Estrogenic and Antiestrogenic Activity of Monophenolic Analogues of Tamoxifen, (*Z*)-2-[*p*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine

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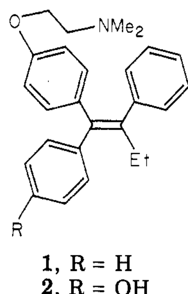
Five hydroxylated analogues of tamoxifen [**1**, (*Z*)-2-[*p*-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine] and its geometric isomer were prepared by reaction of protected hydroxy-α-ethyldeoxybenzoins with 4-[2-(dimethylamino)ethoxy]phenylmagnesium bromide, followed by acid-catalyzed dehydration–deprotection and chromatographic separation of isomer mixtures. Estrogen receptor binding affinity and estrogenic and antiestrogenic activity of each of the compounds were determined in the rat, in comparison with 4-hydroxytamoxifen (**2**). The new compounds had a wide range of receptor binding affinities, with that of 3-hydroxytamoxifen (**6c**), the most strongly bound, approaching that of estradiol. The trans isomers **6a,b** were more strongly bound than were the cis isomers **7a,b**. Antiestrogenic activity was seen in all compounds except **7b**. This was also true for estrogenic activity, except that in **6c** this activity was also substantially reduced. Maximal antiestrogenic effectiveness of **6c** occurred at a 10-fold greater daily dose (50 μg/rat) than that required for maximal effect of **2**.

Estrogen antagonist activity has been found in both steroidal and nonsteroidal estrogen analogues.[1] The most significant group of nonsteroidal antiestrogens is the triarylethylenes, of which tamoxifen (1) is representative.[2]



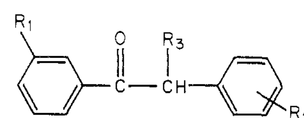
1, R = H
2, R = OH

The antiestrogenic properties of 1 have led to its clinical use in treatment of malignant tumors, especially estrogen receptor positive breast cancer.[3-5] Also, 1 and related compounds are of theoretical importance in mechanistic studies of estrogen action.[6,7]

All of the clinically useful nonsteroidal antiestrogens contain a triphenylethylene nucleus in which one of the benzene rings contains a para-substituted polar residue, and the remaining benzene rings are trans to each other.[8]

In animal studies, estrogen receptor affinity and antiestrogenic potency were greater in trans isomers relative to those of respective cis isomers[8] and were substantially increased by addition of phenolic hydroxyl groups.[9-12] Thus, these effects were greater in 2, compared to those of its cis isomer and to those of 1.[9,13] However, 2 and its cis isomer are the only monophenolic analogues of 1 now known. We have prepared isomers of these compounds in which the phenolic hydroxy group has been substituted at the other accessible positions in the unsubstituted benzene rings. These compounds were tested for their estrogen receptor affinities and antiestrogenic effects in the rat uterus. Also, the estrogenic effects of these compounds have been determined, since 1, 2, and their geometric isomers have shown partial estrogenic activity in the rat.[9]

Chemistry. Alkylation of **3a–c** with ethyl iodide gave **4a–c**, which underwent acid-catalyzed O-demethylation to furnish monophenols **5a–c**, characterization of which is summarized in Table I. Attempted preparation of the THP ethers of these compounds using *p*-toluenesulfonic acid as catalyst was not successful. Reaction of these



3, R_3 = H; **4**, R_3 = Et

	R_1	R_2
a	H	*p*-OMe
b	H	*m*-OMe
c	OMe	H

compounds with dihydropyran in the presence of H_2SO_4 was successful. These ethers were allowed to react with 4-[2-(dimethylamino)ethoxy]phenylmagnesium bromide, followed by acid-catalyzed deprotection and dehydration, to furnish the phenolic analogues of 1 (Scheme I). Compound **6c** could not be separated from its geometric isomer by analytical or preparative TLC. Separation was done by fractional crystallization. In contrast, the components of the other two isomer mixtures differed substantially in chromatographic mobility. Thus, **6b** and **7b** could be separated on a small scale by preparative TLC, followed

