Synthesis of 2-hydroxyestriol monoglucuronides and monosulfates

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The ring A monoglucuronides and monosulfates of 2-hydroxyestriol were synthesized from 2-hydroxyestriol 16,17-diacetate by means of the Koenigs-Knorr reaction with methyl α-acetobromoglucuronate and sulfation with sulfur trioxide-pyridine complex, respectively. The conjugated positions of these compounds were definitely established by conversion to 2-hydroxyestriol monomethyl ethers by methylation, then enzymatic hydrolysis. The ring D monoglucuronides and monosulfates of 2-hydroxyestriol were also prepared from 2-hydroxyestriol 2,3-dibenzyl ether by glucuronidation and sulfation in a similar fashion followed by debenzylation, respectively. The positions of conjugation were established on the basis of their ^{1}H-nuclear magnetic resonance spectral data. (*Steroids* **55:** 128–132, 1990)

Keywords: steroids; 2-hydroxyestriol; catechol estrogen; glucuronide; sulfate; Koenigs-Knorr reaction

Introduction

In recent years, considerable attention has been focused on the physiologic significance of the formation of catechol estrogens in living animals.[1,2] The metabolic conjugation of catechol estrogens in animals[3–5] has been investigated by several groups. In our previous papers, we reported the in vitro and in vivo bioconversion of 4-hydroxyestrogens using synthetic monoglucuronides and monosulfates of 4-hydroxyestrogens as reference compounds.[6–8] Also, we disclosed the excretion of 4-hydroxyestriol 4-, 3-, and 16-glucuronides and 4-sulfate in rat bile after oral administration of 4-hydroxyestriol.[9] Accordingly, it appears to be of interest to clarify a difference in the metabolic conjugation between 4-hydroxyestriol and 2-hydroxyestriol. The authentic specimens are prerequisites for the unambiguous characterization of conjugation metabolites. The present report deals with the convenient synthesis of 2-hydroxyestriol monoglucuronides and monosulfates.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-4 automatic polarimeter. ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded on JEOL FX-100 and GX-500 spectrometers at 100 and 500 MHz using tetramethylsilane as an internal standard. Abbreviations used are s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectral (MS) measurements were carried out on a JEOL JMS-O1SG-2 mass spectrometer equipped with a JAM-2000 computer. Negative ion mass spectra (NIMS) were obtained on a JMS DX-303 HF mass spectrometer, and high-resolution mass spectra (high-MS) on a JEOL DX-303 mass spectrometer. The apparatus used for high-pressure liquid chromatography (HPLC) was a Waters ALC/GPC 202 high-performance liquid chromatograph equipped with a 440 UV detector (Millipore Waters Assoc., Milford, MA, USA) monitoring the absorbance at 254 nm. The HPLC was carried out on Develosil ODS-5 (5 μm; 15 cm × 0.4 cm ID) (Nomura Chemical Co., Kyoto, Japan) and μ-Bondasphere 5 μCN (5 μm; 15 cm × 0.39 cm ID) (Millipore Waters Assoc.) columns under ambient conditions at a flow rate of 1 ml/min unless otherwise stated. For column chromatography and preparative thin-layer chromatography (TLC), Silica gel 60 and Silica gel HF254 (E. Merck, AG, Darmstadt, FRG) were used, respectively.

Methyl 2,3,4-tri-O-acetyl-1-O-(16α,17β-diacetoxy-2-hydroxy-1,3,5(10)-estratrien-3-yl)-β-D-glucopyranosiduronate (2), *methyl 2,3,4-tri-O-acetyl-1-O-(16α,17β-diacetoxy-3-hydroxy-1,3,5(10)-estratrien-2-yl)-β-D-glucopyranosiduronate* (3)

Freshly prepared $CdCO_3$[11] (400 mg) was added to a solution of 2-hydroxyestriol 16,17-diacetate (**1**)[10] (200 mg) in anhydrous benzene (15 ml), and the sus-

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Received September 2, 1989; revised October 16, 1989.

pension was concentrated to approximately 10 ml by slow distillation over a period of 30 minutes to remove the moisture. After the addition of methyl α-acetobromoglucuronate (100 mg), the whole was refluxed for 4 hours. Additional amounts of methyl α-acetobromoglucuronate (100 mg) and $CdCO_3$ (200 mg) were then added, and the whole was further refluxed for 16 hours. The precipitate was removed by filtration and washed with benzene. The filtrate and washings were combined and evaporated down. Purification of the oily residue by means of column chromatography on silica gel gave a mixture of **2** and **3** (37 mg).

