Statistics. ED_{50} values were calculated according to the method of Litchfield and Wilcoxon.[7]

Carrageenan-Induced Paw Edema (CPE).[8] Five to ten male rats of the Wistar strain, weighing 120–150 g, were used per dose. Hind paw edema was induced by a subcutaneous injection of a 1% carrageenan aqueous solution into the left hind paw. ED_{50} was determined 3 h after carrageenan injection.

Phenylquinone-Induced Writhing (PQW).[9] Five to fifteen female mice of the ddN strain, weighing 18–22 g, were used per dose. The writhing was induced by an intraperitoneal injection of phenylquinone (0.03%), and the number of writhes was calculated for 15 min.

Ultraviolet-Induced Erythema.[10] Erythema was induced on the depilated skin of the dorsal trunk of female guinea pigs of the Hartlet strain (350–450 g). ED_{50} was determined 3 h after irradiation of ultraviolet light.

Acetic Acid Induced Writhing.[11] The writhing was induced by an intraperitoneal injection of a 1% acetic acid aqueous solution in male Wistar rats (90–120 g).

Silver Nitrate Induced Arthritic Pain.[12] Arthritis was induced by an injection of a 1% silver nitrate aqueous solution into the ankle joint of right hind leg of male Wistar rats (130–180 g).

Antipyretic Assay.[13] Hyperthermia was induced by a subcutaneous injection of a 15% yeast suspension in male Wistar rats (350–500 g).

Gastric Ulcer Assay.[14] Male Wistar rats were used. The rats, fasted for 24 h, were sacrificed 6 h after a single oral administration of test compounds, and the stomach was removed and macroscopically observed. The dose (UD_{50}) producing ulcers in 50% of the rats was calculated according to the regression line of each compound.

Acute Lethal Toxicity. LD_{50} was determined from the 7-day mortality in male Wistar rats (150–230 g).

Acknowledgment. We thank Dr. M. Shimizu, Director of Research and Development Headquarters, for his encouragement. Thanks are also due to the members of the Analytical Center of these laboratories for the elemental analyses and spectral measurements.

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1,1,2-Triphenylbut-1-enes: Relationship between Structure, Estradiol Receptor Affinity, and Mammary Tumor Inhibiting Properties

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1,1,2-Triphenylbut-1-enes, which are substituted with acetoxy groups on one, two, or three aromatic rings in the para and/or meta positions, were synthesized. The identity of the occurring *E* and *Z* isomers were established by ^1H NMR spectroscopy. A study on structure–activity relationships was carried out with regard to estradiol receptor affinity and to inhibiting effects on the growth of a postmenopausal human mammary carcinoma implanted in nude mice. The para-substituted compounds generally exhibited a higher receptor affinity and a better antitumor activity than the corresponding meta-substituted ones. The *E* isomers were superior to the respective *Z* isomers in those two properties. The tumor-inhibiting effect of the mono- and disubstituted compounds was better than that of the trisubstituted ones. Except for the trisubstituted compounds, they all showed a good correlation between estradiol receptor affinity and antitumor activity. One of the compounds was also tested on the 9,10-dimethylbenz[*a*]-anthracene-induced, hormone-dependent mammary carcinoma of the Spraque–Dawley rat, and the results corresponded to those obtained in the xenograft tumor.

Many compounds of the triarylethylene type have been tested with regard to their mammary tumor inhibiting properties.[1] One of these compounds, tamoxifen, is now widely used for the treatment of advanced breast cancer.[1] It is of great interest that the *E* isomer of tamoxifen[2] and the metabolite hydroxytamoxifen[2] show contrasting biological properties concerning estradiol receptor affinity, uterotrophic and antiuterotrophic activity, and mammary tumor inhibiting effects. For example, the estradiol receptor affinity of tamoxifen (*Z* configuration) is higher than that of its *E* isomer.[3] Furthermore, compared with ta-

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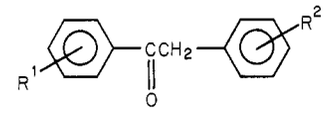
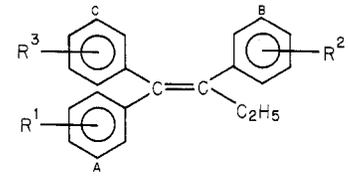
Table I. Methoxy-Substituted 1,2-Diphenylethanones



no.	R^1	R^2	synth method[a]	yield,[b] %	mp,[c] °C	formula
1	H	H	*d*		52-54	$C_{14}H_{12}O$
2[e]	H	4-OCH_3	A	51	96-97	$C_{15}H_{14}O_2$
3	H	3-OCH_3	A	53	oil	$C_{15}H_{14}O_2$
4[e]	4-OCH_3	H	B	61	77	$C_{15}H_{14}O_2$
5[f]	3-OCH_3	H	C	38	oil	$C_{15}H_{14}O_2$
6	4-OCH_3	4-OCH_3	*d*		110-112	$C_{16}H_{16}O_3$
7	3-OCH_3	3-OCH_3	C	35	oil	$C_{16}H_{16}O_3$

