Potential Antiestrogens. Synthesis and Evaluation of Mammary Tumor Inhibiting Activity of 1,2-Dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes

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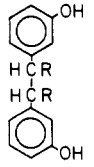
The syntheses of the *meso*-1,2-dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes [alkyl substituent: CH_3 (**19**), C_2H_5 (**20**), C_3H_7 (**22**), C_4H_9 (**23**), *i*-C_4H_9 (**24**), and C_5H_{11} (**25**)] and of *d,l*-3,4-bis(3′-hydroxyphenyl)hexane (**21**) are described. In vitro these compounds inhibited the [^{3}H]estradiol receptor interaction competitively, exhibiting K_a values between 0.20×10^9 (**20**) and 0.11×10^6 M^{-1} (**24**). In vivo the meso compounds reduced the estrone-stimulated mouse uterine growth; the most effective compounds were **20**, **22**, and **23** (53, 50, and 45% inhibition, respectively). Compounds **20** and **22–24** showed weak estrogenic activity in the mouse uterine weight test and in the vaginal cornification test. Compounds **19** (NSC-297169), **20** (NSC-297170), and **22** (NSC-297171) exhibited a dose-dependent growth inhibition on the MCF-7 human breast tumor cell line (10^{-6} to 10^{-9} M). These compounds also showed a marked dose-dependent inhibition on the DMBA-induced, hormone-dependent mammary carcinoma of the Sprague–Dawley rat corresponding to their association constants.

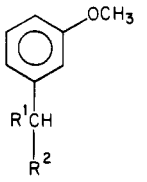
Since the therapy of the disseminated estradiol receptor positive mammary carcinoma of postmenopausal women with antiestrogens such as tamoxifen produces only poor remission rates,[1,2] we have been looking for more effective compounds. The displacement of the phenolic hydroxy groups of the synthetic estrogens *trans*-4,4′-dihydroxy-α,β-dialkylstilbenes into the 3,3′ positions led to antiestrogens of which *trans*-3,3′-dihydroxy-α,β-diethylstilbene (3,3′-DES) showed the strongest inhibitory activity on the 9,10-dimethyl-1,2-benzanthracene (DMBA) induced, hormone-dependent mammary adenocarcinoma of the Sprague–Dawley (SD) rat.[3] The dihydro derivative of the latter compound, *meso*-3,4-bis(3′-hydroxyphenyl)hexane (**20**), exhibited the same antitumor activity.[4] 3,3′-DES and



compd	R	configuration
19	CH_3	meso
20	C_2H_5	meso
21	C_2H_5	d,l
22	C_3H_7	meso
23	C_4H_9	meso
24	i-C_4H_9	meso
25	C_5H_{11}	meso

compound **20**, however, showed weak estrogenic properties in the uterine weight test of the immature mouse. Tetraalkylation of the 1,2 position of the diphenylethane skeleton of compound **20** and of hexestrol resulted in a nearly complete loss of estrogenic activity, accompanied by a marked antitumor activity, which, however, was inferior to that of compound **20**.[5] This paper describes another approach to obtain antiestrogens with antitumor activity lacking estrogenic side effects by variation of the alkyl chains of compound **20**.

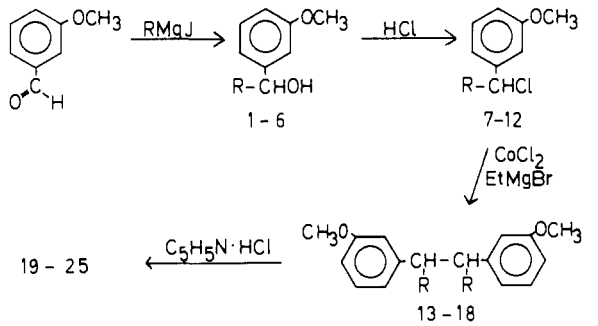
Table I. α-Hydroxy- and α-Chloro-Substituted 1-(3′-Methoxyphenyl)alkanes



no.	R^1	R^2	synth method[a]	yield,[b] %	bp, °C (mmHg)	formula
1	CH_3	OH	A	85	124 (11)	$C_9H_{12}O_2$
2	C_2H_5	OH	A	70	134 (10)	$C_{10}H_{14}O_2$
3	C_3H_7	OH	A	84	136 (10)	$C_{11}H_{16}O_2$
4	C_4H_9	OH	A	79	180 (0.2)	$C_{12}H_{18}O_2$
5	i-C_4H_9	OH	A	68	104 (0.4)	$C_{12}H_{18}O_2$
6	C_5H_{11}	OH	A	90	110 (0.2)	$C_{13}H_{20}O_2$
7	CH_3	Cl	B	73	112 (10)	$C_9H_{11}ClO$
8	C_2H_5	Cl	B	93	87 (0.4)	$C_{10}H_{13}ClO$
9	C_3H_7	Cl	B	93	84 (0.5)	$C_{11}H_{15}ClO$
10	C_4H_9	Cl	B	64	142 (0.2)	$C_{12}H_{17}ClO$
11	i-C_4H_9	Cl	B	53	98 (0.4)	$C_{12}H_{17}ClO$
12	C_5H_{11}	Cl	B	98	136 (0.8)	$C_{13}H_{19}ClO$

