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Selective synthesis of 4-methoxyestrogen from 4-hydroxyestrogen

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Abstract

The introduction of an oxygen atom into the C-6 position of 4-hydroxyestrogen allowed for the selective methylation of the two phenolic hydroxyl groups. When the 6-oxo derivative of 4-hydroxyestrone was benzylated in ethanol, only the 3-monobenzyl ether was obtained without formation of the 4-monobenzyl ether. Moreover, the 6-carbonyl group was further reduced to methylene almost quantitatively in the reaction of 4-acetoxy-6-oxoestrone 3-benzyl ether derivative with sodium borohydride. Therefore, 4-methoxyestrogen was synthesized by essentially combining these two reactions.

Keywords: Catechol estrogen; 4-Hydroxyestrone; 4-Hydroxy-6-oxoestrone; 4-Methoxyestrone; 4-Hydroxyestrone 3-methyl ether; Selective synthesis

1. Introduction

The catecholic 2- and 4-hydroxyestrogens are the major metabolites of primary estrogens. A portion of catechol estrogen is further metabolized to guaiacol estrogen such that one of two hydroxyl groups of the A ring is methylated. The physiological roles of these catechol and guaiacol estrogens have been explored, especially with respect to gonadotropin secretion, carcinogenesis, and competitive inhibition of the methylation of catechol amines by catechol-*O*-methyltransferase. It has been recently reported that guaiacol estrogen is a naturally occurring mammalian tubulin polymerization inhibitor [1,2] and an important source of primary estrogen and of catecholestrogen metabolites in hamster kidney [3].

It is of particular interest that the biotransformation of guaiacol estrogens from catechol estrogens involves the highly selective *O*-monomethylation at the C-2 or C-4 hydroxyl group. 2-Hydroxyestrogen has two indistinguishable phenolic hydroxyl groups with respect to chemical reactions [4]. Incubation of 2-hydroxyestrogen with human or rat liver homogenate in the presence of *S*-adenosyl-L-methionine and Mg^{2+} yields a mixture of the 2- and 3-monomethyl ethers in approximately equal amounts [5]. However, in vivo *O*-methylation of 2-hydroxyestrogen appears specific for the C-2 hydroxyl group and gives only the 2-methoxyestrogen in man [6]. In order to explain the discrepancy between the in vitro and in vivo results, Fishman et al. demonstrated that prior sulfate formation would possibly result in the selective *O*-methylation of 2-hydroxyestrogen [7]. On the other hand, Nakagawa et al. suggested that the C-2 hydroxyl group of 2-hydroxyestrogen is slightly more basic than the C-3 hydroxyl group and hence, the in vitro *O*-methylation at pH 6.0 gives the 2-methyl ether in three times larger amount than the 3-methyl ether [8]. Additionally, they showed that the in vitro *O*-methylation of 6-oxo-2-hydroxyestrogen, which has a carbonyl group at the benzylic position, produces eight times as much 3-methylated as 2-methylated compounds at pH 6.0. As to 4-hydroxyestrogen, the 4-hydroxyl group is much less active than the 3-hydroxyl group with respect to chemical reactivity [9]. In contrast, the incubation of 4-hydroxyestrogen with catechol-*O*-methyltransferase of human liver preferentially gives the 4-methyl ether [10]. Shimada et al. reported in vitro experiments in which sulfation had no direct effect on the selective *O*-methylation of 4-hydroxyestrogen [11]. Consequently, there is contradiction as to whether sulfate formation participates in the selective *O*-methylation of 2- and 4-hydroxyestrogens. Furthermore, in vivo *O*-methylation of 2-hydroxyestrogen occurs at the slightly more nucleophilic hydroxyl group, whereas that of 4-hydroxyestrogen occurs at the less nucleophilic hydroxyl group.

The syntheses of 4-methoxyestrogen [12,13] and 4-hydroxyestrogen 3-methyl ether [12] have already been reported. However, we are interested in the selective synthesis of guaiacol estrogen *via* catechol estrogen as this might

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contribute to the elucidation of the metabolizing mechanism in vivo. Previously, we tried to selectively obtain the 3-monobenzyl ether from 4-hydroxyestrone by reacting it with one equivalent of benzyl chloride in the presence of anhydrous potassium carbonate in ethanol. As a result, we observed the production of the 3-monobenzyl ether and the 4-monobenzyl ether in a ratio of approximately 3:2 [9]. Recently, we found that the 6-oxo derivative of 4-hydroxyestrogen undergoes monobenzylation at only the C-3 hydroxyl group under similar conditions, and the 4-acetoxy-6-oxoestrogen reacts with sodium borohydride to give the corresponding catechol estrogen quantitatively. The present paper describes new synthetic routes leading to 4-methoxyestrogen and 4-hydroxyestrogen 3-methyl ether from 4-hydroxyestrogen with high selectivity.

