methylsilane as the internal standard. UV, IR, and NMR spectra on all intermediates and the final product were consistent with the proposed structure and are not included. TLC was performed on silica gel sheets with a fluorescent indicator. Compounds were visualized using a UV lamp and migrated as single spots. Octanol–water partition coefficients were determined by the method of Leo, Hansch, and Elkins.[18]

Ethyl α-Acetyl-β-(2,5-dimethoxyphenyl)acrylate (2). A mixture of 2,5-dimethoxybenzaldehyde (100 g, 0.6 mol), freshly distilled ethyl acetoacetate [84.5 g, 0.65 mol, bp 73 °C (15 mm)], anhydrous benzene (200 mL), piperidine (6 mL), and glacial acetic acid (12 mL) was refluxed in the presence of a Dean–Stark trap until almost the theoretical amount of water had been collected (~3 h). The reaction mixture was cooled, benzene (300 mL) was added, and the solution was washed successively with water (100 mL), cold 0.1 N hydrochloric acid (200 mL), 5% aqueous sodium bicarbonate (200 mL), and acetic acid–water (1:99, 100 mL). After drying over anhydrous magnesium sulfate, the benzene solution was filtered and concentrated by distillation of the solvent in vacuo. The residue was distilled, yielding 104.0 g (64%) of **2**, bp 169–170 °C (0.3 mmHg). Recrystallization from ethanol–pentane afforded an analytical sample, mp 72–73 °C. Anal. ($C_{15}H_{18}O_5$) C, H.

Ethyl α-Acetyl-β-(2,5-dimethoxyphenyl)propionate (3). The acrylate **2** (38.0 g, 0.15 mol) was dissolved in ethyl acetate (150 mL) and hydrogenated (20–40 psi of H_2) in a Parr low-pressure apparatus using 5% palladium on charcoal catalyst (1.5 g) until approximately the theoretical quantity of hydrogen was absorbed (1–2 h). The catalyst was removed by filtration and the solvent concentrated by distillation in vacuo. The reaction product was purified by vacuum distillation to afford 38.4 g (89%) of **3**, bp 146–148 °C (0.3 mmHg). Anal. ($C_{15}H_{21}O_5$) C, H.

2,4-Diamino-7,8-dihydro-6-(2,5-dimethoxybenzyl)-5-methyl-7-oxopyrido[2,3-*d*]pyrimidine (4). In a flask equipped with a Dean–Stark trap, a mixture of the propionate **3** (21.2 g, 0.079 mol), 2,4,6-triaminopyrimidine (100 g, 0.08 mol), and diphenyl ether (100 mL) was heated rapidly with vigorous stirring to 190 °C and maintained at 195–230 °C until no additional water–ethanol mixture was distilled (~1.5 h). The reaction mixture was cooled to room temperature, methanol (100 mL) was added, and the crude product was collected by filtration. The product was suspended in boiling water (500 mL) and filtered, and the solid was washed with hot water (500 mL) followed by methanol (100 mL) and pentane (50 mL) and then dried to give 17.0 g (62%) of the 7-oxo compound **4** as a yellow brownish solid, mp 325–326 °C. Anal. ($C_{17}H_{19}N_5O_3$) C, H, N.

7-Chloro-2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine (5). To a solution of *N,N*-dimethylformamide (17.5 mL, 0.24 mol) in dry chloroform (100 mL), carefully cooled in an ice bath, a solution of thionyl chloride (28.6 g, 0.24 mol) in dry chloroform was added dropwise so that the temperature remained below 5 °C. When the exothermic reaction resulting from formation of the dimethylformamide–thionyl chloride complex (1:1) subsided, the 7-oxo compound **4** (8.2 g, 0.024 mol) was added over a period of 10–15 min. The reaction mixture was gradually allowed to reach room temperature and then heated under gentle reflux for 3 h. The reaction mixture was cooled and treated with ethanolic base (80 mL) while maintaining the temperature at 25–30 °C by cooling. The brownish solid thus formed was collected by filtration, slurried in 50% aqueous ammonium hydroxide (200 mL), filtered, washed with water (150–200 mL), and dried to afford 3.4 g (40%) of the 7-chloro compound **5**, mp 192–200 °C dec. Recrystallization from aqueous ethanol afforded an analytical sample, mp 193–196 °C dec. Anal. ($C_{17}H_{18}ClN_5O_2 \cdot 0.25H_2O$) C, H, N, Cl.

