H, N. The diester (1.29 g, 2.7 mmol) was added at 0 °C to 15 mL of neat TFA. After 1.5 h at 0 °C and then 2 h at room temperature, the TFA was removed in vacuo, Et_2O was added, and the mixture was evaporated in vacuo (two times). The residual oil was dissolved in saturated aqueous $NaHCO_3$ solution and washed with CH_2Cl_2. The aqueous layer was separated, acidified with 1 N HCl, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was separated, dried over $MgSO_4$, filtered, and evaporated to a yellow oil. The oil was chromatographed on silica gel and eluted with 13% CH_3OH–$CHCl_3$ to give 0.62 g (54%) of 13 as a viscous oil. IR (neat, NaCl) 2981, 1740, 1652, 1320, 1159, 1134 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 7.17 (m, 5 H, Ar H), 3.95–4.80 (m, 5 H, 3 CH, OCH_2), 3.64 (m, 2 H, NCH_2), 3.48 (m, 2 H, CH_2), 1.7–2.8 (m, 6 H, 3 CH_2), 1.46 (dd, 3 H, CH_2), 1.23 (dt, 3 H, CH_3). Anal. ($C_{20}H_{27}NO_7S$) C, H, N.

Method F. 1-[2-[(1-Carboxy-3-phenylpropyl)sulfonyl]-1-oxopropyl]-L-proline (14). A solution of **13** (0.46 g, 1.1 mmol) in 0.25 N methanolic NaOH was allowed to stand at room temperature overnight. Water (3 mL) was added and the reaction allowed to proceed an additional night. The mixture was concentrated in vacuo and 1 N HCl was added until a precipitate formed. The aqueous solution was extracted with EtOAc. The EtOAc layer was separated, dried over $MgSO_4$, filtered, and evaporated to give 0.38 g (87%) of the diacid 14 as a fluffy white solid. IR (KBr) 2947, 1743, 1625, 1160, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.46 (s, 2 H, OH), 7.18 (br s, 5 H, Ar H), 3.3–4.6 (m, 5 H, 3 CH, NCH_2), 1.8–3.0 (m, 8 H, 4 CH_2), 1.63 (br d, 3 H, CH_3). Anal. ($C_{18}H_{23}NO_7S$) C, H, N.

Acknowledgment. We thank F. A. MacKellar, E. Schoeb, R. B. Scott, and S. Uhlendorf for the physical measurements and V. Brown and G. Sekerak for the microanalyses.

Registry No. 1, 56777-24-3; 2, 59854-13-6; 3, 96965-37-6; 4, 96965-38-7; 5, 96965-39-8; 6, 96965-40-1; 7a (isomer 1), 96965-41-2; 7a (isomer 2), 97058-35-0; 7a (R^1 = *t*-Bu, isomer 1), 96965-53-6; 7a (R^1 = *t*-Bu, isomer 2), 97058-38-3; 7b (isomer 1), 96965-42-3; 7b (isomer 2), 97058-36-1; 7c (isomer 1), 97059-08-0; 7c (isomer 2), 96965-59-2; 8a, 96965-43-4; 8b, 96965-44-5; 8c, 96965-45-6; 8d, 96965-46-7; 8e (R^1 = Et), 96965-55-8; 8f (R^1 = Et), 96965-56-9; 9a (isomer 1), 96965-47-8; 9a (isomer 2), 97059-09-1; 9b (isomer 1), 96965-48-9; 9b (isomer 2), 97058-37-2; 10a, 79625-83-5; 10b, 96998-96-8; 10c, 96965-49-0; 10d, 96965-50-3; 11, 96998-97-9; 12, 96965-57-0; 13, 96965-51-4; 13 (prolyl *tert*-butyl ester), 96965-58-1; 14, 96965-52-5; *AA-OR^1 (*AA = A, R^1 = *t*-Bu), 2812-46-6; *AA-OR^1 (*AA = C, R^1 = *t*-Bu), 80876-00-2; *AA-OR_1 (*AA = D, R^1 = *t*-Bu), 77497-74-6; *AA-OR^1 (*AA = B, R^1 = *t*-Bu), 96965-54-7; ACE, 9015-82-1; (±)-ethyl α-bromobenzenebutanoate, 80828-27-9.

Ring-Substituted 1,1,2,2-Tetraalkylated 1,2-Bis(hydroxyphenyl)ethanes. 4. Synthesis, Estrogen Receptor Binding Affinity, and Evaluation of Antiestrogenic and Mammary Tumor Inhibiting Activity of Symmetrically Disubstituted 1,1,2,2-Tetramethyl-1,2-bis(hydroxyphenyl)ethanes

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The syntheses of symmetrically 2,2′-disubstituted derivatives of 1,1,2,2-tetramethyl-1,2-bis(4-hydroxyphenyl)ethane (1) and of 5,5′-, and 6,6′-disubstituted derivatives of 1,1,2,2-tetramethyl-1,2-bis(3-hydroxyphenyl)ethane (6) are described (1 and 6 are strong antiestrogens with mammary tumor inhibiting activity exhibiting only slight estrogenic properties): (2,2′-substituents) F (2), Cl (3), OCH_3 (4), CH_3 (5); (5,5′-substituents) Cl (7); (6,6′-substituents) F (8), Cl (9), OCH_3 (10), CH_3 (11). The synthesis of 1–11 was accomplished by reductive coupling of the corresponding 2-phenyl-2-propanols with $TiCl_3$ and $LiAlH_4$. The binding affinity of the compounds to the calf uterine estrogen receptor was measured relative to that of [^{3}H]estradiol by a competitive binding assay. With the exception of 7 and 10 all other compounds showed relative binding affinity (RBA) values between 0.5 and 6.4% that of estradiol, 2 (RBA value 6.4), and 8 and 9 (4.0 and 3.5), exceeding those of the corresponding unsubstituted 1 and 6 (3.6 and 3.0). Compounds exhibiting RBA values of >2.5% were evaluated in the mouse uterine weight test. The substituted derivatives showed an increase in uterotrophic and a decrease in antiuterotrophic activity compared to 1 and 6. Compound 2 showed a strong, dose-dependent inhibition on the DMBA-induced hormone-dependent mammary tumor of the SD-rat, exceeding that of the parent compound 1. At a dose of 5 mg/kg per day, 2 reduced total tumor area by 47% and caused a complete remission in 74% of the tumors.