2-Hydroxyestriol 3-glucuronide (**4**), *2-hydroxyestriol 2-glucuronide* (**5**)

The mixture of **2** and **3** (30 mg) was dissolved in 5% methanolic KOH (5 ml) and allowed to stand at room temperature for 7 hours. The reaction mixture was neutralized with 5% HCl and diluted with H_2O (100 ml), and the organic solvent was evaporated off. The aqueous solution was percolated through a column packed with Amberlite XAD-4 resin (Rohm and Hass Co., Philadelphia, PA, USA) (25 cm × 1 cm ID). The column was washed with H_2O and the desired compounds were eluted with MeOH. The dried eluate was subjected to HPLC on Develosil ODS-5 using 0.5% NaH_2PO_4 (pH 3.0)/CH_3CN (5 : 1) as a mobile phase. The HPLC gave two peaks on the chromatogram in a ratio of approximately 1 : 1. The desired fractions were collected and evaporated down. An aqueous solution of each residue was applied to a Dowex 50W-X8 (Na^+ form) (5 cm × 1 cm ID) column. The eluate was dried and recrystallized from aqueous MeOH to give **4** (6 mg) and **5** (6 mg) as colorless amorphous substances, respectively. Compound **4** had the following features: mp, 210 C (dec.); $[\alpha]_D^{16}$ −26.6° (c = 0.1, MeOH/H_2O (7 : 1)); ^{1}H-NMR (CD_3OH) δ, 0.78 (1H, s, 18-CH_3), 3.66 (1H, d, J = 9 Hz, 17α-H), 4.05 (1H, m, 16β-H), 6.55 (1H, s, 4-H), 7.18 (1H, s, 1-H). The following values were determined for compound **5**: mp, 227 to 230 C; $[\alpha]_D^{18}$ −31.7° (c = 0.1, MeOH/H_2O (7 : 1)); analysis calculated, $C_{24}H_{32}O_{10}.2H_2O$: C, 55.81; H, 7.02; found: C, 55.44; H, 6.23; ^{1}H-NMR (CD_3OD) δ, 0.77 (3H, s, 18-CH_3), 6.70 (1H, s, 4-H), 6.82 (1H, s, 1-H): NIMS m/z; 479 $(M-H)^-$.

2,3-Dibenzyloxy-1,3,5(10)-estratriene-16α,17β-diol (**14**)

Anhydrous K_2CO_3 (100 mg) and benzyl chloride (0.1 ml) were added to a solution of **1** (200 mg) in anhydrous EtOH (20 ml), and the suspension was refluxed for 24 hours. The precipitate was removed by filtration and washed with CH_2Cl_2. The filtrate and washings were combined and evaporated down. The residue was treated with 5% methanolic KOH (30 ml) at 70 C for 1 hour, and the resulting solution was diluted with H_2O (100 ml) and extracted with AcOEt. The organic phase was evaporated down and the oily residue was purified by column chromatography on silica gel with benzene/AcOEt (30 : 1). The dried eluate was recrystallized from MeOH to give **14** (153 mg) as colorless needles. The following values were determined: mp, 142 to 144 C; $[\alpha]_D^{30}$ +56.0° (c = 0.1, $CHCl_3$); analysis calculated, $C_{32}H_{36}O_4$: C, 79.31; H, 7.49; found: C, 78.86; H, 7.41; ^{1}H-NMR ($CDCl_3$), 0.79 (3H, s, 18-CH_3), 3.56 (1H, d, J = 6 Hz, 17α-H), 4.14 (1H, m, 16β-H), 5.08 (4H, s, 2 × $OCH_2C_6H_5$), 6.64 (1H, s, 4-H), 6.86 (1H, s, 1-H), 7.14 to 7.48 (10H, m, 2 × $OCH_2C_6H_5$).

