

## Studies on Steroid Conjugates. IV. Synthesis of Catechol Estrogen Monoglucuronides<sup>1)</sup>

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2-Hydroxyestradiol 2- and 3-monoglucuronides (X, V) were prepared from the suitably protected catechol estrogen derivatives employing Koenigs-Knorr reaction as shown in Chart 1. These synthetic glucuronides underwent readily hydrolysis to furnish 2-hydroxyestradiol and free glucuronic acid when incubated with beef-liver  $\beta$ -glucuronidase preparation.

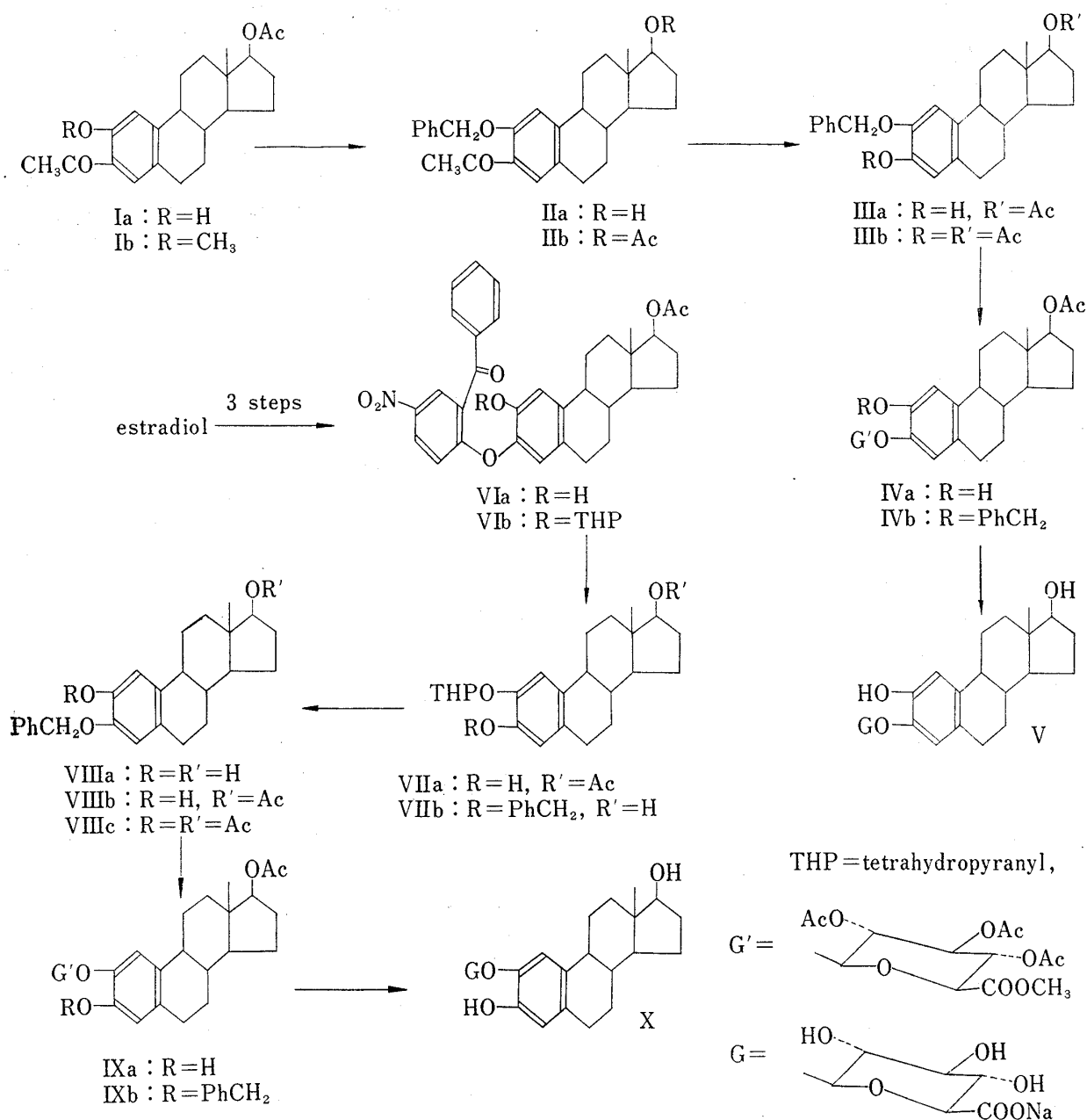
The isolation of a phenolic steroid having a methoxy group at C-2 from the human urine was first reported by Gallagher and his co-worker in 1957.<sup>3)</sup> They suggested that this novel biotransformation would proceed in two separate stages, namely hydroxylation and subsequent O-methylation. It is of particular interest that *in vivo* methylation does take place exclusively at C-2 in contrast with the fact that two phenolic hydroxyl groups of the catechol estrogen are virtually indistinguishable. Recently Fishman, *et al.* demonstrated that the sulfate formation would possibly participate in the selective O-methylation at C-2.<sup>4)</sup> In connection with this problem the biochemical significance of the glucuronic acid conjugation appears to be a quite attractive subject. The present paper deals with the synthesis of two isomeric 2- and 3-monoglucuronides of 2-hydroxyestradiol.