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(1) V. R. Mukku, J. L. Kirkland, and G. M. Stancel, *Trends Pharmacol. Sci.*, **2**, 98 (1981).
(2) V. C. Jordan, C. J. Dix, K. E. Naylor, G. Prestwich, and L. Rowsby, *J. Toxicol. Environ. Health*, 4, 363 (1978).
(3) W. L. McGuire, P. P. Carbone, M. E. Sears, and G. C. Escher in "Estrogen Receptors and Human Breast Cancer", W. L. McGuire, P. P. Carbone, and E. P. Volmer, Eds., Raven Press, New York, 1976, pp 1–7.
(4) C. Fabian, L. Sternson, M. El-Serafi, L. Cain, and E. Hearne, *Cancer*, **48**, 876 (1981).
(5) R. L. Sutherland and L. C. Murphy, *Eur. J. Cancer*, **16**, 1141 (1980).
(6) B. S. Katzenellenbogen and E. R. Ferguson, *Endocrinology*, **97**, 1 (1975).
(7) K. B. Horwitz and W. L. McGuire, *J. Biol. Chem.*, **253**, 8185 (1978).
(8) (a) S. Ernst, G. Hite, J. S. Cantrell, A. Richardson, Jr., and H. D. Benson, *J. Pharm. Sci.*, **65**, 148 (1976). (b) J. R. Skidmore, A. L. Walpole, and J. Woodburn, *J. Endocrinol.*, **52**, 289 (1972).
(9) V. C. Jordan, B. Haldemann, and K. E. Allen, *Endocrinology*, **108**, 1353 (1981).
(10) T. Tatee, K. E. Carlson, J. A. Katzenellenbogen, D. W. Robertson, and B. S. Katzenellenbogen, *J. Med. Chem.*, **22**, 1509 (1979).
(11) T. S. Ruh, L. J. Baudendistel, W. F. Nicholson, and M. F. Ruh, *J. Steroid Biochem.*, 11, 315 (1979).
(12) L. J. Black and R. L. Goode, *Life Sci.*, **26**, 1453 (1980).
(13) V. C. Jordan, M. M. Collins, L. Rowsby, and G. Prestwich, *J. Endocrinol.*, **75**, 305 (1977).

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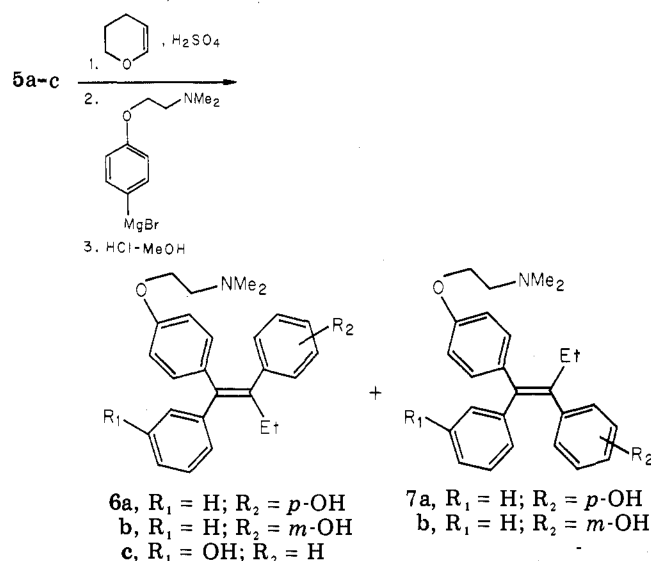
Table I. Characterization of Monophenolic α-Ethyldeoxybenzoins[a]

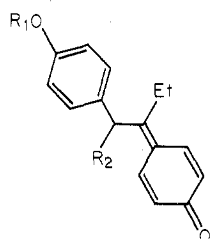
compd	R_1	R_2	yield, %	mp, °C	crystn solvent
5a	H	*p*-OH	82	82-85	cyclohexane
5b	H	*m*-OH	79	64-67	benzene-cyclohexane
5c	OH	H	72	93-95	cyclohexane

[a] The formula for each of these compounds is $C_{16}H_{16}O_2$.

Scheme I



by recrystallization of each isomer. Similar separation of **6a** and **7a** was not successful by this method, however, due to the tendency of these compounds to undergo reisomerization on TLC plates and during crystallization. This in part may have been due to formation, from either of these isomers, of tautomeric equilibrium partner **8a**.



8a, $R_1 = Me_2NCH_2CH_2$; R_2 = Ph
b, R_1 = H; R_2 = Et

Isomerization of *cis*- or *trans*-diethylstilbestrol has been suggested to occur via an analogous intermediate, **8b**.[14] Efficient separation of **6a** and **7a** (also **6b** and **7b**) was carried out by rapid preparative column chromatography.[15,16] Characterization of these compounds is summarized in Table II. ¹H NMR chemical-shift assignments, and thus configurational assignments of the compounds, were based on those reported for configurationally analogous triarylethylenes.[17-19]

Table II. Salient ¹H NMR Spectral and Chromatographic Features of Isomeric Hydroxytamoxifens **6** and **7**[a,b]