2. Experimental

All melting points were taken on a Yanaco micro hot-stage apparatus (Kyoto, Japan) and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter (Tokyo, Japan) in $CHCl_3$. Ultraviolet (UV) spectra were obtained on a Shimadzu UV-3000 (Kyoto, Japan) and infrared (IR) spectra were obtained on a Horiba FT-720 (Tokyo, Japan). ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz, respectively, using tetramethylsilane as an internal standard on a JEOL GSX-400 spectrometer (Tokyo, Japan). Low and high resolution mass spectral (MS, HRMS) measurements were made on a JEOL JMS-700 instrument. Elemental analyses were determined on a Yanaco CHN Corder MT-5. Column chromatography was performed on Merck silica gel 60 (Darmstadt, Germany). Estrone was purchased from Sigma (St. Louis, MO, USA). All other chemicals used were commercially available and were of analytical grade.

*2.1. 3,4-Dihydroxy-17,17-ethylenedioxy-1,3,5(10)-estratrien-6-one diacetate **(2)***

A solution of **1** (297 mg), ethylene glycol (0.060 ml), and *p*-toluenesulfonic acid (4.5 mg) in benzene (50 ml) was refluxed using a Dean-Stark water separator for 5 h. The reaction mixture was washed sequentially with saturated $NaHCO_3$ solution and water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **2** (222 mg, 67%) as colorless needles. mp 177.0–177.5°C. $[\alpha]_D^{20}$ −25.7° (c = 0.28). UV λ_{max} (EtOH) nm (ϵ): 247 (7050), 299 (1940). IR ν_{max} (KBr) cm^{-1}: 1772, 1688 (C = O), 1480 (aromatic). ^{1}H-NMR δ: 0.88 (3H, s, 18-CH_3), 2.22 (1H, dd, J = 17.2, 13.0 Hz, 7α-H), 2.30 (3H, s, 3-$OCOCH_3$), 2.36 (3H, s, 4-$OCOCH_3$), 2.69 (1H, dd, J = 17.2, 3.7 Hz, 7β-H), 3.86–3.98 (4H, m, -OCH_2CH_2O-), 7.33 (1H, d, J = 8.6 Hz, 2-H), 7.36 (1H, d, J = 8.6 Hz, 1-H). MS *m/z*: 428 (M^+), 386 ($[M-Ac+H]^+$), 344 ($[M-2Ac+2H]^+$), 300 ($[M-2Ac+2H-C_2H_4O]^+$). Analysis calculated for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 67.22; H, 6.35.

*2.2. 3,4-Dihydroxy-17,17-ethylenedioxy-1,3,5(10)-estratrien-6-one **(3)***

A solution of **2** (222 mg) in MeOH (30 ml) was treated with 5% $NaHCO_3$ (10 ml), and the mixture was left at room temperature for 6 h. After AcOH (0.5 ml) was added to neutralize excess alkali, MeOH was evaporated under reduced pressure. The reaction mixture was extracted with $CHCl_3$, washed with water, and dried over anhydrous Na_2SO_4. Removal of $CHCl_3$ gave an oily residue, which was purified by column chromatography on silica gel with hexane/AcOEt (9:1) to give **3** (162 mg, 91%) as colorless oil. $[\alpha]_D^{26}$ −39.8° (c = 0.40). UV λ_{max} (EtOH) nm (ϵ): 273 (7570), 358 (2580). IR ν_{max} (KBr) cm^{-1}: 3438 (OH), 1631 (C = O), 1444 (aromatic). ^{1}H-NMR δ: 0.89 (3H, s, 18-CH_3), 2.30 (1H, dd, J = 17.2, 12.8 Hz, 7α-H), 2.74 (1H, dd, J = 17.2, 3.3 Hz, 7β-H), 3.87–3.99 (4H, m, -OCH_2CH_2O-), 5.63 (1H, s, 3-OH), 6.77 (1H, dd, J = 8.4, 1.1 Hz, 1-H), 7.08 (1H, d, J = 8.4 Hz, 2-H), 12.78 (1H, s, 4-OH). MS *m/z*: 344 (M^+). HRMS calculated for $C_{20}H_{24}O_5$: 344.1624. Found: 344.1624.

*2.3. 3-Benzyloxy-4-hydroxy-17,17-ethylenedioxy-1,3,5(10)-estratrien-6-one **(4)***