An initial attempt was focused to the preparation of the 3-glucuronide. The synthetic route leading to the catechol estrogen sulfate developed by Fishman, *et al.*<sup>5)</sup> seemed to be of disadvantage because somewhat tedious elaborations would be required and in addition difficulties would be anticipated in the subsequent steps. Therefore 2-benzyloxyestradiol was chosen as a key intermediate. Treatment of 3-acetylestriatriene-2,17-diol 17-acetate (Ia), derivable from the known 2-methoxy compound (Ib),<sup>6)</sup> with benzyl chloride in the presence of potassium carbonate gave the 2-benzyl ether (IIa), which in turn was converted into the 17-acetate (IIb) by usual acetylation. The Baeyer-Villiger reaction with use of perbenzoic acid in chloroform provided the 3,17-diol diacetate (IIIb) in satisfactory yield. When IIIb was treated with potassium bicarbonate under mild conditions, partial hydrolysis proceeded with success yielding the 3-monohydroxy derivative (IIIa) almost quantitatively.

Examinations were then made on an alternative method with more convenience. Direct benzylation of the free catechol with a limited amount of the reagent resulted in formation of a mixture of the 2- and 3-monobenzyl ethers accompanied with the fully benzylated product. However, separation of these compounds was a tedious work and the desired 2-monobenzyl ether could be obtained only in poor yield.<sup>7)</sup>

- 1) This paper constitutes Part XXXV of the series entitled "Analytical Chemical Studies on Steroids;" Part XXXIV: T. Nambara, Y. Matsuki, T. Kudo, and T. Iwata, *Chem. Pharm. Bull.* (Tokyo), **18**, 626 (1970).
- 2) Location: *Aobayama, Sendai.*
- 3) S. Kraychy and T.F. Gallagher, *J. Am. Chem. Soc.*, **79**, 754 (1957); *idem*, *J. Biol. Chem.*, **229**, 519 (1957).
- 4) J. Fishman, M. Miyazaki, and I. Yoshizawa, *J. Am. Chem. Soc.*, **89**, 7147 (1967); *idem*, *Biochemistry*, **8**, 1669 (1969).
- 5) a) J. Fishman, *J. Am. Chem. Soc.*, **80**, 1213 (1958); b) M. Miyazaki and J. Fishman, *J. Org. Chem.*, **33**, 662 (1968).
- 6) T. Nambara, S. Honma, and S. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 474 (1970).
- 7) T. Nambara, S. Honma, and T. Iwata, to be published.

Introduction of the glucuronyl moiety into IIIa was undertaken employing Koenigs-Knorr reaction in usual way.<sup>8)</sup> The catechol monobenzyl ether and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- $\alpha$ -D-glucopyranuronate being stirred in dry benzene with freshly prepared silver carbonate, condensation reaction did take place to afford methyl (2-benzyloxy-17 $\beta$ -acetoxy-estra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranosid)uronate (IVb) in 17% yield. Elimination of the benzyl group was readily attained by catalytic hydrogenation over palladium-on-charcoal to yield the 2-hydroxy derivative (IVa). Upon treatment with methanolic sodium hydroxide under mild condition the simultaneous removal of the protecting groups in both steroid and sugar moiety proceeded to furnish the desired sodium 3-glucosiduronate (V).



