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Studies on Steroid Conjugates. IX. New Synthesis of Estriol 16- and 17-Monoglucuronides[1]

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A new synthetic route leading to the titled compounds by three steps from estriol (I) has been developed. When estriol 3-benzoate (IIa) and methyl acetobromoglucuronate were stirred in benzene in the presence of silver carbonate, the condensation reaction occurred at C-16 and C-17 yielding two isomeric glucuronide derivatives (IIIa, IVa) in approximately equal amount. Upon alkaline hydrolysis these two were readily converted into the desired estriol monoglucuronides (V, VI). The structures of IIIa and IVa were further confirmed by transforming into the 16,17-ketol derivatives (VII, VIII) by Jones oxidation. The yield and composition of the product in Koenigs–Knorr reaction were significantly influenced by the solvent as was shown in Table I. Application of the present method to the preparation of estriol-6,7-^{3}H 16- and 17-glucuronides has also been described.

Recently the considerable attentions have been drawn to the bio-medical problems associated with the metabolism and physiological role of the estrogen conjugates in the human fetal-placental unit. The synthesis of three possible estriol monoglucuronides was previously accomplished by Koenigs–Knorr reaction of the suitably protected steroid with methyl acetobromoglucuronate.[3,4] Bernstein and his co-workers devised the more convenient method to obtain the 16-glucuronide by introducing a glucuronyl moiety directly into the C-16 position of estriol 3-benzyl ether.[5] The necessity of the isotope-labeled estriol monoglucuronide prompted us to develop the more simple route with the satisfactory yield. In this paper we wish to report a new method for the preparation of the titled compounds by three steps starting from the readily available estriol and its application to the synthesis of the labeled compounds.

First estriol (I) was converted into the 3-benzoate (IIa) by Schotten-Baumann reaction with benzoyl chloride in a fairly good yield.[6] When IIa and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate were stirred in anhydrous benzene in the presence of silver carbonate,[3,4,7] the condensation reaction proceeded almost quantitatively. Although the reaction product appeared to be homogeneous, the careful thin-layer chromatography (TLC) indicated that it consisted of two analogous substances in a ratio of *ca.* 1 to 1. Separation of these two was attained by the repeated fractional crystallization from ether yielding the 16- and 17-glucuronide acetate-methyl esters (IIIa, IVa). The structures of these isomeric glucuronide derivatives were elucidated by leading to the known glucosiduronic

1) This paper constitutes Part LVII of the series entitled "Analytical Chemical Studies on Steroids"; Part LVI: T. Nambara, Y. Matsuki, and N. Miyazaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 842 (1972).
2) Location: *a*) *Aobayama, Sendai*; *b*) *Shimosakunobe, Kawasaki.*
3) T. Nambara and K. Imai, *Chem. Pharm. Bull.* (Tokyo), **15**, 1232 (1967).
4) J.S. Elce, J.G.D. Carpenter, and A.E. Kellie, *J. Chem. Soc.* (*C*), **1967**, 542.
5) J.P. Joseph, J.P. Dusza, and S. Bernstein, *J. Am. Chem. Soc.*, **89**, 5078 (1967); J.P. Joseph, J.P. Dusza, E.W. Cantrall, and S. Bernstein, *Steroids*, **14**, 591 (1969).
6) K. Tsuneda, J. Yamada, K. Yasuda, and H. Mori, *Chem. Pharm. Bull.* (Tokyo), **11**, 510 (1963).
7) H.H. Wotiz, E. Smakula, N.N. Lichtin, and J.H. Leftin, *J. Am. Chem. Soc.*, **81**, 1704 (1959); T. Nambara, Y. Matsuki, and T. Chiba, *Chem. Pharm. Bull.* (Tokyo), **17**, 1636 (1969); T. Nambara and S. Honma, *ibid.*, **18**, 1191 (1970); T. Nambara, Y. Matsuki, and Y. Kawarada, *ibid.*, **19**, 844 (1971).