[a] Capital letters refer to synthetic methods A and B under Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields.

Scheme I



Chemistry. The synthesis of the 1,2-dialkyl-1,2-bis-(3′-hydroxyphenyl)ethanes (**19–25**; Table II) started from 3-methoxybenzaldehyde (Scheme I). The latter com-

(1) H. W. C. Ward, *Br. Med. J.*, 1, 13 (1973).
(2) M. P. Cole, C. T. A. Jones, and I. D. H. Todd, *Br. J. Cancer*, **35**, 270 (1971).
(3) G. Kranzfelder, M. Schneider, E. v. Angerer, and H. Schönenberger, *J. Cancer Res. Clin. Oncol.*, **97**, 167 (1980).
(4) G. Kranzfelder, R. W. Hartmann, E. v. Angerer, H. Schönenberger, and A. E. Bogden, *J. Cancer Res. Clin. Oncol.*, in press.
(5) R. W. Hartmann, G. Kranzfelder, E. v. Angerer, and H. Schönenberger, *J. Med. Chem.*, **23**, 841 (1980).

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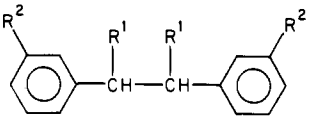
Table II. 3-Methoxy- and 3-Hydroxy-Substituted 1,2-Dialkyl-1,2-diphenylethanes



no.	R^1	R^2	synth method[a]	yield,[b] %	mp or bp, °C (mmHg)	recrystn solvent[c]	formula	assoc constants (K_a),[d] M^{-1}
13[f]	CH_3	OCH_3	C	26	96	F	$C_{18}H_{22}O_2$	
14[f]	C_2H_5	OCH_3	C	31	88	F	$C_{20}H_{26}O_2$	
15[f]	C_3H_7	OCH_3	C	23	80	F	$C_{22}H_{30}O_2$	
16[f]	C_4H_9	OCH_3	C	21	175 (0.2)		$C_{24}H_{34}O_2$	
17[f]	i-C_4H_9	OCH_3	C	26	95	F	$C_{24}H_{34}O_2$	
18[e]	C_5H_{11}	OCH_3	C	42	145-170 (0.1)		$C_{26}H_{38}O_2$	
19[f]	CH_3	OH	D	92	163	G	$C_{16}H_{18}O_2$	0.11×10^8
20[f]	C_2H_5	OH	D	91	182	H	$C_{18}H_{22}O_2$	0.20×10^9
21[g]	C_2H_5	OH	D	44	104	I	$C_{18}H_{22}O_2$	0.91×10^7
22[f]	C_3H_7	OH	D	89	161	H	$C_{20}H_{26}O_2$	0.91×10^8
23[f]	C_4H_9	OH	D	85	158	H	$C_{22}H_{30}O_2$	0.23×10^8
24[f]	i-C_4H_9	OH	D	88	189	H	$C_{22}H_{30}O_2$	0.11×10^6
25[f]	C_5H_{11}	OH	D	46	156	H	$C_{24}H_{34}O_2$	0.33×10^7

[a] Capital letters refer to synthetic methods C and D under Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] F = MeOH; G = acetic acid/H_2O; H = benzene/EtOH; I = CCl_4/ligroin. [d] Calculated from data generated by a Lineweaver-Burk analysis (see ref 16) using a competitive inhibitor model (see ref 4). [e] Mixture of meso and d,l compound. [f] Meso form. [g] d,l form.