8) H.H. Wotiz, E. Smakula, N.N. Lichtin, and J.H. Leftin, *J. Am. Chem. Soc.*, **81**, 1704 (1959); T. Nambara and K. Imai, *Chem. Pharm. Bull.* (Tokyo), **15**, 1232 (1967); T. Nambara, Y. Matsuki, and T. Chiba, *ibid.*, **17**, 1636 (1969).

The synthesis of the isomeric 2-glucuronide required somewhat more involved reaction sequence. An initial attempt to use 2-benzyloxyestradiol diacetate as an intermediate proved to be unsuccessful since the 1,2-transfer of the acetyl group did readily take place with the catechol monoacetate during the processing of catalytic hydrogenolysis.<sup>7)</sup> Therefore 3-(2-benzoyl-4-nitro)phenyl ether of the catechol estrogen (VIa), reported by Fishman<sup>5a)</sup> as a synthetic intermediate leading to 2-methoxyestrogen, was chosen as a starting compound. Additive condensation with 3,4-dihydro-2H-pyran under catalysis by anhydrous *p*-toluenesulfonic acid<sup>9)</sup> and subsequent removal of the protecting group at C-3 with piperidine gave the 2-tetrahydropyranyl ether (VIIa) in satisfactory yield. Usual benzylation followed by selective elimination of the tetrahydropyranyl group on brief treatment with the mineral acid resulted in formation of 2-hydroxyestradiol 3-benzyl ether (VIIIa). Acetylation with acetic anhydride and pyridine gave the 2,17-diacetate (VIIIc), which on mild alkaline hydrolysis was led to the catechol 3-benzyl ether 17-acetate (VIIIb). Condensation of methyl acetobromoglucuronate with VIIIb could be attained under the similar conditions as in the case of the isomeric 3-glucuronide. Upon hydrogenolysis over palladium-on-charcoal the 3-benzyl ether (IXb) underwent debenylation to provide methyl (3-hydroxy-17 $\beta$ -acetoxyestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranosid)uronate (IXa) in reasonable yield. Hydrolytic cleavage with methanolic sodium hydroxide finally gave the desired 2-hydroxyestradiol 2-glucosiduronate (X).

The nuclear magnetic resonance spectra of the acetylated glucuronides were indicative of the formation of the  $\beta$ -glucuronoside structure. The anomeric proton of the sugar moiety appeared at 4.9 ppm as doublet ( $J=6-8$  cps) indicating a *trans*-diaxial relationship to the vicinal 2'-proton. The infrared spectra of the glucuronides exhibited an absorption band at 890  $\text{cm}^{-1}$ , which also supported the configurational assignment of the pyranoside bond to  $\beta$  rather than  $\alpha$ .<sup>10)</sup> In addition definite evidence for the  $\beta$ -glucuronoside linkage in these monoglucuronides was demonstrated by characterizing 2-hydroxyestradiol and D-glucuronic acid after incubation with beef-liver  $\beta$ -glucuronidase.

It is hoped that the availability of the catechol estrogen monoglucuronides may serve to throw light on the mechanism of *in vivo* O-methylation.

#### Experimental<sup>11)</sup>

**3-Acetylestria-1,3,5(10)-triene-2,17 $\beta$ -diol 17-Acetate (Ia)**—A solution of Ib (2.0 g) in AcOH (80 ml)–48% HBr (20 ml) was heated on water-bath for 2 hr. The reaction mixture was poured into ice-water and extracted with ether. The organic layer was washed with 5%  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$  and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . After evaporation of solvent the crude product obtained was recrystallized from MeOH to give Ia (1.5 g) as colorless needles. mp 138–140°,  $[\alpha]_D^{19} +62.6^\circ$  ( $c=0.22$ ). *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{28}\text{O}_4$ : C, 74.13; H, 7.92. Found: C, 73.88; H, 7.54.

**2-Benzyloxy-3-acetylestria-1,3,5(10)-trien-17 $\beta$ -ol (IIa)**—To a solution of Ia (1.5 g) and benzyl chloride (1.5 ml) in EtOH (100 ml) was added anhydrous  $\text{K}_2\text{CO}_3$  (4.0 g) and the solution was refluxed for 8 hr. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$ , washed with  $\text{H}_2\text{O}$  and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . After evaporation of solvent the crude product obtained was recrystallized from hexane-benzene to give IIa (1.26 g) as colorless needles. mp 121–123°,  $[\alpha]_D^{18} +78.9^\circ$  ( $c=0.15$ ). *Anal.* Calcd. for  $\text{C}_{27}\text{H}_{32}\text{O}_3$ : C, 80.16; H, 7.97. Found: C, 80.38; H, 7.97.

