (6b). The product was separated from the starting material by preparative thin layer chromatography on silica gel impregnated with $AgNO_3$ and was further purified by LH-20 column chromatography. Reverse isotope dilution with the carrier 16α-hydroxyestrone showed the product to be 100% radiohomogeneous, with a specific activity of approximately 56 Ci/mmol.

In order to elicit an antibody with high specificity to the ring D structure we sought to prepare a steroid hapten which could be linked to the carrier protein at the C-6 position. Initial attempts to prepare the 6-carboxymethyloxime of 16α-hydroxyestrone by forming the corresponding oxime of the 17-thioketal derivative 3d and then desulfurizing failed because the carboxymethyloxime group was invariably cleaved in the latter process. We resorted, therefore, to an alternative route to generate the desired carboxyl derivative which would be suitable for linkage with the carrier protein. Hydrolysis of the acetate groups in 3b and reaction of the product with t-butyl dimethylsilyl chloride provided the corresponding bis derivative 7a. Reduction of the 6-keto group with $NaBH_4$ yielded the 6α-hydroxy derivative 7b which underwent smooth desulfurization with mercuric chloride and calcium carbonate to

give the 17-keto product 8a. Reaction of the latter with succinic anhydride gave the hemisuccinate 8b, from which the t-butyl dimethylsilyl ether protecting groups were readily removed selectively with dilute HCl to provide the desired 6-carboxy derivative 8c. The two required substances for producing the desired antibodies and for measuring their binding were therefore made available.

ACKNOWLEDGMENT

This work was supported by grant 5 PO1 CA22795 from the National Cancer Institute.

REFERENCES

1. Present address: Faculty of Pharmaceutical Sciences, Higashi Nippon Gakuen University, Ishikari Tobetsu, Hokkaido 061-02, Japan
2. Fishman, J., Bradlow, H.L., and Gallagher, T.F., J. BIOL. CHEM. 235, 3104 (1960).
3. Marrian, G.F., Watson, E.J.D., and Panattoni, M., BIOCHEM. J. 65, 12 (1957).
4. Brown, J.B. and Marrian, G.F., J. ENDOCRINOL. 15, 307 (1957).
5. Fishman, J., Zumoff, B., Hellman, L., and Gallagher, T.F., STEROIDS 11, 337 (1968).
6. Gurpide, E., Schwers, J., Welch, M.T., and Lieberman, S. J. CLIN. ENDOCRINOL. METAB. 26, 1355 (1966).
7. Tulchinsky, D., Frigoletto, F.D., Ryan, K.J., and Fishman, J. J. CLIN. ENDOCRINOL. METAB. 40, 560 (1975).
8. Rotti, K., Stevens, J., Watson, D., and Longcope, C., STEROIDS 25, 807 (1975).
9. Clark, J.H., Paszko, Z., and Peck, E.J., ENDOCRINOLOGY 100, 91 (1977).
10. Adlercreutz, H. and Martin, F., ACTA ENDOCRINOL. 83, 410 (1970).
11. Adlercreutz, H., Trikkourer, M.J., and Hanneman, D.H., J. STEROID BIOCHEM. 5, 211 (1974).
12. Zumoff, B., Fishman, J., Gallagher, T.F., and Hellman, L., J. CLIN. INVEST. 47, 20 (1968).
13. Lahita, R., Bradlow, H.L., Kunkel, H.G., and Fishman, J., ARTHRITIS & RHEUM. 22, 1195 (1979).
14. Fishman, J. and Martucci, C., J. CLIN. ENDOCRINOL. METAB. 51, 611 (1980).
15. Fishman, J., J. AM. CHEM. SOC. 82, 6143 (1960).
16. Leeds, N.S., Fukushima, D.K., and Gallagher, T.F. J. AM. CHEM. SOC. 76, 2943 (1954).
17. Hobkirk, R. and Nilsen, M., CAN. J. BIOCHEM. 55, 769 (1977).
18. Wintersteiner, O., Moore, M., and Cohen, A.I. J. ORG. CHEM. 29, 1325, (1964).