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**156. Toshio Nambara^{*1} and Kazuhiro Imai^{*2}: Syntheses
of Estriol Monoglucuronides.^{*3}***(Pharmaceutical Institute, Tohoku University School of Medicine^{*1} and Faculty
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Syntheses of estriol glucosiduronic acids were carried out employing Koenigs-Knorr reaction. Of these three 16- and 17-glucuronides were prepared from estrone 3-benzyl ether, and 3-isomer from estriol 16,17-diacetate as shown in Chart 1. All the glucuronides thus obtained underwent hydrolysis with beef liver β -glucuronidase to furnish estriol and glucuronic acid.

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Cohen and Marrian²⁾ isolated first sodium estriol glucosiduronate from human pregnancy urine. The complete structure had been ambiguous until when Neeman and Hashimoto^{3,4)} elucidated its free acid as 3,17 β -dihydroxyestra-1,3,5(10)-trien-16 α -yl- β -D-glucopyranosiduronic acid by degradative means. On the other hand the presence of isomeric estriol 3- and 17-glucuronides in biological fluids has also been reported on the basis of chromatographic behaviors as well as separation of their derivatives.⁵⁻⁸⁾ The present paper deals with the syntheses of estriol 16-, 17- and 3-glucuronides in connection with clinical chemical studies on urinary estrogen conjugates.

For the purpose of protecting the phenolic hydroxyl group estrone (Ia) was transformed into 3-benzyl ether (Ib). Treatment of Ib with isopropenyl acetate and catalytic amount of sulfuric acid afforded Δ^6 -17-ol acetate (II). Subsequent epoxidation of II with perbenzoic acid resulted in formation of 16 α , 17 α -epoxide (III), which in turn was converted with sulfuric acid to 16 α -hydroxy-17-oxo derivative (IVb). The structure of IVb was confirmed by leading to the known 3,16 α -dihydroxyestra-1,3,5(10)-trien-17-one diacetate (Va) on hydrogenation over palladium-charcoal followed by usual acetylation.^{*4}

Condensation of glucuronic acid component with IVb was accomplished by Koenigs-Knorr reaction in the usual way.¹¹⁾ When IVb and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranosiduronate were stirred in dry benzene with freshly prepared

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^{*3} This paper constitutes Part XI of the series entitled "Analytical Chemical Studies on Steroids;" Part X: This Bulletin, in press. A part of this report was presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan (Sendai, October, 1966). During the course of this work we found a communication¹⁾ dealing with syntheses of estriol 16- and 17-glucuronides, but no further details have appeared in the scientific literature.

^{*4} It is sufficiently postulated that 16 α -hydroxy-17-oxo compound would be susceptible to ketol rearrangement with base.^{9,10)} Therefore the attempt for direct benzylation of 3,16 α -dihydroxyestra-1,3,5(10)-trien-17-one was avoided.

- 1) J. S. Elce, J. G. D. Carpenter, A. E. Kellie: *Biochem. J.*, **91**, 30 p (1964).
- 2) S. L. Cohen, G. F. Marrian: *Biochem. J.*, **30**, 57, 2250 (1936).
- 3) M. Neeman, Y. Hashimoto: *J. Am. Chem. Soc.*, **84**, 2972 (1962).
- 4) Y. Hashimoto, M. Neeman: *J. Biol. Chem.*, **238**, 1273 (1963).
- 5) J. G. D. Carpenter, A. E. Kellie: *Biochem. J.*, **84**, 303 (1962).
- 6) C. G. Beling: *Acta endocrinol., Suppl.*, **79**, 9 (1963).
- 7) R. Hähnel: *Anal. Biochem.*, **10**, 184 (1965).
- 8) R. Wilson, G. Eriksson, E. Diczfalusy: *Acta endocrinol.*, **46**, 525 (1964).
- 9) N. S. Leeds, D. K. Fukushima, T. F. Gallagher: *J. Am. Chem. Soc.*, **76**, 2943 (1954).
- 10) J. Fishman: *Ibid.*, **82**, 6143 (1960).
- 11) H. H. Wotiz, E. Smakula, N. N. Lichtin, J. H. Leftin: *Ibid.*, **81**, 1704, 1708 (1959).