**2-Benzyloxy-3-acetylestria-1,3,5(10)-trien-17 $\beta$ -ol Acetate (IIb)**—Usual acetylation of IIa (1.4 g) with pyridine (10 ml) and  $\text{Ac}_2\text{O}$  (1 ml) followed by recrystallization from MeOH gave IIb (1.2 g) as colorless

9) J.F.W. McOmie, "Advances in Organic Chemistry: Methods and Results," Vol. 3, ed. by R.A. Raphael, E.C. Taylor, and H. Wynberg, Interscience Publishers, Inc., New York, 1963, pp. 191–294.

10) V.R. Mattox, J.E. Goodrich, and W.D. Vrieze, *Biochemistry*, **8**, 1188 (1969).

11) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in  $\text{CHCl}_3$  unless otherwise stated. Nuclear magnetic resonance spectra were obtained on Hitachi Model H-60 spectrometer at 60 Mc; the chemical shifts are quoted as ppm downfield from  $(\text{CH}_3)_4\text{Si}$  as an internal standard. For preparative thin-layer chromatography (TLC) Silica gel HF (E. Merck AG) was used as adsorbent.

needles. mp 131—131.5°,  $[\alpha]_D^{25} + 34.4^\circ$  ( $c=0.12$ ). *Anal.* Calcd. for  $C_{29}H_{34}O_4$ : C, 77.99; H, 7.67. Found: C, 77.81; H, 7.79.

**2-Benzoyloxyestra-1,3,5(10)-triene-3,17 $\beta$ -diol Diacetate (IIIb)**—To a solution of IIb (1.43 g) in  $CHCl_3$  (55 ml) was added perbenzoic acid- $CHCl_3$  solution (0.4M, 30 ml) and the solution was allowed to stand at room temperature for 1 week. The reaction mixture was diluted with ether and washed with ice-cooled 5% NaOH,  $H_2O$  and dried over anhydrous  $Na_2SO_4$ . After evaporation of solvent the crystalline product obtained was recrystallized from MeOH to give IIIb (826 mg) as colorless needles. mp 77—79°,  $[\alpha]_D^{25} + 48.9^\circ$  ( $c=0.05$ ). *Anal.* Calcd. for  $C_{29}H_{34}O_5$ : C, 75.30; H, 7.41. Found: C, 74.84; H, 7.28.

**2-Benzoyloxyestra-1,3,5(10)-triene-3,17 $\beta$ -diol 17-Acetate (IIIa)**—To a solution of IIIb (400 mg) in acetone (20 ml) was added a solution of  $KHCO_3$  (400 mg) in MeOH (50 ml)– $H_2O$  (10 ml) and stirred at 30° for 30 hr. The reaction mixture was diluted with ether, washed with  $H_2O$  and dried over anhydrous  $Na_2SO_4$ . After evaporation of solvent the crystalline product obtained was recrystallized from MeOH to give IIIa (272 mg) as colorless needles. mp 161—164°,  $[\alpha]_D^{25} + 61.9^\circ$  ( $c=0.19$ ). *Anal.* Calcd. for  $C_{27}H_{32}O_4$ : C, 77.11; H, 7.67. Found: C, 77.30; H, 7.63.

**Methyl (2-benzoyloxy-17 $\beta$ -acetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranosid)uronate (IVb)**—To a solution of IIIa (275 mg) in anhydrous benzene (30 ml) were added methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- $\alpha$ -D-glucopyranuronate (518 mg) and freshly prepared  $Ag_2CO_3$  (1 g) and stirred for 25 hr in the dark place. During continuation of stirring an additional amount of  $Ag_2CO_3$  (600 mg) was added in several portions. After completion of the reaction the precipitate was filtered off and washed with hot  $CHCl_3$ . After evaporation of the combined filtrates the residue obtained was submitted to preparative TLC using hexane–AcOEt (2:1) as developing solvent. Separation of the desired fraction and recrystallization of the eluate from EtOH gave IVb (60 mg) as colorless needles. mp 100—101°,  $[\alpha]_D^{25} - 30.0^\circ$  ( $c=0.10$ ). *Anal.* Calcd. for  $C_{40}H_{48}O_{13}$ : C, 65.20; H, 6.57. Found: C, 64.92; H, 6.33. NMR (5% solution in  $CCl_4$ )  $\delta$ : 0.82 (3H, s, 18- $CH_3$ ), 2.00 (12H, 4 $\times$ -OCOCH<sub>3</sub>), 3.70 (3H, s, -COOCH<sub>3</sub>), 3.92 (1H, pyranose-C<sub>5</sub>-H), 4.52 (1H, t,  $J=8$  cps, 17 $\alpha$ -H), 5.00 (2H, s, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.90—5.30 (4H, pyranose-CH-OCOCH<sub>3</sub>, -C<sub>1</sub>-H), 6.78 (1H, s, aromatic C<sub>4</sub>-H), 7.33 (6H, aromatic C<sub>1</sub>-H, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>).

