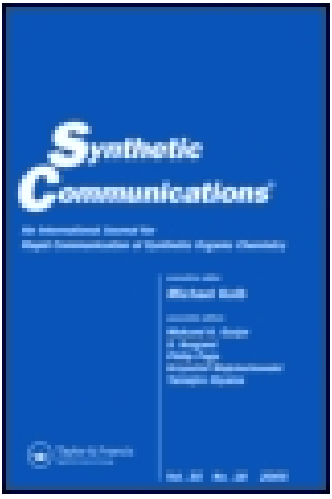

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An Optimized Synthesis of 2-Methoxyestradiol, A Naturally Occurring Human Metabolite with Anticancer Activity

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AN OPTIMIZED SYNTHESIS OF 2-METHOXYESTRADIOL, A NATURALLY OCCURRING HUMAN METABOLITE WITH ANTICANCER ACTIVITY

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Abstract: 2-Methoxyestradiol, a naturally occurring human metabolite with demonstrated anticancer activity, has been synthesized in three steps and 76% yield from the bis(MOM) ether of 2-formylestradiol.

2-Methoxyestradiol **(4)** is a naturally occurring human metabolite which binds to the colchicine binding site of tubulin and acts as a tubulin polymerization inhibitor.[1-4] The cytotoxic effects of 2-methoxyestradiol in cancer cell cultures are associated with uneven chromosome distribution, faulty spindle formation, inhibition of DNA synthesis, and an increase in the number of abnormal metaphases.[5,6] In addition to effects on tubulin polymerization and microtubule stability, the inhibition of calmodulin activity by 2-methoxyestradiol has also been suggested as a possible mechanism for metaphasal arrest.[7] Recent interest in 2-methoxyestradiol has been reinforced by its ability to inhibit angiogenesis, the growth of new blood vessels required for the growth of solid tumors,[8,9] and studies

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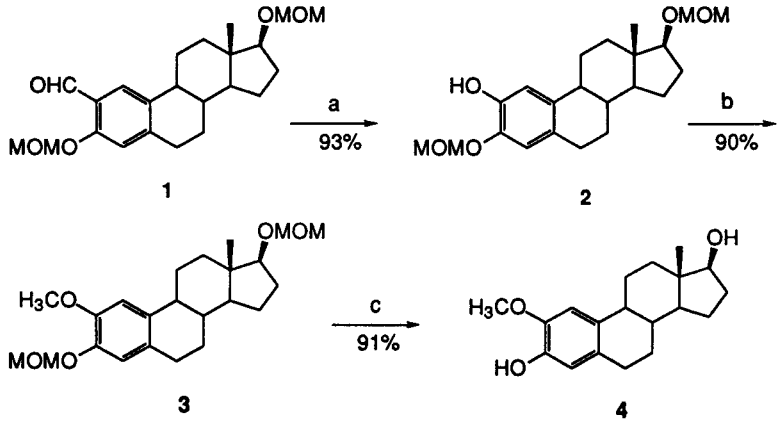
of the mechanism of the antiangiogenic effect have indicated that the promotion of endothelial cell apoptosis may be involved.[10] The antiangiogenic effect of 2-methoxyestradiol may also be responsible for its ability to suppress, in vivo, type II collagen-induced arthritis.[11] Moreover, 2-methoxyestradiol has been found to inhibit the growth of human breast cancer implants in severe combined immunodeficient (SCID) mice.[9] These results have increased the demand for 2-methoxyestradiol and related compounds for additional preclinical studies, and also for possible future clinical testing as anticancer agents in humans. The present communication describes an optimized, practical preparation of 2-methoxyestradiol from the bis(methoxymethyl) ether **1** of 2-formylestradiol[12,13] in three steps with an overall yield of 76%. The synthesis, outlined in Scheme 1, complements the previously published routes[14-20] and provides additional flexibility for the synthesis of biologically active 2-methoxyestradiol analogs.[21-23] The synthesis utilizes the protected 2-formylestradiol **1** as the starting material and proceeds to the final product in three practical steps with an overall yield of 76%. Many derivatives of the aldehyde are possible, thus increasing the versatility of the route for the preparation of 2-methoxyestradiol analogs of biological interest.

The starting material **1** is readily obtained as a single regioisomer from estradiol in 83% yield.[12,13] Baeyer-Villiger oxidation of **1** with *m*-chloroperbenzoic acid and sodium hydrogenphosphate in methylene chloride, followed by hydrolysis of the resulting formate with sodium hydroxide, yielded the phenolic intermediate **2** in 93% yield. In this reaction, the sodium hydrogenphosphate functions as a buffer in order to prevent the acidic decomposition of the bis(MOM) ethers, which are sensitive to acid, but are generally stable in the pH > 4 range. Treatment of the phenol **2** with 10 equivalents of anhydrous potassium carbonate and a catalytic amount of tetra-*n*-butylammonium iodide in DMF provided an anion which was alkylated with excess methyl iodide to afford the intermediate ether **3** in 90% yield.

Scheme 1[a]



[a](a) (1) MCPBA, Na_2HPO_4, CH_2Cl_2, 23 °C (20 h); (2) NaOH, CH_3OH, 23 °C (2 h). [b]CH_3I, K_2CO_3, DMF, 23 °C (25 h). [c]HCl (6 N), THF, 23 °C (6 h).