acids (V, VI) upon hydrolysis with methanolic sodium hydroixde, respectively. The structural assignment was further confirmed by transforming IIIa and IVa into the 16,17-ketol glucuronide derivatives (VII, VIII) by Jones oxidation.[8] Inspection of the nuclear magnetic resonance (NMR) spectra revealed that a 17α-proton signal in VIII appeared at 4.03 ppm as a singlet, while a 16β-proton signal in VII at 4.48 ppm as a multiplet. The present method has proved to be much more advantageous than the others, since two isomeric 16- and 17-glucuronides are simultaneously obtainable only by three steps starting from estriol. It should be emphasized that there can be seen a marked difference in the results between Koenigs-Knorr reaction of the 3-benzoate and that of the 3-benzyl ether, though no plausible explanation is now available.

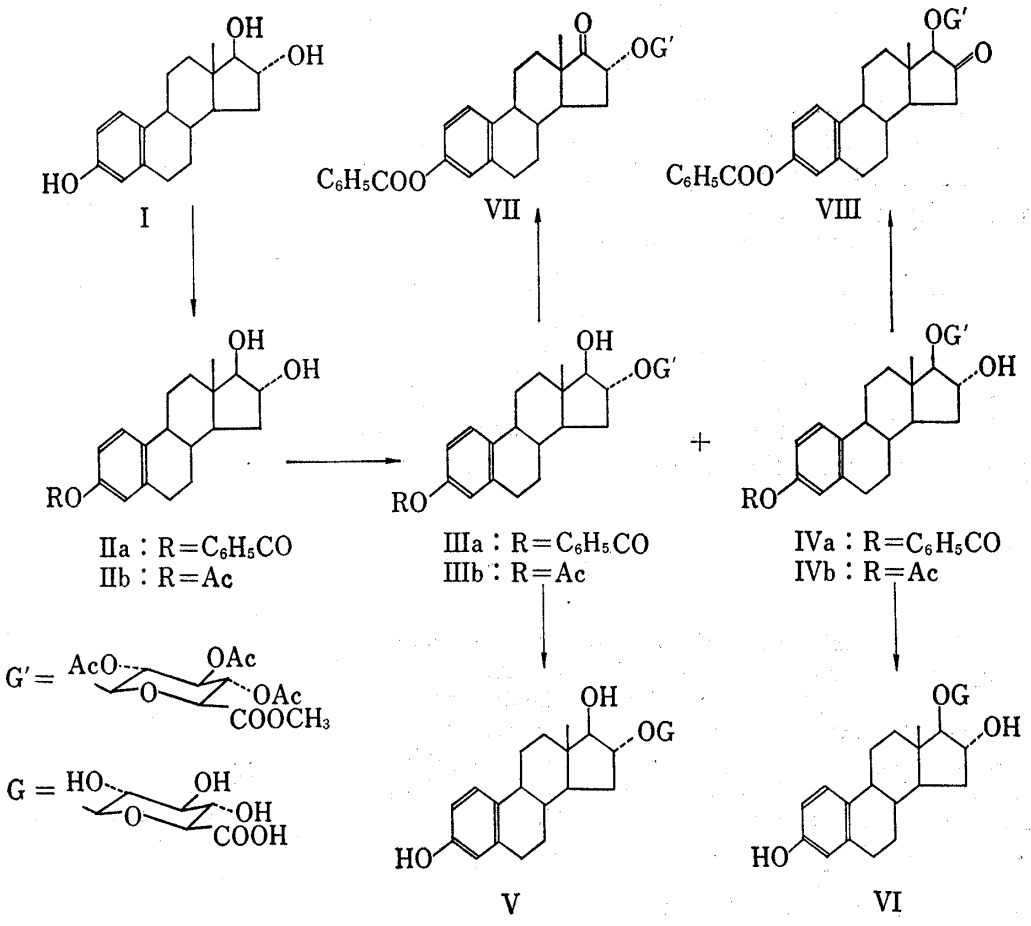

Chart 1

These findings led us to examine the effects of the protecting group at C-3 and the reaction solvent on the yield of the desired compounds. Attempt to prepare estriol 3-acetate (IIb) by usual Schotten–Baumann reaction with acetyl chloride was unsuccessful, because acetyl chloride was rapidly decomposed. The reaction of sodium salt of estriol with acetyl chloride in anhydrous condition afforded the desired 3-acetate in satisfactory yield. The use of estriol 3-acetate (IIb) as a starting material gave almost the same results as in the case of the 3-benzoate. However, it was surprising that the result was significantly influenced by the reaction solvent. When chloroform or carbontetrachloride was employed as the solvent, the condensation reaction occurred preferentially at C-17 rather than at C-16. The use of dioxane or tetrahydrofuran (THF) gave no detectable amount of the condensation product. The results on the solvent effect are collected in Table I.