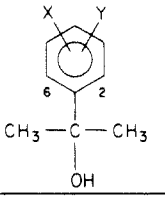
In the search for new structures of mammary tumor inhibiting antiestrogens, modifications on the synthetic estrogen hexestrol were performed. Displacement of the phenolic OH groups,[1] variation of the alkyl chains in the 1,2-positions,[2] and tetraalkylation in the 1,1,2,2-positions of the 1,2-diphenylethane skeleton[3] led to a number of active compounds. The most effective representatives are metahexestrol, metabutestrol, tetramethylHES (1), and metatetramethylHES (6) (Chart I). The tetramethylated 1,2-diphenylethanes exhibited the strongest antiestrogenic activity.[3] In contrast to metahexestrol they showed only slight[3] or no[4] estrogenic properties, depending on the test system. Compounds 1 and 6 are of great interest for the treatment of hormone-dependent breast cancer, for they showed marked inhibitory activity on the established DMBA-induced mammary carcinoma of the SD-rat.[3]

In contrast to the partial antiestrogens metahexestrol and tamoxifen (Nolvadex), tetramethylHES (1) and metatetramethylHES (6) seem to unfold their mammary tumor inhibiting activity by means of their antiestrogenic potency. They antagonized the tumor growth stimulating

(1) Kranzfelder, G.; Hartmann, R. W.; von Angerer, E.; Schönenberger, H.; Bogden, A. E. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 165.
(2) Hartmann, R. W.; Buchborn, H.; Kranzfelder, G.; Schönenberger, H.; Bogden, A. E. *J. Med. Chem.* **1981**, *24*, 1192.
(3) Hartmann, R. W.; Kranzfelder, G.; von Angerer, E.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 841.
(4) Gschwendt, M.; Rincke, G.; Schuster, T. *Mol. Cell. Endocrinol.* **1982**, *26*, 231.

0022-2623/85/1828-1295$01.50/0

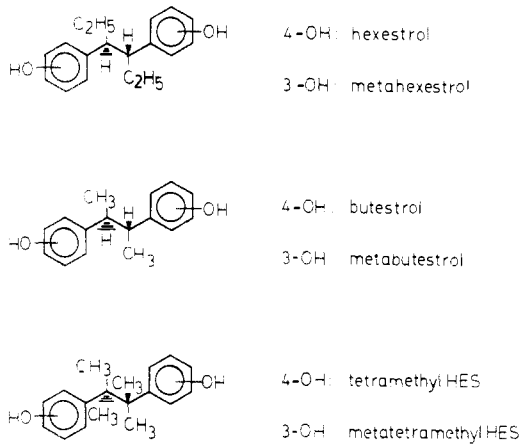
Table I. Substituted 2-Phenyl-2-propanols



compd	X	Y	synth method[a]	yield,[b] %	mp, °C	formula
1b[c]	H	4-OCH_3	A	74	oil	$C_{10}H_{14}O_2$
2b	2-F	4-OCH_3	A	81	oil	$C_{10}H_{13}FO_2$
3b	2-Cl	4-OCH_3	A	83	yellow oil	$C_{10}H_{13}ClO_2$
4b	2-OCH_3	4-OCH_3	A	76	yellow oil	$C_{11}H_{16}O_3$
5b	2-CH_3	4-OCH_3	A	73	oil	$C_{11}H_{16}O_2$
6b[c]	H	3-OCH_3	A	70	34	$C_{10}H_{14}O_2$
7b	5-Cl	3-OCH_3	A	88	yellow oil	$C_{10}H_{13}ClO_2$
8b	6-F	3-OCH_3	A	79	oil	$C_{10}H_{13}FO_2$
9b	6-Cl	3-OCH_3	A	89	87–88	$C_{10}H_{13}ClO_2$
10b	6-OCH_3	3-OCH_3	A	86	oil	$C_{11}H_{16}O_3$
11b	6-CH_3	3-OCH_3	A	80	yellow oil	$C_{11}H_{16}O_2$

[a] A refers to synthetic method A under the Experimental Section. [b] Yield of analytically pure (TLC) product; no effort made to optimize yields. [c] See ref 3.

Chart I



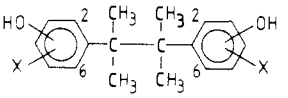
and inhibiting effects of the synthetic estrogen diethylstilbestrol on DMBA tumor bearing, ovariectomized rats, whereas the partial antiestrogens increased both effects.[5,6]

A further enhancement of the antitumor activity of the 1,1,2,2-tetramethyl-1,2-diphenylethanes **1** and **6** could be realized by synthesizing derivatives with a higher affinity for the estradiol receptor (E_2R).

This paper is the fourth part of an extensive structure–activity study dealing with the influence of a symmetrical substitution of the two aromatic rings of 1,2-dialkylated or 1,1,2,2-tetraalkylated 1,2-bis(hydroxyphenyl)ethanes on E_2R binding affinity and estrogenic, antiestrogenic, and mammary tumor inhibiting properties. In the preceding publications the effects of ring substituents on the biological activity of hexestrol,[7] metahexestrol,[8] butestrol, and metabutestrol[9] have been described.

In these studies we were able to show that it is possible to enhance the E_2R binding affinity of the 1,2-diphenylethanes by appropriate substitution,[7-9] provided that the substituents are in ortho position to the ethane bridge.[7,8] But, the increase of binding affinity for the E_2R often was accompanied by an enhancement of the estrogenic properties as well.[8,9] A correlation of E_2R binding affinity and estrogenic activity, however, was not found.

