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A new, practical synthesis of 2-methoxyestradiols☆

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Abstract

An efficient and practical approach to synthesize moderate to large amounts of 2-methoxyestradiol (2-ME2) is described. The key step in the synthesis is the regioselective introduction of an acetyl group at the C-2 position of estradiol using a zirconium tetrachloride mediated Fries rearrangement carried out on estradiol diacetate. The seven step synthetic procedure readily gave 2-ME2 in 49% overall yield. Application of this method to the synthesis of 2-methoxy-7α-methylestradiol is also described.

Keywords: Steroids; 2-Methoxyestradiol; Zirconium tetrachloride; Fries rearrangement; Antiangiogenesis

1. Introduction

The sequential biochemical hydroxylation and methylation of the natural hormone estradiol (E2) gives rise to the endogenous mammalian metabolite 2-methoxyestradiol (2-ME2) [1]. 2-ME2 is a natural metabolite of estrogen devoid of uterotropic or estrogenic activity in vivo. Recent studies [2] have shown that 2-ME2 inhibits the cellular machinery involved in replicating cancer cells, specifically microtubules. In addition, 2-ME2 has been demonstrated to act as an antiangiogenic agent that prevents the growth of new blood vessels required to nourish tumors [3]. Initiation of either of these events will cause tumors to shrink but the combination of effects may provide significant advantages over current anticancer therapies. These results have increased the demand for 2-ME2 for preclinical and clinical testing as anticancer agents in humans. Prior syntheses of 2-ME2 [4–11] suffer from low overall yields or require chromatographic purification of intermediates and as such, are not applicable to moderate to large-scale synthesis. The present communication describes a new, practical preparation of 2-ME2 from estradiol in 49% overall yield and its successful application to the synthesis of 2-methoxy-7α-methylestradiol.

2. Experimental

Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a General Electric GE-300 (300 MHz) spectrometer as deuterochloroform ($CDCl_3$) solutions using tetramethylsilane (TMS) as an internal standard ($\delta = 0$) unless noted otherwise. Infared spectra were recorded on a Perkin-Elmer model 1600 FT-IR instrument equipped with a diffuse reflectance accessory using a KBr matrix. Combustion analyses were performed by Midwest Microlabs Ltd. (Indianapolis, IN). Thin-layer chromatography (TLC) analyses were carried out on silica gel GF (Analtech) glass plates (2.5 cm × 10 cm with 250 μM layer and prescored). HPLC analyses were performed using a system composed of the following components: Waters Associates model 6000A pump and model 481 UV–VIS detector, Hewlett-Packard model 3396A integrator and Rheodyne model 7125 injector.

Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Company (Milwaukee, WI). Estradiol was purchased from Schering AG (Berlin, Germany). 7α-Methylestrone was obtained from Ciba-Geigy, now known as Novartis International AG.

2.1. Estra-1,3,5(10)-trien-3,17β-diol diacetate (**2a**)

Under nitrogen, acetic anhydride (50 ml, 264.5 mmol) was added to a solution of estradiol (**1a**, 20.0 g, 73.43 mmol) in

☆ The method presented here is the subject of a pending US patent.

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dry pyridine (200 ml). The reaction mixture was stirred at room temperature overnight (16 h). The next morning, analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The mixture was cooled in an ice bath and the excess acetic anhydride quenched by addition of methanol (25 ml). The mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature. The solvents were removed in vacuo and the solid residue crystallized from hot methanol to give the pure diacetate (**2a**, 24.87 g, 95%): mp = 127–128 °C (lit. [12] 125–126 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 2924, 2874, 1765, and 1734 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.823 (s, 18-CH_3), 2.056 (s, 17-OAc), 2.277 (s, 3-OAc), 4.688 (dd, J_1 = 9.3 Hz, J_2 = 7.5 Hz, 17-H), 6.786 (d, J = 2.7 Hz, 4-H), 6.834 (dd, J_1 = 8.55 Hz, J_2 = 2.7 Hz, 2-H), 7.275 (d, J = 8.55 Hz, 1-H). Analysis calculated for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 73.98; H, 7.99.