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Table I. Effect of Solvent on Koenigs–Knorr Reaction of Estriol 3-Benzoate with Methyl Acetobromoglucuronate

Solvent (ml)	Estriol 3-benzoate (g)	Acetobromo sugar (g)	Ag_2CO_3 (g)	Total yield (%)	Product
Benzene (15)	0.1	0.2	0.25	90	16-G′/17-G′ (1:1)
Toluene (10)	0.1	0.2	0.5	90	16-G′/17-G′ (1:1)
$CHCl_3$ (50)	0.2	0.8	1	90	17-G′
CCl_4 (10)	0.1	0.2	0.5	50	17-G′
Dioxane (15)	0.1	0.2	0.25	0	
THF (15)	0.1	0.2	0.25	0	
Benzene (40)–THF (20)	0.4	0.8	1	70	16-G′/17-G′ (1:1)

These results prompted us to prepare the isotope-labeled estriol 16- and 17-glucuronides from estriol-6,7-^{3}H along the established route described above. The starting compound was first led to the 3-benzoate by Schotten–Baumann reaction. Subsequent Koenigs–Knorr reaction in anhydrous benzene in the presence of silver carbonate provided a mixture of two isomeric glucuronide acetate-methyl esters, whose separation could be efficiently achieved by the preparative TLC. The homogeneity of these two products was confirmed by the reverse isotope dilution method, respectively. Removal of the protecting groups by the alkaline hydrolysis followed by purification on Amberlite XAD-2 resin gave the desired estriol-6,7-^{3}H 16- and 17-glucuronides in reasonable yield.

It is hoped that the present method for the preparation of the 16- and 17-glucuronides starting from estriol with the more ease may serve for the bio-medical studies on the estrogen conjugates.

Experimental[9]

Koenigs-Knorr Reaction of Estriol 3-Benzoate in Benzene——To a solution of estriol 3-benzoate (IIa)[6] (47 g) in anhydrous benzene (7 liter) were added methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (90 g) and Ag_2CO_3 (117 g) and stirred in the dark place for 20 hr. After removal of the precipitate by filtration the filtrate was evaporated *in vacuo* to give an oily residue. Recrystallization from ether gave methyl (3-benzoyloxy-16α-hydroxyestra-1,3,5(10)-trien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IVa) (18.0 g) as colorless needles. mp 203—206°. $[\alpha]_D^{16}$ +11.2° (c=0.09, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{44}O_{13}$: C, 64.39; H, 6.26. Found: C, 64.65; H, 6.37. NMR (5% solution in $CDCl_3$) δ: 0.78 (3H, s, 18-CH_3), 2.03 (9H, s, -OCOCH_3), 3.32 (1H, d, J=5 cps, 17α-H), 3.74 (3H, s, -COOCH_3), 4.00—4.40 (2H, m, 16β-H, pyranose-C_5-H), 4.56 (1H, d, J=7 cps, pyranose-C_1-H), 4.80—5.40 (3H, m, pyranose-CH-OAc). TLC (benzene–AcOEt (1:1)): *Rf* 0.60. The mother liquor was allowed to stand at room temperature for 3 days to yield a crystalline product. Recrystallization from MeOH gave methyl (3-benzoyloxy-17β-hydroxyestra-1,3,5(10)-trien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IIIa) (2.78 g) as colorless needles. mp 208—210°. $[\alpha]_D^{16}$ +9.8° (c=0.10, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{44}O_{13}$: C, 64.39; H, 6.26. Found: C, 64.08; H, 6.33. NMR (5% solution in $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 2.03 (9H, s, -OCOCH_3), 3.67 (1H, d, J=5 cps, 17α-H), 3.74 (3H, s, -COOCH_3), 3.89 (1H, m, 16β-H), 4.04 (1H, m, pyranose-C_5-H), 4.57 (1H, d, J=7 cps, pyranose-C_1-H), 4.80—5.40 (3H, m, pyranose-CH-OAc). TLC (benzene–AcOEt (1:1)): *Rf* 0.65. An additional amount of IIIa (0.47 g) was similarly obtained as the second crop.

