# The synthesis of estriol 16- and 17-monoglucuronide from estriol

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An efficient and convenient procedure for the synthesis of estriol 16- and 17-monoglucuronides from estriol is described. This is achieved by the selective protection and deprotection of the hydroxy groups in estriol, Koenigs-Knorr reactions with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate and subsequent hydrolysis. The products have been characterized by proton nuclear magnetic resonance ($^1H$ NMR), two-dimensional $^1H$ homonuclear shift-correlated spectra (2D-COSY) and mass spectra. The selective Koenigs-Knorr reaction of the alcoholic hydroxyl group in the presence of a phenolic hydroxyl group is also reported. (Steroids 58:452–456, 1993)

## Introduction

As part of our current research program into the development of enzyme immunoassay systems for the home measurement of steroid glucuronides,[1] we require a supply of estriol monoglucuronides. Estriol 16,17-diacetate is easily prepared from estriol by acetylation with acetic anhydride (Ac$_2$O) and a catalytic amount of boron trifluoride etherate in tetrahydrofluran (THF) at room temperature,[2] or in our laboratory* by simply refluxing in acetic acid (HAc) catalyzed with copper(II) acetate monohydrate.[3] Hence estriol 3-glucuronide is comparatively easy to synthesize after coupling of estriol 16,17-diacetate with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate and subsequent hydrolysis. However, estriol 16- or 17-glucuronides are less readily available because of the difficulty in their synthesis. The usual synthesis of estriol 16- and 17-glucuronides from estrone involves many steps including the glucuronidation of 16α-hydroxy-17-oxo-derivatives and reduction of the 17-carbonyl group. The reduction of the 17-carbonyl group often produces significant quantities of the 17α-isomer, which significantly decreases the yield. For example, during synthesis of estriol 16-glucuronide from estrone according to a literature procedure,[4] we obtained about 30% of methyl 3,17α-dihydroxyl-1,3,5(10)-estratrien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (17α-isomer)† during reduction of the 17-carbonyl group.

We now report an alternative method for the synthesis of either estriol 16- or 17-glucuronide from estriol based on the selective protection and deprotection of the hydroxyl groups, glucuronidation, and hydrolysis. The methodology provides a simple procedure and good yields of pure products, which have been characterized by spectroscopy.

## Experimental

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*, In accordance with reference 3, we refluxed estriol in acetic acid catalyzed with copper(II) acetate monohydrate to get estriol 16α,17β-diacetate in 60% yield. IR $\nu_{max}$ 3,414 cm$^{-1}$ (3-OH), 1,742 and 1,713 cm$^{-1}$ (C=O, C-16, 17-diacetate); $^1H$ NMR (CDCl$_3$) δ, 0.84 (s, 3H, 18-CH$_3$), 2.06 (s, 3H, OCOCH$_3$), 2.10 (s, 3H, OCOCH$_3$), 5.00 (d, 1H, J = 5.59 Hz, 17α-H), 5.18 (m, 1H, 16β-H), 5.36 (s, 1H, 3-OH), 6.57–7.14 (m, 3H, aromatic).