pound was converted with alkylmagnesium iodide into the corresponding 3-methoxybenzyl-α-alkyl alcohols (1–6; Table I). The 3-methoxybenzyl-α-alkyl chlorides (7–12; Table I) were obtained by passing hydrogen chloride into a solution of the corresponding benzyl alcohols in ligroin. The reductive coupling of the benzyl chlorides according to Wilds and McCormack[6] using $CoCl_2$ and ethylmagnesium bromide gave a mixture of the corresponding *meso*- and *d,l*-1,2-dialkyl-1,2-bis(3′-methoxyphenyl)-ethanes, as was proved by TLC, HPLC, and ^1H NMR spectroscopy. In the ^1H NMR spectrum of the meso compounds the signals of the methine protons are shifted upfield, whereas the singlets of the methoxy protons are shifted downfield compared to the *d,l* form.[7] With the exception of compound 18, the separation of the meso diastereomers was accomplished by fractional crystallization from methanol (13–15 and 17) and fractional distillation (16), respectively (Table II). The cleavage of the ethers of the meso compounds 13–17 and of compound 18 (mixture of meso and *d,l* forms) was accomplished with pyridinium hydrochloride. In the case of compounds 13–17, the corresponding *meso*-1,2-dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes 19, 20, and 22–24 were obtained. Compound 18 was obtained as a mixture of *meso*- and *d,l*-1,2-dipentyl-1,2-bis(3′-hydroxyphenyl)ethane. Its meso diastereomer (25) was separated by fractional crystallization from benzene/EtOH. The separation of *d,l*-1,2-diethyl-1,2-bis(3′-hydroxyphenyl)ethane (21) was accomplished by fractional crystallization of the mixture of diastereomers from CCl_4/ligroin, obtained after cleavage of the ethers of a mixture of meso and *d,l* forms. Compounds 19, 20, and 22–25 were free of *d,l* isomers, and compound 21 was free of meso isomer, as was proved by TLC and HPLC [TLC (SiO_2; toluene–ethyl acetate, 9:1) R_f for 20, 0.29; for 21, 0.18; HPLC (RP 18) (MeOH–H_2O, 3:1) t_R for 20, 7.2 min; for 21, 8.2 min (for further details, see Experimental Section, General Procedures)].

(6) A. L. Wilds and W. B. McCormack, *J. Org. Chem.*, 14, 45 (1949).

(7) The NMR assignments were made by synthesizing the ethyl derivatives meso- and *d,l*-14 separately by catalytic hydrogenation of the corresponding *cis*- and *trans*-3,4-bis(3′-methoxyphenyl)-3-hexenes (Hartmann et al., unpublished results).

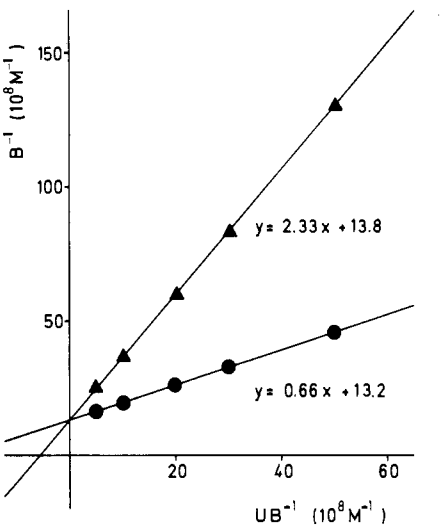


Figure 1. Competitive inhibition of the estradiol receptor interaction by compound 23: (●–●) [^3H]estradiol; (▲–▲) [^3H]-estradiol and 23 (10^{-7} M).

Biological Properties. In vitro the homologous 1,2-dialkyl-1,2-diphenylethanes inhibited the estradiol receptor interaction competitively (Figure 1). The receptor affinities of the straight-chain *meso*-1,2-dialkyl-1,2-diphenylethanes increased from the methyl to the ethyl derivative (19 and 20, respectively; Table II). The replacement of the ethyl group by a propyl, butyl, and pentyl substituent (22, 23, and 25, respectively) led to an increasing loss of receptor affinity. The isobutyl derivative (24) exhibited the lowest K_a value. The affinity of the *d,l* compound 21 for the estrogen receptor was about 20 times smaller than that of the meso analogue (20).

In vivo compounds 20 and 22–24 exhibited uterotrophic activity in the mouse uterine weight test, but high doses were necessary to reach their maximum uterotrophic effects (Table III). None of these compounds reached the maximum uterotrophic effect of estrone (Table III). Partial estrogens such as nafoxidine were also found to be unable to produce full uterotrophic response.[3] The meso compounds 19 and 25 and the *d,l* compound 21 did not show any significant stimulation of the uterine weight (data