3,17β-Dihydroxyestra-1,3,5(10)-trien-16α-yl-β-D-glucopyranosiduronic Acid (V)——To a solution of IIIa (36 mg) in MeOH (9 ml) was added 1 N NaOH (1.8 ml) and allowed to stand at room temperature overnight. The resulting solution was concentrated *in vacuo*. The residue obtained was dissolved in H_2O (10 ml), percolated through a column of Amberlite XAD-2 resin (80 ml). After washing with distilled water (300 ml) the desired substance was eluted with 50% EtOH. The eluate was dissolved in H_2O saturated

9) All melting points were taken on a micro hot-stage apparatus and are uncorrected. For the preparative TLC silica gel HF (E. Merck AG) was used as an adsorbent. Infrared (IR) spectra were run on Hitachi Model EPI-G2 spectrometer. NMR spectra were recorded on Hitachi Model R-20A spectrometer at 60 Mc using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet and m=multiplet.

with *n*-BuOH and pH of this solution was adjusted to 1 with 5% HCl saturated with *n*-BuOH. After allowing to stand overnight in refrigerator the resultant precipitate was collected by filtration and dried. Recrystallization from aq. MeOH gave V (18 mg) as colorless needles. mp 222—224° (decomp.) (Reported: mp 224—225° (decomp.)).[3]

3,16α-Dihydroxyestra-1,3,5(10)-trien-17β-yl-β-D-glucopyranosiduronic Acid (VI)——A solution of IVa (24 mg) in MeOH (9 ml) was treated with 1 N NaOH (1.8 ml) in the manner as described in V. Chromatographic purification on Amberlite XAD-2 resin followed by recrystallization from H_2O saturated with BuOH and then from aq. MeOH gave VI (10 mg) as colorless needles. mp 241° (decomp.) (Reported: mp 235—240° (decomp.)).[3]

Methyl(3-Benzoyloxy-17-oxoestra-1,3,5(10)-trien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (VII)——IIIa (92 mg) was treated with Jones reagent (0.14 ml) in acetone (9.2 ml) in the manner as described in VIII. Recrystallization from MeOH gave VII (33 mg) as colorless needles. mp 254—255°. $[\alpha]_D^{25}$ +67.9° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{42}O_{13}$: C, 64.58; H, 5.99. Found: C, 63.94; H, 5.91. NMR (5% solution in $CDCl_3$) δ: 0.93 (3H, s, 18-CH_3), 2.03 (9H, s, -OCOCH_3), 3.74 (3H, s, -COOCH_3), 4.04 (1H, m, pyranose-C_5-H), 4.48 (1H, m, 16β-H), 4.78 (1H, d, J=7 cps, pyranose-C_1-H), 4.90—5.40 (3H, pyranose-CH-OAc). IR ν_{max}^{KBr} cm^{-1}: 1740, 1755 (C=O), 890 (pyranose-C_1-H).

Methyl(3-Benzoyloxy-16-oxoestra-1,3,5(10)-trien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (VIII)——To a solution of IVa (200 mg) in acetone (20 ml) was added Jones reagent (0.3 ml) under ice-cooling and stirred for 1.5 hr. The resulting solution was diluted with H_2O and extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product was recrystallized from MeOH to give VIII (173 mg) as colorless needles. mp 181—182°. $[\alpha]_D^{25}$ −78.2° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{42}O_{13}\cdot H_2O$: C, 62.97; H, 6.12. Found: C, 63.18; H, 6.04. NMR (5% solution in $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 2.03 (9H, s, -OCOCH_3), 3.74 (3H, s, -COOCH_3), 4.03 (1H, s, 17α-H), 4.04 (1H, m, pyranose-C_5-H), 4.78 (1H, d, J=7 cps, pyranose-C_1-H), 4.90—5.40 (3H, pyranose-CH-OAc). IR ν_{max}^{KBr} cm^{-1}: 1740, 1755 (C=O), 890 (pyranose-C_1-H).