	6a	7a	6b	7b	6c
	¹H NMR Chem Shift, δ				
CCH_3 triplet	0.93	0.93	0.90	0.91	0.85
NCH_3	2.33	2.40	2.22	2.28	2.18
NCH_2 triplet	2.70	2.81	2.57	2.67	*c*
OCH_2 triplet	3.95	4.10	3.92	4.07	3.88
OH	5.47[d]	4.92[d]	*c*	*c*	*c*
	Chromatographic Data				
TLC: R_f	0.20	0.40	0.27	0.41	0.28
GLC: t_R,[e,f] min	5.7	6.9	4.7	5.7	5.0

[a] The CCH_2 quartet in these compounds was obscured by other peaks. [b] Spectra were recorded in $CDCl_3$ (**6a**, **7a**), 15% Me_2SO-d_6 in acetone-d_6 (**6b**, **7b**), and Me_2SO-d_6 (**6c**). [c] Unambiguous assignment was not possible. [d] Addition of D_2O resulted in disappearance of this broad peak. [e] Determined at 240 °C on a 0.125 in. × 5 ft. stainless-steel column packed with 3% OV-1 on 80-100 mesh Gas Chrom Q using nitrogen (30 mL/min) as carrier gas. [f] The retention time of **2** was 6.0 min.

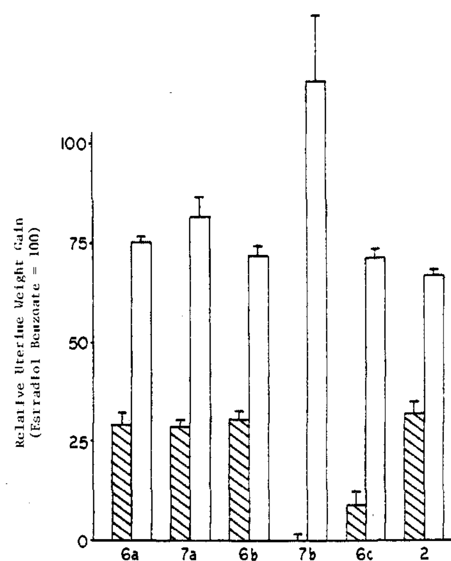


Figure 1. Uterotropic effect of the hydroxytamoxifens (50 μg daily) when administered without (▧) and simultaneously with (□) estradiol benzoate. Standard errors are indicated by brackets. The average uterine weight from animals receiving 0.5 μg per day of estradiol benzoate alone was 107 mg and that from animals receiving vehicle only was 25 mg.

Biological Results and Discussion. In Vivo Studies. The hydroxytamoxifens were assayed for uterotropic activity in the 3-day immature uterine weight test,[9,20] in comparison with estradiol benzoate. The ability of these compounds to antagonize estradiol benzoate induced uterine weight gain was determined similarly. Results are summarized relative to the effect of estradiol benzoate administered alone (Figure 1) and as a ratio of uterine weight to body weight (Table III). The ability of **6c** to antagonize uterine weight gain as a function of dose was

(14) V. W. Winkler, M. A. Nyman, and R. S. Egan, *Steroids*, 17, 197 (1971).
(15) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
(16) E. K. Fifer, personal communication.
(17) D. J. Collins and J. J. Hobbs, *Aust. J. Chem.*, **27**, 1545 (1974).
(18) D. J. Collins, J. J. Hobbs, and C. W. Emmens, *J. Med. Chem.*, 14, 952 (1971).
(19) K. E. Allen, E. R. Clark, and V. C. Jordan, *Br. J. Pharmacol.*, 71, 83 (1980).
(20) R. W. Hartmann, G. Kranzfelder, E. von Angerer, and H. Schönenberger, *J. Med. Chem.*, **23**, 841 (1980).

Table III. Estrogenic and Antiestrogenic Activity of the Hydroxytamoxifens in the 3-Day Rat Uterine Weight Gain Assay

compd	uterotropic effect[a,b]	antiuterotropic effect[a,c]	% inhibn[d]
6a	12.0 ± 0.4	16.5 ± 0.5	29.7
7a	11.0 ± 0.9	15.7 ± 0.7	34.3[e]
6b	10.1 ± 0.7	15.7 ± 0.8	34.3[e]
7b	5.7 ± 0.3	19.7 ± 1.8	11.0
6c	6.7 ± 0.5	16.7 ± 0.5	28.5
2	11.8 ± 0.7	15.2 ± 0.2	37.2[e]
estradiol benzoate	24.0 ± 2.0	21.6 ± 2.1	
control	5.6 ± 0.6	4.4 ± 0.1	