Table III. Antiestrogenic and Estrogenic Activity of Compounds 19, 20, and 22-25[g] in Mouse Uterine Weight Test and Vaginal Cornification Test

	antiuterotrophic test			vaginal cornification test[e]	uterotrophic test	
compd	dose,[a] μg	effect,[b] means ± SD	% inhibn[c,d]		dose,[a] μg	effect,[b] means ± SD
19	0	11.2 ± 1.5				
	0.1	38.5 ± 2.6				
estrone	0.1	37.5 ± 4.0				
19	0	10.5 ± 1.6				
	5	36.5 ± 5.1	7		no effect	
estrone	0.1	38.4 ± 5.1				
19	0	9.9 ± 1.5				
	50	34.3 ± 2.2	12			
	500	29.0 ± 3.5	31[f]			
estrone	0.1	37.7 ± 5.5				
20	0	12.5 ± 2.6		A	0	10.7 ± 1.8
	2.5	29.5 ± 3.0	27[f]	A	0.8	11.8 ± 2.1
	5	23.5 ± 2.8	53[f]	A	8	14.4 ± 1.1
estrone	0.1	35.7 ± 3.1				
20	0	10.0 ± 0.9				
	25	28.1 ± 0.8	48[f]	A	24	18.5 ± 3.5
	50	28.3 ± 1.5	47[f]	B	80	30.5 ± 4.0
	500	30.9 ± 3.2	40[f]	B	800	30.8 ± 4.9
estrone	0.1	44.8 ± 6.8		C	0.4	39.5 ± 4.1
22				A	0	11.4 ± 1.4
				A	3	13.6 ± 1.6
	0	9.9 ± 0.8		A	9	13.9 ± 1.5
	0.1	36.3 ± 7.6	5	A	90	17.6 ± 2.0
	5	30.4 ± 6.5	26	B	300	28.4 ± 3.6
	50	25.9 ± 4.9	43[f]	B	900	28.7 ± 2.4
	500	23.9 ± 3.3	50[f]	B	1500	28.0 ± 3.1
estrone	0.1	37.7 ± 5.5		C	0.4	40.3 ± 3.9
23				A	0	10.5 ± 1.2
	0	10.5 ± 1.6		A	30	11.2 ± 2.8
	0.1	37.5 ± 3.6	3	A	100	12.6 ± 2.5
	3.3	33.9 ± 2.0	16	A	300	16.5 ± 1.7
	100	25.9 ± 3.1	45[f]	B	900	30.8 ± 3.8
	1000	38.1 ± 3.7	1	B	2700	35.2 ± 3.4
estrone	0.1	38.4 ± 5.1		C	0.4	39.7 ± 4.6
24	0	11.4 ± 0.9		A	0	10.0 ± 1.3
	2.7	38.1 ± 3.6	4	A	2	11.8 ± 3.0
	5	34.1 ± 3.9	18	A	20	14.5 ± 1.6
	27	33.2 ± 2.8	22	A	60	19.0 ± 2.8
	80	31.5 ± 2.9	28[f]	B	180	32.6 ± 3.1
	240	31.7 ± 4.6	27[f]	B	533	31.3 ± 2.7
estrone	0.1	39.2 ± 3.6		C	0.4	39.7 ± 4.6
25	0	10.5 ± 1.6				
	0.1	37.1 ± 4.5	5			
	3.3	39.5 ± 4.7			no effect	
	100	34.2 ± 2.9	15			
	1000	34.6 ± 2.4	14			
estrone	0.1	38.4 ± 5.1				

[a] Dose per animal and day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Percent inhibition = $100 - (E_{S,T} - E_V)/(E_S - E_V) \times 100$; 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] Predominant components of vaginal smear: A = leucocytes (diestrus); B = leucocytes (≤50%), nucleated and cornified, nucleated cells (characteristic of antiestrogens; see ref 8); C = cornified nonnucleated cells (estrus). [f] Significant ($\alpha = 0.01$). [g] Compound 21 was neither uterotrophic nor antiuterotrophic (data not given).

not given). These results were confirmed by the vaginal cornification test (Table III). Compounds **20** and **22–24** led to a cornification of the vaginal epithelium in doses in which they had shown considerable uterotrophic activity. Partial estrogens were found to produce identical vaginal smears.[8] The microscopical picture of smears of mice treated with the nonuterotrophic compounds **19**, **21**, and **25** was typical of diestrus.

Compounds **19**, **20** and **22–24** significantly inhibited the estrone-stimulated uterine growth of the immature mouse in the Dorfman test (Table III). Compounds **20**, **22**, and **23** exhibited an activity comparable to nafoxidine.[3,9] It is remarkable that inhibitory effects were obtained in doses in which these compounds had no or only weak uterotrophic activity.

On the MCF-7 human breast tumor cell line the hexestrol derivatives **19**, **20**, and **22** showed antitumor properties. They exhibited a significant, dose-related growth inhibition with a stronger effect than tamoxifen (Table IV). Compound **23** did not show any significant inhibitory activity (data not given). Growth inhibitory effects of compounds **19**, **20**, and **22** were somewhat diminished by in-

(8) L. Terenius, *Steroids*, 17, 653 (1971).