2,4-Diamino-6-(2,5-dimethoxybenzyl)-5 methylpyrido[2,3-*d*]pyrimidine (7). The pure 7-chloro compound **5** (0.3 g, 0.8 mmol) was dissolved in absolute ethanol (200 mL), and potassium hydroxide (0.2 g) and 5% palladium on charcoal catalyst (0.2 g) were added. Hydrogenolysis was conducted in a Parr low-pressure apparatus with the hydrogen pressure between 35 and 40 psi. The reduction required about 36 h. The reaction mixture was filtered to remove the catalyst, and the solvent was eliminated by evaporation under reduced pressure. The residue was dissolved in a small amount of ethanol, and water was added to produce 0.1 g (38%) of **7** as a yellow powder, mp 252–254 °C. An analytical sample from a similar preparation was recrystallized from aqueous ethanol–hydrochloric acid as the hydrochloride salt, mp 283–286 °C. Anal. ($C_{17}H_{19}N_5O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl.

Cytotoxicity in Cell Culture. To determine the cytotoxicity of the antifolates in cell culture, 1×10^5 cells were seeded in 35-mm Petri dishes (W-256, S-180) or 15-mL culture tubes (L1210) using Dulbecco's modified Eagle medium containing 10% fetal calf serum. Drugs were added 6 h later. Following a growth period of 72 h, monolayers of W256 and S180 were trypsinized and cell counts determined using a Coulter counter. Results are expressed as ED_{50}, the concentration of drug required for 50% inhibition of cell growth.

Antitumor Activity. Walker 256 carcinosarcoma was implanted subcutaneously in the right flank of male Sprague–Dawley rats. Drug treatment commenced on the 3rd day following implantation of tumors and continued for 5 successive days. Tumor-bearing animals received BW301U at a dose of either 25 mg/kg daily or 15 mg/kg twice daily. Drug was administered intraperitoneally as a suspension in methylcellulose. Tumor volumes and body weights were monitored daily.

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Synthesis and Biological Activity of Some 15-Oxaestranes

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The estrogenic activity of orally administered 15-oxaestrone was evaluated by the uterotropic assay in rats and was found to be 12 times greater than that of estrone. In addition, several analogues of 15-oxaestrone were prepared and their estrogenic potency was determined.

The preparation of 15-oxaestrone[1] (**1**) has been described previously. We now report the synthesis of various analogues derived from **1**, as well as the estrogenic activity found for these compounds.

Chemistry. Various derivatives of the natural hormone estrone have been prepared in an attempt to obtain compounds which would possess greater activity than the parent compound when administered orally. One such derivative, 17α-ethynylestradiol, has been shown in hu-

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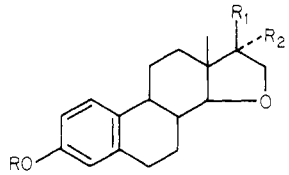
Table I. Uterotropic Activity

compd	dose range, ng	regression line[a] slope (b)	regression line[a] Y intercept (a)	doubling dose, ng	ratio (estrone = 1.0)
estrone	400-4000	59.7	-120.2	989	1.0
estradiol	400-4000	61.5	-121.2	839	1.2
estriol	1000-40 000	17.6	1.8	1688	0.6
ethynylestradiol	100-1000	80.0	-120.2	172	5.8
mestranol	100-1000	57.9	-65.3	138	7.2
1	40-400	47.7	-32.1	80	12.4
2	20-200	34.5	-7.8	84	11.8
3	100-1000	36.7	-23.9	177	5.6
4	40-1000	58.8	-52.2	77	12.8

[a] $Y = b \log x + a$, where x = daily dose in nanograms and Y = mean uterine weight.

mans to be extraordinarily active by mouth,[2a,b] as is its 3-methyl ether (mestranol). Furthermore, the 3-cyclopentyl ether of estrone has also been reported to be considerably more active orally than the parent hormone.[3] With this in mind, the 3-cyclopentyl ether 4 was prepared



1, R = H; $R_1 + R_2$ = O
2, R = R_2 = H; R_1 = OH
3, R = CH_3; R_1 = OH; R_2 = C≡CH
4, R = c-C_5H_{10}; $R_1 + R_2$ = O

in the conventional manner by heating 15-oxaestrone (1) with lithium methoxide and cyclopentyl bromide. In addition, the 15-oxa analogue of mestranol, 3, was synthesized via the introduction of the 17α-ethynyl group, followed by etherification with diazomethane. Reduction of 15-oxaestrone (1) with lithium tri-*tert*-butoxyaluminum hydride afforded 15-oxaestradiol (2).

Biological Activity. The results are given in Table I together with the data for 3-hydroxyestra-1,3,5(10)-triene-16,17β-diol (estriol), 17α-ethynyl-1,3,5(10)-estratriene-3,17-diol (ethynylestradiol), and 17α-ethynyl-3-methoxy-1,3,5(10)-estratrien-17-ol (mestranol). When administered by the oral route, 15-oxaestrone (1) was found to be 12 times as potent as estrone and more than twice that of ethynylestradiol. The 15-oxaestradiol (2) and 1 are almost equally potent, as is the case for estrone and estradiol. Surprisingly, the introduction of a 17-ethynyl moiety to give compound 3 caused a decrease in activity, while the formation of the 3-cyclopentyl ether derivative, i.e., compound 4, appeared to have little effect.

