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Studies on Steroid Conjugates. XIII. Synthesis of 2,4,17α-d_3-Estriol 3-Glucuronoside[1]

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As a series of our studies on the steroid conjugates the direct analysis of female hormone glucuronosides by mass chromatography has previously been undertaken. Quantitative determination by this technique required the deuterated estrogen glucuronoside as an internal standard. The present paper deals with preparation of 2,4,17α-d_3-estriol 3-glucuronoside starting from the readily available 16α-hydroxyestrone.

An initial effort was directed to a convenient synthetic route to estriol 3-glucuronoside from estriol by utilizing the method previously established.[3] Partial hydrolysis of estriol triacetate (Ia) with potassium bicarbonate under the mild conditions afforded the 16,17-diacetate (Ib) in a satisfactory yield. Introduction of the glucuronyl moiety was accomplished by Koenigs-Knorr reaction in the usual manner.[4] When Ib and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate were stirred in dry benzene with freshly prepared silver carbonate, condensation reaction took place to provide methyl (16α,17β-diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (II) in 9% yield. Hydrolytic cleavage with methanolic sodium hydroxide gave the desired sodium 3-glucosiduronate (III).

On the basis of the model experiment preparation of the labeled compound was then carried out. Treatment of 16α-hydroxyestrone (IV) with lithium aluminum deuteride in anhydrous tetrahydrofuran, followed by usual acetylation afforded 17α-d_1-estriol triacetate (Vb), which on alkaline hydrolysis was led to 17α-d_1-estriol (Va). Substitution of deuterium on the aromatic ring was undertaken by utilizing reductive dehalogenation with a deuterated reagent. Being stirred with N-bromosuccinimide, Va underwent facile bromination at C-2 and C-4 yielding 17α-d_1-2,4-dibromoestriol (VIa),[5] which in turn was converted into the triacetate (VIb). An initial attempt for dehalogenation with lithium aluminum deuteride resulted in failure, since the yielded estriol showed no significant incorporation of deuterium. Recently Bosin, *et al.* disclosed that a deuterium atom can be introduced into an aromatic system by treatment of the appropriate aryl halide with sodium borodeuteride in the presence of transition metal as a catalyst.[6] In actuality VIb underwent reduction with sodium borodeuteride-palladium chloride providing 2,4,17α-d_3-estriol triacetate (VIIb) in a satisfactory yield. Subsequently selective deacetylation was effected by brief exposure to a bicarbonate solution to furnish 2,4,17α-d_3-estriol 16,17-diacetate (VIIa).

1) Part XII: T. Nambara, Y. Matsuki, J. Igarashi, Y. Kawarada, and M. Kurata, *Chem. Pharm. Bull.* (Tokyo), **22**, 2242 (1974). This paper also constitutes Part LXXVI of the series entitled "Analytical Chemical Studies on Steroids"; Part LXXV: T. Nambara, S. Ikegawa, T. Ishizuka, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **22**, 2656 (1974). The following trivial names are used in this paper: estrone, 3-hydroxyestra-1,3,5(10)-trien-17-one; estriol, estra-1,3,5(10)-triene-3,16α,17β-triol.

2) Location: *Aobayama, Sendai.*

3) *a*) T. Nambara and K. Imai, *Chem. Pharm. Bull.* (Tokyo), **15**, 1232 (1967); *b*) T. Nambara, Y. Matsuki, and Y. Kawarada, *ibid.*, **19**, 844 (1971).

4) H.H. Wotiz, E. Smakula, N.N. Lichtin, and J.H. Leftin, *J. Am. Chem. Soc.*, **81**, 1704 (1959).

5) R.H. Albrecht and D.D. Hagerman, *Steroids*, **19**, 177 (1971).

6) T.R. Bosin, M.G. Raymond, and A.R. Buckpitt, *Tetrahedron Letters*, **1973**, 4699.