(9) L. Terenius, *Acta Endocrinol.*, 64, 47 (1970).

Table IV. Growth Inhibition of the Estrogen Responsive MCF-7 Human Breast Tumor Cell Line by Tamoxifen and Compounds 19, 20, and 22

				competitive inhibn experiment			
				concn, M			
compd	concn, M	cell no. × 10^3,[a] means ± SD	% T/C[b]	test compd	E_2[c]	cell no. × 10^3,[a] means ± SD	% T/C[b]
control		43.9 ± 1.2				6.6 ± 0.7	
tamoxifen	10^{-6}	32.9 ± 2.8	75[d]				
	10^{-7}	34.6 ± 1.4	79[d]				
	10^{-8}	38.0 ± 2.2	87				
19	10^{-6}	32.2 ± 2.4	71[d]	10^{-7}		6.1 ± 0.1	92
	10^{-7}	33.6 ± 3.6	77[d]	10^{-7}	10^{-7}	7.1 ± 0.3	107
	10^{-8}	35.9 ± 3.0	82	10^{-7}	10^{-8}	8.0 ± 0.3	121
	10^{-9}	39.8 ± 1.4	91				
20	10^{-6}	11.9 ± 1.7	27[d]	10^{-7}		4.7 ± 0.2	70[d]
	10^{-7}	27.7 ± 6.0	63[d]	10^{-7}	10^{-7}	5.6 ± 0.6	84
	10^{-8}	27.9 ± 1.6	64	10^{-7}	10^{-8}	5.1 ± 0.2	78
	10^{-9}	32.1 ± 1.6	73				
22	10^{-6}	23.3 ± 1.3	53[d]	10^{-7}		4.3 ± 0.2	66[d]
	10^{-7}	29.7 ± 7.4	68[d]	10^{-7}	10^{-7}	5.6 ± 0.3	84
	10^{-8}	34.3 ± 1.1	78	10^{-7}	10^{-8}	6.1 ± 0.1	93
	10^{-9}	30.7 ± 1.5	71				

[a] Cell number based on coulter counts on day 10. Multiply by 40 for actual number of cells/plate. [b] Percent T/C = test compound/control. [c] E_2 = 17β-estradiol. [d] Significant (α = 0.001).

Table V. Effect of Compounds 19, 20, 22, and 23 and Ovariectomy on the DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat

		no. of tumors		% of tumors with				% change of	
compd	dose,[a] mg	B[b]	NT[c]	CR[d]	PR[e]	NC[f]	P[g]	body wt[h,i]	tumor area[j,i]
control		17	42	0	2	20	78	4.1	610
19	18	21	16	14	11	24	51	2.4	188[k]
	36	22	12	29	9	38	24	1.6[l]	105[k]
	72	26	22	34	25	16	25	0.9[l]	6[k]
22	2.25	22	17	25	8	13	54	0.4[l]	271[k]
	4.5	16	6	23	14	27	36	−1.1[k]	109[k]
	9	22	1	52	22	9	17	−2.7[k]	−24[k]
ovariectomy		18	2	90	10	0	0	3.6	−99[k]
control		36	35	7	18	37	38	0.2	247
20	1	37	29	21	15	35	29	−1.6	47[k]
	2	30	23	19	30	30	21	−1.5	11[k]
	4	37	11	46	25	19	10	−5.3[k]	−75[k]
control		22	53	0	5	21	74	2.1	570
23	8.5	21	37	2	9	24	65	1.5	534
	17	27	45	3	6	36	55	1.7	389[l]
	34	22	30	6	9	27	58	0.3	260[l]
ovariectomy		23	8	74	23	3	0	3.6	−96[k]

[a] Dose per kilogram of body weight and day. The animals received a single dose daily from Monday to Thursday and a double dose on Friday. [b] At the beginning of the test. [c] Occurring during the test. [d] CR = complete remission, tumor not palpable. [e] PR = partial remission, reduction of initial tumor size ⩾50%. [f] NC = no change, tumor size 51–150% of initial tumor size. [g] P = progression, tumor size >150% of initial tumor size. [h] Average on the 7th day of therapy. [i] The U test according to Wilcoxon, Mann, and Whitney was used. [j] Average on the 28th day of therapy. [k] Significant (α = 0.01). [l] Significant (α = 0.05).

creasing the concentration of estradiol in the cell culture medium (Table IV). Tamoxifen inhibition can also be overcome by estradiol as has been reported by others.[10-12]