2.2. 7α-Methylestra-1,3,5(10)-triene-3,17β diacetate (2b)

Following the procedure outlined for the synthesis of **2a**, 7α-methylestradiol (**1b**, 10.0 g, 34.91 mmol) was reacted with acetic anhydride (25 ml, 264.5 mmol) in dry pyridine (100 ml) to give the pure diacetate (**2b**, 11.57 g, 89.4%): mp = 143–144 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2973, 2917, 2883, 1766, and 1734 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.829 (s, 18-CH_3), 0.841 (d, J = 7.2 Hz, 7-CH_3), 2.060 (s, 17-OAc), 2.276 (s, 3-OAc), 4.699 (dd, J_1 = 8.6 Hz, J_2 = 7.6 Hz, 17-H), 6.779 (d, J = 2.4 Hz, 4-H), 6.842 (dd, J_1 = 8.33 Hz, J_2 = 2.4 Hz, 2-H), 7.287 (d, J = 8.33 Hz, 1-H). Analysis calculated for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.40; H, 8.13.

2.3. 2-Acetylestra-1,3,5(10)-triene-3,17β-diol 17-acetate (3a)

Under nitrogen, solid zirconium tetrachloride (60 g, 257.5 mmol) was added to a solution of the diacetate (**2a**, 20.0 g, 56.11 mmol) in dry dichloromethane (1.5 l). The suspension was stirred at room temperature for 48 h. At the end of that time, analysis by TLC (1% acetone in CH_2Cl_2) indicated a complete reaction. The brown-yellow suspension was cooled in an ice bath and quenched by the slow addition of water (250 ml) with stirring. The yellow mixture was stirred at 0 °C for 1 h, diluted further with water (500 ml) and extracted with methylene chloride (3×). The organic extracts were washed with water (2×), filtered through anhydrous sodium sulfate, combined, and concentrated in vacuo to give 19.8 g crude product as a yellow solid. Crystallization of this material from hot methanol gave the pure product (**3a**, 16.8 g, 84%) as a light yellow solid: mp = 198–200 °C (lit. [5] 202–204 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 3231, 2924, 1727, 1642, and 1619 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.844 (s, 18-CH_3), 2.069 (s, 17-OAc), 2.604 (s, 2-Ac), 4.703 (dd, J_1 = 9.3 Hz, J_2 = 7.5 Hz, 17-H), 6.693 (s, 4-H), 7.597 (s, 1-H), 12.041 (s, 3-OH). Analysis calculated for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.02; H, 7.92.

2.4. 2-Acetyl-7α-methylestra-1,3,5(10)-triene-3,17β-diol 17-acetate (3b)

Following the procedure outlined for the synthesis of **3a**, zirconium tetrachloride (33 g, 141.6 mmol) was added to a solution of the diacetate (**2b**, 11.0 g, 29.7 mmol) in dry dichloromethane (1 l). The suspension was stirred at room temperature for 48 h. At the end of that time, NMR analysis of a small aliquot taken to dryness indicated only ∼33% reaction. Additional $ZrCl_4$ (33 g, 141.6 mmol) was added and the reaction was stirred at room temperature for an additional 3 days. At the end of that time, NMR analysis indicated a complete reaction. The reaction mixture was cooled in an ice bath, diluted with water (∼200 ml) and stirred at 0 °C for 1 h. The mixture was then extracted with dichloromethane (3×). The organic fractions were washed with water (2×), filtered through Na_2SO_4, combined and concentrated in vacuo to give 11.3 g crude product as a yellow foam. Crystallization of this material from hot methanol gave 7.0 g of a light yellow solid. The mother liquors were concentrated and purified via Flash column chromatography (methylene chloride) to give an additional 0.6 g product. Total yield (**3b**, 7.6 g, 69%): mp = 145–147 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3230, 2948, 1726, 1640, and 1619 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.840 (d, J = 7.2 Hz, 7-CH_3), 0.852 (s, 18-CH_3), 2.070 (s, 17-OAc), 2.604 (s, 2-Ac), 4.712 (dd, J_1 = 9.45 Hz, J_2 = 7.65 Hz, 17-H), 6.684 (s, 4-H), 7.628 (s, 1-H), 12.003 (s, 3-OH). Analysis calculated for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.45; H, 8.10.