†, The reduction of methyl 2,4-dibromo-17-oxo-1,3,5(10)-estratrien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate gave 30% of methyl 3,17α-dihydroxyl-1,3,5(10)-estratrien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (17α-isomer). High EIMS m/z, 604.2563 (M)$^+$ (calculated for C$_{31}$H$_{40}$O$_{12}$, 604.2520); $^1H$ NMR δ, 0.72 (s, 3H, 18-CH$_3$), 2.04 (s, 6H, 2OCOCH$_3$), 2.06 (s, 3H, OCOCH$_3$), 3.73 (d, 1H, J = 4.39 Hz, 17β-H), 3.77 (s, 3H, COOCH$_3$), 4.07 (d, 1H, J = 4.39 Hz, 5'-H), 4.38 (m, 1H, 16β-H), 4.46 (d, 1H, J = 8.06 Hz, 1'-H), 5.05 (t, 1H, 2'-H), 5.18-5.33 (m, 2H, 3' and 4'-H), 6.55–7.17 (m, 3H, aromatic).
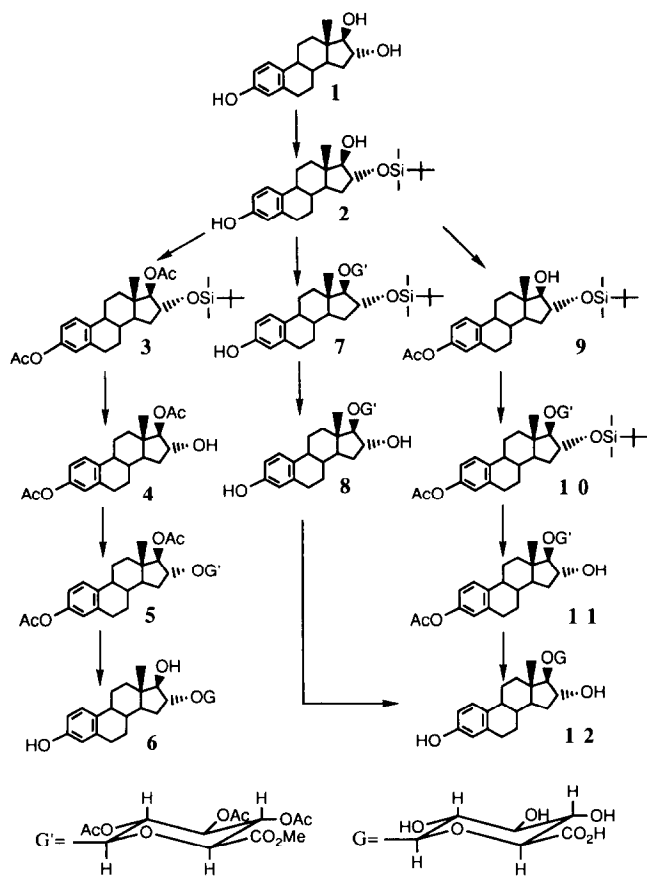
**Scheme 1**

triplet, m, multiplet, J, coupling constant). Mass spectra (MS) were recorded on a VG 70-250S double focusing magnetic sector mass spectrometer. Microanalyses were performed by the micro-analytical laboratory, University of Otago, Dunedin, New Zealand. Column chromatography was carried out with silica gel (Merck Kieselgel 60, 230-400 mesh). Thin-layer chromatography (TLC) was performed using precoated silica gel plates (Merck Kieselgel 60 $F_{254}$) and the spots were visualized by UV fluorescence or by spraying with 10% concentrated sulfuric acid in ethanol and heating at 120°C for 3 minutes. The general synthetic scheme employed in this work for the synthesis of estriol 16- and 17-glucuronides is outlined in Scheme 1:

## (a) Selective protection and deprotection of estriol and its derivatives

**16α - (tert - Butyldimethylsilyloxy) - 1,3,5(10) - estratrien - 3,17β - diol (2) and 3,17β-diacetoxy-16α-(tert-butyldimethylsilyloxy)-1,3,5(10)-estratrien (3).** Compounds 2 and 3 were prepared from estriol 1 by selective protection of the 16α-hydroxyl group with tert-butyldimethylsilyl chloride, followed by acetylation with $Ac_2O$ and pyridine according to established literature procedures[5] in 82% and 93% yield, respectively. For compound 2 (white solid), mp, 193-194 C; reported mp, 194 C (lit. 5). IR $\nu_{max}$ 3,563 cm$^{-1}$ (OH), 3,316 cm$^{-1}$ (OH); $^1$H NMR (CDCl$_3$) δ, 0.06 (s, 6H, $-$SiMe$_2$), 0.73 (s, 3H, 18-CH$_3$), 0.87 (s, 9H, $-$SiCMe$_3$), 3.30 (s, 1H, 17β-OH), 3.46 (d, 1H, J = 5.49 Hz, 17α-H), 4.06 (m, 1H, 16β-H), 4.66 (s, 1H, 3-OH), 6.49-7.08 (m, 3H, aromatic); For compound 3 (fine crystals) mp, 110-112 C; (no melting point