The most active inhibitors of the estradiol receptor interaction (19, 20, 22, and 23) were tested on the DMBA-induced, hormone-dependent mammary carcinoma of the rat (Table V, Figure 2). Based on the dosage of compound 20 [1, 2, and 4 (mg/kg) sc/day], which had shown considerable tumor inhibitory activity,[4] compounds 19, 22, and 23 were administered in doses corresponding to the association constants. Compound 19, 20, 22, and 23 exhibited a dose-dependent inhibition of the tumor growth. Only the effect of 23 at the lowest dose (8.5 mg/kg) was not significant. Even at higher doses, compound 23 was only weakly active. Compounds 19 and 20 exhibited a strong antitumor activity in all doses. Compound 22 produced strong inhibitory effects only with the 4.5- and 9-mg doses (Figure 2).

Discussion

The differences in the association constants of the 1,2-dialkyl-1,2-diphenylethanes are caused by the steric influence of the alkyl substituents, as well as by their hydrophobic property. In the series of the *meso*-1,2-dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes, compound 20 is the homologue with the maximum binding affinity for the estradiol receptor.

The tumor inhibiting effect on the DMBA-induced rat mammary carcinoma correlates with the receptor affinity.

(10) M. Lippman, G. Bolan, and K. Huff, *Cancer Res.*, 36, 4595 (1976).
(11) C. K. Osborne and M. E. Lippman, *Breast Cancer: Adv. Res. Treat.*, 2, 103–154 (1978).
(12) W. B. Butler, W. H. Kelsey, and N. Goran, *Cancer Res.*, 41, 82 (1981).

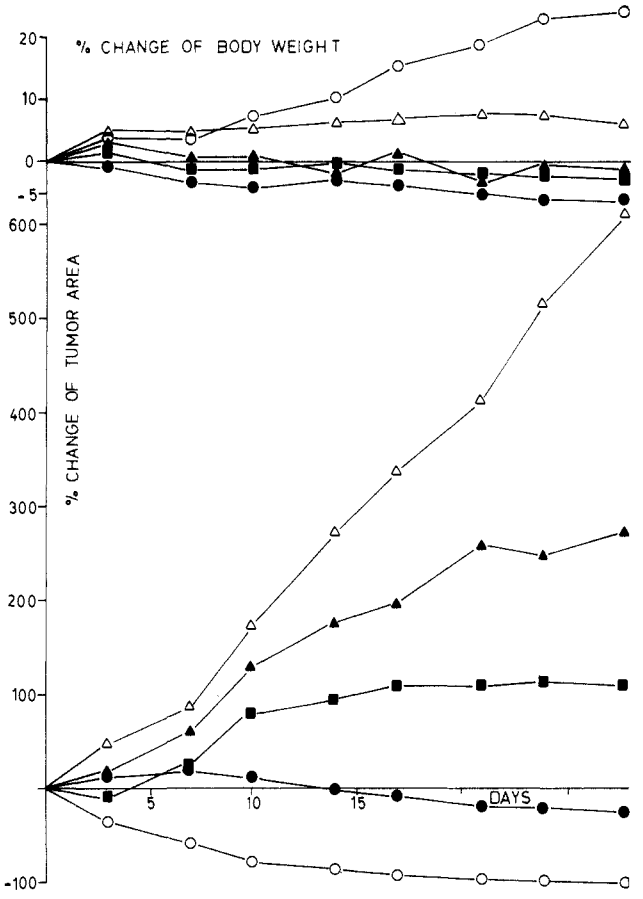


Figure 2. The effect of ovariectomy and **22** on tumor area and body weight of the SD rat bearing DMBA-induced, hormone-dependent mammary tumors: control (△–△); ovariectomy (○–○); **22**, from Monday to Thursday 2.25 (mg/kg) sc/day, on Friday 4.5 mg/kg sc (▲–▲); **22**, from Monday to Thursday 4.5 (mg/kg) sc/day, on Friday 9 mg/kg sc (■–■); **22**, from Monday to Thursday 9 (mg/kg) sc/day, on Friday 18 mg/kg sc (●–●).