2.5. 2-Acetyl-3-benzyloxyestra-1,3,5(10)-trien-17β-ol 17-acetate (4a)

Under nitrogen, benzyl bromide (16 ml, 134.5 mmol) was added to a mixture of the 2-acetyl compound (**3**, 16.0 g, 43.19 mmol) and anhydrous potassium carbonate (25 g, 180.9 mmol) in dry dimethylformamide (500 ml). The mixture was heated to 60 °C over the weekend (62 h). At the end of that time, analysis by TLC (CH_2Cl_2) indicated about a 75% completion of reaction. Additional benzyl bromide (16 ml, 134.5 mmol) and potassium carbonate (25 g, 180.9 mmol) were added and the reaction heated at 60 °C for a further 4 h. At the end of that time, analysis by TLC indicated a complete reaction. The reaction mixture was cooled to room temperature, poured into ice water (∼1.5 l) and stirred until the ice melted. The resulting precipitate was collected by filtration and washed well with water until the filtrate was neutral. The light yellow solid crude product was crystallized from methylene chloride/methanol to give the pure product (**4a**, 17.62 g, 91.4%) as a white solid: mp = 172–174 °C (lit. [5] 170–172 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 2936, 2920, 1730, 1663, and 1604 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.823 (s,

18-CH_3), 2.059 (s, 17-OAc), 2.576 (s, 2-Ac), 4.680 (dd, $J_1 = 8.85$ Hz, $J_2 = 7.35$ Hz, 17-H), 5.122 (s, benzyl CH_2), 6.735 (s, 4-H), 7.343–7.456 (m, benzyl aromatic), 7.708 (s, 1-H). Analysis calculated for $C_{29}H_{34}O_4$: C, 78.00; H, 7.67. Found: C, 77.80; H, 7.68.

2.6. 2-Acetyl-3-benzyloxy-7α-methylestra-1,3,5(10)-trien-17β-ol 17-acetate (**4b**)

Following the procedure outlined for the synthesis of **4a**, benzyl bromide (7 ml, 58.85 mmol) was added to a mixture of the 2-acetyl compound (**3b**, 7.0 g, 18.89 mmol) and anhydrous potassium carbonate (11 g, 79.6 mmol) in dry dimethylformamide (250 ml). The reaction mixture was then heated to 60 °C overnight. Analysis by TLC (CH_2Cl_2) indicated an incomplete reaction. Additional benzyl bromide (10 ml, 84.1 mmol) and potassium carbonate (11 g, 79.6 mmol) were added and the reaction continued for another 24 h. Analysis by TLC at that time indicated a complete reaction. The reaction mixture was cooled to room temperature, filtered, diluted with water (∼1 l) and extracted with dichloromethane (3×). The organic fractions were washed with water (2×) saturated sodium bicarbonate solution (1×), filtered through sodium sulfate, combined and concentrated in vacuo to give 15 g of a yellow oily solid. Crystallization of this material from hot methanol gave the pure benzyl ether (**4b**, 6.7 g, 77%): mp = 179–181 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2927, 1734, 1663, and 1603 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.832 (s, 18-CH_3), 0.842 (d, $J = 5.7$ Hz, 7-CH_3), 2.060 (s, 17-OAc), 2.572 (s, 2-Ac), 4.694 (dd, $J_1 = 9.16$ Hz, $J_2 = 7.95$ Hz, 17-H), 5.117 (s, benzyl CH_2), 6.731 (s, 4-H), 7.335–7.459 (m, benzyl aromatic), 7.723 (s, 1-H). Analysis calculated for $C_{30}H_{36}O_4$: C, 78.23; H, 7.88. Found: C, 78.09; H, 7.90.