Benzyl chloride (0.093 ml) and anhydrous K_2CO_3 were added to a solution of **3** (55.7 mg) in EtOH (60 ml), and the mixture was refluxed for 7 h. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. Crystallization from MeOH gave **4** (52.1 mg, 74%) as colorless needles. mp 135.0–135.9°C. $[\alpha]_D^{26}$ −43.0° (c = 0.40). UV λ_{max} (EtOH) nm (ϵ): 253 (11 400), 319 (3280). IR ν_{max} (KBr) cm^{-1}: 3438 (OH), 1637 (C = O), 1452 (aromatic). ^{1}H-NMR δ: 0.88 (3H, s, 18-CH_3), 2.31 (1H, dd, J = 17.1, 13.0 Hz, 7α-H), 2.74 (1H, dd, J = 17.1, 3.4 Hz, 7β-H), 3.86–3.99 (4H, m, -OCH_2CH_2O-), 5.16 (2H, s, 3-$OCH_2C_6H_5$), 6.70 (1H, dd, J = 8.6, 1.0 Hz, 1-H), 7.02 (1H, d, J = 8.6 Hz, 2-H), 7.27–7.46 (5H, m, 3-$OCH_2C_6H_5$), 13.00 (1H, s, 4-OH). MS *m/z*: 434 (M^+), 343 ($[M-C_6H_5CH_2]^+$), 299 ($[M-C_6H_5CH_2-C_2H_4O]^+$), 91 ($C_6H_5CH_2^+$). Analysis calculated for $C_{27}H_{30}O_5$: C, 74.63; H, 6.96. Found: C, 74.66; H, 6.84.

*2.4. 3-Benzyloxy-4-hydroxy-17,17-ethylenedioxy-1,3,5(10)-estratrien-6-one acetate **(5)***

Treatment of **4** (130 mg) with acetic anhydride (10 ml) and pyridine (10 ml) in the usual manner and recrystallization from MeOH gave **5** (135 mg, 95%) as colorless plates. mp 176.9–180.0°C. $[\alpha]_D^{26}$ −28.4° (c = 0.64). UV λ_{max} (EtOH) nm (ϵ): 270 (10 100), 354 (3980). IR ν_{max} (KBr) cm^{-1}: 1765, 1676 (C = O), 1484 (aromatic). ^{1}H-NMR δ: 0.87 (3H, s, 18-CH_3), 2.21 (1H, dd, J = 17.1, 13.2 Hz,

7α-H), 2.37 (3H, s, 4-OCOCH$_3$), 2.69 (1H, dd, J = 17.1, 3.5 Hz, 7β-H), 3.86–3.98 (4H, m, -OCH$_2$CH$_2$O-), 5.10 (2H, s, 3-OC*H*$_2$C$_6$H$_5$), 7.13 (1H, d, J = 8.8 Hz, 2-H), 7.21 (1H, d, J = 8.8Hz, 1-H), 7.28–7.38 (5H, m, 3-OCH$_2$C$_6$*H*$_5$). MS *m/z*: 476 (M^+), 434 ([M-Ac+H]$^+$), 343 ([M-Ac+H-C$_6$H$_5$CH$_2$]$^+$), 91 (C$_6$H$_5$CH$_2^+$). Analysis calculated for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 72.93; H, 6.69.

2.5. 3-Benzyloxy-17,17-ethylenedioxy-1,3,5 (10)-estratrien-4-ol **(6)**

Sodium borohydride (175 mg) was added to a solution of **5** (108 mg) in MeOH (50 ml), and the mixture was stirred at room temperature for 30 min. Excess reagent was destroyed by addition of a few drops of AcOH, and MeOH was removed under reduced pressure. The reaction mixture was extracted with $CHCl_3$, washed with water, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **6** (79.3 mg, 83%) as colorless rods. mp 137.4–138.5°C. $[\alpha]_D^{27}$ +17.7° (c = 0.44). UV λ_{max} (EtOH) nm (ε): 279 (2350). IR ν_{max} (KBr) cm^{-1}: 3458 (OH), 1493 (aromatic). ^{1}H-NMR δ: 0.88 (3H, s, 18-CH$_3$), 2.63 (1H, m, 6β-H), 2.93 (1H, dd, J = 17.6, 5.3 Hz, 6α-H), 3.86–3.98 (4H, m, -OCH$_2$CH$_2$O-), 5.08 (2H, s, 3-OC*H*$_2$C$_6$H$_5$), 5.71 (1H, s, 4-OH), 6.76 (1H, d, J = 8.6 Hz, 2-H), 6.79 (1H, d, J = 8.8 Hz, 1-H), 7.32–7.43 (5H, m, 3-OCH$_2$C$_6$*H*$_5$). ^{13}C-NMR δ: 143.2 (s, C-4), 143.1 (s, C-3), 136.8 (s, C1 of Ph), 134.6 (s, C-10), 128.7 (d, C3 and C5 of Ph), 128.2 (d, C4 of Ph), 127.7 (d, C2 and C6 of Ph), 123.6 (s, C-5), 119.5 (s, C-17), 116.0 (d, C-1), 109.3 (d, C-2), 71.2 (t, 3-OCH$_2$Ph), 65.2 and 64.6 (t, -OCH$_2$CH$_2$O-), 49.4 (d, C-14), 46.1 (s, C-13), 43.8 (d, C-9), 38.5 (d, C-8), 34.2 (t, C-16), 30.8 (t, C-12), 26.5 (t, C-7), 26.2 (t, C-11), 23.3 (t, C-6), 22.4 (t, C-15), 14.3 (q, C-18). MS *m/z*: 420 (M^+), 329 ([M-C$_6$H$_5$CH$_2$]$^+$), 91 (C$_6$H$_5$CH$_2^+$). Analysis calculated for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 77.15; H, 7.65.

