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A VERSATILE SYNTHESIS OF 2-METHOXYESTRADIOL, AN ENDOGENOUS METABOLITE OF ESTRADIOL WHICH INHIBITS TUBULIN POLYMERIZATION BY BINDING TO THE COLCHICINE BINDING SITE

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Abstract: A versatile synthesis of 2-methoxyestradiol (**7**) is described. Formylation of estradiol (**1**) with hexamethylenetetramine gave 2-formylestradiol (**3**). Benzylation of the two hydroxyl groups of **3** under basic conditions with benzyl bromide afforded the dibenzyl ether **4**. 2-Methoxyestradiol (**7**) was then obtained from **4** by Baeyer-Villiger oxidation, methylation of the phenol, and debenzylaion.

Hydroxylation of estradiol by hepatic cytochrome P-450 enzymes followed by *O*-methylation with catechol *O*-methyltransferase leads to 2-methoxyestradiol (**7**), an endogenous metabolite of estradiol which has been detected in human urine.[1,2] 2-Methoxyestradiol is also a metabolite of 17α-ethynylestradiol, a synthetic estrogen present in many oral contraceptives.[3] Current interest in 2-methoxyestradiol has been stimulated by its cytotoxicity in several cancer cell cultures.[4,5] This cytotoxic effect is associated with abnormal chromosome distribution, faulty spindle formation, inhibition of mitosis, and an increase in abnormal metaphases. Recently it has been discovered that 2-methoxyestradiol either inhibits tubulin polymerization or results in polymer with altered stability properties and morphology, depending on the reaction conditions, by binding to the colchicine binding site.[6] This identification of a natural ligand for the colchicine binding site of tubulin indicates that 2-methoxyestradiol or a closely related metabolite could possibly be functioning as a natural regulator of tubulin polymerization and function.

The cytotoxicity of 2-methoxyestradiol in cancer cell cultures and its antitubulin activity raise the possibility that it could serve as a lead compound for the design and synthesis of a new series of anticancer agents. Current *in vitro* studies have indicated that 2-methoxyestradiol inhibits angiogenesis (the creation of new blood vessels), which is essential for the growth of solid tumors.[7-9] When investigated *in vivo*, 2-methoxyestradiol was found to be a potent inhibitor of neovascularization of solid tumors and to inhibit their growth when administered orally in mice.[7] In view of these exciting results, we have sought to develop a short and versatile synthesis of this metabolite which could also serve for the preparation of a variety of related compounds of potential value in the investigation of the structural parameters associated with the antitumor and antitubulin

activity of 2-methoxyestradiol. This might also result in the eventual preparation of new compounds with more favorable biological properties for potential therapeutic use as anticancer agents. Although a number of 2-methoxyestradiol syntheses have been reported, they are not suitable for the present purposes either because they are either too long and inefficient or are lacking in sufficient versatility to suit our needs for analog development.[10-13]

Formylation of estradiol (**1**) with two equivalents of hexamethylenetetramine in refluxing trifluoroacetic acid for 4 hours gave 4-formylestradiol (**2**) in 13% yield and 2-formylestradiol (**3**) as a white crystalline material, mp 230-232 °C (ethanol) (lit.[14] 231-233 °C), in 25% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.79 (s, 1 H), 9.82 (s, 1 H), 7.41 (s, 1 H), 6.71 (s, 1 H), 4.90 (t, *J* = 7.8 Hz, 1 H), 2.91 (m, 2 H), 2.34 (m, 3 H), 1.95 (m, 2 H), 1.78 (m, 3 H), 1.52 (m, 6 H), 0.90 (s, 3 H); LRCIMS *m/z* (isobutane) 301 (MH^+, 5.71), 283 (MH-H_2O, 100).

Treatment of **3** with 10 equivalents of NaH and a catalytic amount of tetrabutylammonium iodide in anhydrous DMF yielded a dianion which was treated with 7.1 equivalents of benzyl bromide to afford intermediate **4** as a white solid, mp 124-125 °C (chloroform-hexane), in 64% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.49 (s, 1 H), 7.79 (s, 1 H), 7.38 (m, 10 H), 6.75 (s, 1 H), 5.15 (s, 2 H), 4.58 (s, 2 H), 3.51 (t, *J* = 8.4 Hz, 1 H), 2.88 (m, 2 H), 2.36 (m, 1 H), 2.09 (m, 4 H), 1.87 (m, 1 H), 1.41 (m, 7 H), 0.87 (s, 3 H); LRCIMS (isobutane) *m/z* 481 (MH^+, 34). Anal. calcd for $C_{33}H_{36}O_3$: C, 82.46; H, 7.55. Found: C, 82.16; H, 7.74.