Methyl 2,3,4-tri-O-acetyl-1-O-(2,3-dibenzyloxy-17β-hydroxy-1,3,5(10)-estratrien-16α-yl)-β-D-glucopyranosiduronate (**15**), *methyl 2,3,4-tri-O-acetyl-1-O-(2,3-dizenzyloxy-16α-hydroxy-1,3,5(10)-estratrien-17β-yl)-β-D-glucopyranosiduronate* (**16**)

To a solution of **14** (200 mg) in anhydrous benzene (15 ml) were added freshly prepared Ag_2CO_3 (300 mg) and methyl α-acetobromoglucuronate (100 mg), and the whole was refluxed for 24 hours in a dark place. Additional amounts of methyl α-acetobromoglucuronate (100 mg) and Ag_2CO_3 (150 mg) were added, and the whole was further refluxed for 24 hours. The precipitate was removed by filtration and washed with benzene. The filtrate and washings were combined and evaporated down. The oily residue was purified by preparative TLC or HPLC on μ-Bondasphere 5 μCN (15 cm × 0.39 cm ID). The desired fractions were collected and the eluate was recrystallized from hexane-ether to give **15** and **16** as colorless needles in a ratio of 7 : 1. Compound **15** had the following values: mp, 202 to 204 C; $[\alpha]_D^{25}$ +25.0° (c = 0.1, $CHCl_3$); analysis calculated $C_{45}H_{52}O_{13}$, C, 67.49; H, 6.54; found, C, 67.50; H, 6.40; ^{1}H-NMR ($CDCl_3$) δ, 0.80 (3H, s, 18-CH_3), 2.05 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 3.67 (1H, d, J = 6 Hz, 17α-H) 3.78 (3H, s, $COOCH_3$), 3.92 (1H, m, 16β-H), 4.10 (1H, d, J = 10 Hz, 5'-H), 4.60 (1H, d, J = 8 Hz, 1'-H), 5.03 (1H, t, J = 15 Hz, 2'-H), 5.14 (4H, s, 2 × $OCH_2C_6H_5$), 5.22 to 5.32 (2H, m, 3'-H, 4'-H), 6.67 (1H, s, 4-H), 6.91 (1H, s, 1-H), 7.26 to 7.46 (10H, m, 2 × $OCH_2C_6H_5$); MS (FAB) m/z, 800 $(M)^+$. The following values were determined for compound **16**: mp, 227 to 229 C; $[\alpha]_D^{25}$ +24.0° (c = 0.1, $CHCl_3$); high MS m/z, 800.3420 $[M]^+$ (calculated for $C_{45}H_{52}O_{13}$, 800.3408); ^{1}H-NMR ($CDCl_3$) δ, 0.75 (3H, s, 18-CH_3), 2.04 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 3.35 (1H, d, J = 6 Hz, 17α-H) 3.78 (3H, s, $COOCH_3$), 4.12 (1H, d, J = 7.5 Hz, 5'-H), 4.25 (1H, m, 16β-H), 4.60 (1H, d, J = 7.5 Hz, 1'-H), 5.10 (4H, s, 2 × $OCH_2C_6H_5$), 5.24 to 5.32 (2H, m, 3'-, 4'-H), 6.69 (1H, s, 4-H), 6.89 (1H, s, 1-H), 7.27 to 7.46 (10H, m, 2 × $OCH_2C_6H_5$).