2.7. 3-Benzyloxyestra-1,3,5(10)-triene-2,17β-diol diacetate (**5a**)

Under nitrogen, *meta*-chloroperbenzoic acid (77%, 17.0 g, 75 mmol) was added to a mixture of the 2-acetyl benzyl ether (**4a**, 17.4 g, 39 mmol) and disodium phosphate (14 g, 98 mmol) in methylene chloride (800 ml). The reaction mixture was stirred overnight at room temperature. After that time, analysis by TLC (2% acetone in CH_2Cl_2) indicated a complete reaction. The mixture was diluted with water (1 l) and extracted with methylene chloride (3×). The organic fractions were washed with water (1×), 10% Na_2SO_3 solution (1×) and half saturated sodium bicarbonate solution (1×), filtered through Na_2SO_4, combined and concentrated in vacuo. The residue was crystallized from methanol to give the pure 2-acetoxy derivative (**5a**, 14.85 g, 82.4%) as a white crystalline solid: mp = 151–153 °C (lit. [5] 157–159 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 929, 1758, 1733, and 1615 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.818 (s, 18-CH_3), 2.054 (s, 17-OAc), 2.259 (s, 2-OAc), 4.682 (dd, $J_1 = 8.85$ Hz, $J_2 = 7.65$ Hz, 17-H), 5.039 (s, benzyl CH_2), 6.713 (s, 4-H), 6.957 (s, 1-H), 7.301–7.381 (m, benzyl aromatic). Analysis calculated for $C_{29}H_{34}O_5$: C, 75.30; H, 7.41. Found: C, 75.54; H, 7.49.

2.8. 3-Benzyloxy-7α-methylestra-1,3,5(10)-triene-2,17β-ol diacetate (**5b**)

Following the procedure outlined for the synthesis of **5a**, *meta*-chloroperbenzoic acid (77%, 6.5 g, 29 mmol) was added to a mixture of the 2-acetyl compound (**4b**, 6.5 g, 14.1 mmol) and disodium phosphate (4.2 g, 29.6 mmol) in dry dichloromethane (300 ml). The mixture was stirred overnight at room temperature, after which time, analysis by TLC (2% acetone in CH_2Cl_2) indicated a complete reaction. The reaction mixture was transferred to a separatory funnel and washed with 10% sodium sulfite solution (1×), water (1×), and 50% saturated sodium bicarbonate solution (1×). The organic fractions were filtered through sodium sulfate, combined and concentrated in vacuo to give 7.1 g of yellow foam. Crystallization of this material from methanol containing 1% water gave 5.47 g of product as a white solid. The mother liquors were concentrated in vacuo and the residue purified via flash chromatography (1% acetone in CH_2Cl_2) to give an additional 0.59 g of product. Total yield (**5b**, 6.06 g, 90%): mp = 127–128 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2943, 1763, 1722, and 1615 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.819 (s, 18-CH_3), 0.841 (d, $J = 7.2$ Hz, 7-CH_3), 2.054 (s, 17-OAc), 2.253 (s, 2-OAc), 4.695 (dd, $J_1 = 8.7$ Hz, $J_2 = 7.8$ Hz, 17-H), 5.029 (dd, $J_1 = 13.81$ Hz, $J_2 = 11.71$ Hz, benzyl CH_2), 6.703 (s, 4-H), 6.965 (s, 1-H), 7.304–7.385 (m, benzyl aromatic). Analysis calculated for $C_{30}H_{36}O_5$: C, 75.60; H, 7.61. Found: C, 75.42; H, 7.58.