[a] Uterine wet weight (milligrams)/body weight (grams) × 100 (±SE). [b] Dose of hydroxytamoxifens = 50 μg per animal per day; dose of estradiol benzoate = 0.5 μg per animal per day. [c] Estradiol benzoate (0.5 μg per animal per day) and the test compound (50 μg per animal per day) administered simultaneously. [d] Percent inhibition = $100 - [100(E_{t,s} - E_v)/(E_s - E_v)]$; E_s = effect of estradiol benzoate; $E_{t,s}$ = effect of estradiol benzoate and the test compound administered simultaneously; E_v = effect of vehicle. [e] Significantly different from controls at $p < 0.05$.

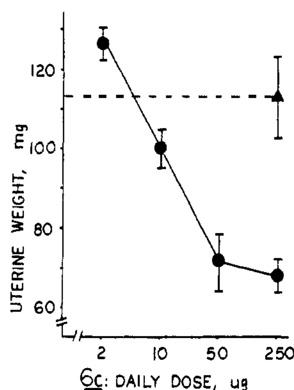


Figure 2. Uterotropic effect of estradiol benzoate (0.5 μg daily) administered alone (▲) and simultaneously with increasing doses of **6c** (●). Standard errors are indicated by brackets. The average uterine weight from animals receiving vehicle only was 25 mg.

determined as shown in Figure 2.

In Vitro Studies. The ability of the hydroxytamoxifens to compete with [^{3}H]estradiol for cytoplasmic estrogen receptors from mature rat uterus was determined by adaption of the dextran-coated charcoal adsorption technique.[13,21,23] Comparative data were also obtained for **1** and **2**. Salient results are summarized in Figure 3.

For a compound to have estrogenic and/or antiestrogenic activity, binding to cytosol estrogen receptors is thought to be a requirement.[6,21] As shown in Figure 3, none of the new hydroxytamoxifens were as strongly bound as was 4-hydroxytamoxifen (**2**), although affinity of the 3-hydroxy isomer of this compound (**6c**) approached that of estradiol. In the 3′- and 4′-hydroxytamoxifens, each of the trans isomers had greater affinity than either of the cis isomers. Since these studies were done at a single incubation temperature (4 °C), these comparisons must be interpreted with care, because binding affinity of triarylethylenes has been shown in some cases to vary differentially with incubation temperature.[23,24]

With the exception of **7b**, all of the hydroxytamoxifens had estrogenic and antiestrogenic activity (Figure 1). Corresponding data in Table III indicated that these effects were independent of overall weight gain. In related studies with dialkylstilbestrols and saturated analogues, activity was retained in compounds in which the hydroxy groups were moved to the 3- and 3′-positions.[20,25] In this study, compounds with the hydroxyl group in position 4′ (**6a**) or 3′ (**6b**) also had activities of similar magnitude. Moving the hydroxy group from position 4 (**2**) to position 3 (**6c**) resulted in a slight decrease in antiestrogenic activity, accompanied by a large decrease in estrogenic activity (Figure 1, Table III). Antiestrogenic activity of **6c** was maximal at a daily dose of 50 μg per animal (Figure 2). Thus, this compound is less potent than **2**, with which a 5 μg daily dose resulted in maximal activity in the same bioassay.[9]

As shown in Table III, at doses of 50 μg per animal per day, the 4′-hydroxytamoxifens (**6a** and **7a**) had about equal estrogenic and antiestrogenic effects, while at similar dose levels of the 3′-hydroxytamoxifens both activities were greater for the trans isomer (**6b**). The equivalent effects of **6a** and **7a** may have been due to isomerization via **8**, in vivo. To determine whether isomerization was occuring, we attempted to reisolate **7a** (**6a**) from uteri of animals which received **7a**. Accumulation of phenolic metabolites of radiolabeled triarylethylene antiestrogens in uterine tissues of rats given these compounds has been reported.[26,27] However, neither **7a** nor **6a** was detectable in thin-layer chromatograms of uterine extracts. This indicated the need for more sensitive analytical conditions.

Biological activity of **1**, as well as several other triarylethylene antiestrogens, has been suggested to be mediated in part through phenolic metabolites.[21,22,26,27] Thus, administration of **1** resulted in the accumulation of **2**, as well as another hydroxylated metabolite for which structural characterization was incomplete, in tissues containing estrogen receptors.[27] The isomers of **2** described in this paper may aid in elucidation of the structure of this and other possible phenolic metabolites of **1** and thus also may aid in clarifying the way in which **1** exerts its biological effects.