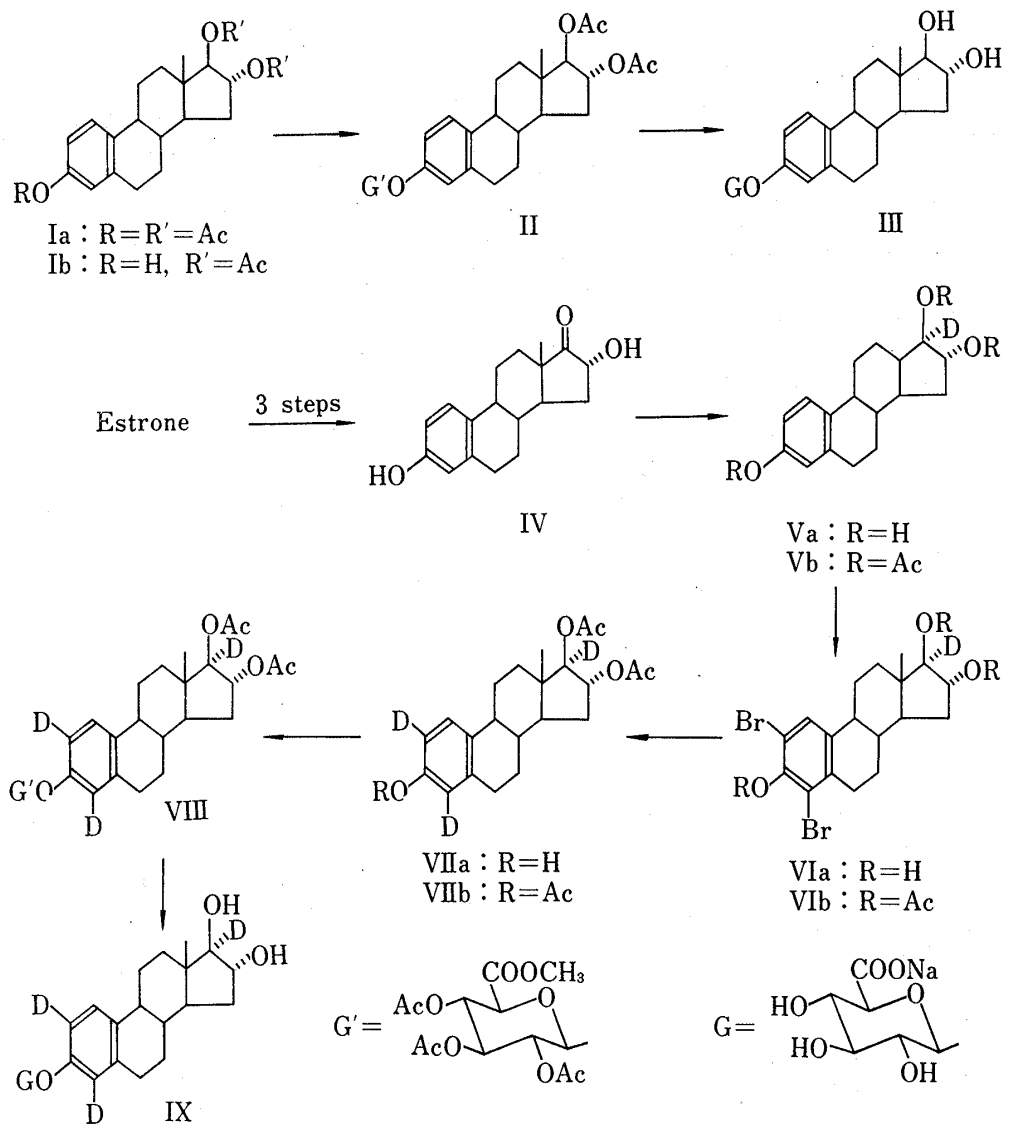

Chart 1

Bernstein and his coworker proposed the use of cadmium carbonate as a more suitable catalyst for preparation of the aryl glucuronoside by Koenigs-Knorr reaction.[7] Indeed condensation reaction of VIIa with methyl acetobromoglucuronate in the presence of this catalyst proceeded more easily to afford the 3-glucuronoside acetate-methyl ester (VIII) in an excellent yield. Upon treatment with methanolic sodium hydroxide under mild conditions VIII underwent simultaneous removal of the protecting groups in both steroid and sugar moieties yielding the desired sodium 2,4,17α-d_3-estriol 3-glucosiduronate (IX). Inspection of mass and nuclear magnetic resonance (NMR) spectra revealed that all the deuterated compounds were labeled at the desired positions with satisfactory isotopic purity.

It is hoped that the deuterated estriol glucuronoside thus obtained will serve as a suitable internal standard for determination of estrogen glucuronosides in the biological fluid by mass chromatography.

7) R.B. Conrow and S. Bernstein, *J. Org. Chem.*, **36**, 863 (1971).