2.6. 3-Benzyloxy-4-hydroxy-1,3,5(10)-estratrien-17-one **(7)**

5% HCl (3 ml) was added to a solution of **6** (95.2 mg) in MeOH (20 ml), and the mixture was stirred at room temperature for 1 h. Saturated aqueous $NaHCO_3$ was added to neutralize the HCl, and most of the MeOH was removed under reduced pressure. The mixture was extracted with $CHCl_3$, and the extract was washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **7** (78.7 mg, 92%) as colorless needles. mp 152.2–153.0°C. ^{1}H-NMR δ: 0.90 (3H, s, 18-CH$_3$), 2.67 (1H, m, 6β-H), 2.99 (1H, dd, J = 17.4, 5.3 Hz, 6α-H), 5.09 (2H, s, 3-OC*H*$_2$C$_6$H$_5$), 5.76 (1H, s, 4-OH), 6.78 (2H, s, 1-H and 2-H), 7.32–7.43 (5H, m, 3-OCH$_2$C$_6$*H*$_5$). MS *m/z*: 376 (M^+), 285 ([M-C$_6$H$_5$CH$_2$]$^+$), 91 (C$_6$H$_5$CH$_2^+$). The product was identical in all respects to that (mp 150.5–152.5°C) prepared by Shimada et al. [14].

2.7. 3-Benzyloxy-4-methoxy-1,3,5(10)-estratrien-17-one **(8)**

30% KOH solution (0.5 ml) was added slowly to a stirred solution of **7** (78.7 mg) in MeOH (10 ml) containing dimethyl sulfate (0.4 ml) at 0°C, and the mixture was heated at 40°C for 10 h. After most of the MeOH was evaporated under reduced pressure, the reaction mixture was extracted with $CHCl_3$, washed with water, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **8** (77.5 mg, 95%) as colorless needles. mp 116.5–117.0°C. (Reported mp 117–118°C [9]). ^{1}H-NMR δ: 0.90 (3H, s, 18-CH$_3$), 2.73 (1H, m, 6β-H), 3.05 (1H, dd, J = 17.8, 5.3 Hz, 6α-H), 3.86 (3H, s, 4-OCH$_3$), 5.10 (2H, s, 3-OC*H*$_2$C$_6$H$_5$), 6.80 (1H, d, J = 8.6 Hz, 2-H), 6.96 (1H, d, J = 8.6 Hz, 1-H), 7.28–7.47 (5H, m, 3-OCH$_2$C$_6$*H*$_5$). MS *m/z*: 390 (M^+), 299 ([M-C$_6$H$_5$CH$_2$]$^+$), 91 (C$_6$H$_5$CH$_2^+$).

2.8. 3-Hydroxy-4-methoxy-1,3,5(10)-estratrien-17-one **(9)**

A solution of **8** (52.0 mg) in EtOH (50 ml) was shaken with 5% palladium-on-carbon (20 mg) under a stream of hydrogen at room temperature for 7 h. The reaction mixture was filtered, and the filtrate was concentrated to an oily residue, which was purified by chromatography on silica gel using hexane/AcOEt (4:1). The eluate was recrystallized from MeOH to give **9** (35.8 mg, 90%) as colorless needles. mp 195.2–206.5°C. ^{1}H-NMR δ: 0.92 (3H, s, 18-CH$_3$), 2.76 (1H, m, 6β-H), 3.02 (1H, dd, J = 17.6, 5.4 Hz, 6α-H), 3.79 (3H, s, 4-OCH$_3$), 5.52 (1H, s, 3-OH), 6.80 (1H, d, J = 8.6 Hz, 2-H), 6.98 (1H, d, J = 8.6 Hz, 1-H). MS *m/z*: 300 (M^+), 269 ([M-OCH$_3$]$^+$).

2.9. 3,4-Dihydroxy-1,3,5(10)-estratriene-6,17-dione **(10)**

Solid $NaHCO_3$ (48 mg) and water (3 ml) were added to a solution of **1** (136 mg) in MeOH (100 ml), and the mixture was stirred at room temperature for 8 h. The reaction mixture was acidified by adding a few drops of AcOH (0.1 ml), and most of the MeOH was removed under reduced pressure. The mixture was extracted with $CHCl_3$, washed with water, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **10** (104 mg, 98%) as colorless needles. mp 193.4–194.5°C. $[\alpha]_D^{24}$ +70.0° (c = 0.53). UV λ_{max} (EtOH) nm (ε): 272 (9300), 361 (3120). IR ν_{max} (KBr) cm^{-1}: 3435 (OH), 1728, 1631 (C = O), 1439 (aromatic). ^{1}H-NMR δ: 0.92 (3H, s, 18-CH$_3$), 2.39 (1H, dd, J = 17.2, 12.8 Hz, 7α-H), 2.85 (1H, dd, J = 17.2, 3.7 Hz, 7β-H), 5.74 (1H, s, 3-OH), 6.77 (1H, dd, J = 8.4, 1.3 Hz, 1-H), 7.10 (1H, d, J = 8.4 Hz, 2-H), 12.75 (1H, s, 4-OH). MS *m/z*: 300 (M^+). HRMS Calculated for $C_{18}H_{20}O_4$: 300.1362. Found: 300.1362. Analysis calculated for $C_{18}H_{20}O_4 \cdot 1/5H_2O$: C, 71.13; H, 6.77. Found: C, 71.23; H, 6.86.