[a] Capital letters refer to synthetic methods A-C under Experimental Section. [b] Yield of pure product; no effort was made to optimize yields. [c] All solid compounds were crystallized from EtOH. [d] Compounds were purchased from EGA Steinheim, West Germany. [e] See ref 13. [f] See ref 14.

Table II. Methoxy-Substituted 1,1,2-Triphenylbut-1-enes[a]



compd	R^1	R^2	R^3	yield,[b] %	mp, °C	formula[c]
15[d]	H	H	H	45	78	$C_{22}H_{20}$
(*Z*)-16[e,f]	H	H	4-OCH_3	16	119-121	
(*E*)-16[e,f]	4-OCH_3	H	H	15	101-102	$C_{23}H_{22}O$
(*Z*,*E*)-17[g]	H	H	3-OCH_3	33		$C_{23}H_{22}O$
	3-OCH_3	H	H			
18	H	4-OCH_3	H	43	122	$C_{23}H_{22}O$
19	H	3-OCH_3	H	41	94	$C_{23}H_{22}O$
20	4-OCH_3	H	4-OCH_3	35	122	$C_{24}H_{24}O_2$
21	3-OCH_3	H	3-OCH_3	29	93	$C_{24}H_{24}O_2$
(*Z*,*E*)-22[d,g]	H	4-OCH_3	4-OCH_3	34		$C_{24}H_{24}O_2$
	4-OCH_3	4-OCH_3	H			
(*Z*,*E*)-23[h]	H	3-OCH_3	3-OCH_3	45	oil	$C_{24}H_{24}O_2$
	3-OCH_3	3-OCH_3	H			
24[i]	4-OCH_3	4-OCH_3	4-OCH_3	34	87	$C_{25}H_{26}O_3$
25	3-OCH_3	3-OCH_3	3-OCH_3	28	oil	$C_{25}H_{26}O_3$
(*Z*)-26	4-OCH_3	4-OCH_3	3-OCH_3	31	95	
(*E*)-26[j]	3-OCH_3	4-OCH_3	4-OCH_3			$C_{25}H_{26}O_3$
(*Z*,*E*)-27[h]	4-OCH_3	3-OCH_3	3-OCH_3	37	oil	$C_{25}H_{26}O_3$
	3-OCH_3	3-OCH_3	4-OCH_3			

[a] Synthetic methods D-F under Experimental Section. [b] Calculated on the corresponding 1,2-diphenylethanones. [c] All solid compounds were analyzed for C and H within ±0.40% of the calculated values. [d] See ref 15. [e] The mixture of *E*,*Z* isomers was separated by fractional crystallization. [f] See ref 11. [g] The mixture of *E*,*Z* isomers was not separated. [h] The mixture of *E*,*Z* isomers was neither purified nor separated. [i] See ref 16. [j] In several experiments, only the *Z* isomer was formed.

moxifen, the affinity of the metabolite hydroxytamoxifen to the estrogen receptor is considerably increased by the additional hydroxy group.[4] Although tamoxifen is a less potent antiestrogen than hydroxytamoxifen, it exhibits a stronger inhibiting effect on the growth of the 9,10-dimethylbenz[*a*]anthracene (DMBA) induced mammary carcinoma of the rat than hydroxytamoxifen.[5]

The strong dependence of the estradiol receptor affinity and of the antitumor effect on the number and position of the substituents on the aromatic rings induced us to carry out a detailed study on structure–activity relationships with regard to receptor affinity and antitumor activity in the class of the 1,1,2-triphenylbut-1-enes. For that purpose, we synthesized compounds of this type, which are substituted with acetoxy groups on one, two, or three aromatic rings in the para and/or meta positions. The meta-substituted compounds were prepared because shifting of the hydroxy groups in diethylstilbestrol from the para to the meta position led to compounds with antiuterotrophic and mammary tumor inhibiting properties.[6]

Chemistry. The acetoxy-substituted 1,1,2-triphenylbut-1-enes (**28–39**) were synthesized according to the method of Dodds et al.[7] starting with the corresponding 1,2-diphenylethanones (**1–7**). Compounds **1** and **6** are commercially available. Compounds **2** and **3** were prepared by Grignard reaction of the corresponding methoxyphenylacetyl chlorides with phenylmagnesium bromides at low temperature using THF as solvent.[8] Compound