silver carbonate, methyl (3-benzyloxy-17-oxoestra-1,3,5(10)-trien-16 α -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (V) was afforded in 23% yield. Then reduction of 17-oxo group was carried out using metal hydride under the various conditions. It has already been substantiated that sodium borohydride reduces ester group as well as C-1-oxo function of glucuronate providing sorbitol.¹²⁾ In fact, even on brief treatment with sodium borohydride the more polar compound than the expected was yielded as major product, which was indicated by thin-layer chromatography (TLC). However, potassium borohydride reduction in dimethylformamide at -10° to -6° followed by fractional recrystallization furnished the desired 17 β -hydroxy derivative (IVa). From the mother liquor the epimeric 17 α -hydroxy compound (IVb) was isolated, whose structure was confirmed by leading to 17-epiestriol. Debenzylation of C-3-substituent was readily attained by hydrogenesis and thereupon, methyl (3,17 β -dihydroxyestra-1,3,5(10)-trien-16 α -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (VII) was obtained. Removal of the protecting groups in glucuronic acid moiety of VII was accomplished by transesterification with sodium methoxide. The sodium salt thus prepared being dissolved in water saturated with *n*-butanol, adjusted to pH 1 with hydrochloric acid and allowed to stand in refrigerator, estriol 16-glucuronide (VIII) was provided as colorless needles.

The second project was focused on the preparation of estriol 17-glucuronide, and IVb was chosen as a starting compound. Since acyl migration would possibly take place during the course of subsequent reduction with metal hydride,¹³⁻¹⁵⁾ 16-hydroxyl function was blocked with *tert*-butyl rather than with acyl group. On treatment with isobutene in the presence of sulfuric acid¹⁶⁾ IVb was transformed with ease into 16 α -*tert*-butoxy derivative (IX), which was reduced with potassium borohydride to the corresponding 17-hydroxy derivative. The reduction product proved to consist of two epimeric 17-hydroxy compounds (Xa, Xb) in the ratio of 3:1, whose separation was achieved by means of preparative-scale thin-layer chromatography. It seems very likely that the rear side attack of the reagent would be sterically interfered probably due to the presence of bulky substituent at C-16 α . Now, the attempt was made on the condensation of Xa with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranosiduronate employing Koenigs-Knorr reaction. Upon the similar treatment as described above methyl (3-benzyloxy-16 α -*tert*-butoxyestra-1,3,5(10)-trien-17 β -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XI) was furnished in 25% yield. Elimination of *tert*-butyl group was achieved on brief exposure to trifluoroacetic acid providing 16 α -hydroxy derivative (XII), which in turn was converted to methyl (3,16 α -dihydroxyestra-1,3,5(10)-trien-17 β -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XIII) by usual hydrogenesis over palladium-charcoal. When XIII was hydrolyzed with methanolic sodium hydroxide and the resultant sodium salt was treated in the same manner as mentioned above, the desired estriol 17-glucuronide (XIV) was furnished as colorless needles.

As the third program the preparation of the remained isomer, namely estriol 3-glucuronide was undertaken. For this purpose estriol 16,17-diacetate (XV), which has already been reported by Tsuneda, *et al.*, was taken as a starting material. Condensation with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranosiduronate by Koenigs-Knorr reaction did not proceed so readily as in the cases of isomeric 16- and 17-glucuronides and in consequence, methyl (16 α ,17 β -diacetoxystestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XVI) was obtained in poor yield.

12) M. L. Wolfrom, K. Anno : *Ibid.*, **74**, 5883 (1952).

13) P. Wieland, K. Heusler, A. Wettstein : *Helv. Chim. Acta*, **41**, 1657 (1958).