2.9. 3-Benzyloxyestra-1,3,5(10)-triene-2,17β-diol (**6a**)

Under nitrogen, a solution of sodium hydroxide (1N, 100 ml, 100 mmol) was added to a solution of the diacetate (**5a**, 14.6 g solid + residue from mother liquors, assume 38.5 mmol). The mixture was heated to 60 °C for 2 h. At that time, analysis by TLC (5% acetone in CH_2Cl_2) indicated an incomplete reaction. Additional sodium hydroxide solution (100 ml, 100 mmol) was added and the reaction continued for an additional hour. Analysis by TLC at that time indicated a complete reaction. The mixture was cooled to room temperature and acetic acid (glacial, 8 ml, 139.2 mmol) was added. The mixture was diluted with water (1.5 l) and the resulting precipitate collected by filtration. After air drying, the solid was dissolved in methylene chloride, filtered through Na_2SO_4 and concentrated in vacuo to give 13.1 g crude product. Trituration of this material with ether gave the pure product (**6a**, 12.56 g, 86.2% from **4a**): mp = 218–220 °C (lit. [5] 227–228 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 3524, 3280, 2919, and 1605 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.776 (s, 18-CH_3), 3.728 (t, $J = 9.15$ Hz, 17-H), 5.061 (s, benzyl CH_2), 5.464 (s, OH),

6.649 (s, 4-H), 6.905 (s, 1-H), 7.359–7.422 (m, benzyl aromatic). Analysis calculated for $C_{25}H_{30}O_3 \cdot 1/10H_2O$: C, 78.95; H, 8.00. Found: C, 79.09; H, 7.97.

2.10. 3-Benzyloxy-7α-methylestra-1,3,5(10)-triene-2,17β-diol (6b)

Under nitrogen, a solution of sodium hydroxide (1N, 30 ml, 30 mmol) was added to a solution of the diacetate **5b** in methanol (300 ml). The reaction mixture was stirred at room temperature and monitored by TLC (2% acetone in CH_2Cl_2) which indicated an incomplete reaction after 2 h. Additional sodium hydroxide solution (30 ml) was added and the reaction was heated to 60 °C. Analysis by TLC indicated a complete reaction after 1 h. The mixture was allowed to cool to room temperature and then concentrated in vacuo. The residue was diluted with water and the solution was adjusted to a pH ∼ 5 (pH paper) with glacial acetic acid. The resulting precipitate was collected by filtration, washed well with water and air-dried to give 5.1 g light purple solid. This material was dissolved in ethyl acetate (∼300 ml), dried over sodium sulfate, filtered through Celite and concentrated in vacuo. The residue was crystallized from ether/heptane to give the pure diol (**6b**, 4.5 g, 92%) as a light purple solid: mp = 146.5–147.5 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3514, 2955, 2902, and 1607 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.777 (s, 18-CH_3), 0.831 (d, J = 7.5 Hz, 7-CH_3), 3.739 (m, 17-H), 5.053 (dd, J_1 = 13.1 Hz, J_2 = 10.96 Hz, benzyl CH_2), 6.636 (s, 4-H), 6.904 (s, 1-H), 7.351–7.425 (m, benzyl aromatic). Analysis calculated for $C_{26}H_{32}O_3 \cdot 1/10H_2O$: C, 79.19; H, 8.23. Found: C, 79.05; H, 8.13.

2.11. 2-Methoxy-3-benzyloxyestra-1,3,5(10)-trien-17β-ol (7a)

Under nitrogen, solid lithium hydroxide monohydrate (1.4 g, 32.7 mmol) and dimethyl sulfate (2.8 ml, 29.59 mmol) were added to a solution of the diol (**6a**, 10.0 g, 26.42 mmol) in dry THF (150 ml). The reaction mixture was heated to reflux and monitored by TLC (3% acetone in CH_2Cl_2) which indicated a complete reaction after 3 h. The mixture was cooled to room temperature and solvents removed in vacuo. The residue was taken up in methylene chloride and washed with water (2×) and brine (1×). The organic fractions were filtered through Na_2SO_4, combined and concentrated in vacuo to give 10.2 g of a foam. Crystallization of this material from benzene/ether gave the pure product (**7a**, 9.61 g, 92.7%): mp = 113–114 °C (lit. [5] 89–90 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 3318, 2925, 2862, and 1606 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.784 (s, 18-CH_3), 3.733 (t, J = 8.7 Hz, 17-H), 3.861 (s, OCH_3), 5.104 (s, benzyl CH_2), 6.623 (s, 4-H), 6.851 (s, 1-H), 7.29–7.46 (m, benzyl aromatic). Analysis calculated for $C_{26}H_{32}O_3$: C, 79.56; H, 8.22. Found: C, 79.37; H, 8.21.