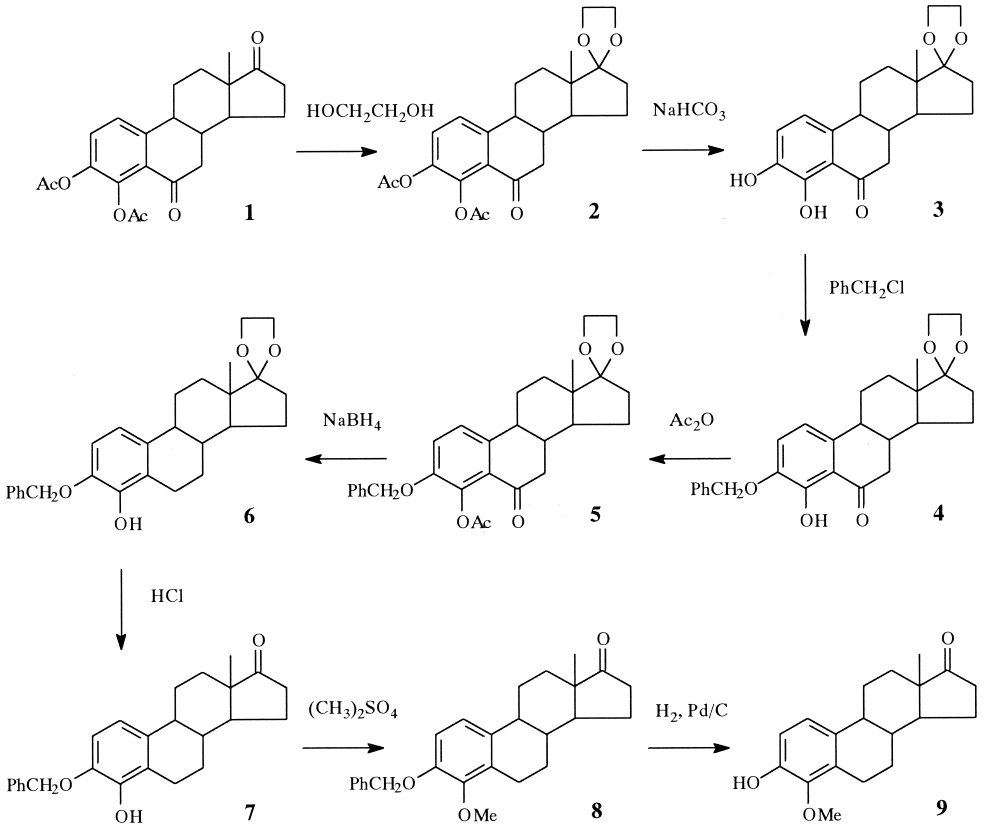

Fig. 1. Scheme for synthesis of 4-methoxyestrone.

*2.10. 3-Methoxy-4-hydroxy-1,3,5(10)-estratriene-6,17-dione **(11)***

An excess amount of ethereal diazomethane solution was added to a solution of **10** (94.2 mg) in MeOH (30 ml), and the mixture was left at room temperature for 13 h. Excess reagent was destroyed by addition of a few drops of AcOH, and MeOH was removed under reduced pressure. The crude product obtained was chromatographed on silica gel using hexane/AcOEt (4:1) to give the 3,4-dimethyl ether (16.7 mg, 16%) and **11** (64.6 mg, 65%) as colorless rods. mp 204.3–205.0°C. $[\alpha]_D^{23}$ +73.8° (c = 0.52). UV λ_{max} (EtOH) nm (ϵ): 270 (7530), 355 (2870). IR ν_{max} (KBr) cm^{-1}: 3442 (OH), 1732, 1626 (C = O), 1442 (aromatic). ^{1}H-NMR δ: 0.93 (3H, s, 18-CH_3), 2.40 (1H, dd, J = 17.1, 13.0 Hz, 7α-H), 2.86 (1H, dd, J = 17.1, 3.4 Hz, 7β-H), 3.90 (3H, s, 3-OCH_3), 6.79 (1H, dd, J = 8.6, 1.0 Hz, 1-H), 7.04 (1H, d, J = 8.5 Hz, 2-H), 12.95 (1H, s, 4-OH). MS *m/z*: 314 (M^+). Analysis calculated for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found: C, 72.56; H, 7.02.