2,3-Dibenzyloxy-17β-hydroxy-1,3,5(10)-estratien-16α-yl-β-D-glucopyranosiduronic acid (**17**)

Compound **17** was prepared from **15** (27 mg) in the manner described for **4** and **5**. The crude product was recrystallized from MeOH to give **17** (18 mg) as a col-

orless amorphous substance with the following features: mp, 228 to 231 C; $[\alpha]_D^{20}$ +114.0° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ; 0.80 (3H, s, 18-CH_3), 5.07 (4H, s, 2 × O$CH_2C_6H_5$), 6.69 (1H, s, 4-H), 6.88 (1H, s, 1-H), 7.26 to 7.46 (10H, m, 2 × O$CH_2C_6H_5$); MS (FAB, NI) m/z, 659 $(M-H)^-$.

2,3-Dibenzyloxy-16α-hydroxy-1,3,5(10)-estratrien-17β-yl-β-D-glucopyranosiduronic acid (**18**)

Compound **18** was prepared from **16** (9 mg) in the manner described for **4** and **5**. The crude product was recrystallized from MeOH to give **18** (6 mg) as a colorless amorphous substance with the following values: mp, 211 to 214 C (dec.); $[\alpha]_D^{20}$ −47.9° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ; 0.84 (3H, s, 18-CH_3), 5.04 (4H, s, 2 × O$CH_2C_6H_5$), 6.64 (1H, s, 4-H), 6.84 (1H, s, 1-H), 7.25 to 7.50 (10H, m, 2 × O$CH_2C_6H_5$); MS (FAB, NI) m/z, 659 $(M-H)^-$.

2-Hydroxyestriol 16-glucuronide (**19**)

A solution of **17** (10 mg) in MeOH (10 ml) was shaken with 5% Pd/C (15 mg) under a hydrogen gas stream for 24 hours. The catalyst was removed by filtration and the filtrate was evaporated down. The crude product was recrystallized from MeOH to give **19** (4.6 mg) as a colorless amorphous substance that had the following values: mp, 251 to 254 C (dec.); $[\alpha]_D^{20}$ −189.0° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ; 0.81 (3H, s, 18-CH_3) 6.54 (1H, s, 4-H), 6.75 (1H, s, 1-H); MS (FAB, NI) m/z, 479 $(M-H)^-$.

2-Hydroxyestriol 17-glucuronide (**20**)

Compound **20** was prepared from **18** (6.6 mg) in the manner described for **19**. The crude product was recrystallized from MeOH to give **20** (4.4 mg) as a colorless amorphous substance. The following values were determined: mp, 180 to 183 C (dec.); $[\alpha]_D^{20}$ −172.0° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ; 0.88 (3H, s, 18-CH_3), 4.21 (1H, m, 16β-H), 4.33 (1H, d, J = 8 Hz, 17α-H), 6.46 (1H, s, 4-H), 6.70 (1H, s, 1-H); MS (FAB, NI) m/z, 479 $(M-H)^-$.

2-Hydroxyestriol 3-sulfate (12), 2-hydroxyestriol 2-sulfate (**13**)

Freshly prepared sulfur trioxide-pyridine complex (400 mg) was added to a solution of 2-hydroxyestriol 16,17-diacetate (**1**) (300 mg) in pyridine (3 ml), and the whole was stirred under ice cooling for 2 hours, then at room temperature for 3 hours. Excess reagent was decomposed by the addition of 5% $NaHCO_3$. The resulting solution was passed through an Amberlite XAD-4 column (20 cm × 1 cm ID) in the manner described above. The eluate containing the 16,17-diacetate monosulfates (**10, 11**) was treated with 5% methanolic KOH (20 ml) for 12 hours at room temperature. The reaction mixture was neutralized with 5% HCl and diluted with H_2O (150 ml). The aqueous solution was purified by Amberlite XAD-4 in the usual manner. The crude product was subjected to HPLC on Develosil ODS-5 (15 cm × 0.4 cm ID) using 0.5 AcONa (pH 5.0)-CH_3CN-THF (10 : 1 : 1). The desired fractions were collected and evaporated down. The crude products were recrystallized from MeOH to give **12** (26 mg) and **13** (26 mg) as colorless amorphous substances, respectively. Compound **12** had the following features: mp, 238 to 241 C (dec.); $[\alpha]_D^{22}$ −103.4° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ; 0.78 (3H, s, 18-CH_3), 3.49 (1H, d, J = 6 Hz, 17α-H), 4.07 (1H, m, 16β-H), 6.88 (1H, s, 4-H), 7.00 (1H, s, 1-H); MS (FAB, NI) m/z, 383 $(M-H)^-$. The following values were found for compound **13:** mp, 237 to 241 C (dec.); $[\alpha]_D^{22}$ −57.7° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ, 0.78 (3H, s, 18-CH_3), 3.46 (1H, d, J = 6 Hz, 17α-H), 4.04 (1H, m, 16β-H), 6.58 (1H, s, 4-H), 7.15 (1H, s, 1-H); MS (FAB, NI) m/z, 383 $(M-H)^-$.