2.12. 2-Methoxy-3-benzyloxy-7α-methylestra-1,3,5(10)-trien-17β-ol (7b)

Following the procedure outlined for the synthesis of **7a**, reaction of the diol (**6b**, 4.37 g, 11.13 mmol) with lithium hydroxide monohydrate (0.6 g, 14.01 mmol) and dimethyl sulfate (1.1 ml, 11.6 mmol) in dry THF (60 ml) gave the pure 2-methoxy product (**7b**, 3.5 g, 77.3%) after crystallization from methanol/water: mp = 68–70 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3415, 2953, and 1607 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.783 (s, 18-CH_3), 0.818 (d, J = 6.9 Hz, 7-CH_3), 3.745 (t, J = 8.25 Hz, 17-H), 3.854 (s, OCH_3), 5.095 (s, benzyl CH_2), 6.607 (s, 4-H), 6.842 (s, 1-H), 7.292–7.464 (m, benzyl aromatic). Analysis calculated for $C_{27}H_{34}O_3 \cdot 1/10H_2O$: C, 79.41; H, 8.44. Found: C, 79.44; H, 8.52.

2.13. 2-Methoxyestradiol (8a)

A mixture of the 3-benzyl ether (**7a**, 1.0 g, 2.55 mmol) and 5% palladium on charcoal (1.0 g) in ethanol (50 ml) was hydrogenated in a Parr shaker apparatus at 38 psi hydrogen pressure for 16 h. The mixture was filtered through Celite and concentrated in vacuo to give 0.72 g residue. Crystallization of this material from CH_2Cl_2/hexanes gave the pure product (**8a**, 0.65 g, 84.4%) as a white crystalline solid. Analysis by HPLC (Waters Associates NovaPak C_{18}, 66% H_2O/34% CH_3CN at 1.0 ml/min, UV = 288 nm) indicated this material to be greater than 99% pure (R_T = 10.73 min) with no detection of 4-methoxyestradiol (R_T = 9.35 min) or estradiol (R_T = 8.51 min): mp = 183–185 °C (lit. [10] 188–190 °C); FT-IR (KBr, diffuse reflectance) ν_{max}: 3424, 3202, 2905, 2863, and 1607 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.787 (s, 18-CH_3), 3.734 (t, J = 8.1 Hz, 17-H), 3.858 (s, OCH_3), 5.433 (s, OH), 6.641 (s, 4-H), 6.795 (s, 1-H). Analysis calculated for $C_{26}H_{32}O_3 \cdot 2/7CH_2Cl_2$: C, 70.99; H, 8.21. Found: C, 70.98; H, 8.22.

2.14. 2-Methoxy-7α-methylestradiol (8b)

Following the procedure outlined for the synthesis of **8a**, a mixture of the benzyl ether (**7b**, 3.4 g, 8.36 mmol) in ethanol (100 ml) was hydrogenated over 5% Pd/C (3.4 g) in a Parr shaker apparatus at a hydrogen pressure of 38 psi for 16 h. Filtration followed by concentration in vacuo gave 2.8 g solid residue. Crystallization of this material from methanol gave the pure 2-methoxy compound (**8b**, 2.25 g, 85%) as a white crystalline solid: mp = 159–161 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3414, 3296, 2955, 2888, and 1608 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.789 (s, 18-CH_3), 0.820 (d, J = 6.9 Hz, 7-CH_3), 3.737 (t, J = 8.25 Hz, 17-H), 3.855 (s, OCH_3), 4.769 (br.s, OH), 6.623 (s, 4-H), 6.789 (s, 1-H). Analysis calculated for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 76.05; H, 9.04.