reported[5]). IR $\nu_{max}$ 1,766 and 1,744 cm$^{-1}$ (C=O, C-3,17-diacetate); $^1$H NMR (CDCl$_3$) δ, 0.03 (d, 6H, $-$SiMe$_2$), 0.77 (s, 3H, 18-CH$_3$), 0.88 (s, 9H, $-$SiCMe$_3$), 2.09 (s, 3H, OCOCH$_3$), 2.28 (s, 3H, OCOCH$_3$), 4.30 (m, 1H, 16β-H), 4.84 (d, 1H, J = 5.59 Hz, 17α-H), 6.78-7.28 (m, 3H, aromatic).

**3-Acetoxy-16α-(tert-butyldimethylsilyloxy)-1,3,5(10)-estratrien-17β-ol (9).** Protection of the 3-hydroxyl group in compound 2 was performed by an acetylation procedure similar to that used in the preparation of compound 3, expect that the reaction was stirred at 0 C (ice-cooled bath)[2] for 0.5 hours instead of at room temperature for 40 hours. After extracting with ethyl acetate (EtOAc) (2 × 20 ml), the organic layer was dried over magnesium sulfate (MgSO$_4$) overnight. Removal of solvent under reduced pressure afforded a white solid that was purified by flash chromatography using hexane/EtOAc (3 : 1) as eluent to give compound 9 as a white solid (48 mg, 87% yield). mp, 190-192 C; Analysis calculated: C$_{26}$H$_{40}$O$_4$Si · 1/2H$_2$O: C, 68.83; H, 9.11; Found: C, 68.93; H, 8.91; IR, $\nu_{max}$ 3,617 and 3,594 cm$^{-1}$ (17β-OH), 1,754 cm$^{-1}$ (C = O, 3-acetate); $^1$H NMR (CDCl$_3$) δ, 0.06 (s, 3H, $-$SiMe), 0.07 (s, 3H, $-$SiMe), 0.75 (s, 3H, 18-CH$_3$), 0.89 (s, 9H, $-$SiCMe$_3$), 2.25 (s, 3H, 3-OCOCH$_3$), 3.44 (s, 1H, 17β-OH), 3.54 (d, J = 5.86 Hz, 1H, 17α-H), 4.06 (m, 1H, 16β-H), 6.75-7.26 (m, 3H, aromatic).

**3,17β-Diacetoxy-1,3,5(10)-estratrien-16α-ol (4).** A typical experimental procedure for the desilylation of the 16α-tert-butyldimethylsilyl group was as follows:[6] Compound 3 (239 mg) was dissolved in 15 ml of solvent mixture (HAc/H$_2$O/THF, 3 : 1 : 1), and stirred for 40 hours at room temperature. After adding 100 ml of ether (Et$_2$O), the organic layer was separated, washed with ice-cooled NaHCO$_3$ and water, and dried over MgSO$_4$. Removal of solvent under reduced pressure afforded a white solid that was purified by flash chromatography using hexane/EtOAc (2 : 1) as eluent to give compound 4 as a white solid (177 mg, 97% yield). mp, 140 C; reported mp, 132 C (lit. 5). IR $\nu_{max}$ 3,503 cm$^{-1}$ (16β-OH), 1,751 and 1,719 cm$^{-1}$ (C=O, 3,17-diacetate); $^1$H NMR (CDCl$_3$) δ, 0.86 (s, 3H, 18-CH$_3$), 2.15 (s, 3H, OCOCH$_3$), 2.28 (s, 3H, OCOCH$_3$), 3.76 (s, 1H, 16α-OH), 4.16 (m, 1H, 16β-H), 4.28 (d, J = 4.48 Hz, 1H, 17α-H), 6.80-7.28 (m, 3H, aromatic).