**Methyl (2-hydroxy-17 $\beta$ -acetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranosid)uronate (IVa)**—A solution of IVb (35 mg) in EtOH was shaken with 5% Pd/C (15 mg) under a current of  $H_2$  for 18 hr at room temperature. After removal of catalyst by filtration the filtrate was concentrated to give a crystalline product. Recrystallization from MeOH gave IVa (18 mg) as colorless needles. mp 188—189.5°,  $[\alpha]_D^{25} 0^\circ$  ( $c=0.26$ ). *Anal.* Calcd. for  $C_{33}H_{42}O_{13}$ : C, 61.29; H, 6.55. Found: C, 61.44; H, 6.58. NMR (10% solution in  $CDCl_3$ )  $\delta$ : 0.83 (3H, s, 18- $CH_3$ ), 2.02, 2.06 (12H, 4 $\times$ -OCOCH<sub>3</sub>), 3.74 (3H, s, -COOCH<sub>3</sub>), 4.16 (1H, d,  $J=8$  cps, pyranose-C<sub>5</sub>-H), 4.63 (1H, t,  $J=8$  cps, 17 $\alpha$ -H), 4.98 (1H, d,  $J=6$  cps, pyranose-C<sub>1</sub>-H), 5.10—5.40 (3H, pyranose-CH-OCOCH<sub>3</sub>), 6.64 (1H, s, aromatic C<sub>4</sub>-H), 6.85 (1H, s, aromatic C<sub>1</sub>-H). IR  $cm^{-1}$  (KBr): 886.

**Sodium (2,17 $\beta$ -dihydroxyestra-1,3,5(10)-trien-3-yl- $\beta$ -D-glucopyranosid)uronate (V)**—To a solution of IVa (13 mg) in hot MeOH (0.5 ml) was added methanolic 1N NaOH (0.1 ml) and allowed to stand at room temperature for 18 hr. After addition of benzene the resulting solution was concentrated *in vacuo*. A yellow sirup obtained was dissolved in  $H_2O$  (5 ml) and adsorbed on Amberlite XAD-2 resin (8 ml). After washing with  $H_2O$  (20 ml) the desired substance was eluted with EtOH. Recrystallization of the eluate from MeOH gave V (7 mg) as colorless needles. mp 245—248° (decomp.). *Anal.* Calcd. for  $C_{24}H_{31}O_9Na \cdot 1\frac{1}{2}H_2O$ : C, 56.13; H, 6.67. Found: C, 56.51; H, 7.13.

**2-(2-Tetrahydropyranyloxy)estra-1,3,5(10)-triene-3,17 $\beta$ -diol 17-Acetate (VIIa)**—To a solution of VIa (2.2 g) in benzene (10 ml) were added 3,4-dihydro-2H-pyran (2.1 ml) and anhydrous *p*-TsOH (10 mg) and the resulting solution was stirred at room temperature for 24 hr. To this solution was added piperidine (50 ml) and refluxed for 1 hr. The reaction mixture was diluted with benzene, washed with  $H_2O$  and dried over anhydrous  $Na_2SO_4$ . After evaporation of solvent a reddish brown oil obtained was dissolved in hexane–benzene (1:1) and was chromatographed on  $Al_2O_3$  (20 g). Eluate obtained with hexane–benzene (1:1) was discarded and the subsequent eluate with benzene was collected. Recrystallization from hexane–benzene gave VIIa (620 mg) as colorless leaflets. mp 93—96°,  $[\alpha]_D^{25} + 57.8^\circ$  ( $c=0.12$ ). *Anal.* Calcd. for  $C_{25}H_{34}O_5$ : C, 72.43; H, 8.27. Found: C, 71.90; H, 8.16.