It may be interesting to speculate that the enhanced oral activity shown by compound 1 may be due in part to the inability of 1 to serve as a substrate for metabolic hydroxylation at C-16, a known pathway for the deactivation of estrone.[4]

Experimental Section

The compounds gave satisfactory analyses for C and H. The UV, IR, and NMR spectra were in agreement with the proposed structures. The melting points are uncorrected.

15-Oxa-1,3,5(10)-estratriene-3,17β-diol (2). A solution of 0.5 g (1.8 mmol) of 1 in 10 mL of dry THF was added dropwise with stirring to a cooled (0 °C) solution of 1.35 g of lithium tri-*tert*-butoxyaluminum hydride in 50 mL of dry THF. After 15 min at 0 °C, $CHCl_3$ (100 mL) was added and the mixture washed with cold 1 N HCl. The solution was then dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude semisolid was crystallized from Me_2CO–H_2O to give 0.4 g (80%) of 2: mp 180–182 °C; $[\alpha]^{25}_D$ +74.91° (c 0.9, CH_3OH). Anal. ($C_{17}H_{22}O_3$) C, H.

15-Oxa-17α-ethynyl-3-methoxy-1,3,5(10)-estratrien-17β-ol (3). To a dioxane solution (48 mL) saturated with acetylene at 0 °C was added 4.8 g of lithium acetylide–ethylenediamine, followed by the dropwise addition (30 min) of 0.8 g (2.9 mmol) of 1 dissolved in 16 mL of dry dioxane. During the addition and for 40 min thereafter, acetylene was bubbled through the reaction mixture. The reaction was then stirred at room temperature for 3.5 h, after which time 100 mL of 20% HCl was added slowly with stirring at 0 °C. The mixture was then extracted with ether, and the ether solution was washed with 0.1 N HCl and water and dried ($MgSO_4$). The solvent was then removed under reduced pressure and the residue allowed to react for 3 days with an ethereal solution of diazomethane. When the reaction was complete (TLC), the solvent and excess diazomethane were blown off by a stream of nitrogen, and the resulting product was dissolved in a minimum of C_6H_6 and passed through 10 g of silica gel. Elution with C_6H_6 afforded 0.75 g of crude material, which when triturated with C_6H_{14} gave 0.57 g of 2, mp 112–124 °C. Drying at 95 °C (0.1 mm) for 18 h afforded 0.5 g (55.2%) of 3: mp 140–142 °C; $[\alpha]^{25}_D$ 0° (c 0.9, $CHCl_3$). Anal. ($C_{20}H_{24}O_3$) C, H.

15-Oxa-3-(cyclopentyloxy)-1,3,5(10)-estratrien-17-one (4). A solution of 0.29 g (1.07 mmol) of 1, 3 mL of absolute C_2H_5OH, 1 mL of cyclopentyl bromide, and 1.5 mL of a lithium methoxide–CH_3OH solution (10.23%, sp gr 0.853) was refluxed for 18 h. The solutions was then poured into 50 mL of H_2O and the mixture extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried ($MgSO_4$) and most of the solvent removed under reduced pressure. The residue was passed through 3 g of neutral alumina (grade 1) and the product crystallized from CH_2Cl_2–Et_2O to give 0.2 g (54.7%) of 4: mp 198–201 °C; $[\alpha]^{25}_D$ +117.85° (c 1.1, $CHCl_3$). Anal. ($C_{22}H_{28}O_3$) C, H.

Pharmacological Methods. The oral estrogenic activity of the compounds was determined by uterotropic assay in rats. Compounds were administered by gavage once daily for 3 consecutive days in 0.2 mL/day sesame oil. Eight to ten Charles River CD 21-day-old rats were employed in each dosage group. All rats were autopsied on the day after the last treatment day, and weights of uteri were determined after blotting on paper toweling. Mean uterine weights were plotted against the logarithm of dose to determine the dose–response curve, and slopes and intercepts of the linear portion of the curve were computed for each compound. All of the oxaestranes were complete agonists. The maximal responses obtained were equal to, or greater than, that obtained with ethynylestradiol. Exact relative potencies, in the absence of parallel dose–response lines, could not be determined. The lack of parallelism was perhaps not unexpected, since these compounds probably vary significantly in their pharmacokinetic profiles. Relative potencies were estimated based upon the arbitrary selection of the 100% uterine weight increase response level for comparison of doses required. This response level was selected because it was unequivocally located on the linear portion of the dose–response curve for all of the compounds and clearly well removed from the sigmoid portion of the curves. Control uterine weight was taken as the mean of 89 rats from 6 experiments (29.3 mg).

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