**Methyl 3,16α-dihydroxy-1,3,5(10)-estratrien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (8).** The desilylation reaction was performed by a procedure similar to that used in the preparation of compound 4. Compound 8 was obtained from compound 7 (220 mg) as colorless crystals (160 mg, 85% yield). mp, 216-218 C; reported mp, 207-212 C (lit. 7). Analysis calculated, C$_{31}$H$_{40}$O$_{12}$ · H$_2$O: C, 59.79; H, 6.79; Found: C, 59.71; H, 6.60; $^1$H NMR (CDCl$_3$) δ, 0.74 (s, 3H, 18-CH$_3$), 2.04 (s, 3H, OCOCH$_3$), 2.05 (s, 3H, OCOCH$_3$), 2.08 (s, 3H, OCOCH$_3$), 3.34 (d, J = 5.22 Hz, 1H, 17α-H), 3.71 (s, 1H, 16α-OH), 3.77 (s, 3H, COOCH$_3$), 4.11 (d, J = 9.33 Hz, 1H, 5'-H), 4.22 (m, 1H, 16β-H), 4.59 (d, J = 7.83 Hz, 1H, 1'-H), 5.09 (t, 1H, 2'-H), 5.25-5.30 (m, 2H, 3' and 4'-H), 5.77 (s, 1H, 3-OH), 6.56-7.11 (m, 3H, aromatic); EIMS m/z, 604 (M)$^+$.

**Methyl 3-acetoxy-16α-hydroxyl-1,3,5(10)-estratrien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (11).** The desilylation reaction was again performed by a procedure similar to that used in the preparation of 4. Compound 11 was obtained from compound 10 (50 mg) as a white solid (33 mg, 78% yield). mp, 225-227 C; reported mp, 222-224 C (lit. 8). $^1$H NMR (CDCl$_3$) δ, 0.76 (s, 3H, 18-CH$_3$), 2.04 (s, 3H, OCOCH$_3$), 2.05 (s, 3H, OCOCH$_3$), 2.08 (s, 3H, OCOCH$_3$), 2.28 (s, 3H, 3-OCOCH$_3$), 3.34 (d, J = 5.13 Hz, 1H, 17α-H), 3.49 (s, 1H, 16α-OH), 3.78 (s, 3H,

COOCH₃), 4.12 (d, J = 9.53 Hz, 1H, 5'-H), 4.26 (m, 1H, 16β-H), 4.60 (d, J = 8.06 Hz, 1'-H), 5.09 (t, 1H, 2'-H), 5.23–5.31 (m, 2H, 3'- and 4'-H), 6.78–7.27 (m, 3H, aromatic).

## (b) Glucuronidation reactions

**Methyl 3,17β-diacetoxy-1,3,5(10)-estratrien-16α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (5).** A typical experimental procedure for the coupling reaction of estriol 3.17β-diacetate **4** with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate was as follows.[9] Dried CdCO₃ (315 mg, 1.83 mmol) was added to a solution of compound **4** (170 mg, 0.46 mmol) in anhydrous toluene (50 ml), and the suspension was concentrated to approximately 30 ml by distillation to remove the moisture. After distillation, the solution of methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (725 mg, 1.83 mmol) in anhydrous toluene (20 ml) was added dropwise by syringe, keeping the addition rate the same as the rate of distillation. Finally, the whole reaction mixture was refluxed for 2.5 hours. The precipitate was then removed by filtration and washed with CH₂Cl₂. The filtrate and washings were combined and evaporated under reduced pressure. Purification of the oily residue by flash chromatography using hexane/EtOAc (1 : 1) as eluent gave compound **5** as a white solid (250 mg, 80% yield). mp, 170–173 C; Analysis calculated: C₃₅H₄₄O₁₄ · 2/3H₂O: C, 59.99; H, 6.52; Found: C, 59.96; H, 6.24; ¹H NMR (CDCl₃) δ, 0.75 (s, 3H, 18-CH₃), 2.02 (s, 3H, OCOCH₃), 2.03 (s, 3H, OCOCH₃), 2.05 (s, 3H, OCOCH₃), 2.06 (s, 3H, OCOCH₃), 2.28 (s, 3H, 3-OCOCH₃), 3.77 (s, 3H, COOCH₃), 3.96 (d, J = 9.70 Hz, 1H, 5'-H), 4.25 (m, 1H, 16β-H), 4.56 (d, J = 7.46 Hz, 1H, 1'-H), 4.95–4.98 (m, 2H, 2' and 17α-H), 5.22–5.25 (m, 2H, 3' and 4'-H), 6.77–7.26 (m, 3H, aromatic).