Chart II



compd	X (4-OH)	compd	X (3-OH)
1	H	6	H
2	2-F	7	5-Cl
3	2-Cl	8	6-F
4	2-OCH_3	9	6-Cl
5	2-CH_3	10	6-OCH_3
		11	6-CH_3

In this paper the syntheses, the determination of the E_2R affinities, and the evaluation of estrogenic, antiestrogenic, and mammary tumor inhibiting properties[10] of 2,2′-disubstituted tetramethylHES [1,1,2,2-tetramethyl-1,2-bis(4-hydroxyphenyl)ethane] compounds and 5,5′- and 6,6′-disubstituted metatetramethylHES [1,1,2,2-tetramethyl-1,2-bis(3-hydroxyphenyl)ethane] derivatives will be described.

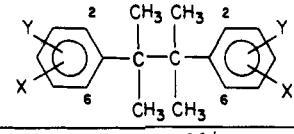
Chemistry. The synthesis of compounds 1–11 (Table II) was accomplished by coupling the correspondingly substituted 2-(methoxyphenyl)-2-propanols **1b–11b** (Table I) using $TiCl_3/LiAlH_4$ according to the method of McMurry andd Silvestri[11] and subsequent ether cleavage of compounds **1a–11a** (methods B and C, Scheme I).

The 5- and 6-substituted 2-(3-methoxyphenyl)-2-propanols **7b–11b** were synthesized like **6b**[3] by Grignard reaction of the corresponding methyl esters **6c–11c** with CH_3MgI (method A, Scheme I, Table I). Compounds **7c–11c** were obtained by esterification of the benzoic acids, which were prepared as previously described.[8]

The synthesis of the 2-substituted 2-(4-methoxyphenyl)-2-propanols **2b–5b** was accomplished like the one of **1b**[3] by Grignard reaction of the acetophenones **1c–5c**

(5) Hartmann, R. W. *Eur. J. Cancer Clin. Oncol.* **1983**, *19*, 959.
(6) For a recent review of the pharmacology and mode of action of tetramethylHES see: Hartmann, R. W. *Drugs Future* **1985**, *10*, 48.
(7) Hartmann, R. W.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.* **1983**, *26*, 1137.
(8) Hartmann, R. W.; Heindl, A.; Schönenberger, H. *J. Med. Chem.* **1984**, *27*, 577.
(9) Hartmann, R. W.; Heindl, A.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.* **1984**, *27*, 819.
(10) These experiments are part of our basic screening procedure for the development of mammary tumor inhibiting antiestrogens: see ref 15.
(11) McMurry, J. E.; Silvestri, M. *J. Org. Chem.* **1975**, *40*, 2678.

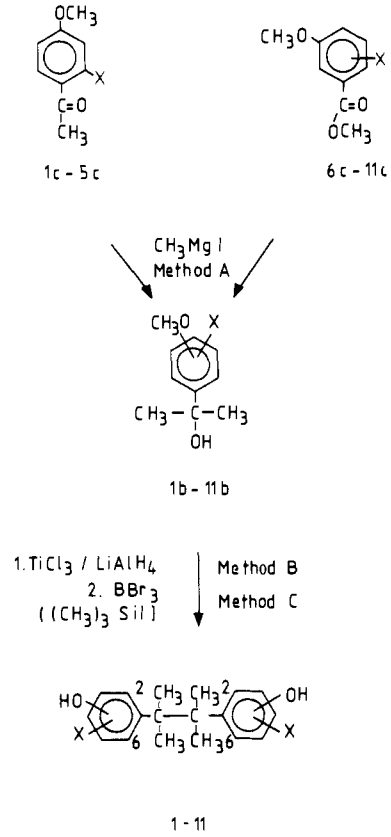
Table II. Substituted 2,3-Dimethyl-2,3-diphenylbutanes



compd	X	Y	synth method[a]	yield,[b] %	mp, °C	recryst solvent[c]	formula[d]
1a[e]	H	4-OCH_3	B	60	184	F	$C_{20}H_{26}O_2$
1[e]	H	4-OH	C	89	210	G	$C_{18}H_{22}O_2$
2a	2-F	4-OCH_3	B	40	158–159	F	$C_{20}H_{24}F_2O_2$
2	2-F	4-OH	C	88	168–169	H	$C_{18}H_{20}F_2O_2$
3a	2-Cl	4-OCH_3	B	36	137–138	E	$C_{20}H_{24}Cl_2O_2$
3	2-Cl	4-OH	C	78	170–172	H	$C_{18}H_{20}Cl_2O_2$
4a	2-OCH_3	4-OCH_3	B	43	115–116	E	$C_{22}H_{30}O_4$
4	2-OCH_3	4-OH	C	38	184–185	D	$C_{20}H_{26}O_4$
5a	2-CH_3	4-OCH_3	B	35	105–106	E	$C_{22}H_{30}O_2$
5	2-CH_3	4-OH	C	83	190–192	H	$C_{20}H_{26}O_2$
6a[e]	H	3-OCH_3	B	80	116	F	$C_{20}H_{26}O_2$
6[e]	H	3-OH	C	91	200	G	$C_{18}H_{22}O_2$
7a	5-Cl	3-OCH_3	B	66	141–142	F	$C_{20}H_{24}Cl_2O_2$
7	5-Cl	3-OH	C	73	218–219	H	$C_{18}H_{20}Cl_2O_2$
8a	6-F	3-OCH_3	B	51	130–131	F	$C_{20}H_{24}F_2O_2$
8	6-F	3-OH	C	79	167	H	$C_{18}H_{20}F_2O_2$
9a	6-Cl	3-OCH_3	B	38	134	F	$C_{20}H_{24}Cl_2O_2$
9	6-Cl	3-OH	C	76	168	H	$C_{18}H_{20}Cl_2O_2$
10a	6-OCH_3	3-OCH_3	B	39	158	E	$C_{22}H_{30}O_4$
10	6-OCH_3	3-OH	C	31	229	D	$C_{20}H_{26}O_4$
11a	6-CH_3	3-OCH_3	B	35	72	E	$C_{22}H_{30}O_2$
11	6-CH_3	3-OH	C	77	161	H	$C_{20}H_{26}O_2$

[a] Capital letters refer to synthetic methods B and C under the Experimental Section. [b] Yield of analytically pure product; no effort made to optimize yields. [c] D = EtOH/H_2O; E = MeOH; F = toluene/ligroin; G = 80% acetic acid; H = benzene. [d] All compounds were analyzed for C and H within ±0.40% of the calculated values. [e] See ref 3.