3. Results and discussion

For the projected syntheses of a series of 2-ME2 derivatives, a method for the preparation of a large amount of 2-ME was required. While reviewing the prior syntheses, we were attracted in particular to the procedure of Nambara et al. [5] whereby estradiol 3-methyl ether 17-acetate is converted to 2-ME2 utilizing Friedel–Crafts and Baeyer–Villiger reactions as key steps. Nambara reports a 50% overall yield for this process, however, the experimental data he gives indicates a much lower yield (22%). We felt we could improve this procedure by incorporating the recently discovered zirconium tetrachloride-mediated Fries rearrangement [13] in place of the Friedel–Crafts acylation. Nambara reported that the aluminum chloride mediated Fries rearrangement carried out on estrone 3-acetate gave an unsatisfactory yield (54%).

2-ME2 and 2-methoxy-7α-methylestradiol were synthesized as outlined in Fig. 1. Estradiol was converted to the 3,17β-diacetate (**2a**) in 95% yield by reaction with acetic anhydride in pyridine. Reaction of diacetate **2a** with zirconium tetrachloride in methylene chloride at room temperature following the procedure of Harroven [13] gave the 2-acetyl derivative **3a** in 84% yield. Formation of the 3-benzyl ether derivative **4a** was carried out in 91.4% yield by reaction of **3a** with benzyl bromide and potassium carbonate in dimethylformamide. Subsequent Baeyer–Villiger oxidation followed by base hydrolysis gave the 2,17β-dihydroxy derivative **6a** in 86.2% overall yield. The phenolic hydroxyl group of **6a** was then selectively methylated in 92.7% yield by reaction with lithium hydroxide-hydrate and dimethyl sulfate in tetrahydrofuran following the procedure of Basak et al. [14]. Removal of the benzyl ether of **7a** was accomplished in 84.4% yield by hydrogenation over Pd/C at 38 psi. The overall yield of 2-ME2 from estradiol was 49%. All the intermediates **2a**–**7a** are solids purified by simple crystallization.

In a similar fashion, 7α-methylestradiol (**1b**) was converted to 2-methoxy-7α-methylestradiol (**8b**) in 25.8% yield. The lower overall yield for the preparation of **8b** was due to decreased yields in the Fries rearrangement (**2b**–**3b**), benzylation (**3b**–**4b**), and methylation (**6b**–**7b**) steps. All intermediates **2b**–**8b** were also solids, purifiable by crystallization. However, in some steps, column chromatography was carried out on the mother liquors in order to increase the overall yield.

The key step in this scheme is the zirconium tetrachloride-mediated Fries rearrangement [13] of diacetate **2a** to give the 2-acetyl derivative **3a** in 84% yield after crystallization. This reaction was carried out at room temperature regioselectively with no indication of acyl migration to the C-4 position. As a method of C-2 functionalization, this method is superior to halogenation, which gives mixtures of the 2- and 4-monosubstituted halides as well as the 2,4-disubstituted dihalides as crude reaction mixtures [6,7,9,11].

Pert and Ridley [15] have reported the regioselective 2-formylation of the 3,17β-bis(methoxymethyl) ether of estradiol by reaction with sec-butyl lithium at low temperature followed by quenching with anhydrous dimethylformamide. This procedure was adopted by Wang and