14) D. Taub, R. D. Hoffsommer, N. L. Wendler : *J. Am. Chem. Soc.*, **81**, 3291 (1959).

15) C. H. Kuo, D. Taub, N. L. Wendler : *J. Org. Chem.*, **28**, 1619 (1963).

16) H. C. Beyerman, G. J. Heiszwolf : *Rec. trav. chim.*, **84**, 203 (1965).

Employing these authentic samples isolation of estriol glucuronides from pregnancy urine are in progress. In addition the studies on the biosyntheses of estriol glucuronides with UDPGA-transferase system are being conducted in these laboratories and will be reported in near future.

Experimental*5

3-Benzoyloxyestra-1,3,5(10)-trien-17-one (Ib)—To a solution of estrone (3 g.) and benzyl chloride (5 g.) dissolved in anhyd. EtOH (200 ml.) was added K_2CO_3 (6 g.), and the mixed solution was refluxed on water bath for 5 hr. The reaction mixture was diluted with $CHCl_3$ (250 ml.), washed with H_2O and dried over anhyd. Na_2SO_4 . After evaporation of solvent the crude product obtained was recrystallized from EtOH to give Ib (3.64 g.) as colorless leaflets. m.p. 128.5~130°, UV λ_{max}^{EtOH} m μ (log ϵ): 277 (3.08), 286 (3.04). *Anal.* Calcd. for $C_{25}H_{28}O_2$: C, 83.29; H, 7.83. Found: C, 83.26; H, 7.68 (Reported m.p. 136°).¹⁷⁾

3-Benzoyloxyestra-1,3,5(10),16-tetraen-17-ol Acetate (II)—To a solution of Ib (3.6 g.) in isopropenyl acetate (25 ml.) was added catalyst solution (5 ml. of isopropenyl acetate and 0.1 ml. of conc. H_2SO_4) (1 ml.) and the reaction mixture was boiled under reflux for 1 hr., and approximately 20 ml. was distilled off for 1 hr. Additional isopropenyl acetate (15 ml.) and catalyst solution (0.1 ml.) were added and concentrated to one-half of its volume by slow distillation over another 2 hr. The residue was diluted with ether, washed with ice-cooled 5% $NaHCO_3$, H_2O and dried over anhyd. Na_2SO_4 . After evaporation of solvent the residue was dissolved in hexane (500 ml.) and filtered through Al_2O_3 (10 g.). Eluate with hexane-benzene (9:1 to 2:1) was concentrated to give crystalline product. Recrystallization from EtOH gave II (2.52 g.) as colorless leaflets. m.p. 150~152°, UV λ_{max}^{EtOH} m μ (log ϵ): 278 (3.26), 287 (3.22). $[\alpha]_D^{31.5} + 48.1^\circ$ (c=0.43). *Anal.* Calcd. for $C_{27}H_{30}O_3$: C, 80.56; H, 7.51. Found: C, 80.73; H, 7.36.

3-Benzoyloxy-16 α ,17 α -epoxyestra-1,3,5(10)-trien-17 β -ol Acetate (III)—To a solution of II (5.8 g.) in $CHCl_3$ (90 ml.) was added perbenzoic acid- $CHCl_3$ solution (0.21M) (103 ml.) and the mixed solution was allowed to stand in refrigerator overnight. The reaction mixture was diluted with $CHCl_3$ (200 ml.), washed with 5% $NaHCO_3$, H_2O and dried over anhyd. Na_2SO_4 . After evaporation of solvent the semicrystalline residue was submitted to further step without purification. A part of the crude product was recrystallized from MeOH-acetone to give III as colorless needles. m.p. 137~147° (sublimate at 120.5~124°), UV λ_{max}^{EtOH} m μ (log ϵ): 278 (3.32), 287 (3.29). $[\alpha]_D^{31.5} + 48.50^\circ$ (c=0.21). *Anal.* Calcd. for $C_{27}H_{30}O_4$: C, 77.48; H, 7.23. Found: C, 77.56; H, 7.27.