Koenigs-Knorr Reaction of Estriol 3-Benzoate in Chloroform——A suspension of IIa (200 mg), Ag_2CO_3 (1 g), and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (800 mg) in $CHCl_3$ (50 ml) was stirred in the dark place for 24 hr at room temperature. After removal of the precipitate by filtration the filtrate was evaporated *in vacuo* to give an oily residue. Recrystallization from ether gave IVa (137 mg) as colorless needles. mp 202—206°.

Estriol 3-Acetate (IIb)——To a suspension of estriol (I) (576 mg) in MeOH (2 ml) was added a solution of NaOH (80 mg) in 90% MeOH (2 ml) and stirred for 10 min at room temperature. The resulting solution was evaporated *in vacuo* to give Na salt of I (713 mg) as colorless solid. mp 265° (decomp.). To a suspension of this salt in benzene (20 ml)–tetrahydrofuran (10 ml) was added dropwise a solution of AcCl (0.72 ml) in benzene (10 ml) and stirred for 15 min at room temperature. The reaction mixture was poured into ice–water and extracted with ether–CH_2Cl_2 (2:1). The organic layer was washed with 5% Na_2CO_3 and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product was purified by the preparative TLC. Recrystallization of the eluate from benzene gave IIb (286 mg) as colorless prisms. mp 184—187°. $[\alpha]_D^{21}$ +54.4° (c=0.85, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.83. Found: C, 72.89; H, 7.62.

Koenigs-Knorr Reaction of Estriol 3-Acetate in Benzene——IIb (150 mg) was treated with Ag_2CO_3 (150 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (200 mg) in benzene (22.5 ml) in the manner as described above. The preparative TLC using MeOH–benzene (15:85) as developing solvent followed by recrystallization from MeOH–ether gave a mixture of methyl (3-acetoxy-17β-hydroxyestra-1,3,5(10)-trien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IIIb) and methyl (3-acetoxy-16α-hydroxyestra-1,3,5(10)-trien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IVb) (122 mg) as colorless leaflets. Inspection by TLC using benzene–AcOEt (1:1) as solvent indicated that the product consisted of IIIb (Rf 0.70) and IVb (Rf 0.63) in a ratio of *ca.* 1 to 1. Treatment of the mixture with methanolic NaOH in the manner as described above gave V and VI, which were identified by TLC. NMR (3.4% solution in $CDCl_3$) δ: 0.76 (1.5 H, s, 18-CH_3), 0.80 (1.5 H, s, 18-CH_3), 2.04 (9H, s, pyranose-OCOCH_3), 2.27 (3H, s, 3-OCOCH_3), 3.33 (0.5 H, d, J=5 cps, 17α-H), 3.68 (0.5 H, d, J=5 cps, 17α-H), 3.76 (3H, s, -COOCH_3), 3.90 (0.5 H, m, 16β-H), 4.00—4.40 (1.5 H, m, 16β-H, pyranose-C_5-H), 4.58 (1H, d, J=7 cps, pyranose-C_1-H), 4.80—5.40 (3H, m, pyranose-CH-OAc). Fractional crystallization from MeOH gave IVb as colorless needles. mp 222—224°. *Anal.* Calcd. for $C_{33}H_{42}O_{13}\cdot\frac{1}{2}H_2O$: C, 60.45; H, 6.61. Found: C, 60.62, 60.29; H, 6.58, 6.45. Hydrolysis of IVb with methanolic NaOH gave VI.