Scheme I



with CH_3MgI (method A, Scheme I, Table I). Compounds **2c–5c** were obtained by Friedel–Crafts acetylation of the correspondingly 3-substituted anisols with acetic anhydride and $AlCl_3$ in CS_2.

The ether cleavage of compounds **1a–3a**, **5a–9a**, and **11a** (Table II) was successfully performed with BBr_3 (method C, Scheme I). But, this procedure as well as a further method using EtSH/NaH in DMF[12] failed with the tetramethoxy compounds **4a** and **10a**.[13] The cleavage with $(CH_3)_3SiI$ in acetonitrile according to the method of Olah et al.[14] selectively produced the 4,4′-phenol. The methoxy groups standing in the ortho positions to the ethane bridge were not attacked by this voluminous agent, as was proved by ^1H NMR: The signals of the OCH_3 substituents standing in the 2,2′- or 6,6′-positions, which are shifted upfield compared to those standing in the 3,3′- or 4,4′-positions, are still present in compounds **4** and **10** (Table II).

Table III. Relative Binding Affinity (RBA) of Compounds 1–11 for Calf Uterine Estrogen Receptor

compd	RBA value[a]	compd	RBA value[a]
1	3.6	**6**	3.0
2	6.4	**7**	0.01
3	3.4	**8**	4.0
4	0.45	**9**	3.5
5	2.6	**10**	<0.01
		11	0.9

[a] Relative binding affinity for the calf uterine estrogen receptor = ratio of molar concentrations of 17β-estradiol (E_2) and inhibitor required to decrease the amount of bound [^{3}H]E_2 by 50% × 100.

Biological Properties. The biological experiments presented in the following paragraphs are part of our standard screening procedure for the development of mammary tumor inhibiting antiestrogens.[15]

The relative binding affinity (RBA) for the E_2R of compounds 1–11 was determined by using a competitive binding assay with calf uterine cytosol, 17β-[^{3}H]estradiol,

(12) Feutrill, G. I.; Mirrington, R. N. *Aust. J. Chem.* **1972**, *25*, 1719.
(13) In the case of the BBr_3 method the 2-(dihydroxyphenyl)-propanes were obtained; in the case of the EtSH/NaH method the unchanged methoxy compounds were isolated.
(14) Olah, G. A.; Narang, S. C.; Balaram Gupta, B. G.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247.
(15) Hartmann, R. W. *Cancer Treat. Rev. (Suppl. A)* **1984**, *11*, 155.

and the dextran-coated charcoal technique.[3] In the semilogarithmic plot of receptor-bound [^{3}H]estradiol vs. concentration of inhibitor, the curves of all derivatives were parallel to the binding curve of estradiol (figure not given). Therefore, it has to be assumed that these compounds are competitive inhibitors of the interaction of estradiol with its receptor.

The difference in the RBA values of the parent unsubstituted compounds **1** and **6** is less pronounced compared to that of hexestrol and metahexestrol[8] and that of butestrol and metabutestrol,[9] respectively (Table III).

Except for the 5-Cl and 6-OCH_3 derivatives of metatetramethylHES (compds **7** and **10**), which showed only weak binding affinity, the disubstituted compounds of **1** and **6** do not differ very much in their RBA values, exhibiting decreased as well as increased binding affinities for the E_2R; i.e., the binding affinity of the parent compound is not changed dramatically by introducing the symmetrical substituents. This is in contrast to the correspondingly substituted hexestrol, metahexestrol, butestrol, and metabutestrol derivatives.[7-9]

While metatetramethylHES, bearing the F substituents in the 4,4′-positions, showed a decrease in the E_2R binding affinity,[3] the F groups standing in the 2,2′- or 6,6′-positions increase the binding affinities of the two 1,1,2,2-tetramethyl-1,2-diphenylethanes by 80 (**2**) and 33% (**8**), respectively.

In the case of the chlorine-substituted derivatives, only compound **9** showed an increased binding affinity, whereas compound **3** exhibited an RBA value not significantly different from that of the parent compound.

The methoxy substituents led to a strong decrease of receptor affinity in the tetramethylHES class (**4**) and to a dramatic diminution in the metatetramethylHES series (**10**).

Similar results were obtained with the CH_3 substituents: Compound **5** showed a slight decrease and compound **11** a strong diminution of the E_2R binding affinity compared to the corresponding unsubstituted parent compound.

In the case of the CH_3 and OCH_3 substituents it becomes apparent that the receptor affinity decreasing effect of corresponding substituents is stronger in the metatetramethylHES series than in the tetramethylHES class. This also has been observed in the metahexestrol and the hexestrol class.[8,7]

The displacement of the chlorine substituents from the 6,6′-positions (**9**) into the 5,5′-positions (**7**) led to a significant decrease of the E_2R binding affinity. A very small binding affinity has already been observed in the case of 1,1,2,2-tetramethyl-1,2-bis(3,5-dihydroxyphenyl)ethane,[3] and has been described for the 5,5′-disubstituted OH- and Cl-metahexestrol derivatives.[8]

The most active inhibitors of the E_2R interaction, i.e. compounds exhibiting a RBA value of at least 2.5, were tested for their uterotrophic and antiuterotrophic activity in the immature mouse as a measure of their estrogenicity and antiestrogenicity.