**Methyl 3-hydroxy-16α-(*tert*-butyldimethylsilyloxy)-1,3,5-(10)-estratrien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (7).** The coupling reaction was performed by a procedure similar to that used in the preparation of **5**, except that a 2 : 1 ratio of methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate to the compound **2** was used instead of a 4 : 1 mole ratio as for compound **4** and there was no refluxing time after addition of the methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate. Compound **7** was obtained from compound **2** (370 mg, 0.92 mmol) as a light-yellow solid (285 mg, 47%). Attempts to crystallize compound **7** were unsuccessful because the *tert*-butyldimethylsilyl group of compound **7** was easily removed in solvent methanol (MeOH) by heating gently and standing at room temperature for 24 hours. Hence compound **8** was obtained as pure crystals in this way. The IR, ¹H NMR, and melting point were identical with product **8** made by deprotection of **7** under weak acid conditions (HAc/H₂O/THF, 3 : 1 : 1).

**Methyl 3-acetoxy-16α-(*tert*-butyldimethylsilyloxy)-1,3,5-(10)-estratrien-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (10).** The coupling reaction was performed by a procedure similar to that used in the preparation of **5**. Compound **10** was obtained from compound **9** (45 mg, 0.10 mmol) as a white solid (53 mg, 69%). mp, 212–214 C; Analysis calculated, C₃₉H₅₆O₁₃Si: C, 61.55; H, 7.42; Found: C, 61.39; H, 7.37; ¹H NMR (CDCl₃) δ, 0.04 (s, 3H, −SiMe), 0.08 (s, 3H, −SiMe), 0.72 (s, 3H, 18-CH₃), 0.88 (s, 9H, −SiCMe₃), 2.02 (s, 3H, OCOCH₃), 2.03 (s, 3H, OCOCH₃), 2.06 (s, 3H, OCOCH₃), 2.28 (s, 3H, 3-OCOCH₃), 3.66 (d, J = 5.5 Hz, 17α-H), 3.73 (s, 3H, COOCH₃), 3.98 (d, J = 9.89 Hz, 1H, 5'-H), 4.10 (m, 1H, 16β-H), 4.64 (d, J = 7.7 Hz, 1'-H), 5.02 (t, 1H, 2'-H), 5.22–5.26 (m, 2H, 3' and 4'-H), 6.78–7.28 (m, 3H, aromatic). FABMS m/z, 761 (M)⁺.

## (c) Hydrolysis

**3,17β-Dihydroxy-1,3,5(10)-estratrien-16α-yl-β-D-glucopyranosiduronic acid (Estriol 16-glucuronide) (6).** A typical hydrolysis experimental procedure for the preparation of estriol 16-glucuronide was as follows. NaOH (1 ml; 0.5 M) was added to the solution of compound **5** (48 mg, 0.07 mmol) in MeOH (10 ml), and stirred for 24 hours at room temperature. When TLC showed no starting material **5**, the reaction mixture was titrated to pH = 8 and concentrated to approximately 3 ml by distillation under reduced pressure. On standing overnight a white solid (sodium salt) was obtained. The crystals were filtered and washed with a little MeOH, and dissolved in MeOH/H₂O (3 : 2). Titration to pH = 2.5 and standing overnight gave compound **6** as fine crystals (free acid) (27 mg, 65%). mp, 223–224 C; reported mp, 223–224 C (lit. 10); Analysis calculated: C₂₄H₃₂O₉ · 4H₂O: C, 53.72; H, 7.51; Found: C, 53.77; H, 6.82; FABMS m/z, 464 (M)⁺. ¹H NMR chemical shift values: see Table 1.