3-Benzoyloxyestra-1,3,5(10)-triene-16α,17β-diol-6,7-^{3}H (Estriol-6,7-^{3}H 3-Benzoate)——To a solution of estriol-6,7-^{3}H (50 μCi; specific activity 8.7 mCi/mg) and cold estriol (1.12 mg) in 2% NaOH (0.55 ml) was added C_6H_5COCl (30 μl) and stirred at room temperature for 30 min. The resulting solution was diluted with H_2O and extracted with AcOEt (5 ml×2). The organic layer was washed with 5% HCl, 5% $NaHCO_3$ and H_2O, successively and dried over anhydrous Na_2SO_4. After usual work-up the residue was submitted to the preparative TLC using AcOEt–hexane–99% EtOH (25:23:2) as solvent. The adsorbent corresponding to the spot was eluted with AcOEt, and the eluate was diluted with the non-labeled estriol 3-benzoate (20.7 mg) as carrier and recrystallized repeatedly from MeOH to the constant specific activity as follows:

Recrystallization No.	from	Weight (mg)	Specific activity (dpm/mg)
1	MeOH	1.165	5204
2	MeOH	1.889	5142
3	MeOH	1.387	5205

Koenigs-Knorr Reaction with Estriol-6,7-^{3}H 3-Benzoate——To a solution of estriol-6,7-^{3}H 3-benzoate (36.2 μCi; specific activity 32.7 μCi/mg) in benzene (1 ml) were added methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (10 mg) and freshly prepared Ag_2CO_3 (10 mg) and stirred in the dark place for 24 hr. The reaction mixture was diluted with benzene (10 ml) and then filtered. The filtrate and washings were combined and evaporated *in vacuo*. The residue obtained was submitted to the preparative TLC using benzene–ether (1:1) as solvent. After multiple development the adsorbent corresponding to the less polar radioactive spot was eluted with AcOEt to give 3-benzoylestriol-6,7-^{3}H 16-glucuronide acetate-methyl ester. Radioactivity yield 30.0%. Homogeneity was confirmed by reverse isotope dilution method employing IIIa (21.4 mg) as carrier as follows:

Recrystallization No.	from	Weight (mg)	Specific activity (dpm/mg)
1	acetone–hexane	1.445	4204
2	acetone–hexane	1.469	4029
3	acetone–hexane	1.151	4082

The adsorbent corresponding to the more polar radioactive spot was eluted with AcOEt to give 3-benzoylestriol-6,7-^{3}H 17-glucuronide acetate-methyl ester. Radioactivity yield 33.4%. Homogeneity was confirmed by reverse isotope dilution method employing IVa (21.5 mg) as carrir as follows:

Recrystallization No.	from	Weight (mg)	Specific activity (dpm/mg)
1	acetone–hexane	1.134	5094
2	acetone–hexane	1.467	5040
3	acetone–hexane	1.338	5025

Estriol-6,7-^{3}H 16-Glucuronide——To a solution of 3-benzoylestriol-6,7-^{3}H 16-glucuronide acetate-methyl ester (0.44 μCi; specific activity 18.1 μCi/mg) in MeOH (1 ml) was added 1 N NaOH (0.2 ml) and allowed to stand at room temperature for 24 hr. The resulting solution was concentrated *in vacuo* below 50°. The residue obtained was dissolved in H_2O and percolated through a column of Amberlite XAD-2 (5 ml). After washing with distilled water (20 ml) the desired substance was eluted with EtOH (25 ml). Evaporation of the effluent gave estriol-6,7-^{3}H 16-glucuronide (0.38 μCi).

Estriol-6,7-^{3}H 17-Glucuronide——Treatment of 3-benzoylestriol-6,7-^{3}H 17-glucuronide acetate-methyl ester (0.47 μCi; specific activity 18.1 μCi/mg) in the manner as described above gave estriol-6,7-^{3}H 17-glucuronide (0.39 μCi).

Radioactive Counting——Counting of ^{3}H was carried out on a Packard Tri-Carb Model 3380 liquid scintillation counter. Samples were counted in Bray's scintillator,[10] which was composed of dioxane (880 ml), MeOH (100 ml), ethylene glycol (20 ml), naphthalene (60 g), 2,5-diphenyloxazole (4 g) and 1,4-bis (5-phenyloxazolyl)benzene (200 mg).

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