2,3-Dibenzyloxy-1,3,5(10)-estratriene-16α,17β-diol 16-sulfate (**21**), *2,3-dibenzyloxy-1,3,5(10)-estratriene-16α,17β-diol 17-sulfate* (**22**)

Compounds **21** and **22** were prepared from **14** (300 mg) in the manner described for **12** and **13**. The mixture of **21** and **22** was purified by preparative TLC using benzene-AcOEt (6 : 1) as a developing solvent. Elution of the adsorbent corresponding to each spot with AcOEt and recrystallization of the eluate from acetone gave **21** (47 mg) and **22** (5 mg) as colorless amorphous substances, respectively. The following values were found for compound **21:** mp, 186 to 188 C; $[\alpha]_D^{22}$ −142.9° (c = 0.1, MeOH); NMR (CD_3OD) δ; 0.80 (3H, s, 18-CH_3), 3.76 (1H, d, J = 6 Hz, 17α-H), 4.38 (1H, m, 16β-H), 5.10 (4H, s, 2 × O$CH_2C_6H_5$), 6.70 (1H, s, 4-H), 6.90 (1H, s, 1-H), 7.28 to 7.47 (10H, m, 2 × O$CH_2C_6H_5$); MS (FAB, NI) m/z, 563 $(M-H)^-$, 472 $(M-H-91\ (C_6H_5CH_2))^-$, 381 $(M-H-182\ (2 \times C_6H_5CH_2))$. Compound **22** had the following values: mp, 206 to 209 C; $[\alpha]_D^{18}$ −42.9° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ, 0.85 (3H, s, 18-CH_3), 3.78 (1H, d, J = 6 Hz, 17α-H), 4.69 (1H, m, 16β-H), 5.10 (4H, s, 2 × O$CH_2C_6H_5$), 6.65 (1H, s, 4-H), 6.85 (1H, s, 1-H), 7.31 to 7.47 (10H, m, 2 × O$CH_2C_6H_5$); MS (FAB, NI) m/z, 563 $(M-H)^-$.

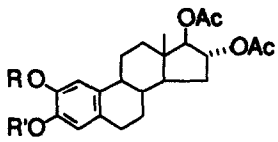
2-Hydroxyestriol 16-sulfate (**24**)

Compound **24** was prepared from **21** (20 mg) in the manner described for **19**. Recrystallization of the crude product from MeOH gave **24** (15 mg) as a colorless amorphous substance with the following features: mp, 201 to 204 C (dec.); $[\alpha]_D^{22}$ +87.3° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ, 0.79 (1H, s, 18-CH_3), 6.48 (1H, s, 4-H), 6.71 (1H, s, 1-H); MS (FAB, NI) m/z, 383 $(M-H)^-$.

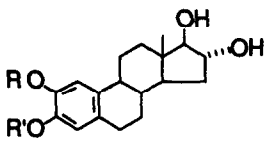
2-Hydroxyestriol 17-sulfate (**25**)

Compound **25** was prepared from **22** (5 mg) in the manner described for **19**. Recrystallization of the crude product from MeOH gave **25** (4 mg) as a colorless amorphous substance with the following values: mp,

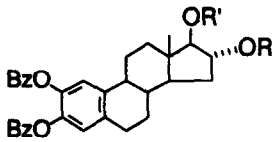
223 to 226 C; $[\alpha]_D^{18}$ −65.6° (c = 0.1, MeOH); ^{1}H-NMR (CD_3OD) δ, 0.83 (3H, s, 18-CH_3), 3.68 (1H, d, J = 6 Hz, 17α-H), 4.64 (1H, m, 16β-H), 6.45 (1H, s, 4-H), 6.70 (1H, s, 1-H); MS (FAB, NI) m/z, 383 $(M-H)^-$.