**3,16α-Dihydroxy-1,3,5(10)-estratrien-17β-yl-β-D-glucopyranosiduronic acid (Estriol 17-glucuronide) (12).** The hydrolysis reaction was performed by a procedure similar to that used in the preparation of **6**. Compound **12** was obtained from compound **8** and **11** as fine crystals in 65% yield respectively. mp, 245–247 C; reported mp, 241 C (lit. 8); Analysis calculated: C₂₄H₃₂O₉ · 3.5H₂O: C, 54.64; H, 7.45; Found: C, 54.19; H, 7.37; FABMS m/z, 465 (MH)⁺. ¹H NMR chemical shift values: see Table 1.

**Hydrolysis of the estriol glucuronides (6) and (12) with β-glucuronidase.** A solution of the estriol glucuronide **6** or **12** (15 mg) and β-glucuronidase (E.C.3.2.1.31 Sigma Co., 5 mg, 14000 Fishman Units) in 0.1-M acetate buffer (pH = 4.6, 30 ml) was incubated at 38 C for 24 hours. The incubated mixture was extracted with EtOAc (3 × 30 ml), washed with water, dried (MgSO₄), and evaporated to give a white solid. The solid was crystallized from aqueous MeOH (1 : 1) to give estriol (7 mg) and (6 mg) as colorless plates, mp, 279–284 C, from enzymatic digestion of **6** and **12** respectively. The products were identical with an authentic sample of estriol in every respect.

## Results and discussion

The procedure described here (see Scheme 1) represents a simple way to synthesize estriol 16- and 17-monoglucuronides in good overall yields (39% for estriol 16-glucuronide, 25% for estriol 17-glucuronide) and purity. The glucuronidation reactions with protected estriol derivatives give higher yields than from comparable 16α-hydroxy-17-oxo-derivatives, because there is no adjacent carbonyl group in the substrate; hence the free hydroxyl group is more nucleophilic in the coupling reactions.[7,11] Since the stereochemistry at carbon-16 and carbon-17 is known in the D-ring when estriol is used as starting material, the problem of obtaining the 17α-isomer during reduction of the 17-carbonyl group is avoided. Also, it should be noted that determination of the absolute stereochemistry at these positions in the 5-membered D-ring is difficult by spectroscopic techniques.[12]

The present method has advantages over previous procedures using estriol as a starting material. For example, the direct glucuronidation of estriol 3-benzoate or the 3-benzyl ether forms a mixture of 16- and 17-glucuronides, from which the 16-glucuronide can be

**Table 1** ¹H NMR chemical shift values for the Estriol 16- and 17-glucuronides

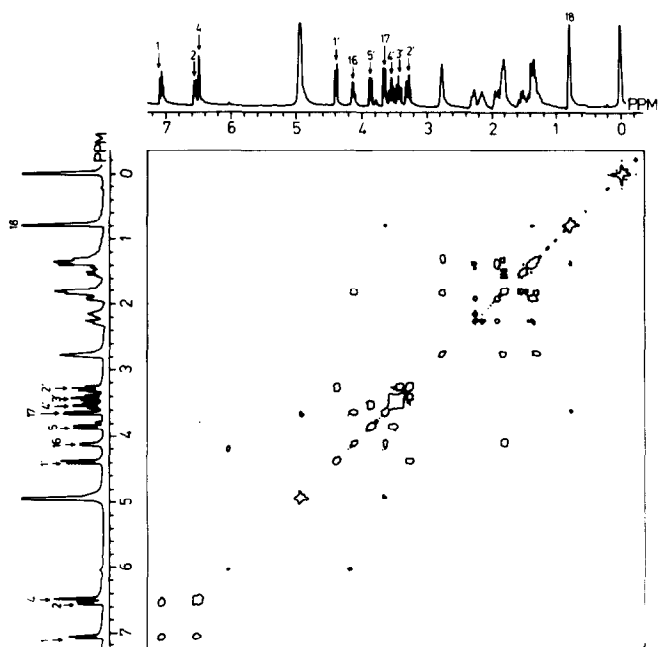| H | Estriol 16-glucuronide (6) ppm | Estriol 17-glucuronide (12) ppm | Difference in ppm |
|---|---|---|---|
| 18-CH₃ | 0.79 (s, 3H) | 0.87 (s, 3H) | +0.08 |
| 17α-H | 3.64 (d, J = 5.49 Hz) | 3.48 (d, J = 4.76 Hz) | −0.16 |
| 16β-H | 4.10 (m, 1H) | 4.18 (m, 1H) | +0.08 |
| 1,2,4-3H (aromatic) | 6.47–7.07 (m) | 6.46–7.06 (m) | — |
| 1'-H | 4.36 (d, J = 7.69 Hz) | 4.43 (d, J = 7.69 Hz) | +0.07 |
| 2'-H | 3.25–3.34 (m) | 3.25–3.34 (m) | — |
| 3'-H | 3.41 (t) | 3.41 (t) | 0 |
| 4'-H | 3.54 (t) | 3.54 (t) | 0 |
| 5'-H | 3.79 (d, J = 9.53 Hz) | 3.88 (d, J = 9.53 Hz) | +0.09 |