We assume that the tetra-*n*-butylammonium iodide functions as a solid-liquid phase-transfer catalyst to increase the reaction rate. Deprotection of **3** with 6 N hydrochloric acid in THF at room temperature led to 2-methoxyestradiol (**4**) in 91% yield.

Experimental

General. Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. ^1H NMR spectra were recorded at 300 MHz. Microanalyses were performed at the Purdue Microanalysis Laboratory. Flash column chromatography was performed on 230-400 mesh silica gel from Scientific Adsorbents, Inc. Chemicals and solvents were analytical grade and used without further purification.

2-Hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol (2). A solution of *m*-chloroperbenzoic acid (1.0 g, 57-86%, 3.3-4.9 mmol) in methylene chloride

(10 mL) was added dropwise to a solution of **1** (1.0 g, 2.6 mmol) in methylene chloride (20 mL) containing sodium hydrogenphosphate (1.2 g, 8.4 mmol). The resulting mixture was stirred at room temperature, and the reaction was followed by silica gel TLC (hexane:ethyl acetate 5:1 by volume). After 20 h, the reaction mixture was poured into ice water (50 mL) and the products were extracted with methylene chloride (3 x 40 mL). The methylene chloride layers were washed with satd sodium bicarbonate (50 mL) and brine (2 x 50 mL), combined and dried over sodium sulfate, and evaporated to dryness to afford a yellowish oil. The oil was dissolved in methanol (20 mL) and the solution was deoxygenated by bubbling nitrogen. After bubbling, 1.0 M sodium hydroxide (5 mL, 5 mmol) was added to the solution and the resulting mixture was stirred at room temperature for 2 h. The solution was neutralized to pH 7, the methanol was removed under reduced pressure, and the residue was transferred into ethyl acetate (50 mL) and water (50 mL). The ethyl acetate was dried over sodium sulfate and evaporated to dryness. Column chromatography of the residue on silica gel (hexane:ethyl acetate 5:1 by volume) gave compound **2** as a colorless oil (896 mg, 93%). ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 1 H), 6.79 (s, 1 H), 5.76 (s, 1 H, OH), 5.16 (s, 2 H), 4.66 (ABq, J = 6.6 Hz, 2 H), 3.61 (t, J = 8.5 Hz, 1 H), 3.54 (s, 3 H), 3.38 (s, 3 H), 2.77 (m, 2 H), 2.30-1.20 (m, 13 H), 0.81 (s, 3 H); CIMS (isobutane) *m/z* 376 (MH^+, 34.5), 315 (100). Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57. Found: C, 70.44; H, 8.60.

2-Methoxy-3,17β-*O*-bis(methoxymethyl)estradiol (3). A solution of **2** (680 mg, 1.81 mmol) in anhydrous DMF (20 mL) containing anhydrous potassium carbonate (2.5 g, 18.1 mmol) was stirred at room temperature under argon for 10 min. Iodomethane (1.25 mL = 2.85 g, 20.1 mmol) was introduced into the reaction mixture, followed by the addition of tetra-*n*-butylammonium iodide (30 mg). The resulting mixture was stirred at room temperature for 5 h and then

another portion of iodomethane (1 mL, 2.28 g, 16.1 mmol) was introduced. After a total of 20 h, the reaction mixture was poured into brine (80 mL) and the products were extracted with ethyl acetate (3 x 40 mL). The ethyl acetate layers were washed with brine (50 mL), combined, dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue (hexane:ethyl acetate 5:1 by volume) gave compound **3** (635 mg, 90%), which was crystallized from methanol to afford white crystals: mp 68-70 °C. ^{1}H NMR ($CDCl_3$) δ 6.87 (s, 1 H), 6.85 (s, 1 H), 5.20 (s, 2 H), 4.67 (ABq, J = 6.6 Hz, 2 H), 3.86 (s, 3 H), 3.62 (t, J = 8.5 Hz, 1 H), 3.52 (s, 3 H), 3.38 (s, 3 H), 2.79 (m, 2 H), 2.30-1.20 (m, 13 H), 0.82 (s, 3 H); CIMS (isobutane) *m/z* 391 (MH^+, 100), 329 (48). Anal. Calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.76. Found: C, 70.99; H, 8.88.

2-Methoxyestradiol (4). To a solution of **3** (510 mg, 1.30 mmol) in THF (10 mL) was added 6 N HCl (6 mL) at room temperature and the resulting solution was stirred at room temperature for 6 h. The reaction mixture was poured into brine (50 mL) and the products were extracted with ethyl acetate (3 x 40 mL). The ethyl acetate layers were washed with satd sodium bicarbonate (30 mL) and brine (50 mL), combined, dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue (methylene chloride:ethyl acetate 9:1 by volume) gave compound **1** (360 mg, 91%), which was crystallized from ethyl acetate/hexane to afford white crystals: mp 188-190 °C (lit.[11] mp 188-190 °C). ^{1}H NMR ($CDCl_3$) δ 6.81 (s, 1 H), 6.65 (s, 1 H), 5.45 (s, 1 H, OH), 5.20 (s, 2 H), 3.86 (s, 3 H), 3.74 (t, J = 8.5 Hz, 1 H), 2.79 (m, 2 H), 2.35-1.15 (m, 13 H), 0.80 (s, 3 H); CIMS (isobutane) *m/z* 303 (MH^+, 100), 285 (MH^+ - 18, 52). Anal. Calcd for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.37; H, 8.57.

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