It becomes apparent from Table IV that there is no correlation between receptor affinity and uterotrophic activity within this class of compounds.

The slight uterine growth stimulating effect in high doses of the parent compounds **1** and **6** is considerably increased by introduction of the substituents. In general the substituted compounds showed a weak uterotrophic activity in small doses, whereas in high doses stronger uterine growth stimulating effects were obtained. But, with the exception of compound **3** the maximum effect of true estrogens like estrone was not reached by the compounds. This is typical of partial estrogens such as nafoxidine.[16]

Table IV. Estrogenic Activity of Compounds **1**–**3**, **5**, **6**, **8**, and **9** in the Mouse Uterine Weight Test

compd	dose,[a] μg	effect,[b] mean ± SD
1	0	9.2 ± 2.7
	8	10.6 ± 2.0
	24	15.7 ± 1.7
	80	15.8 ± 2.8
	250	13.3 ± 2.0
	1000	14.3 ± 2.3
estrone	0.4	45.3 ± 4.7
2	0	16.4 ± 1.3
	1	27.0 ± 4.0
	10	38.8 ± 3.8
	100	42.6 ± 3.5
	1000	38.5 ± 3.3
estrone	0.4	51.4 ± 5.3
3	0	13.7 ± 2.2
	1	35.8 ± 5.2
	10	51.5 ± 2.0
	100	32.7 ± 1.4
	1000	35.2 ± 3.1
estrone	0.4	44.1 ± 4.0
5	0	11.1 ± 1.8
	1	16.6 ± 1.8
	10	22.2 ± 3.0
	100	28.0 ± 1.7
	1000[c]	38.0 ± 3.3
estrone	0.4	47.3 ± 2.7
6	0	9.2 ± 2.7
	8	9.8 ± 2.0
	24	15.7 ± 2.2
	80	15.4 ± 4.7
	250	14.6 ± 2.2
	1000	13.4 ± 1.7
estrone	0.4	45.3 ± 4.7
8	0	9.7 ± 1.9
	1	15.4 ± 1.8
	10	16.6 ± 2.0
	100	22.6 ± 2.2
	1000	35.6 ± 2.8
estrone	0.4	43.9 ± 2.8
9	0	11.1 ± 1.8
	1	14.7 ± 3.2
	10	22.6 ± 2.7
	100	33.0 ± 3.6
	1000	35.7 ± 4.7
estrone	0.4	47.3 ± 2.7

[a] Dose per animal per day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Applied as a suspension (see the Experimental Section).

The halogen-substituted compounds **2**, **3**, **8**, and **9** showed stronger uterine growth stimulating activity than did the CH_3 compound **5**. In spite of reduced RBA values the chlorine derivatives (**3** and **9**) exhibited stronger uterotrophic activity than the fluorine derivatives (**2** and **8**).

It is striking that the increase of the uterotrophic effects was stronger with the halogen-substituted tetramethylHES derivatives **2** and **3** than with the correspondingly substituted metatetramethylHES derivatives **8** and **9**. Only in the case of the fluorine compound this might be due to the increased receptor affinity of compound **2** compared to compound **8**, since in the case of the chlorine derivatives there was no significant difference in the receptor affinities.

The antiestrogenic activity of the test compounds was determined by the inhibition of the uterine growth stimulated by estrone (Table V).

All test compounds exhibited similar antiuterotrophic effects, reaching inhibition values between 22 and 43%.

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Table V. Antiestrogenic Activity of Compounds **2**, **3**, **5**, **8**, and **9** in the Mouse Uterine Weight Test

compd	dose,[a] μg	effect,[b] mean ± SD	% inhibn[c,d]
2	0	13.8 ± 2.0	
	0.1	47.7 ± 5.6	
	1	49.9 ± 4.9	
	10	41.0 ± 4.9	20[e]
	100	35.9 ± 2.7	35[e]
	1000	34.2 ± 4.1	40[e]
estrone	0.4	47.8 ± 3.2	
3	0	13.7 ± 2.2	
	5	48.4 ± 3.4	
	50	38.9 ± 3.5	17
	500	35.2 ± 3.8	29[e]
estrone	0.4	44.1 ± 4.0	
5	0	13.7 ± 2.2	
	5	51.2 ± 2.9	
	50	50.0 ± 4.4	
	500	37.3 ± 3.9	22
estrone	0.4	44.1 ± 4.0	
8	0	13.7 ± 2.9	
	5	48.6 ± 3.7	
	50	37.2 ± 3.4	29[e]
	500	32.5 ± 2.9	43[e]
estrone	0.4	46.6 ± 4.5	
9	0	13.7 ± 2.9	
	5	44.2 ± 5.9	7
	50	34.9 ± 6.2	36[e]
	500	37.8 ± 6.0	27[e]
estrone	0.4	46.6 ± 4.5	

[a] Dose per animal per day. [b] Uterus dry weight (mg)/body weight (g) × 100. [c] % inhibn = 100 − $[(E_{S,T} - E_V)/(E_S - E_V)] \times 100$; E_S = effect of estrone standard; $E_{S,T}$ = effect of standard under simultaneous application of test substance; E_V = effect of vehicle. [d] The U-test according to Wilcoxon, Mann, and Whitney was used. [e] Significant ($\alpha = 0.01$).

The two derivatives that had shown the highest RBA values in the receptor test, **2** and **8**, showed the strongest antiuterotrophic activity. But, in general there is no correlation between receptor affinity and antiuterotrophic property. It is striking that the disubstituted compounds did not reach the inhibition values of the unsubstituted parent compounds tetramethylHES and metatetramethylHES (both compds 74%[3]). Probably this decrease of antagonistic activity is due to the increased uterotrophic property of the disubstituted 1,1,2,2-tetramethyl-1,2-diphenylethanes.