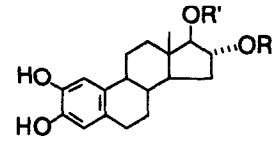
1 : R = R' = H
2 : R = H, R' = G'
3 : R = G', R' = H
10 : R = H, R' = SO_3H
11 : R = SO_3H, R' = H



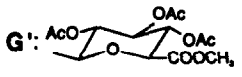
4 : R = H, R' = G
5 : R = G, R' = H
6 : R = CH_3, R' = G
7 : R = G, R' = CH_3
8 : R = CH_3, R' = H
9 : R = H, R' = CH_3
12 : R = H, R' = SO_3H
13 : R = SO_3H, R' = H



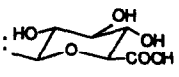
14 : R = R' = H
15 : R = G', R' = H
16 : R = H, R' = G'
17 : R = G, R' = H
18 : R = H, R' = G
21 : R = SO_3H, R' = H
22 : R = H, R' = SO_3H
23 : R = R' = SO_3H



19 : R = G, R' = H
20 : R = H, R' = G
24 : R = SO_3H, R' = H
25 : R = H, R' = SO_3H



G' :



G :

Bz : $C_6H_5CH_2$-

Results and discussion

Our initial effort was directed to the preparation of 2-hydroxyestriol ring A monoglucuronides and monosulfates. Introduction of a glucuronyl moiety into 2-hydroxyestriol 16,17-diacetate (**1**)[10] was undertaken by means of the Koenigs-Knorr reaction using cadmium carbonate as a catalyst.[11] Condensation of **1** with methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (methyl α-acetobromoglucuronate) in anhydrous benzene readily proceeded to afford two positional isomers, methyl 2,3,4-tri-O-acetyl-1-O-(16α,17β-diacetoxy-2-hydroxy-1,3,5(10)-estratrien-3-yl)-β-D-glucopyranosiduronate (**2**) and methyl 2,3,4-tri-O-acetyl-1-O-(16α,17β-diacetoxy-3-hydroxy-1,3,5(10)-estratrien-2-yl)-β-D-glucopyranosiduronate (**3**), in an approximately equal amount. The removal of protecting groups in ring D and sugar moieties in **2** and **3** was attained by hydrolysis with methanolic sodium hydroxide under mild conditions, yielding a mixture of the isomeric ring A monoglucuronides. These two were efficiently resolved by HPLC to give the desired 3-glucuronide (**4**) and 2-glucuronide (**5**), respectively. The β-glucuronoside structure in these monoglucuronides was ascertained by characterizing 2-hydroxyestriol liberated when incubated with the β-glucuronidase (*Helix pomatia*) preparation.

To establish the positions of the glucuronyl residue in **4** and **5**, these two were transformed into 2-hydroxyestriol 2- and 3-methyl ethers (**8, 9**) for direct comparison with the authentic specimens. On treatment with methyl iodide and potassium carbonate in dimethylformamide followed by alkaline hydrolysis, **4** and **5** were led to 2-methoxyestriol 3-glucuronide (**6**) and 2-hydroxyestriol 2-glucuronide 3-methyl ether (**7**). Enzymatic hydrolysis of **6** and **7** with β-glucuronidase afforded 2-methoxyestriol (**8**) and 2-hydroxyestriol 3-methyl ether (**9**), which were unequivocally identified by means of HPLC using the authentic specimens.