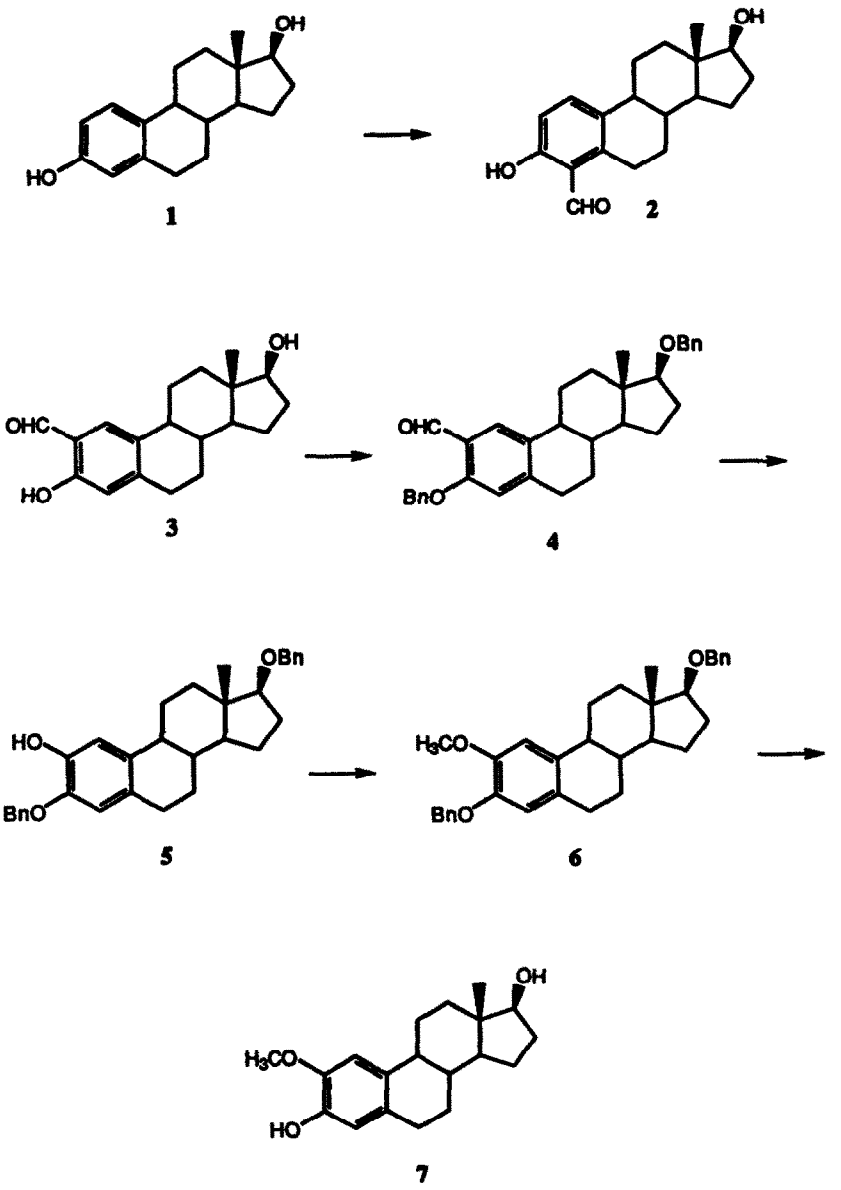
Baeyer-Villiger oxidation of **4** with 1.2 equivalents of MCPBA and a catalytic amount of TsOH in CH_2Cl_2 for 3 hours gave the phenol **5** as a colorless oil in 58% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (m, 10 H), 6.90 (s, 1 H), 6.64 (s, 1 H), 5.45 (s, 1 H), 5.06 (s, 2 H), 4.57 (s, 2 H), 3.50 (t, *J* = 8.0 Hz, 1 H), 2.76 (m, 2 H), 2.11 (m, 3 H), 1.43 (m, 10 H), 0.87 (s, 3 H). D_2O exchanged ^{1}H NMR showed that the peak at δ 5.45 ppm was exchangeable. LRCIMS (isobutane) *m/z* 469 (MH^+, 100).