The most active antiestrogens, the fluorine compounds **2** and **8**, were tested for their mammary tumor inhibiting activity using the DMBA-induced, hormone-dependent mammary carcinoma of the SD-rat.

The two compounds showed different activities on this experimental tumor. Whereas **8** led only to a slight, nonsignificant retardation of the tumor growth, **2** exhibited a strong antitumor activity (Figure 1; Table VI).

The tetramethylHES derivative **2** applied in a dose of 5 mg/kg per day reduced tumor area by 47%. In this dose, 74% of the tumors showed a complete remission and 10% a partial remission.

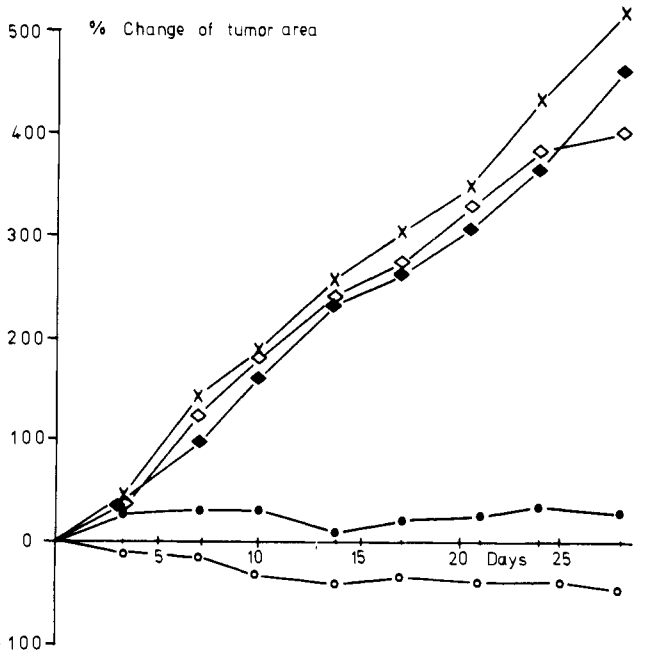

Figure 1. Effects of compounds **2** and **8** on the tumor area of the SD-rat bearing DMBA-induced, hormone-dependent mammary tumors. Control (×). **2**: 2 mg/kg per day (●), 5 mg/kg per day (○). **8**: 2 mg/kg per day (♦), 5 mg/kg per day (◊).

Mammary tumor inhibiting data of the unsubstituted parent compounds are as follows (percent change of tumor area): control, 573%; tetramethylHES, (4 mg) 275%, (20 mg) 11% (complete remission 40%, partial remission 20%); metatetramethylHES, (4 mg) 375%, (20 mg) 168% (complete remission 11%, partial remission 28%).[3]

Discussion

In contrast to the corresponding hexestrols[7,8] and butestrols[9] the substituted 1,1,2,2-tetramethyl-1,2-diphenylethanes did not show strong differences in their binding affinities for the E_2R compared to the unsubstituted parent compounds.

As experiments with molecular models show, an unhindered rotation on the Ar–C bonds and the bond between the benzylic C atoms is found in the case of the butestrols and hexestrols, but not in the case of the 1,1,2,2-tetramethyl-1,2-diphenylethanes. The latter compounds exhibit a compact structure in the center of the molecule, thus causing an immobilization of the phenyl rings. Because of these facts the molecule cannot adjust to the E_2R binding site in the way necessary to an optimum interaction. This is the explanation of the relatively small E_2R binding affinities of tetramethylHES and metatetramethylHES compared to those of the other parent 1,2-diphenylethanes. In the case of the hexestrols and butestrols the introduction of additional groups in the ortho position to the ethane bridge hinders rotation on the

Table VI. Effect of **2** and **8** on the DMBA-Induced Mammary Carcinoma of the Sprague–Dawley Rat

compd	dose,[a] mg/kg per day	no. of animals	no. of tumors[b]	new tumors	complete remission,[c] %	partial remission,[d] %	static tumors,[e] %	progr tumors,[f] %	change of body wt,[g] %	change of tumor area,[h,i] %
control		9	26	26	8	2	14	77	+2.5	+515
2	2	9	25	3	64	4	14	18	−3.1	+22[j]
2	5	9	25	6	74	10	3	13	−4.0	−47[j]
8	2	9	23	21	0	7	16	77	+3.5	+441[k]
8	5	8	23	22	0	4	7	89	+2.9	+396[k]

[a] Dissolved in olive oil. [b] At the beginning of the test. [c] Tumor not palpable. [d] Reduction of initial tumor size ≥50%. [e] Tumor size 51–150% of initial size. [f] Tumor size >150% of initial size. [g] Average on the seventh day of therapy. [h] Average on the 28th day of therapy. [i] The U-test according to Wilcoxon, Mann, and Whitney was used. [j] Significant ($p < 0.01$). [k] Not significant ($p > 0.05$).

C–C bonds in the center of the molecule, depending on the van der Waals radii of the substituents. This effect may prevent the formation of the optimum conformation and will consequently lead to a more or less pronounced decrease of the E_2R binding affinity. In the case of the 1,1,2,2-tetramethyl-1,2-diphenylethanes the introduction of substituents—as far as sterically possible—has no effect or no profound effect on the conformation of the parent compound, for rotation is already hindered. This is why the substituents do not strongly influence E_2R binding affinity. The different RBA values are due to electronic, lipophilic, or steric effects of the substituents but not to conformational changes of the molecule.