*2.11. 3-Methoxy-4-hydroxy-17,17-ethylenedioxy-1,3,5(10)-estratrien-6-one **(12)***

A solution of **11** (25.9 mg), ethylene glycol (0.020 ml), and *p*-toluenesulfonic acid (1.3 mg) in benzene (40 ml) was refluxed using a Dean-Stark water separator for 7.5 h. The reaction mixture was washed successively with saturated $NaHCO_3$ solution and water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **12** (22.6 mg, 77%) as colorless rods. mp 173.5–174.3°C. $[\alpha]_D^{25}$ −41.3° (c = 0.45). UV λ_{max} (EtOH) nm (ϵ): 269 (8900), 356 (3420). IR ν_{max} (KBr) cm^{-1}: 3438 (OH), 1631 (C = O), 1456 (aromatic). ^{1}H-NMR δ: 0.89 (3H, s, 18-CH_3), 2.31 (1H, dd, J = 17.5, 13.1 Hz, 7α-H), 2.74 (1H, dd, J = 17.5, 3.7 Hz, 7β-H), 3.88 (3H, s, 3-OCH_3), 3.87–3.99 (4H, m, -OCH_2CH_2O-), 6.78 (1H, dd, J = 8.4, 1.0 Hz, 1-H), 7.02 (1H, d, J = 8.4 Hz, 2-H), 12.98 (1H, s, 4-OH). MS *m/z*: 358 (M^+). Analysis calculated for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.41; H, 7.26.

*2.12. 3-Methoxy-4-hydroxy-17,17-ethylenedioxy-1,3,5(10)-estratrien-6-one acetate **(13)***

Treatment of **12** (62.0 mg) with acetic anhydride (10 ml) and pyridine (10 ml) in the usual manner and recrystallization from MeOH gave **13** (67.7 mg, 98%) as colorless plates. mp 170.9–172.1°C. $[\alpha]_D^{23}$ −19.2° (c = 0.66). UV λ_{max} (EtOH) nm (ϵ): 252 (6870), 320 (2720). IR ν_{max} (KBr) cm^{-1}: 1763, 1684 (C = O), 1485 (aromatic). ^{1}H-NMR δ: 0.88 (3H, s, 18-CH_3), 2.21 (1H, dd, J = 17.1, 13.0 Hz, 7α-H), 2.39 (3H, s, 4-$OCOCH_3$), 2.68 (1H, dd, J = 17.1, 3.7 Hz, 7β-H), 3.85 (3H, s, 3-OCH_3), 3.86–3.98 (4H, m,

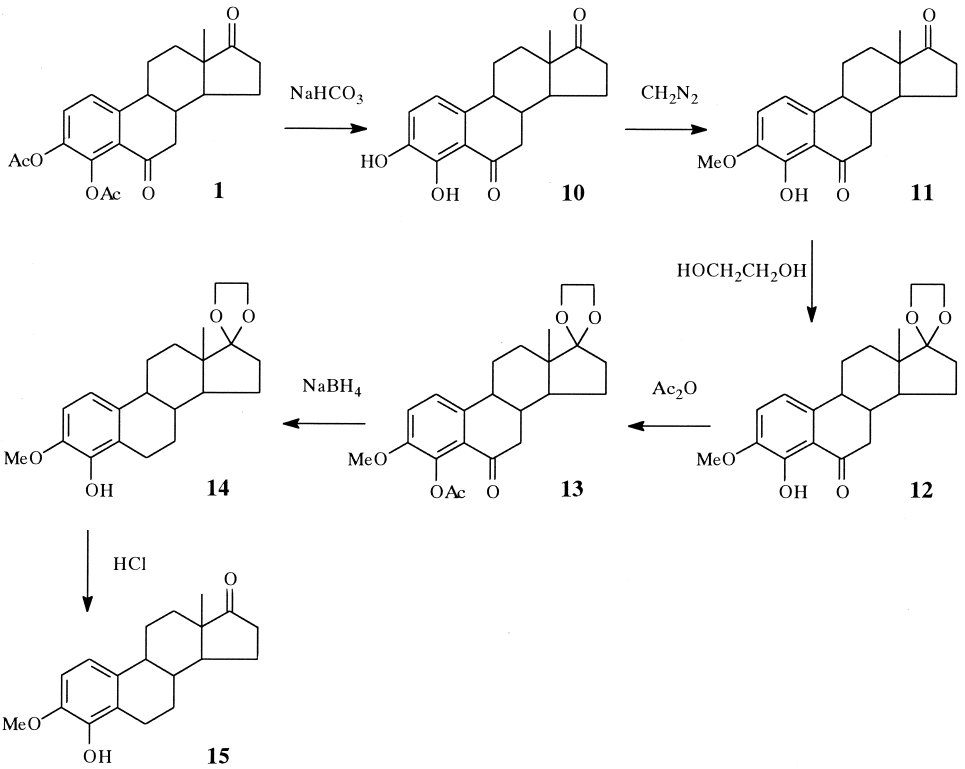

Fig. 2. Scheme for the synthesis of 4-hydroxyestrone 3-methyl ether.