separated by fractional crystallization. However, this method produces a low overall yield of the 16-glucuronide together with some contamination by the 17-isomer.[8,13] Also, it is difficult to get high yields of 3-protected estriols.[2] Although estriol 3-benzoate undergoes selective monoglucuronidation in CHCl₃ activated by Ag₂CO₃, mainly affording the 17-glucuronide,[8] the low yield of the 3-protected estriol is still a problem. Scheme 1 provides a very simple alternative procedure, which gave the 16- or 17-glucuronide in comparatively high yields with preservation of the stereochemistry and location of the glucuronide linkage. By selectively protecting and deprotecting the hydroxyl groups of estriol in a controlled sequence, this method also provides an opportunity for further manipulation of estriol and opens the way for the synthesis of a wide range of estriol derivatives.

It is more convenient to use CdCO₃ as a promoter in the Koenigs-Knorr reactions than Ag₂CO₃, because all the compounds and the solvent can be easily and thoroughly dried by azeotropic distillation to remove moisture before starting the reaction. It is thus easier to control the reaction under scrupulously anhydrous conditions and the reaction shows greater selectivity. For example, the Koenigs-Knorr reaction with 2,3-protected 2-hydroxyestriol using CdCO₃ gave a mixture of the 16α- and 17β-coupling products in a ratio of 7:1.[14] However, there is no previous report about the preferential selection of the Koenigs-Knorr reaction for an alcoholic hydroxyl group in the presence of a phenolic hydroxyl group. In the present work, we synthesized estriol 17-glucuronide by direct glucuronidation of compound 2 (Scheme 1), in which both types of hydroxyl group were available. The result showed that the alcoholic hydroxyl group is much more reactive in this compound than the phenolic hydroxyl group despite the fact that there was a bulky *tert*-butyldimethylsilyl group at the 16α-OH position. Hence estriol 17-glucuronide can be prepared without the necessity to protect the 3-OH group.

An interesting observation made during our synthesis was the ease of deprotection of the *tert*-butyldimethylsilyl group in some of these compounds. The usual

reagent for the cleavage of a silyl ether is tetra-*n*-butyl-ammonium fluoride. However in the current work, removal of the *tert*-butyldimethylsilyl group using weak acid (HAc/H₂O/THF) gave a much higher yield than that by fluoride ion as described in the literature.[5] It is possible that tetra-*n*-butyl-ammonium fluoride functions as a strong base in THF and causes 1,2-migration of the acyl group.[15] We also noted that the *tert*-butyl-dimethylsilyl group in compound 7 could be removed simply by dissolution in MeOH and gentle heating.