Regardless of their influence on E_2R binding affinity, the substituents increase the uterotrophic activity of the parent compounds. An explanation for this phenomenon might be the finding that *meso*-3,4-bis(4-hydroxyphenyl)-3,4-dimethylhexane shows also a considerable increase of uterotrophic activity compared to tetramethylHES.[3] One can imagine that a substituent standing in the ortho position to the 1,2-diphenylethane bridge and the CH_3 group at the benzylic C atom mimic an Et group at the same position. Since the discovery that the replacement of the 1,2-diethyl groups by isopropyl groups in metahexestrol destroys the partial antiestrogenic activity and generates a "true" estrogen[17] and the finding that tetramethylation in the 1,2-positions of 1,2-bis(4-hydroxyphenyl)ethane produces the strong antiestrogen tetramethylHES,[3] it is known that the alkyl chains in the center of the 1,2-diphenylethanes strongly influence agonistic and antagonistic properties of the corresponding compounds.

In the case of the 1,2-dimethyl-1,2-diethyl-1,2-diphenylethane compound[3] the increase of uterotrophic activity was accompanied by a decrease of antiuterotrophic activity.[3] The compounds evaluated in this study showed the same effect. This correlation generally holds in the 1,2-diphenylethane class as is shown by the hexestrol,[7] metahexestrol,[8] butestrol,[9] and metabutestrol[9] derivatives.

The aim of this study, namely an increase of the mammary tumor inhibiting activity of the 1,1,2,2-tetramethyl-1,2-diphenylethanes, was reached by introducing F substituents in the 2,2′-positions of tetramethylHES. 1,1,2,2-Tetramethyl-1,2-bis(2-fluoro-4-hydroxyphenyl)-ethane (**2**) exhibits an approximately 10-fold stronger antitumor activity compared to the parent compound (**2**, in a dose of 2 mg/kg, shows the same tumor-inhibiting effect as tetramethylHES in a dose of 20 mg/kg).

Probably this increase of mammary tumor inhibiting activity is partially caused by the enhanced E_2R binding affinity. A correlation between binding affinity for the E_2R and antitumor activity has been described in a series of 1,2-dialkylated 1,2-bis(3-hydroxyphenyl)ethanes.[2] An enhancement of the E_2R binding affinity, however, need not necessarily cause increased tumor-inhibiting properties. This is shown by **8**, which exhibits in spite of an increased RBA value no better antitumor activity compared to metatetramethylHES.

The introduction of the fluorine substituents, however, leads to a decrease of antiuterotrophic and an increase of uterotrophic activity. It is striking that there is no potent mammary tumor inhibiting antiestrogen exhibiting no or only marginal estrogenic side effects. All strongly active compounds (e.g., tamoxifen and metahexestrol) show at least slight estrogenic activity. On the other hand, strong antiestrogens with no or only marginal estrogenic activity (e.g., tetramethylHES and LY 117018[18]) exhibit only moderate or weak antitumor activity. This finding may lead to the conclusion that a certain amount of residual estrogenic activity is essential for strong antitumor activity of antiestrogens.

The question whether **2** unfolds its mammary tumor inhibiting activity like its parent compound as an antiestrogen[5] or like metahexestrol and tamoxifen as an estrogen[5] remains to be elucidated.

Experiments with **2** on further experimental tumor models are presently performed.

Experimental Section

General Procedures. TLC of each compound was performed on Merck F 254 silica gel plates. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universität Regensburg. The structures of all compounds were confirmed by their IR (Beckman AccuLab 3) and ^1H NMR spectra (Varian EM 390, 90 MHz).

Synthetic methods A–C are representatives for compounds reported in Tables I and II.

Method A. 2-(2-Fluoro-4-methoxyphenyl)-2-propanol (2b). Methyl iodide (17.75 g, 0.125 mol) was dissolved in ether and the resultant mixture added dropwise with stirring to magnesium turnings (3.04 g, 0.125 mol) in 15 mL of dry ether. The mixture was heated to reflux for 0.5 h. A solution of 2-fluoro-4-methoxyacetophenone (**2c**; 16.82 g, 0.1 mol) in ether was added dropwise with stirring. After heating to reflux for 2 h, the mixture was cooled and poured on ice. The resulting precipitate was dissolved by the addition of a NH_4Cl solution. The ethereal layer was separated, and the aqueous layer was extracted with ether. The combined ethereal extracts were washed with solutions of $NaHSO_3$, $NaHCO_3$, and water and dried over anhydrous Na_2SO_4. The solvent was removed and the resulting oil distilled under high vacuum to give 14.9 g of **2b**.

Method B. 2,3-Bis(2-fluoro-4-methoxyphenyl)-2,3-dimethylbutane (2a). $TiCl_3$ (4.63 g, 0.03 mol) was placed under N_2 in a flask with 150 mL of dry glyme. $LiAlH_4$ (0.38 g, 0.01 mol) was quickly added to the stirred $TiCl_3$ slurry. The resulting black suspension was stirred for 10 min. Compound **2b** (1.84 g, 0.01 mol) was dissolved in 10 mL of dry glyme and the resultant mixture added dropwise with stirring. The mixture was heated to reflux and kept there for 16 h. After cooling, the reaction mixture was quenched by the addition of 2 N HCl, diluted with H_2O, and extracted with ether. The ether extract was washed ($NaHCO_3$ and H_2O) and dried ($MgSO_4$). The solvent was removed, and the resulting crude product was crystallized from toluene/ligroin to give 0.67 g of **2a**.

Method C. 2,3-Bis(2-fluoro-4-hydroxyphenyl)-2,3-dimethylbutane (2). A solution of **2a** (3.34 g, 0.01 mol) in 250 mL of dry CH_2Cl_2 was cooled to −60 °C. Under nitrogen, BBr_3 (7.52 g, 0.03 mol) was added with stirring. After 0.5 h the freezing mixture was removed, and the reaction mixture was kept at room temperature for 4 h. MeOH (50 mL) was added, and the mixture was shaken with 2 N NaOH. After neutralization of the aqueous layer with 3 N HCl the solution was extracted with ether. After removal of the ether, the crude product was repeatedly recrystallized from benzene to give 2.69 g of **2**.