Experimental Section

Melting points are uncorrected. All of the new compounds gave elemental analysis data within ±0.3% of calculated values, as determined by Atlantic Microlab, Inc., Atlanta, GA. Reaction progress and purity of products were checked by analytical TLC using 5 × 20 cm silica gel GF_{254} plates (0.25 mm thickness), with benzene–triethylamine (9:1, v/v) as developing solvent. Developed plates were viewed under light of 254-nm wavelength. ^{1}H NMR spectra (60 MHz) were run in $CDCl_3$ unless otherwise indicated, using tetramethylsilane as internal standard. Reactions involving air-sensitive materials were carried out under dry nitrogen.

Starting Materials. Compounds **1** and **2** were obtained as gifts from Stuart Pharmaceuticals Division of ICI Americas, Wilmington, DE. Compound **3a** was prepared by reaction of *p*-methoxyphenylacetyl chloride[28] with benzene in 86% yield: mp 93.5–94 °C (lit.[28] 96 °C); **3b** was similarly prepared from *m*-methoxyphenylacetyl chloride[29] (76%): bp 137–140 °C (0.20

(21) E. R. Ferguson and B. S. Katzenellenbogen, *Endocrinology*, **100**, 1242 (1977).
(22) J.-L. Borgna and H. Rochefort, *Mol. Cell. Endocrinol.*, **20**, 71 (1980).
(23) A. E. Wakeling and S. R. Slater, *Cancer Treat. Rep.*, **64**, 741 (1980).
(24) L. J. Black, C. D. Jones, and R. L. Goode, *Mol. Cell. Endocrinol.*, **22**, 95 (1981).
(25) M. Schneider, E. von Angerer, G. Kranzfelder, and H. Schönenberger, *Arch. Pharm. (Weinheim, Ger.)*, **313**, 919 (1980).
(26) B. S. Katzenellenbogen, E. J. Pavlik, D. W. Robertson, and J. A. Katzenellenbogen, *J. Biol. Chem.*, **256**, 2908 (1981).
(27) J.-L. Borgna and H. Rochefort, *J. Biol. Chem.*, **256**, 859 (1981).
(28) G. Drefahl, M. Hartmann, and H. Grosspietsch, *Chem. Ber.*, **91**, 755 (1958).

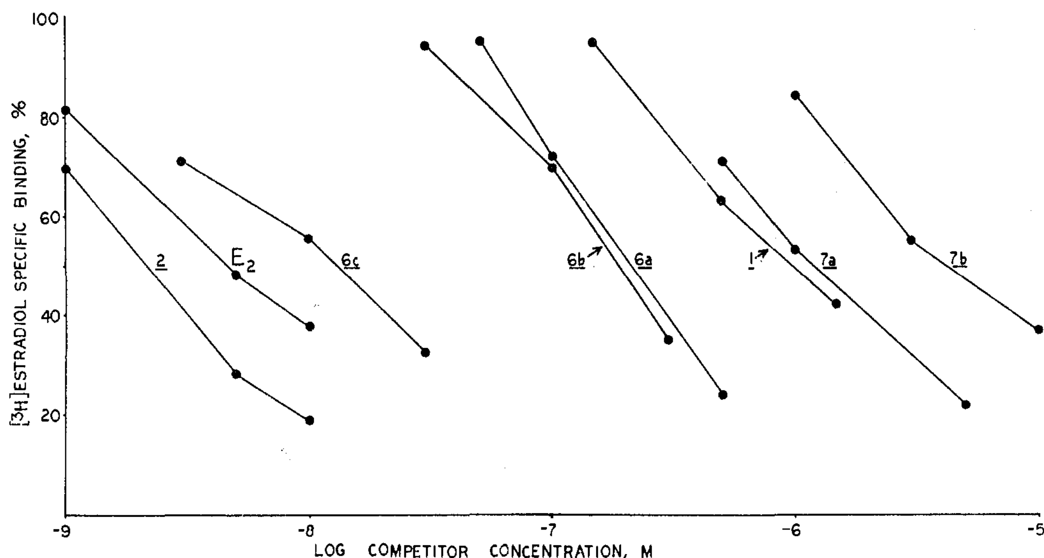


Figure 3. Effect of increasing concentrations of **1**, **2**, **6a–c**, **7a,b**, and estradiol (E_2) on the binding of ^{3}[H]estradiol to uterine cytosol from mature rats. Specifically bound radioactivity (disintegrations per minute) is plotted as a percentage of that in control incubations.