3-Benzoyloxy-16 α -hydroxyestra-1,3,5(10)-trien-17-one (IVb)—To a solution of the crude product (III) in MeOH (20 ml.)-acetone (3 ml.) was added 6N H_2SO_4 (4 ml.) and the mixed solution was allowed to stand at room temperature for 72 hr. The reaction mixture was diluted with AcOEt (200 ml.), washed with 5% $NaHCO_3$, H_2O and dried over anhyd. Na_2SO_4 . After evaporation of solvent the residue obtained was recrystallized from EtOH to give IVb (49 mg.) as colorless leaflets. m.p. 178~182°, UV $\lambda_{max}^{dioxane}$ m μ (log ϵ): 278 (3.37), 286 (3.46). $[\alpha]_D^{29.8} + 124^\circ$ (c=0.33). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.69; H, 7.39.

Transformation of IVb to 3,16 α -Dihydroxyestra-1,3,5(10)-trien-17-one Diacetate (IVa)—A solution of IVb (34 mg.) dissolved in EtOH (20 ml.) was shaken with 5% Pd/C (20 mg.) for 20 hr. in the stream of H_2 at room temperature. After removal of catalyst by filtration the filtrate was concentrated to give crystalline residue. Recrystallization from hexane-acetone gave 3,16 α -dihydroxyestra-1,3,5(10)-trien-17-one (18 mg.) as colorless prisms. m.p. 203~223°. Acetylation with pyridine (0.14 ml.) and Ac_2O (0.07 ml.) in the usual manner followed by recrystallization from MeOH furnished IVa (13 mg.) as colorless plates. m.p. 168.5~173°. On further recrystallization from MeOH the sample melted at 174~176°. The mixed m.p. of the mixture with the authentic sample⁹⁾ showed no depression and the IR spectra of two samples were also entirely identical.

Methyl (3-Benzoyloxy-17-oxoestra-1,3,5(10)-trien-16 α -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (V)—To a solution of IVb (430 mg.) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranosiduronate (780 mg.) in anhyd. benzene (70 ml.) was added freshly prepared Ag_2CO_3 (1 g.) and the suspended solution was stirred at room temperature for 20 hr. During the continuation of stirring additional amount of Ag_2CO_3 (3 g.) was added in several portions. The precipitate was removed by filtration and the filtrate was evaporated to give the crystalline residue. Recrystallization from EtOH gave V (183 mg.) as colorless needles. m.p. 217.5~220°, UV $\lambda_{max}^{dioxane}$ m μ (log ϵ): 279 (3.28), 288 (3.25). $[\alpha]_D^{29.8} + 79.7^\circ$ (c=0.25). *Anal.* Calcd. for $C_{33}H_{44}O_{12}$: C, 65.88; H, 6.40. Found: C, 65.70; H, 6.42.

Reduction of V with KBH_4 —To a cooled solution of V (251 mg.) in DMF (20 ml.) was added methanolic solution (1 ml.) of KBH_4 (70 mg.) dropwise, and the mixed solution was allowed to stand at -10~-6° for

*5 All melting points were taken on a micro hot stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solution unless otherwise stated. TLC plate was prepared and activated according to the Stahl's procedure using silica gel H (E. Merck AG) as adsorbent.

17) R. Weisz: C. A., 35, 137 (1941); U. S. Pat., 2,208,915.