In the case of the tetramethoxy compounds **4a** and **10a** ether cleavage was performed with $(CH_3)_3SiI$.

2,3-Bis(4-hydroxy-2-methoxyphenyl)-2,3-dimethylbutane (4). Trimethylsilyl iodide (12.0 g, 0.06 mol) was added dropwise under nitrogen to a stirred solution of **4a** (3.59 g, 0.01 mol) in 200 mL of dry acetonitrile. The mixture was heated to reflux for 24 h. After cooling, the solution was diluted with H_2O and extracted with ether. The ethereal extract was washed ($NaHSO_3$ and H_2O) and then shaken with 5 N NaOH. The aqueous phase was acidified with 3 N HCl and extracted with ether. After washing

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(H_2O) and drying ($MgSO_4$), the solvent was removed and the crude product was recrystallized from EtOH/H_2O to give 1.18 g of **4**.

Biological Methods. Estradiol Receptor Binding Assay. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^{3}H]estradiol. A previously described procedure was used with modifications.[3] Test compounds were incubated with cytosol from calf uteri and [^{3}H]estradiol at 4 °C for 16 h. Incubation was stopped by adding dextran-coated charcoal. After centrifugation, the radioactivity of a 100-μL supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabeled test compounds. Six concentrations of the competitors were tested. They were chosen to provide a linear portion on a semilog plot, crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled estradiol and of test compounds reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activities were determined by stimulation of the uterine growth and the inhibition of the uterine growth stimulated by estrone, respectively, with immature NMRI mice as described previously.[3] Twenty-day-old female mice (weight 14.5 ± 1.2 g, mean ± SD) were randomly distributed into groups of 10 animals. They were subcutaneously injected daily for 3 days with 0.1 mL of olive oil solutions containing the test compound. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, washed, dried, and weighed.

Mammary Tumor Growth Inhibition Test. The method used has been described previously.[3] The tumor-inhibiting effect was determined by using the DMBA-induced, hormone-dependent mammary adenocarcinoma of the SD-rat. Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of 10. Compounds were dissolved in olive oil and applied sc. Measurement of tumor size and determination of body weight were made twice weekly. The therapy was continued for 28 days.

Acknowledgment. Thanks are due to the Deutsche Forschungsgemeinschaft and to the Verband der Chemischen Industrie, Fonds der Chemischen Industrie, who supported this work by grants. The technical assistance of Maria Beer and Gabi Seidl is gratefully acknowledged.

Registry No. 1, 74385-27-6; **1a**, 32445-98-0; **1b**, 7428-99-1; **2**, 96826-17-4; **2a**, 96826-32-3; **2b**, 96826-25-4; **2c**, 74457-86-6; **3**, 96826-18-5; **3a**, 96826-33-4; **3b**, 96826-26-5; **3c**, 41068-36-4; **4**, 96826-19-6; **4a**, 96826-34-5; **4b**, 96826-27-6; **4c**, 829-20-9; **5**, 96826-20-9; **5a**, 96826-35-6; **5b**, 91968-30-8; **5c**, 24826-74-2; **6**, 74385-30-1; **6a**, 74385-22-1; **6b**, 55311-42-7; **7**, 96826-21-0; **7a**, 96826-36-7; **7b**, 96826-28-7; **7c**, 96826-41-4; **8**, 96826-22-1; **8a**, 96826-37-8; **8b**, 96826-29-8; **8c**, 96826-42-5; **9**, 96844-92-7; **9a**, 96826-38-9; **9b**, 96826-30-1; **9c**, 54810-63-8; **10**, 96826-23-2; **10a**, 96826-39-0; **10b**, 72667-90-4; **10c**, 2150-40-5; **11**, 96826-24-3; **11a**, 96826-40-3; **11b**, 96826-31-2; **11c**, 73502-03-1; methyl iodide, 74-88-4.

Supplementary Material Available: ^{1}H NMR data (Table VII) of compounds **1a**–**11a** and **1**–**11** (3 pages). Ordering information is given on any current masthead page.

Synthetic and Conformational Studies on Anatoxin-a: A Potent Acetylcholine Agonist

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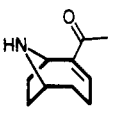
Anatoxin-a is a powerful nicotinic acetylcholine receptor agonist. Its recently reported synthesis[6] has been further optimized to provide anatoxin-a of >99% optical purity in 10% overall yield. The geometry of solid anatoxin-a has been determined by X-ray crystallography of its hydrochloride. The solution conformation has been determined by 500-MHz ^{1}H NMR spectroscopy, utilizing 2D NMR methods and homonuclear decouplings. For further comparisons, force field calculations have been employed to evaluate the differences in energy between the various conformations available for anatoxin-a. The molecule is seen to adopt the same ring conformation both in solution and in the crystal. Comparison of this conformation with the models proposed for acetylcholine receptor activation shows good agreement and allows for further inferences concerning the stereodiscrimination by the receptor.

Undisturbed transmission of the neuronal impulse over the synaptic cleft between two consecutive nerve cells is essential for normal operation of the nervous system. Impairments in acetylcholine-mediated neurotransmission can lead to severe consequences including myasthenia gravis, Parkinson's disease, and Alzheimer's disease. Therefore, the development of new efficient drugs with powerful cholinergic activity has gained increased impetus.

Good understanding of the geometrical requirements for agonist–receptor recognition is vital for rational design of new drugs with enhanced potency.[1] Inspection of various nicotinic acetylcholine agonists prompted the proposal[2] of a model for activation of the nicotinic acetylcholine receptor (nAChR).[3] This model subsequently has been refined to account for the observed stereodiscrmination at the receptor site.[4]

Anatoxin-a (1) is a low molecular weight alkaloid originally isolated from the fresh water blue-green alga *Anabaena flos-aquae* (Lyngb) de Breb.[5] The efficacy of 1 in stimulating the nAChR is greater than that of the



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natural neurotransmitter acetylcholine. The anatoxin-a

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