mmHg); ^{1}H NMR ($CDCl_3$) δ 3.52 (s, 3, OCH_3), 4.07 (s, 2, CH_2), 6.54–8.05 (m, 9, aromatic). The semicarbazone of **3b** separated from methanol as white crystals, mp 162–164.5 °C. Anal. ($C_{16}H_{17}N_3O_2$) C, H, N. Compound **3c** was prepared in 82% yield by reaction[30] of *m*-methoxybenzonitrile and benzylmagnesium bromide: bp 140–154 °C (0.2 mmHg) [lit.[30] 169–170 °C (3 mmHg)]. Compounds **4a–c** were in turm prepared from **3a–c** by reaction with NaH in DMF, followed by alkylation with ethyl iodide. **4a**: yield 92%, mp 38 °C (lit.[18] mp 38–39 °C). **4b**: yield 85%; bp 130–136 °C (0.20 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 8 Hz, 3, CCH_3), 1.70–2.50 (m, 2, CH_2), 3.58 (s, 3, OCH_3), 4.40 (t, J = 8 Hz, 1, CH), 6.48–8.11 (m, 9, aromatic). The oxime of **4b** was crystallized from methanol, mp 124–126 °C. Anal. ($C_{17}H_{19}NO_2$) C, H, N. Compound **4c**: yield 79%; bp 132–140 °C (0.05 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 8 Hz, 3, CCH_3), 1.57–2.52 (m, 2, CH_2), 3.66 (s, 3, OCH_3), 4.40 (t, J = 8 Hz, 1, CH), 6.51–7.68 (m, 9, aromatic). The oxime of **4c** crystallized from methanol–water, mp 94–95.5 °C. Anal. ($C_{17}H_{19}NO_2$) C, H, N.

Preparation of 5a–c. General Procedure. A solution of **4** (20 mmol) in 100 mL of acetic acid and 5.6 mL of 48% HBr (50 mmol) was stirred and refluxed for 4 h (**4c** required 9 h). After solvent was removed in vacuo, the residue was neutralized with aqueous $NaHCO_3$. The mixture was extracted with 200 mL of ether. The organic phase was washed with two 100-mL portions of water, dried (Na_2SO_4), and concentrated in vacuo. Compound **5b** was purified by distillation: bp 160–163 °C (0.30 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 8 Hz, 3, CCH_3), 1.64–2.34 (m, 2, CH_2), 4.40 (t, J = 8 Hz, 1, CH), 6.61–7.75 (m, 7, aromatic), 7.92 (dd, J = 8 and 2 Hz, 2, aromatic H ortho to C=O). Trituration of crude **5a** or **5c** with petroleum ether (100 mL) afforded amorphous solids with ^{1}H NMR spectra similar to that of **5b**. They were used without further purification. Analytical samples of **5a–c** were prepared and characterized as summarized in Table I.

Tetrahydropyranyl Ethers of 5a–c. To a solution of 4.8 g (20 mmol) of the phenol in 10 mL of dihydropyran was added a trace of 98% H_2SO_4. The mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with 100 mL of benzene and washed successively with 50 mL of 10% Na_2CO_3 and three 50-mL portions of water. The solution was dried (Na_2CO_3) and concentrated in vacuo. The THP ethers of **5a,b** were isolated as white powders after stirring with petroleum ether. The THP ether of **5c** would not solidify upon similar treatment. Infrared spectra of these compounds had no OH absorption.

4-[2-(Dimethylamino)ethoxy]bromobenzene. A 50% mineral oil dispersion of sodium hydride (11.5 g, 0.24 g-atom) was washed with two 50-mL portions of hexane. To this was added in portions a suspension of 18.6 g (0.129 mol) of (dimethylamino)ethyl chloride hydrochloride in 100 mL of dry DMF containing 20 g (0.116 mol) of *p*-bromophenol. The stirred reaction mixture was cooled in ice during the addition. Then the mixture was heated to 85–90 °C for 3 h, during which time an additional 80 mL of DMF was added. The mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo. The residue was dissolved in methanol and reconcentrated. The residue was dissolved in 200 mL of ether and extracted successively with 100 mL of 10% aqueous NaOH and three 100-mL portions of water. The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The product was obtained (85%) by distillation: bp 82–84 °C (0.10 mmHg) [lit.[31] bp 147–155 °C (15 mmHg)].