**2-(2-Tetrahydropyranyloxy)-3-benzoyloxyestra-1,3,5(10)-trien-17 $\beta$ -ol (VIIb)**—To a solution of VIIa (211 mg) and benzyl chloride (0.1 ml) dissolved in EtOH (20 ml) was added anhydrous  $K_2CO_3$  (400 mg) and refluxed for 8 hr. 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 hexane–benzene to give VIIb (203 mg) as colorless needles. mp 79—80°,  $[\alpha]_D^{25} + 91.9^\circ$  ( $c=0.05$ ). *Anal.* Calcd. for  $C_{30}H_{38}O_4$ : C, 77.89; H, 8.28. Found: C, 77.73; H, 8.09.

**3-Benzoyloxyestra-1,3,5(10)-triene-2,17 $\beta$ -diol (VIIIa)**—To a solution of VIIb (80 mg) in MeOH (5 ml) was added conc. HCl (0.5 ml) and allowed to stand at room temperature for 2.5 hr. The precipitated crystalline product was collected by filtration and was recrystallized from MeOH to give VIIIa (54 mg) as colorless needles. mp 227—228°,  $[\alpha]_D^{25} + 57.1^\circ$  ( $c=0.09$ ). *Anal.* Calcd. for  $C_{25}H_{30}O_3$ : C, 79.33; H, 7.99. Found: C, 79.07; H, 7.98.

**3-Benzoyloxyestra-1,3,5(10)-triene-2,17 $\beta$ -diol Diacetate (VIIIc)**—Usual acetylation of VIIIa (1.5 g) with pyridine (15 ml) and Ac<sub>2</sub>O (15 ml) followed by recrystallization from MeOH gave VIIIc (1.5 g) as colorless needles. mp 158—159°,  $[\alpha]_D^{25} +35.9^\circ$  ( $c=0.15$ ). *Anal.* Calcd. for C<sub>29</sub>H<sub>34</sub>O<sub>5</sub>: C, 75.30; H, 7.41. Found: C, 75.15; H, 7.00.

**3-Benzoyloxyestra-1,3,5(10)-triene-2,17 $\beta$ -diol 17-Acetate (VIIIb)**—To a solution of VIIIc (500 mg) in acetone (20 ml) was added a solution of KHCO<sub>3</sub> (400 mg) in MeOH (50 ml)—H<sub>2</sub>O (10 ml) and stirred at room temperature for 18 hr. The reaction mixture was diluted with ether, washed with H<sub>2</sub>O and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After evaporation of solvent the crystalline product obtained was recrystallized from MeOH to give VIIIb (453 mg) as colorless needles. mp 149—151°,  $[\alpha]_D^{25} +56.4^\circ$  ( $c=0.04$ ). *Anal.* Calcd. for C<sub>27</sub>H<sub>32</sub>O<sub>4</sub>: C, 77.11; H, 7.67. Found: C, 76.67; H, 7.59.

**Methyl(3-benzyloxy-17 $\beta$ -acetoxystera-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranosid)uronate (IXb)**—To a solution of VIIIb (400 mg) in anhydrous benzene (20 ml) were added 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- $\alpha$ -D-glucopyranuronate (500 mg) and freshly prepared Ag<sub>2</sub>CO<sub>3</sub> (1 g) and stirred for 28 hr in the dark place. During continuation of stirring an additional amount of Ag<sub>2</sub>CO<sub>3</sub> (600 mg) was added in several portions. After completion of the reaction the precipitate was filtered off and washed with hot CHCl<sub>3</sub>. After evaporation of the combined filtrates the residue obtained was submitted to preparative TLC using hexane-AcOEt (2:1) as developing solvent. Separation of the desired fraction and recrystallization of the eluate from EtOH gave IXb (60 mg) as colorless needles. mp 161—163°,  $[\alpha]_D^{18} -5.9^\circ$  ( $c=0.17$ ). *Anal.* Calcd. for C<sub>40</sub>H<sub>48</sub>O<sub>13</sub>: C, 65.20; H, 6.57. Found: C, 65.14; H, 6.47. NMR (7% solution in CCl<sub>4</sub>)  $\delta$ : 0.83 (3H, s, 18-CH<sub>3</sub>), 1.98 (12H, 4 $\times$ -OCOCH<sub>3</sub>), 3.70 (3H, s, -COOCH<sub>3</sub>), 3.90 (1H, pyranose-C<sub>5</sub>-H), 4.30—4.80 (1H, 17 $\alpha$ -H), 4.95 (2H, s, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.90—5.30 (4H, pyranose-CH-OCOCH<sub>3</sub>, -C<sub>1</sub>-H), 6.55 (1H, s, aromatic C<sub>4</sub>-H), 7.05 (1H, s, aromatic C<sub>1</sub>-H), 7.32 (5H, s, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>).