The dose of each compound necessary to produce a defined inhibitory effect—for example, the dose required to keep the tumor area constant—can be calculated from the effects of the three doses tested (see Table V and Figure 2). These calculated doses of compounds **19**, **20**, and **22** show the same graduation like their association constants. The only outlier is compound **23**. It is striking that receptor affinity and tumor inhibitory activity of the estrogen diethylstilbestrol found under the same experimental conditions in our laboratory roughly fit in this correlation ($K_a = 0.83 \times 10^9$ M^{-1}; change of tumor area: 0.1 mg, -38%; 0.05 mg, 58%). Inhibitory effects on the MCF-7 cell line are also in accordance with the receptor affinities of the inhibitors and with the results on the DMBA-induced rat mammary tumors. In both tests, no effect was obtained with compound **23**. The result of the competitive inhibition experiment does not conclusively establish that the tumor growth inhibitory activity of these compounds is the result of a specific competitive binding at the receptor level, rather than a nonspecific toxicity.

This study indicates that in the series of homologous 1,2-dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes, 3,4-bis(3′-hydroxyphenyl)hexane is the most effective compound regarding estradiol receptor affinity and mammary tumor inhibiting activity. Binding affinities for the estradiol receptor may be a reliable instrument for predicting mammary tumor inhibiting activity.

The mechanism of the antitumor activity of the 1,2-dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes is not yet clear. The tumor inhibiting effect may be due to either antiestrogenic or estrogenic activity of these compounds.[13] It is a well-known fact that estrogens also exhibit tumor growth inhibitory activity tested on the DMBA-induced rat mammary carcinoma.[14] Experiments are presently being carried out to elucidate the mode of action of the 1,2-dialkyl-1,2-bis(3′-hydroxyphenyl)ethanes. They will be published subsequently.

Experimental Section

General Procedures. TLC of each compound was performed on Merck F 254 silica gel plates. HPLC was accomplished using an Altex 110 A pump (flow rate 1 mL/min) and a Kontron Uvikon 720 LC spectrometer. A LiChrosorb Si 60, 5 μm (Merck) column and a RP 18, 10 μm (Altex) column were used [solvent systems CH_2Cl_2–hexane (1:1) and MeOH–H_2O (3:1), respectively]. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the microlaboratory of the University of Munich. Analyses are indicated by the symbols of the elements and were within ±0.4% of the theoretical values. The structures of all compounds were confirmed by their IR (Beckman AccuLab 3) and ^1H NMR spectra (Varian T-60).

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

Method A. 1-(3′-Methoxyphenyl)-1-ethanol (1). 3-Methoxybenzaldehyde (13.6 g, 0.1 mol) was dissolved in dry ether and was added dropwise with stirring to a solution of methylmagnesium iodide (20.8 g, 0.125 mol) in 50 mL of ether. 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 reduced pressure to give 12.9 g of **1**.

Method B. 1-Chloro-1-(3′-methoxyphenyl)ethane (7). HCl gas was passed into a solution of compound **1** (15.2 g, 0.1 mol) in 200 mL of ligroin at -5 °C until no more H_2O was formed. The ligroin layer was separated and dried. The solvent was removed and the resulting oil was distilled under reduced pressure to yield 12.5 g of **7**.

Method C. *meso*-2,3-Bis(3′-methoxyphenyl)butane (13). Anhydrous $CoCl_2$ (1.04 g, 8 mmol) was added at room temperature with stirring to a solution of ethylmagnesium bromide (20.0 g, 0.15 mol) in 100 mL of ether. A solution of compound **7** (17.1 g, 0.1 mol) in 50 mL of dry ether was added dropwise. After the vigorous evolution of gas had stopped, the black mixture was poured into ice and HCl and extracted with ether. The ethereal extracts were washed and dried, the solvent was removed, and the residual oil was fractionally crystallized from MeOH. The yield of **13** was 3.52 g.

Method D. *meso*-2,3-Bis(3′-hydroxyphenyl)butane (19). Compound **13** (2.70 g, 0.01 mol) and anhydrous pyridinium hydrochloride (6.93 g, 0.06 mol) were slowly heated up to 220 °C with stirring. The melt was poured into ice–water, and the resulting precipitate was separated with ether. The ethereal extract was washed with water and was extracted with aqueous NaOH. The aqueous layer was acidified with HCl and the product separated with ether. The ethereal extract was dried, the solvent was removed, and the crude product was recrystallized from dilute acetic acid to yield 2.23 g of **19**.

Biological Methods. Estradiol Receptor Binding Assay. The association constants of the inhibitor–receptor complexes were determined by the displacement of [^3H]estradiol. The

(13) In this context it is of interest that uterotrophic and antiuterotrophic activity may vary with the applied dose as well as with the frequency of administration. Estriol was found to act as an estrogen antagonist after a single injection as well as a potent estrogen without antagonistic properties when present continuously [J. H. Clark, Z. Paszko, and E. J. Peck, *Endocrinology*, **100**, 91 (1977)].