4.5 hr. After decomposition of excess KBH_4 with AcOH the reaction mixture was diluted with AcOEt (200 ml.), washed with H_2O and dried over anhyd. Na_2SO_4 . Upon evaporation of solvent the crystalline residue (152 mg.) was obtained. Fractional recrystallization from EtOH gave methyl (3-benzyloxy-17 β -hydroxyestra-1,3,5(10)-trien-16 α -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (VIa) (88 mg.) as colorless needles. m.p. 244~247°(decomp.), $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 278 (3.27), 287 (3.24), $[\alpha]_{\text{D}}^{20} + 15.1^\circ$ (c=0.49). *Anal.* Calcd. for $\text{C}_{38}\text{H}_{46}\text{O}_{12}$: C, 65.69; H, 6.67. Found: C, 65.53; H, 6.71. The mother liquor was submitted to the preparative TLC using benzene-ether (1:1) as developing solvent. Elution of the adsorbent corresponding to the spots (Rf 0.44 and 0.60) followed by recrystallization from EtOH gave additional VIa (6.7 mg.) and methyl (3-benzyloxy-17 α -hydroxyestra-1,3,5(10)-trien-16 α -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (VIb) (9.3 mg.), respectively. VIb: m.p. 200~204.5°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 278 (3.30), 286 (3.27). $[\alpha]_{\text{D}}^{16.4} + 23.0^\circ$ (c=0.45). *Anal.* Calcd. for $\text{C}_{38}\text{H}_{46}\text{O}_{12}$: C, 65.69; H, 6.67. Found: C, 65.89; H, 6.81.

Transformation of VIb to 17-Epiestriol—A solution of VIb (10.7 mg.) dissolved in EtOH (25 ml.) was shaken with 5% Pd/C (10 mg.) for 7 hr. in the stream of H_2 . On usual work-up the product thus obtained was recrystallized from hexane- CHCl_3 to give amorphous substance (6 mg.) (TLC: Rf 0.60, benzene-ether (1:1)). To a solution of this product in MeOH (15 ml.) was added MeONa solution (0.7 g. of Na dissolved in 25 ml. of MeOH) (5 drops) and the solution was refluxed for 3 hr. in the stream of N_2 . After allowing to stand at room temperature overnight the reaction mixture was concentrated, diluted with H_2O and adjusted to pH 1 with HCl . The solution was extracted with CHCl_3 -*iso*- $\text{C}_3\text{H}_7\text{OH}$ (2:1) (60 ml. $\times$ 3) and the organic layer was washed with H_2O and dried over anhyd. Na_2SO_4 . After evaporation of solvent the residue was submitted to hydrolysis with β -glucuronidase in the usual manner and the extract with AcOEt was subjected to TLC employing ether-acetone (19:1) as developing solvent. The sample showed a characteristically colored spot at Rf 0.69, which proved to be identical with that of the authentic 17-epiestriol.

Methyl (3,17 β -Dihydroxyestra-1,3,5(10)-trien-16 α -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (VII)—A solution of VIa (114 mg.) dissolved in EtOH (80 ml.) was shaken with 5% Pd/C (140 mg.) for 24 hr. in the stream of H_2 at room temperature. After removal of catalyst by filtration the filtrate was concentrated to give crystalline residue. Recrystallization from hexane-acetone gave VII (53 mg.) as colorless needles. m.p. 228~229°(decomp.), UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 281 (3.27), 289 (3.23). $[\alpha]_{\text{D}}^{17.6} + 17.6^\circ$ (c=0.70). *Anal.* Calcd. for $\text{C}_{31}\text{H}_{40}\text{O}_{12}$: C, 61.58; H, 6.67. Found: C, 61.53; H, 6.62.