**Methyl(3-hydroxy-17 $\beta$ -acetoxystera-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranosid)uronate (IXa)**—A solution of IXb (40 mg) in EtOH was shaken with 5% Pd/C (10 mg) under a current of H<sub>2</sub> for 18 hr at room temperature. After removal of catalyst by filtration the filtrate was concentrated to give a crystalline product. Recrystallization from MeOH gave IXa (18 mg) as colorless needles. mp 179—180.5°,  $[\alpha]_D^{17} -29.2^\circ$  ( $c=0.10$ ). *Anal.* Calcd. for C<sub>33</sub>H<sub>42</sub>O<sub>13</sub>: C, 61.29; H, 6.55. Found: C, 61.72; H, 6.63. NMR (4% solution in CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, s, 18-CH<sub>3</sub>), 2.03, 2.10 (12H, 4 $\times$ -OCOCH<sub>3</sub>), 3.73 (3H, s, -COOCH<sub>3</sub>), 4.12 (1H, d,  $J=8$  cps, pyranose-C<sub>5</sub>-H), 4.55 (1H, t,  $J=8$  cps, 17 $\alpha$ -H), 4.90 (1H, d,  $J=8$  cps, pyranose-C<sub>1</sub>-H), 5.10—5.45 (3H, pyranose-CH-OCOCH<sub>3</sub>), 6.62 (1H, s, aromatic C<sub>4</sub>-H), 6.86 (1H, s, aromatic C<sub>1</sub>-H). IR cm<sup>-1</sup> (KBr): 890.

**Sodium(3,17 $\beta$ -dihydroxyestra-1,3,5(10)-triene-2-yl- $\beta$ -D-glucopyranosid)uronate (X)**—To a solution of IXa (13 mg) in hot MeOH (0.5 ml) was added methanolic 1N NaOH (0.1 ml) and allowed to stand at room temperature for 18 hr. After addition of benzene the resulting solution was concentrated *in vacuo*. A yellow sirup obtained was dissolved in H<sub>2</sub>O (5 ml) and adsorbed on Amberlite XAD-2 resin (8 ml). After washing with H<sub>2</sub>O (20 ml) the desired substance was eluted with EtOH. Recrystallization of the eluate from MeOH gave X (3.3 mg) as colorless powders. mp >300°. *Anal.* Calcd. for C<sub>24</sub>H<sub>31</sub>O<sub>9</sub>Na $\cdot$ 3 $\frac{1}{2}$ H<sub>2</sub>O: C, 52.45; H, 6.97. Found: C, 52.32; H, 6.86.

**Enzymatic Hydrolysis of 2-Hydroxyestradiol 2- and 3-Monoglucuronides with  $\beta$ -Glucuronidase**—To an aq. solution (4 ml) of 2-hydroxyestradiol monoglucuronide (*ca.* 2 mg) were added 0.1M acetate buffer (pH 4.7, 20 ml) and beef-liver  $\beta$ -glucuronidase (Tokyo Zōkikagaku Co., Ltd) (10000 Fishman Unit/ml) (0.8 ml) and the mixed solution was incubated at 37° for 24 hr. The incubated fluid was saturated with NaCl and extracted with ether. The organic layer was washed with 5% NaHCO<sub>3</sub> and H<sub>2</sub>O, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and then concentrated *in vacuo*. A portion of the residue thus obtained was submitted to TLC employing silica gel H (E. Merck AG) as adsorbent and benzene-MeOH (7:3) as developing solvent. The test sample exhibited a spot at *Rf* 0.67, which proved to be identical with that of the authentic sample (2-hydroxyestradiol). Free glucuronic acid liberated was characterized by the method of Fishman, *et al.*<sup>12)</sup> with use of naphthoresorcinol as coloring reagent.

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