Although estriol monoglucuronides have been reported as the human metabolites of estradiol, and synthesized by different methods, spectroscopic determination of the exact position and the configuration of the glycosidic linkage still awaits the availability of appropriate standards. In the present work the ¹H NMR and mass spectra unambiguously identified estriol 16- and 17-glucuronides. The conjugated position and configuration of the glucuronide linkage in the two isomers were established on the basis of ¹H NMR and 2D-COSY spectral data. The 2D-COSY spectra allowed the assignment of the 1'-H to 5'-H of the glucuronic acid moiety and unambiguously identified the 16β- and 17α-protons. Figures 1 and 2 clearly show the correlations of 1'-H/2'-H, 2'-H/3'-H, 3'-H/4'-H, 4'-H/5'-H and 16β-H/17α-H. The 17α- and 16β-protons signals in the 16α-glucuronide (compound 6, Scheme 1) appeared as a doublet at 3.64 ppm and as a multiplet at 4.10 ppm, respectively, while those in the 17β-glucuronide (Compound 12, Scheme 1) appeared as a doublet at 3.48 ppm and as a multiplet at 4.18 ppm. These chemical shift values are in good agreement with those reported for the 16α- and 17β-glucuronides of 2,3-protected 2-hydroxyestriol.[14] The transrelationship of the 1'-H and 2'-H in the glucuronic acid was demonstrated by the large coupling constants for both isomers (J₁,₂ = 7.69 Hz)[16] and subsequent hydrolysis of the glucuronides with β-glucuronidase confirmed the β-linkage of the glucuronic acid with estriol. There were only small differences in ¹H NMR chemical shift values between the two isomers (Table 1). The major differences were that the position of the 16β-H, 18-CH₃, 1'-H, and 5'-H signals were shifted downfield nearly 0.1

**Figure 1**  2D-COSY spectrum of estriol 16-glucuronide. The figure shows the proton correlations of 1'/2', 2'/3', 3'/4', 4'/5', 16β/17α; and in the sequence 1'-16β-5'-17α-4'-3'-2'.
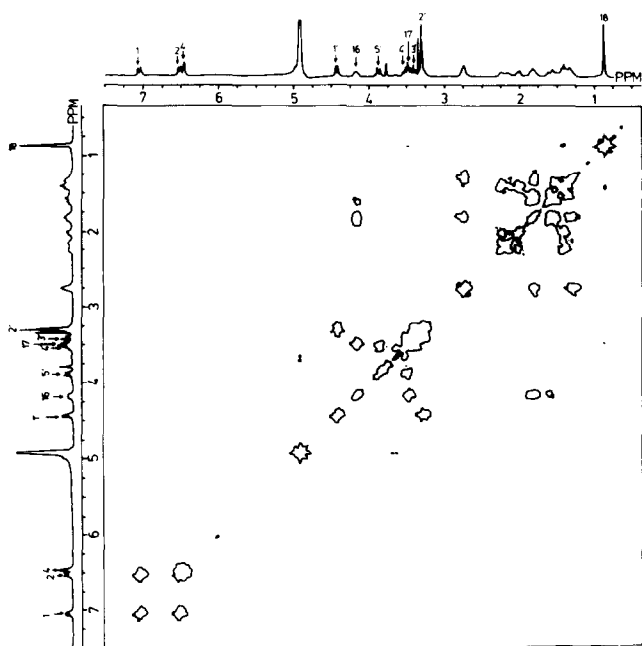


**Figure 2**  2D-COSY spectrum of estriol 17-glucuronide. The figure shows the proton correlations of 1'/2', 2'/3', 3'/4', 4'/5', 16β/17α; and in the sequence 1'-16β-5'-4'-17α-3'-2'.

ppm in the 17β-isomer compared with the 16α-isomer. However, the 17α-proton was shifted upfield by about 0.2 ppm as clearly shown in Figures 1 and 2. In the 16α-glucuronide, the 17α-proton occurred as a doublet

situated downfield of the 4'-H position (Figure 1), but in the 17β-glucuronide, the 17α-proton was upfield of the 4'-H position (Figure 2). This small difference is useful in distinguishing between the two isomers.

## Acknowledgments

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