3,17 β -Dihydroxyestra-1,3,5(10)-trien-16 α -yl- β -D-glucopyranosiduronic Acid (VIII)—To a solution of VII (50 mg.) in MeOH (30 ml.) was added freshly prepared MeONa solution (1 ml.) (0.7 g. of Na in 25 ml. of MeOH) and refluxed in the stream of N_2 for 2 hr. The reaction mixture was concentrated *in vacuo* below 30°. After addition of anhyd. MeOH (1 ml.) and allowing to stand in a refrigerator overnight the precipitate was separated by centrifuging. The crude Na salt was dissolved in H_2O saturated with *n*- BuOH and pH of this solution was adjusted to 1.0 with aq. HCl saturated with *n*- BuOH . A gel thus yielded gradually crystallized to give VIII (8.2 mg.) as colorless needles on cooling in a refrigerator. m.p. 224~225°(decomp.), UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 281 (3.29). $[\alpha]_{\text{D}}^{26.6} 0^\circ$ (c=0.21, EtOH), *Anal.* Calcd. for $\text{C}_{24}\text{H}_{32}\text{O}_9 \cdot \text{H}_2\text{O}$: C, 59.74; H, 7.10. Found: C, 59.46; H, 7.07. Naturally-occurring estriol 16-glucuronide isolated from human pregnancy urine was reported to show m.p. 223~224°(decomp.).^{3,4)} Na salt: VII (38 mg.) was dissolved in methanolic 0.2N NaOH (0.5 ml.) and the solution was allowed to stand at room temperature overnight. Colorless prisms separated were filtered, washed with MeOH and dried at 120° for 14 hr. Yield 8 mg. m.p. 246~252°(decomp.). *Anal.* Calcd. for $\text{C}_{23}\text{H}_{30}\text{O}_9\text{Na}_2 \cdot 2\text{H}_2\text{O}$: C, 54.75; H, 6.13. Found: C, 54.14; H, 6.31.

3-Benzyloxy-16 α -tert-butoxyestra-1,3,5(10)-trien-17-one (IX)—To a solution of VIb (554 mg.) in CH_2Cl_2 (5 ml.) were added CH_2Cl_2 (5 ml.) containing conc. H_2SO_4 (2 drops) and isobutene (0.5 ml.) under ice-cooling, and the mixed solution was allowed to stand at room temperature overnight. The reaction mixture was diluted with CHCl_3 (250 ml.), washed with 5% NaHCO_3 , H_2O and dried over anhyd. Na_2SO_4 . After evaporation of solvent the residue obtained was chromatographed on Al_2O_3 (5 g.). Elution with hexane-benzene (2:1) and recrystallization of the eluate from EtOH gave IX (105 mg.) as colorless needles. m.p. 177~180°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 278 (3.28), 288 (3.26). $[\alpha]_{\text{D}}^{18.7} + 210.8^\circ$ (c=0.31). *Anal.* Calcd. for $\text{C}_{29}\text{H}_{36}\text{O}_3$: C, 80.51; H, 8.39. Found: C, 80.26; H, 8.14.

Reduction of IX with KBH_4 —To a solution of IX (32 mg.) in CH_2Cl_2 (2 ml.) was added 95% ethanolic solution (4 ml.) of KBH_4 (18 mg.) under ice-cooling. The reaction mixture was allowed to stand at room temperature for several days. After decomposition of the excess reagent with AcOH the reaction mixture was diluted with CHCl_3 (100 ml.), washed with H_2O and dried over anhyd. Na_2SO_4 . On usual work-up the crude product obtained was submitted to the preparative TLC using benzene-ether as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.32~0.68) and recrystallization of the eluate from hexane-acetone gave 3-benzyloxy-16 α -tert-butoxyestra-1,3,5(10)-trien-17 β -ol (Xa) (12 mg.) as colorless needles. m.p. 136~138°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 278 (3.32), 287 (3.29). $[\alpha]_{\text{D}}^{19.4} + 15.3^\circ$ (c=0.49). *Anal.* Calcd. for $\text{C}_{29}\text{H}_{38}\text{O}_3$: C, 80.14; H, 8.81. Found: C, 80.07; H, 8.80. Elution of the adsorbent corresponding to the spot (Rf 0.68~0.85) and recrystallization of the eluate from acetone-hexane gave 3-benzyloxy-16 α -tert-butoxyestra-1,3,5(10)-trien-17 α -ol (Xb) (4.2 mg.) as colorless needles. m.p. 178~180°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 278 (3.31), 286 (3.26). $[\alpha]_{\text{D}}^{19.7} + 49.0^\circ$ (c=0.36). *Anal.* Calcd. for $\text{C}_{29}\text{H}_{38}\text{O}_3$: C, 80.14; H, 8.81. Found: C, 80.00; H, 8.63.