Preparation of Isomeric Hydroxytamoxifens (6 and 7). The synthesis and purification of **6a** and **7a** are typical. To a stirred mixture of Mg turnings (0.16 g, 6.6 mg-atoms) in 2 mL of THF were added a few drops each of ethyl bromide and ethylene bromide, plus an iodine crystal. Within a few minutes a mild exothermic reacton ensued. Then a solution of 1.4 g (5.5 mmol) of 4-[2-(dimethylamino)ethoxy]bromobenzene in 5 mL of THF (containing a few drops of the alkyl bromides) was added at such a rate as to maintain an intense reaction. After completion of addition, the reaction mixture was refluxed for 1 h and cooled to room temperature, and a solution of 1.5 g (4.6 mmol) of the THP ether of **5a** in 7 mL of THF was added dropwise. After addition, the mixture was refluxed for 2 h and cooled, and 0.84 mL of saturated aqueous NH_4Cl was added. The suspension was stirred for 30 min. A paste was made of this by addition of a few grams of Super Cel. This was poured onto a pad of Super Cel and washed well with THF. The filtrate was concentrated in vacuo to give a golden oil. This was dissolved in 5 mL of methanol, and 6 mL of 4 N HCl was added. After stirring for 30 min, the mixture was concentrated and partitioned between 50 mL of 10% aqueous HCl and 50 mL of ether. The aqueous phase was extracted with a second 50-mL portion of ether. The ether extracts were discarded. The aqueous phase was extracted with three 50-mL portions of chloroform–methanol (4:1, v/v). The organic extracts were combined, dried (Na_2SO_4), and concentrated in vacuo to give 1.2 g of the hydrochloride salt. This was dissolved in 30 mL of methanol and filtered, and the solution was alkalinized (pH 9) with 5% aqueous $NaHCO_3$. After concentrating in vacuo, 70 mL of methylene chloride was added, and the mixture was heated at 70 °C for 15 min. After cooling, the organic phase was washed with three 30-mL portions of water, dried (Na_2SO_4), and concentrated in vacuo to give a brown glass, which was triturated

(29) W. Wiegrebe, D. Sasse, H. Reinhart, and L. Faber, *Z. Naturforsch. B*, **25**, 1408 (1970).
(30) M. Ikeda, K. Hirao, Y. Okuno, N. Numao, and O. Yonemitsu, *Tetrahedron*, **33**, 489 (1977).
(31) L. Novák and M. Protiva, *Collect. Czech. Chem. Commun.*, **30**, 3752 (1965).

with petroleum ether to give 0.6 g (29%) of a mixture of **6a** and **7a**. Mixtures of **6b** and **7b**, as well as **6c** and its geometric isomer, were prepared in the same manner in respective yields of 42 and 38%.

Rapid Preparative Chromatographic Purification of 6 and 7. A mixture of 576 mg of **6a** and **7a** was dissolved in 5 mL of benzene-triethylamine (12:1, v/v) and chromatographed on 60 g of silica gel (Brinkmann, 0.040-0.063 mm). Elution with 3 L of the above solvent (75 mL/min), followed by concentration of the eluent, gave a white solid, which was suspended in benzene and filtered to give 274 mg of **7a**: mp 132-134 °C. Further elution of the column with 1 L of benzene-triethylamine-methanol (12:1:3, v/v), at 75 mL/min, followed by workup as before, gave 272 mg of **6a**, mp 144-149 °C. Anal. ($C_{26}H_{29}NO_2$) C, H, N for each isomer.

Similarly, 1 g of a mixture of **6b** and **7b** was chromatographed on 60 g of silica gel using benzene-triethylamine (15:1, v/v), followed by benzene-acetone-triethylamine (15:10:1, v/v) as eluting solvents. This gave 160 mg of **7b**, which was recrystallized from benzene-hexane: mp 153.5-155 °C; the more polar isomer, **6b** (396 mg), was recrystallized from benzene, mp 166-167.5 °C. Anal. ($C_{26}H_{29}NO_2$) C, H, N for each isomer.

Chromatography of 1.31 g of impure **6c** using benzene-triethylamine (10:1, v/v) as eluting solvent furnished 1.1 g of a white solid, which was crystallized from benzene-hexane to give 388 mg of **6c** as white crystals, mp 160-162 °C. Anal. ($C_{26}H_{29}NO_2$) C, H, N.