-OCH_2CH_2O-), 7.14 (1H, d, J = 8.6 Hz, 2-H), 7.28 (1H, dd, J = 8.6, 2.0 Hz, 1-H). MS *m/z*: 400 (M^+), 358 ([M-Ac+H]$^+$). Analysis calculated for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 68.95; H, 7.01.

2.13. 3-Methoxy-17,17-ethylenedioxy-1,3,5 (10)-estratrien-4-ol ***(14)***

Sodium borohydride (110 mg) was added to a solution of **13** (59.4 mg) in MeOH (50 ml), and the mixture was stirred at room temperature for 50 min. Excess reagent was destroyed by addition of a few drops of AcOH, and MeOH was removed under reduced pressure. The reaction mixture was extracted with $CHCl_3$, washed with water, and dried, over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **14** (40.4 mg, 79%) as colorless plates. mp 157.3–157.8°C. $[\alpha]_D^{23}$ +33.6° (c = 0.59). UV λ_{max} (EtOH) nm (ϵ): 280 (1990). IR ν_{max} (KBr) cm^{-1}: 3431 (OH), 1493 (aromatic). ^{1}H-NMR δ: 0.88 (3H, s, 18-CH_3), 2.62 (1H, m, 6β-H), 2.93 (1H, dd, J = 17.2, 5.3 Hz, 6α-H), 3.86 (3H, s, 3-OCH_3), 3.87–3.98 (4H, m, -OCH_2CH_2O-), 5.65 (1H, s, 4-OH), 6.71 (1H, d, J = 8.6 Hz, 2-H), 6.81 (1H, d, J = 8.6Hz, 1-H). ^{13}C-NMR δ: 144.0 (s, C-3), 142.8 (s, C-4), 134.2 (s, C-10), 123.4 (s, C-5), 119.5 (s, C-17), 116.0 (d, C-1), 107.9 (d, C-2), 65.3 and 64.6 (t, -OCH_2CH_2O-), 56.0 (q, 3-OCH_3), 49.4 (d, C-14), 46.1 (s, C-13), 43.8 (d, C-9), 38.5 (d, C-8), 34.3 (t, C-16), 30.8 (t, C-12), 26.5 (t, C-7), 26.2 (t, C-11), 23.3 (t, C-6), 22.4 (t, C-15), 14.3 (q, C-18). MS *m/z*: 344 (M^+). Analysis calculated for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.29; H, 8.14.

2.14. 3-Methoxy-4-hydroxy-1,3,5 (10)-estratrien-17-one ***(15)***

5% HCl (1.6 ml) was added to a solution of **14** (47.5 mg) in MeOH (20 ml), and the mixture was stirred at room temperature for 1.5 h. Saturated aqueous $NaHCO_3$ was added to neutralize the HCl, and most of the MeOH was removed under reduced pressure. The mixture was extracted with $CHCl_3$, and the extract was washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product obtained was recrystallized from MeOH to give **15** (33.4 mg, 81%) as colorless leaflets. mp 207.5–208.1°C. ^{1}H-NMR δ: 0.90 (3H, s, 18-CH_3), 2.67 (1H, m, 6β-H), 2.98 (1H, dd, J = 17.6, 5.5 Hz, 6α-H), 3.86 (3H, s, 3-OCH_3), 5.73 (1H, s, 4-OH), 6.72 (1H, d, J = 8.6 Hz, 2-H), 6.80 (1H, dd, J = 8.6, 1.9 Hz, 1-H). MS *m/z*: 300 (M^+).

3. Results and discussion

4-Hydroxyestrone was prepared from estrone according to Stubenrauch and Knuppen [15] and transformed into the diacetate with acetic anhydride and pyridine in the usual manner. Oxidation of the diacetate with a pyridinium chlorochromate - celite mixture in benzene [16] provided the