Transformation of Xb to 17-Epiestriol—Xb was submitted to catalytic hydrogenation over 5% Pd/C and then treated with trifluoroacetic acid in the same manner as in the case of Xa. The crude product thus obtained proved to be identical with the authentic 17-epiestriol by TLC comparison.

Methyl (3-Benzoyloxy-16 α -tert-butoxyestra-1,3,5(10)-trien-17 β -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XI)—To a solution of Xa (296 mg.) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranosiduronate (320 mg.) in anhyd. benzene (55 ml.) was added freshly prepared Ag₂CO₃ (1 g.) and the suspended solution was stirred at room temperature for 12 hr. During the continuation of stirring additional amount of Ag₂CO₃ (1 g.) was added in several portions. The precipitate was filtered off and the filtrate was evaporated to dryness *in vacuo*. The crude product obtained was submitted to preparative TLC using hexane-ether (1:2) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.26~0.40) and recrystallization of the eluate from MeOH gave XI (128 mg.) as colorless needles. m.p. 153~156°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 278 (3.31), 286 (3.28). $[\alpha]_{\text{D}}^{27.8}$ -289°(c=0.20). *Anal.* Calcd. for C₄₂H₅₄O₁₂: C, 67.18; H, 7.25. Found: C, 67.23; H, 7.40.

Methyl (3-Benzoyloxy-16 α -hydroxyestra-1,3,5(10)-trien-17 β -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XII)—XI (116 mg.) was dissolved in trifluoroacetic acid (1 ml.) under cooling in ice-water and allowed to stand for 1 hr. After evaporation of solvent the crude product was recrystallized from aq. EtOH to give XII (95 mg.) as colorless prisms. m.p. 169~179°. This crystalline product was submitted to preparative TLC using benzene-ether (1:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.24~0.46) and recrystallization of the eluate from MeOH gave XII (61 mg.) as colorless fiber. m.p. 183~184.5°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 279 (3.30), 287 (3.26). $[\alpha]_{\text{D}}^{30.8}$ +0.86°(c=0.23). *Anal.* Calcd. for C₃₈H₄₆O₁₂: C, 65.69; H, 6.67. Found: C, 65.65; H, 6.53.

Methyl (3,16 α -Dihydroxyestra-1,3,5(10)-trien-17 β -yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XIII)—A solution of XII (56 mg.) dissolved in EtOH (50 ml.) was shaken with 5% Pd/C (100 mg.) for 48 hr. in the stream of H₂ at room temperature. After removal of catalyst by filtration the filtrate was concentrated to afford crystalline residue. Recrystallization from acetone-hexane gave XIII (27 mg.) as colorless needles. m.p. 207~212°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 281 (3.35), 288 (3.30). $[\alpha]_{\text{D}}^{25.4}$ -63.5°(c=0.20). *Anal.* Calcd. for C₃₁H₄₀O₁₂·H₂O: C, 59.79; H, 6.80. Found: C, 60.05; H, 6.56.

3,16 α -Dihydroxyestra-1,3,5(10)-trien-17 β -yl- β -D-glucopyranosiduronic Acid (XIV)—A solution of XIII (50 mg.) dissolved in methanolic 0.2N NaOH (1.5 ml.) was allowed to stand at room temperature for 3 hr. and then in refrigerator for 20 hr. Upon concentration of the reaction mixture the crystalline residue was obtained. The crude product was dissolved in H₂O saturated with *n*-BuOH and pH of this solution was adjusted to 1.0 with aq. HCl saturated with *n*-BuOH. After allowing to stand overnight in refrigerator the resultant precipitate was centrifuged, dried and recrystallized from MeOH to give XIV (11 mg.) as colorless needles. Further recrystallization from MeOH gave the analytical sample. m.p. 235~240°(decomp.), UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ): 281 (3.29). $[\alpha]_{\text{D}}^{16.5}$ -116°(c=0.26, MeOH). *Anal.* Calcd. for C₂₄H₃₂O₉·H₂O: C, 59.74; H, 7.10. Found: C, 59.49; H, 6.98.