Experimental[8]

Estra-1,3,5(10)-triene-3,16α,17β-triol 16,17-Diacetate (Ib)——To a solution of estriol triacetate (Ia) (2.61 g) in EtOH (100 ml) was added 6% $KHCO_3$ (20 ml) and stirred at room temperature for 3 days. The resulting solution was neutralized with 5% HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was submitted to column chromatography on Al_2O_3 (60 g). Elution with benzene and recrystallization of the eluate from ether–hexane gave Ib (1.35 g) as colorless needles. mp 178—179°. $[\alpha]_D^{18}$ −27.8° (c=0.13). *Anal.* Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 70.66; H, 7.83. NMR (5% solution in $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 2.06 (6H, s, 16α-, 17β-$OCOCH_3$), 4.94 (1H, d, J=5 Hz, 17α-H), 5.15 (1H, m, 16β-H). Tsuneda, *et al.* prepared this compound by the different procedure (reported mp 171—173°).[9]

Methyl (16α,17β-Diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (II)——Treatment of Ib (2 g) with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (4 g) and freshly prepared Ag_2CO_3 (4 g) in anhydrous benzene (300 ml) in the manner as described in the previous paper,[3a] followed by preparative thin-layer chromatography (TLC) and recrystallization from MeOH gave II (326 mg) as colorless needles. mp 197—198.5°. $[\alpha]_D^{18}$ −20.5° (c=0.12). *Anal.* Calcd. for $C_{35}H_{44}O_{14}$: C, 61.04; H, 6.44. Found: C, 61.03; H, 6.50. NMR (5% solution in $CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 2.03 (15H, s, $-OCOCH_3$), 3.70 (3H, s, $-COOCH_3$), 4.13 (1H, m, pyranose-C_5–H), 4.94 (1H, d, J=5 Hz, 17α-H), 5.15 (IH, m, 16β-H), 5.19 (1H, d, J=7 Hz, pyranose-C_1-H), 5.00—5.40 (3H, m, pyranose-C<u>H</u>–OAc). IR ν_{max}^{KBr} cm^{-1}: 888 (pyranose-C_1–H). Elce, *et al.* prepared this compound by the different procedure (reported mp 192—194°).[10]

Sodium (16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-yl-β-D-glucopyranosid)uronate (III)——To a solution of II (100 mg) in MeOH (12 ml) was added 1N NaOH (2.4 ml) and allowed to stand at room temperature overnight. The precipitate was collected by filtration, washed with H_2O and MeOH, and recrystallized from MeOH to give III (56 mg) as colorless needles. mp 270—272° (decomp). *Anal.* Calcd. for $C_{24}H_{31}O_9Na\cdot 2H_2O$: C, 55.16; H, 6.75. Found: C, 55.09; H, 7.67. Elce, *et al.* prepared this compound by the different procedure (reported mp 272.5—281° (decomp)).[10]

17α-d_1-Estra-1,3,5(10)-triene-3,16α,17β-triol (Va)——To a solution of 3,16α-dihydroxyestra-1,3,5(10)-trien-17-one (IV) (1.2 g) in anhydrous tetrahydrofuran (80 ml) was added $LiAlD_4$ (700 mg) portionwise and stirred at room temperature for 1 hr. After addition of moist AcOEt to decompose the excess reagent the organic layer was separated, washed with 20% Rochelle salt solution and H_2O, and evaporated. The crystalline product obtained was treated with Ac_2O (10 ml)–pyridine (20 ml) at room temperature overnight. The crude product was submitted to column chromatography on silica gel (50 g). Elution with benzene and recrystallization of the eluate from acetone–hexane gave 17α-d_1-estra-1,3,5(10)-triene-3,16α,17β-triol triacetate (Vb) (1 g) as colorless prisms. mp 126—127°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. Mass spectrum showed the isotopic composition of 98% d_1. A solution of Vb (1 g) dissolved in 3% methanolic KOH was allowed to stand at room temperature for 1 hr. The resulting solution was neutralized with 5% HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization from acetone gave Va (800 mg) as colorless needles. mp 283—283.5°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression.

17α-d_1-2,4-Dibromoestra-1,3,5(10)-triene-3,16α,17β-triol Triacetate (VIb)——To a solution of Va (650 mg) in anhydrous EtOH (180 ml) was added N-bromosuccinimide (1 g) and stirred at room temperature for 2 hr. After evaporation of solvent an oily residue obtained was dissolved in $CHCl_3$ (150 ml) and shaken with H_2O (25 ml). The precipitated 17α-d_1-2,4-dibromoestra-1,3,5(10)-triene-3,16α,17β-triol (VIa) was separated by filtration, washed with H_2O, and dried. mp 282—283.5° (decomp) (reported mp 276—277° (decomp)).[5] VIa was treated with Ac_2O (5 ml)–pyridine (10 ml) in the usual manner to give VIb as pale yellow amorphous substance. mp 104—107°. NMR (4% solution in $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 1.98, 2.03 (6H, s, 16α-, 17β-$OCOCH_3$), 2.31 (3H, s, 3-$OCOCH_3$), 5.05 (1H, broad d, J=7 Hz, 16β-H), 7.38 (1H, s, 1-H). Analytical sample could not be obtained and therefore the product was submitted to further elaboration without purification.