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procedure used has been described previously.[3,5,15] Calf uterine cytosol (final concentration of protein 5 mg/mL), the dextran-coated charcoal (DCC) method, and the plotting method according to Lineweaver and Burk[16] were applied.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activities were determined by stimulation of the uterine growth[17] and the inhibition of the uterine growth stimulated by estrone,[18] respectively, using immature NMRI mice as described previously.[3,5] 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. Furthermore, the estrogenic activity was assayed using the vaginal cornification test. The methods applied were similar to that described in the literature.[9] Female NMRI mice weighing about 15 g were ovariectomized. The completeness of castration was checked 10 days after ovariectomy (mainly leucocytes). The animals were primed with a single injection of estradiol 3-benzoate (0.2 μg). Only animals responding with vaginal estrus (mainly cornified cells) were used. The animals were randomly distributed into groups of three and were subcutaneously injected daily for 3 days with 0.1 mL of the test compound dissolved in olive oil. Vaginal smears were taken 6, 24, 30, 48, and 54 h after the last injection by flushing the vagina with 10 μL of physiological saline. The smears were evaluated according to the conventional Allen–Doisy test.[19]

Breast Tumor Cell Proliferation Inhibition Test. A rapidly growing culture of MCF-7 cells which had been cultivated in McCoy's 5a medium supplemented with fetal bovine serum was used. The serum had been stripped of endogenous estrogens by treatment with DCC for 0.5 h at 45 °C. The cells were washed with phosphate-buffered saline (PBS) and were dispersed with 0.25% trypsin–EDTA solution, counted, and diluted to 10^6 cells/mL in serum-free medium. Test compounds were dissolved in ethanol and serially diluted with complete medium to obtain the appropriate concentrations. The final concentration of ethanol in the assay plates did not exceed 0.5%. The cell culture medium containing the appropriate concentration of test compound was inoculated with cell suspension, yielding 10^4 cells/mL. This mixture was placed into 60-mm culture plates at 5 mL/plate, yielding a final inoculum of 5×10^4 cells/plate. On days 3, 5, 7, 10, and 13, three replicate plates for each test or control group were washed with PBS, and the cells were dispersed with trypsin–EDTA and were counted on the Coulter counter, three counts per plate. In a second experiment the compounds were retested at an inhibitory concentration of 10^{-7} M without and with estradiol (10^{-7} and 10^{-8} M) added. The same procedure was used as described above.

Mammary Tumor Growth Inhibition Test. The methods used have been described previously.[3,5] The tumor-inhibiting effect was determined 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 ten. 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.

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Membrane Effects of Antiinflammatory Agents. 1. Interaction of Sulindac and Its Metabolites with Phospholipid Membrane, a Magnetic Resonance Study

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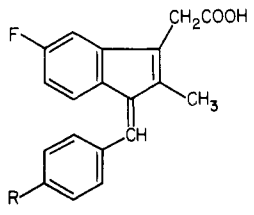
High-resolution proton NMR and spin-label ESR spectroscopies have been applied to examine the interaction of the nonsteroidal antiiflammatory drug sulindac (1) and its active sulfide metabolite (2) and inactive sulfone metabolite (3) with phospholipid membranes. Only weak interactions were observed with 1 and 3, but a strong interaction with 2 was indicated both by specific changes in the proton transverse relaxation rate ($1/T_2$) of different substituents in 2 and by a unique shift in membrane transition temperature in the presence of 2 as measured by the ESR technique. Since the structural differences of these compounds are confined to a single polar substituent, i.e., the oxidation state of the sulfur atom, the strong interaction of the sulfide metabolite (2) with the neutral phospholipid membrane is ascribed to its high partition coefficient in the lipid membrane and its ability to penetrate into the lipid bilayer with the carboxyl group remaining at the polar membrane surface. As evidenced from the ESR spectra of two spin-labels, C_5- and C_{12}-doxylstearic acid, no significant change of the membrane fluidity was induced by the interaction of 2 with phospholipid vesicles.

The antiinflammatory agent sulindac (1) is a new type



1 (sulindac), R = $S(=O)CH_3$
2 (active metabolite), R = SCH_3
3 (inactive metabolite), R = $S(=O)_2CH_3$

of reversible prodrug.[1] In vivo it is reversibly reduced to an active sulfide metabolite (2) and irreversibly oxidized to an inactive sulfone metabolite (3). Like many antiinflammatory agents, sulindac sulfide (2) inhibits the biosynthesis of prostaglandins by a membrane-associated enzyme, the arachidonic acid cyclooxygenase.[2] Since many inflammatory processes are cell-surface phenomena, the possible effects of these structures on model membrane systems were investigated in our laboratories.[3]

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