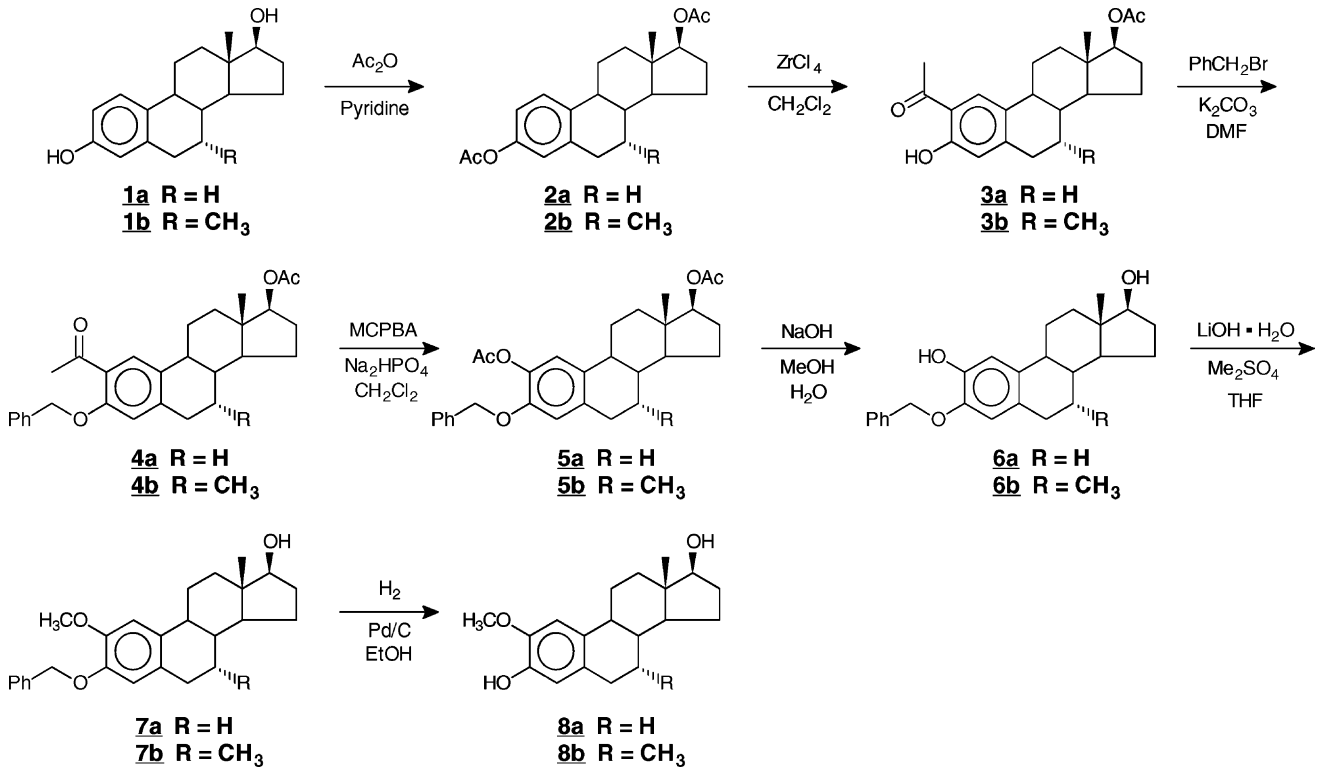

Fig. 1. Synthesis of 2-ME2 and its 7α-methyl derivative.

Cushman [10] into a five-step synthesis of 2-ME2 with an overall yield of 63%. However, the first three intermediates in this synthesis are oils that require chromatographic purification [10,16], making this approach unattractive for a moderate to large-scale synthesis.

Agoston et al. [17] have reported that all commercially available preparations of 2-ME2 are either less than 98% pure or contain undesirable steroid contaminants that are of concern for pharmaceutical uses. Using HPLC, the authors identified estradiol and 4-methoxyestradiol as the major contaminants. The 4-methoxyestradiol contaminant arises from small amounts of 4-brominated intermediate that is carried through the reaction sequence. The estradiol contaminant arises upon the reaction of the 2-brominated intermediate with sodium methoxide in the presence of a copper catalyst. A small amount (1–2%) of the reactive copper complex is quenched by a hydride rather than a methoxide. Since the procedure we presented here does not rely on the halogenation method, no indication of these contaminants is detected in the final product. Analysis by HPLC indicated a purity of >99%.

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