Receptor Binding Assay. The [^{3}H]estradiol (58 Ci/mmol) used in this assay was obtained from Amersham Corp.; radiochemical purity was checked by TLC. Uteri from Sprague-Dawley rats (200-250 g) were homogenized (1 uterus/2 mL) in ice-cold 10 mM Tris buffer, pH 7.4, which contained 1.5 mM EDTA and 3 mM sodium azide (TEA buffer). The homogenate was centrifuged at 100000*g* for 1 h at 4 °C. Incubation mixtures contained 200-μL aliquots of the supernatant, 10 μL of a solution of 1.1 × 10^{-7} M [^{3}H]estradiol in dimethylacetamide, and 10 μL of unlabeled competitor in 1:1 dimethylacetamide-TEA buffer. Ten concentrations of competitor were used ranging from 1 × 10^{-9} to 5 × 10^{-5} M. Control incubations contained 10 μL of solvent alone, and nonspecific binding was determined in similarly prepared incubations which contained 1 × 10^{-5} M estradiol. Incubations were performed in triplicate, in 5-mL polypropylene centrifuge tubes, at 2-4 °C for 4 h. Then a suspension of 400 μL of dextran-coated charcoal [0.1% dextran (Sigma no. D-1390), 1% acid-washed Norit A in TEA buffer] was added, and the incubation was continued for 15 min at 2-4 °C. Tubes were then centrifuged at 1000*g* for 10 min, and 400-μL aliquots were dissolved in 5 mL of Scintiverse (Fisher). Bound [^{3}H]estradiol was determined by liquid scintillation spectrometry. Quench corrections were made by the external standard method.

Uterotropic Assay for Estrogenic Activity. Immature Wistar female rats (21 days old, 25-35 g) were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, IN. They were divided randomly into groups of at least six animals. To 0.1-mL aliquots of fresh solutions of estradiol benzoate (0.25 mg/mL) and of each of the hydroxytamoxifens (25 mg/mL) in dimethylacetamide was added 5 mL of peanut oil. The resulting solutions (0.1 mL) were administered sc once daily for 3 days. Control animals received vehicle alone. On the 4th day, the animals were killed by decapitation. The uteri were dissected, and fat and connective tissue were removed. After blotting lightly to remove intraluminal fluid, uteri were weighed to the nearest 0.1 mg. Body weights were also recorded.

Uterotropic Assay for Antiestrogenic Activity. This was carried out exactly as described above, except that animals receiving the test compounds also received 0.5 μg/0.1 mL of estradiol benzoate, administered separately at different injection sites. One group of control animals received 0.5 μg of estradiol benzoate and vehicle; the other received two injections of vehicle.

Stability of Peanut Oil Solutions of the Hydroxytamoxifens. Several of the solutions prepared as described above were analyzed periodically by ultraviolet spectrometry (300-400 nm) to make sure the triarylethylenes were not being adsorbed on the glass surfaces of the containers in which they were kept. No changes in absorption intensities were seen. For example, those of **6a** (λ_{max} 306 nm, log ε 3.96) and **7a** (λ_{max} 305 nm, log ε 3.97) did not vary over a period of 7 days.

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Carboxyimamidate, a Low-Molecular-Weight Polyelectrolyte with Antitumor Properties and Low Toxicity

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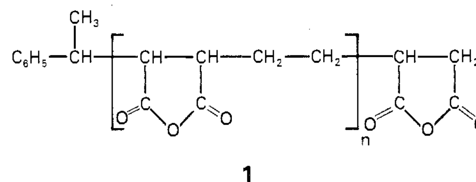
A new polyelectrolyte was synthesized and evaluated for antitumor activity. The product is a derivative of ethylene/maleic anhydride copolymer of low molecular weight ($M_n \approx 1100$). The anhydride groups were first converted to the half-amide, half-ammonium salt by reaction with ammonia. A percentage (14-25 wt %) of these groups was further converted to the imide by heating. The product, carboxyimamidate (Carbethimer, N-137) inhibited the growth of a number of solid tumors in vivo. Sensitive tumor models included Lewis lung carcinoma, Madison 109 lung carcinoma, M5076 ovarian tumor, colon carcinoma 26, B16 melanoma, and P815 mastocytoma. Activity was dose related between nontoxic dose levels of 300 and 2000 mg/kg ip.

Strong acid polyanionic polymers of either natural or synthetic origin, such as polysulfates, polysulfonates, and polyphosphates, have been reported to be growth inhibitors of transplanted tumors in mice.[1-3] Similar inhibition of tumor growth has been demonstrated for weak acid carboxy-containing polyelectrolytes derived from polyacrylic acid or ethylene/maleic anhydride copolymer (EMA) **1**.[4] These studies compared acute toxicity and inhibition activity of several polymer series over a broad molecular



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weight range (2000-100 000). The effects of total charge density, charge distribution, and carboxylic acid strength

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(1) Regelson, W.; Holland, J. F. *Nature (London)* **1958**, *181*, 46.
(2) Balazs, A.; Holmgren, H. S. *Proc. Soc. Exp. Biol. Med.* **1949**, *72*, 142.

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