The synthesis of 2-hydroxyestriol ring A monosulfates was then undertaken. Treatment of **1** with freshly prepared sulfur trioxide-pyridine complex furnished a mixture of 2-hydroxyestriol 16,17-diacetate monosulfates (**10, 11**) in approximately equal amounts as judged by reversed phase HPLC. After alkaline hydrolysis under mild conditions and subsequent HPLC separation, the mixture provided the desired 2-hydroxyestriol 3- and 2-monosulfates (**12, 13**). These isomeric monosulfates led to the known 2-hydroxyestriol 2- and 3-methyl ethers on methylation with diazomethane and subsequent hydrolysis with sulfatase (*Helix pomatia*).[12]

We next, attempted to synthesize 2-hydroxyestriol ring D monoglucuronides and monosulfates. The Koenigs-Knorr reaction of 2-hydroxyestriol 3,4-dibenzyl ether (**14**) with methyl α-acetobromoglucuronate occurred readily to give the glucuronide acetate-methyl esters (**15, 16**) in a ratio of 7 : 1. These positional isomers were efficiently separated by HPLC on a normal phase column. On hydrolysis with methanolic alkali followed by hydrogenolysis over palladium-on-charcoal, compounds **15** and **16** were transformed into the desired 2-hydroxyestriol 16- and 17-glucuronides (**19, 20**) through **17** and **18** in satisfactory yields.

The conjugated position and configuration of the glucuronoside linkage in **15** and **16** were established on the basis of ^{1}H-NMR spectral data. The 17α-proton and 16β-proton signals in **15** appeared at 3.67 ppm as a doublet and 3.92 ppm as a multiplet, respectively, while those in **16** appeared at 3.35 ppm as a doublet and 4.25 ppm as a multiplet. These data permitted us to assign the structures 16α- and 17β-glucuronide acetate-methyl esters to **15** and **16**, respectively.[13] The anomeric protons of these conjugates appeared at approximately 4.6 ppm as a doublet (J = 7 to 8 Hz), indicating the formation of the β-glucuronoside linkage.

The preparation of ring D monosulfates from **14** was also performed. In a previous report, we described the synthesis of 4-hydroxyestriol 16- and 17-sulfates by dicyclohexylcarbodiimide-mediated sulfation.[13] In the present case, however, the similar reaction with **14** mainly produced the 16-sulfate, together with a small amount of the 17-sulfate. On time-controlled sulfation with sulfur trioxide-pyridine complex in pyridine, **14** was converted into a mixture of 16- and 17-monosulfates and 16,17-disulfate (**21, 22, 23**) in a ratio of 10 : 1 : 5. These three were separated by preparative TLC and

HPLC (μ-Bondasphere CN). Compounds **21** and **22** were then transformed into the desired 2-hydroxyestriol 16-sulfate and 17-sulfate (**24, 25**) by hydrogenolysis over palladium on charcoal. These products underwent enzymatic hydrolysis to provide 2-hydroxyestriol. To elucidate the sulfated positions in **21** and **22**, their ^{1}H-NMR spectral data were inspected. The 17α- and 16β-protons appeared at 3.76 ppm as a doublet and 4.38 ppm as a multiplet in **21**, while those at 3.78 ppm appeared as a doublet and those at 4.69 ppm appeared as a multiplet in **22**. These data permitted us to assign the structures 16- and 17-sulfates to **24** and **25**, respectively.[13]

The ready availability of these authentic specimens will serve for the characterization and determination of catechol estrogen conjugates in biologic fluids. Further studies of the metabolism of 2-hydroxyestriol in living animals are being conducted in these laboratories, and the details will be reported elsewhere.

Acknowledgments

The authors are indebted to the staff of the central analytical laboratory of this institution for elemental analyses and spectral measurements. This work was supported in part by a grant from the Ministry of Eduction, Science and Culture of Japan.

Notes

The following trivial names are used in this report: 2-hydroxyestriol = 1,3,5(10)-estratriene-2,3,16α,17β-tetraol; glucuronide = β-D-glucopyranosiduronic acid.

This paper constitutes Part CCXLVIII of "Studies on Steroids" by T. Nambara. Part CCXLVII: J. Goto, Y. Saisho, T. Nambara (1989). *Anal Sci* **5**:399–402.

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