6-oxo derivative (**1**). The C-17 oxo group of **1** was selectively ketalized by reaction with one equivalent of ethylene glycol in the presence of *p*-toluenesulfonic acid in benzene [17] to produce the 17-monoketal (**2**) (Fig. 1). Simultaneous removal of the acetyl groups at C-3 and C-4 by treatment with sodium bicarbonate in methanol furnished the desired 6-oxo-4-hydroxyestrone 17-monoketal (**3**) in excellent yield. When this compound (**3**) was refluxed with an excess amount of benzyl chloride in the presence of anhydrous potassium carbonate in ethanol, only the 3-monobenzyl ether (**4**) was obtained without formation of the 4-monobenzyl ether or the 3,4-dibenzyl ether. Generally, benzylation of a catechol compound gives the two isomeric monobenzyl ethers and the dibenzyl ether. However, in the present case, no benzylation of the C-4 hydroxyl group of the 4-hydroxylated estrogen (**3**), which had a keto group at the C-6 position, occurred under the conditions used. This selective benzylation could result from a protective effect of the hydrogen bond between the 4-hydroxyl group and 6-oxo group in compound **3** rather than from steric hindrance by the bulky benzyl cation, since the signal of the 4-hydroxyl proton was observed at 12.79 ppm in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3,** and compound **10** was also very preferentially monomethylated at the C-3 hydroxyl group by an excess amount of diazomethane, as described later. Compound **4** was easily acetylated with acetic anhydride and pyridine to give the acetate (**5**). Reduction of 6-oxoestrogens with sodium borohydride usually gives a mixture of the 6α- and 6β-hydroxylated derivatives [18]. While the reduction of 6-oxo-4-hydroxyestrone 3-monobenzyl ether 17-monoketal (**4**) with sodium borohydride gave a mixture of 6-hydroxylated derivatives, that of the acetate (**5**) produced 4-hydroxyestrone 3-monobenzyl ether 17-ketal (**6**) in 83% yield. The structure of **6** was confirmed by assignment of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, which showed the C-6 carbon as a triplet at 23.3 ppm, supporting a methylene moiety at C-6. Another example of a carbonyl group reduction to methylene has been reported with respect to phenolic ketones treated with sodium borohydride [19]. In the present studies, reduction of the carbonyl group to methylene was not observed in compound **4,** but in compound **5,** which had an acetylated 4-hydroxyl group. The protective 17-ketal function was removed by reaction with hydrochloric acid to give 4-hydroxyestrone 3-monobenzyl ether (**7**). Subsequent methylation of **7** with dimethyl sulfate yielded 4-methoxyestrone 3-benzyl ether (**8**). Removal of the benzyl group at C-3 was achieved by catalytic hydrogenation over palladium-on-charcoal yielding the desired 4-methoxyestrone (**9**).

On the other hand, preparation of the isomeric 3-methyl ethers was undertaken by methylation of 6-oxo-4-hydroxyestrone (Fig. 2). Hydrolysis of **1** with sodium bicarbonate formed 6-oxo-4-hydroxyestrone (**10**). In the ^{1}H-NMR spectrum of **10,** the 4-hydroxyl proton signal appeared at 12.75 ppm, supporting hydrogen bonding between the 6-oxo and 4-hydroxyl groups. Methylation of **10** with an excess amount of diazomethane gave the 3-monomethyl ether (**11**) in 65% yield and the 3,4-dimethyl ether in 16% yield without formation of the isomeric 4-monomethyl ether. Selective ketalization of the C-17 oxo group of **11** with one equivalent of ethylene glycol provided the 17-monoketal (**12**). Acetylation with acetic anhydride and pyridine in the usual manner afforded the acetate (**13**). When treated with sodium borohydride, acetate **13** was transformed into 4-hydroxyestrone 3-methyl ether 17-ketal (**14**) in 79% yield. The structure of **14** was confirmed by assignment of the ^{13}C-NMR spectrum, which showed the C-6 methylene carbon as a triplet at 23.3 ppm. Subsequent treatment of **14** with hydrochloric acid gave 4-hydroxyestrone 3-methyl ether (**15**).

In this paper, we discussed the possibility for the conversion of 4-hydroxyestrogen into 4-methoxyestrogen from the viewpoint of synthetic chemistry. The introduction of an oxo function into 4-hydroxyestrogen at the C-6 position produced a large difference in the reactivity of the 3- and 4-hydroxyl groups by hydrogen bonding of the 4-hydroxyl hydrogen and 6-carbonyl oxygen. Therefore, preparation of the 3-monomethyl ether or the 3-monobenzyl ether was possible at this stage. After acetylation of the 4-hydroxyl group, treatment with sodium borohydride produced reductive elimination of the 6-oxo function to yield the 4-hydroxyestrogen 3-monosubstituted ether. As a result, the monomethyl ether of 4-hydroxyestrogen could be selectively synthesized from 4-hydroxyestrogen by applying these reactions.

The predominant formation of 2 (or 4)-methoxyestrogen from estrogen in vivo might be due to sulfate conjugation of estrogen before hydroxylation at C-2 (or C-4) followed by *O*-methylation [20]. If sulfate conjugation is not involved in *O*-methylation of catechols, it seems that selective *O*-methylation of catechol estrogen originates essentially in the chemical difference between the two phenolic hydroxyl groups. A slight difference in basicity of the two hydroxyl groups of 2-hydroxyestrogen might contribute greatly to selective *O*-methylation [21]. However, when a functional group, such as oxygen, is temporarily introduced into the C-6 position of 4-hydroxyestrogen, a large difference in reactivity evolves in its two phenolic hydroxyl groups. Within this 6-oxo derivative, the C-3 phenolic hydroxyl group is more nucleophilic than the C-4 hydroxyl group. Therefore, it seems that this compound inevitably undergoes *O*-methylation of the C-4 hydroxyl group after conjugative protection of the C-3 hydroxyl group. Further studies on the selective methylation of 2-hydroxyestrogen are in progress, and the details will be reported in the near future.

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