Methyl (16 α ,17 β -Diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl- β -D-glucopyranosid)uronate (XVI)—To a solution of estriol 16,17-diacetate (XV) (90 mg.) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranosiduronate (148 mg.) in anhyd. benzene (20 ml.) was added freshly prepared Ag₂CO₃ (132 mg.), and the suspended solution was stirred at room temperature for 24 hr. The precipitate was filtered off and the filtrate was evaporated to dryness *in vacuo*. The crude product thus obtained was submitted to preparative TLC using hexane-ether (1:2) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.25) and recrystallization of the eluate from MeOH gave XVI (9.5 mg.) as colorless needles. m.p. 197~198°, UV $\lambda_{\text{max}}^{\text{dioxane}}$ m μ (log ϵ): 274 (3.11), 282 (3.04). $[\alpha]_{\text{D}}^{25.8}$ -0.20°(c=0.27). *Anal.* Calcd. for C₃₆H₄₄O₁₄: C, 61.04; H, 6.44. Found: C, 60.58; H, 6.19.

16 α ,17 β -Dihydroxyestra-1,3,5(10)-trien-3-yl- β -D-glucopyranosiduronic Acid (XVII)—A solution of XVI (16 mg.) dissolved in methanolic 0.2N NaOH (2 ml.) was allowed to stand at room temperature for 48 hr. The reaction mixture was concentrated to a small volume furnishing crystalline product, which was filtered and washed with MeOH. The crude Na salt was dissolved in H₂O saturated with *n*-BuOH and pH of this solution was adjusted to 1.0 with aq. HCl saturated with *n*-BuOH. A gel thus yielded gradually crystallized to give XVII (7 mg.) as colorless leaflets. m.p. 213~220°(decomp.), UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ): 274 (3.22), 282 (3.15). $[\alpha]_{\text{D}}^{26.6}$ -18.1°(c=0.049, EtOH). *Anal.* Calcd. for C₂₄H₃₂O₉: C, 62.05; H, 6.94. Found: C, 62.79; H, 6.88.

Hydrolysis of Estriol Monoglucuronides with β -Glucuronidase—To an aq. solution (0.3 ml.) containing estriol monoglucuronide (ca. 0.5 mg.) were added 0.1M acetate buffer (pH 4.6, 1 ml.) and beef-liver β -glucuronidase (Tokyo Zōki, Co., Ltd.) (6,000 Fishman Unit/ml., 0.2 ml.) and the mixed solution was incubated at 37° for 20 hr. The incubated fluid was extracted with AcOEt (4 ml. $\times$ 3), washed with H₂O, dried and concentrated *in vacuo*. A portion of the residue thus obtained was submitted to TLC employing ether-acetone (19:1) as developer. The test sample exhibited a characteristically colored spot at Rf 0.16, which proved to be identical with that of the authentic estriol. Another portion of the sample was transformed into trimethylsilyl derivative by treatment with hexamethyldisilazane and trimethylchlorosilane in the usual way, and subjected to gas liquid chromatography employing 1% QF-1 (on Gas Chrom P) as the stationary phase. Retention time of the test sample was found to be identical with that of estriol. Free

glucuronic acid liberated was characterized by the method of Fishman, *et al.*¹⁸⁾ with use of naphthoresorcinol as coloring reagent.

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Added in Proof: After this paper had been submitted for publication, an article (J.S. Elce, J.G.D. Carpenter, A.E. Kellie: J. Chem. Soc. (C), **1967**, 542) appeared, in which the synthesis of estriol monoglucuronides was also described.

¹⁸⁾ W.H. Fishman, S. Green: J. Biol. Chem., **215**, 527 (1955).