2,4,17α-d_3-Estra-1,3,5(10)-triene-3,16α,17β-triol Triacetate (VIIb)——To a solution of VIb (565 mg) in MeOD (10 ml) was added $PdCl_2$ (565 mg) and stirred at 0° under a stream of N_2 gas. To this solution was

8) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. NMR spectra were obtained on a Hitachi Model R-20A spectrometer at 60 MHz employing tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet. Infrared (IR) spectra were run on a JASCO Model IR-S spectrometer. Mass spectra were measured by a Shimadzu Model LKB-9000S spectrometer and a Shimadzu Model GCMSPAC-300 data processing system.

9) K. Tsuneda, J. Yamada, K. Yasuda, and H. Mori, *Chem. Pharm. Bull.* (Tokyo), **11**, 510 (1963).

10) J.S. Elce, J.G.D. Carpenter, and A.E. Kellie, *J. Chem. Soc.*, **1967**, 542.

added $NaBD_4$ (150 mg) portionwise and stirred for 30 min. The reaction mixture was poured into 1% HCl (50 ml) and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was treated with Ac_2O (1 ml)–pyridine (3 ml) in the usual manner. Recrystallization from acetone-hexane gave VIIb (233 mg) as colorless prisms. mp 126—127.5°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. NMR (4% solution in CCl_4) δ: 0.83 (3H, s, 18-CH_3), 1.98, 2.03 (6H, s, 16α-,17β-$OCOCH_3$), 2.18 (3H, s, 3-$OCOCH_3$), 5.03 (1H, broad d, J=7 Hz, 16β-H), 7.13 (1H, s, 1-H). Mass spectrum showed the isotopic composition of 0% d_0, 1% d_1, 6% d_2, 88% d_3, and 4% d_4.

2,4,17α-d_3-Estra-1,3,5(10)-triene-3,16α,17β-triol 16,17-Diacetate (VIIa)——To a solution of VIIb (340 mg) in EtOH (10 ml) was added 30% $KHCO_3$ solution (1 ml) and stirred at 35° for 4 hr. The resulting solution was diluted with ice-water and extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up an oily residue obtained was submitted to preparative TLC on silica gel HF using benzene–AcOEt (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.40) and recrystallization of the eluate from acetone-hexane gave VIIa (190 mg) as colorless prisms. mp 175—176.5°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 2.07, 2.12 (6H, s, 16α-, 17β-$OCOCH_3$), 5.18 (1H, broad d, J=7 Hz, 16β-H), 7.12 (1H, s, 1-H). Mass spectrum showed the isotopic composition of 0% d_0, 2% d_1, 9% d_2, 84% d_3, and 4% d_4.

Methyl (2,4,17α-d_3-16α,17β-Diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (VIII)——To a solution of VIIa (28 mg) in anhydrous toluene (3 ml) was added freshly prepared $CdCO_3$ (30 mg) and concentrated to 2 ml by slow distillation to remove the moisture. To this solution was added methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (60 mg) in anhydrous toluene (1 ml) and refluxed. After 1 and 2 hr an additional portion of the acetobromosugar (60 mg) dissolved in toluene (1 ml) was added, respectively. The precipitate was removed by filtration and washed with toluene. The filtrate and washings were combined and evaporated. An oily residue obtained was submitted to preparative TLC on silica gel HF using benzene–AcOEt (4: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.65) and recrystallization of the eluate from MeOH gave VIII (34 mg) as colorless needles. mp 193.5—194.5°. Mixed melting point on admixture with the non-labeled authentic sample showed no depression. NMR (4% solution in $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 2.05, 2.09 (15H, s, –$OCOCH_3$), 3.72 (3H, s, –$COOCH_3$), 4.20 (1H, m, pyranose-C_5-H), 5.25 (5H, m, 16β-, pyranose-C_1-H, pyranose-CH-OAc), 7.18 (1H, s, 1-H). Mass spectrum showed the isotopic composition of 0% d_0, 3% d_1, 10% d_2, 81% d_3, and 4% d_4.

Sodium (2,4,17α-d_3-16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-yl-β-D-glucopyranosid)uronate (IX)——To a solution of VIII (32 mg) in MeOH (3 ml) was added 1N methanolic NaOH (0.4 ml) and allowed to stand at room temperature for 1.5 hr. The resulting solution was concentrated to a small volume. The crystalline product was collected by filtration and recrystallized from MeOH to give IX (10 mg) as colorless needles. mp 268—271.5° (decomp). Mass spectrum showed the isotopic composition of 0% d_0, 3% d_1, 11% d_2, 80% d_3, and 4% d_4.

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