Treatment of the phenol **5** with 10 equivalents of anhydrous K_2CO_3 and a catalytic amount of tetrabutylammonium iodide in DMF provided an anion which was alkylated with excess methyl iodide to afford the intermediate **6** in 80% yield, also obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (m, 10 H), 6.85 (s, 1 H), 6.63 (s, 1 H), 5.11 (s, 2 H), 4.58 (s, 2 H), 3.87 (s, 3 H), 3.51 (t, *J* = 8.0 Hz, 1 H), 2.74 (m, 2 H), 2.12 (m, 4 H), 1.83 (m, 1 H), 1.44 (m, 8 H), 0.88 (s, 3 H); LRCIMS (isobutane) *m/z* 483 (MH^+, 100).

Hydrogenolysis of the two benzyl groups of **6** over 10% palladium on charcoal in THF gave 2-methoxyestradiol (**7**) in 83% yield as a white solid. Recrystallization of this solid from acetone under argon atmosphere gave 2-methoxyestradiol (**7**) as colorless blades, mp 188-190 °C (lit.[10] mp 188-190 °C): $[\alpha]_D^{25}$ +99°, $CHCl_3$, c = 0.5, (lit.[10] $[\alpha]_D^{21}$ +100°, $CHCl_3$, c = 0.5). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.81 (s, 1 H), 6.65 (s, 1 H), 5.43 (s, br, 1 H), 3.87 (s, 3 H), 3.75 (t, *J* = 8.5 Hz, 1 H), 2.77 (m, 2 H), 2.20 (m, 3 H), 1.99 (m, 1 H), 1.87 (m, 1 H), 1.69 (m, 1 H), 1.35 (m, 8 H), 0.80 (s, 3 H); LRCIMS (isobutane) *m/z* 303 (MH^+, 7), 302 (M^+, 100).

A variety of 2-methoxyestradiol congeners are being prepared by utilization of the versatile intermediates **3**, **4** and **5** in Scheme I.

Scheme I


OH
HO
1
OH
HO
CHO
2
OH
OHC
HO
3
OBn
OHC
BnO
4
OBn
HO
BnO
5
OBn
H3CO
BnO
6
OH
H3CO
HO
7

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References

(1) Breuer, H.; Knuppen, R. *Naturwissenschaften* **1960**, *12*, 280-281.

(2) Gelbke, H. P.; Knuppen, R. *J. Steroid Biochem.* **1976**, *7*, 457-463.

(3) Williams, M. C.; Helton, E. D.; Goldzieher, J. W. *Steroids.* **1975**, *25*, 229-246.

(4) Seegers, J. C.; Aveling, M.-L.; van Aswegen, C. H.; Cross, M.; Koch, F.; Joubert, W. S. *J. Steroid Biochem.* **1989**, *32*, 797-809.

(5) Lottering, M.-L.; Haag, M.; Seegers, J. C. *Cancer Res.* **1992**, *52*, 5926-5932.

(6) D'Amato, R. J.; Lin, C. M.; Flynn, E.; Folkman, J.; Hamel, E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3964-3968.

(7) Fotsis, T.; Zhang, Y.; Pepper, M. S.; Adlercreutz, H.; Montesano, R.; Nawroth, P. P.; Schweigerer, L. *Nature* **1994**, *368*, 237-239.

(8) Folkman, J.; Watson, K.; Ingber, D. E.; Hanahan, D. *Nature* **1989**, *339*, 58-61.

(9) Blood, C. H.; Zetter, B. R. *Biochem. Biophys. Acta.* **1990**, *1032*, 89-118.

(10) Fishman, J. *J. Am. Chem. Soc.* **1958**, *80*, 1213-1216.

(11) Nambara, T.; Honma, S.; Akiyama, S. *Chem. Pharm. Bull.* **1970**, *1970*, 474-480.

(12) Rao, P. N.; Burdett Jr., J. E. *Synthesis* **1977**, 168-169.

(13) Chen, S.-h.; Luo, G.-r.; Wu, X.-s.; Chen, M.; Zhao, H.-m. *Steroids* **1986**, *47*, 63-68.

(14) Pert, D. J.; Ridley, D. D. *Aust. J. Chem.* **1989**, *42*, 405-419.

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