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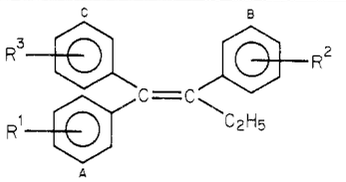
Table III. Acetoxy-Substituted 1,1,2-Triphenylbut-1-enes[a]



compd	R^1	R^2	R^3	yield,[b] %	mp, °C	formula[c]	% RBA[d]
15[e]	H	H	H				0.8
(*Z,E*)-**28**[f]	H	H	4-$OCOCH_3$	69			2.0
(*E*)-**28**[g]	4-$OCOCH_3$	H	H	14	121	$C_{24}H_{22}O_2$	5.7
(*Z*)-**29**[g]	H	H	3-$OCOCH_3$	14	103		0.3
(*Z,E*)-**29**[f]	3-$OCOCH_3$	H	H	67		$C_{24}H_{22}O_2$	0.7
30	H	4-$OCOCH_3$	H	68	118	$C_{24}H_{22}O_2$	3.8
31	H	3-$OCOCH_3$	H	65	74	$C_{24}H_{22}O_2$	1.5
32	4-$OCOCH_3$	H	4-$OCOCH_3$	72	82	$C_{26}H_{24}O_4$	6.8
33	3-$OCOCH_3$	H	3-$OCOCH_3$	67	94	$C_{26}H_{24}O_4$	1.2
(*Z*)-**34**[g]	H	4-$OCOCH_3$	4-$OCOCH_3$	14	106	$C_{26}H_{24}O_4$	1.0
(*E*)-**34**[g]	4-$OCOCH_3$	4-$OCOCH_3$	H	52	171		5.9
(*Z*)-**35**[h]	H	3-$OCOCH_3$	3-$OCOCH_3$	29	79	$C_{26}H_{24}O_4$	0.2
	3-$OCOCH_3$	3-$OCOCH_3$	H	31	76		1.1
36	4-$OCOCH_3$	4-$OCOCH_3$	4-$OCOCH_3$	76	133	$C_{28}H_{26}O_6$	4.2
37	3-$OCOCH_3$	3-$OCOCH_3$	3-$OCOCH_3$	31	100	$C_{28}H_{26}O_6$	1.5
(*Z*)-**38**[h]	4-$OCOCH_3$	4-$OCOCH_3$	3-$OCOCH_3$	63	121	$C_{28}H_{26}O_6$	3.0
(*E*)-**38**[i]	3-$OCOCH_3$	4-$OCOCH_3$	4-$OCOCH_3$				
(*Z*)-**39**[h]	4-$OCOCH_3$	3-$OCOCH_3$	3-$OCOCH_3$	29	126	$C_{28}H_{28}O_6$	2.7
(*E*)-**39**[h]	3-$OCOCH_3$	3-$OCOCH_3$	4-$OCOCH_3$	28	oil		1.8

[a] Synthetic method G under Experimental Section. [b] Calculated on the corresponding methoxy-substituted 1,1,2-triphenyl-but-1-enes. [c] All compounds were analyzed for C and H within 0.40% of the calculated values. [d] RBA = $[E_2]/[I] \times 100$; [E2] and [I] are the molar concentrations of nonradioactive E_2 and inhibitor required to decrease the bound radioactivity by 50%. E_2 = 17β-estradiol. [e] See Table II. [f] *Z,E* = mixture of *Z* and *E* isomers (1:1). [g] The mixture of *E* and *Z* isomers was separated by fractional crystallization. [h] The mixture of *E* and *Z* isomers was separated by chromatography on silica gel. [i] The *E* isomer was also not formed by isomerization during ether cleavage and acetylation.

4 was synthesized by Friedel–Crafts acylation of anisole with phenylacetyl chloride. Compounds **5** and **7** were prepared by a Grignard reaction from 3-methoxybenzamide and the corresponding benzylmagnesium chloride[9] (Table I).

The 1,2-diphenylethanones (**1–7**) were alkylated with ethyl iodide to give the 1,2-diphenylbutanones **8–14** (Scheme I). Compounds **8–14** were converted with the respective phenylmagnesium bromide into the corresponding tertiary carbinols, which were dehydrated using a mixture of sulfuric acid and acetic acid[10] to give the methoxy-substituted 1,1,2-triphenyl-but-1-enes (Table II). Only in the case of compound **16** was the resulting mixture of the *E* and *Z* isomer separated by fractional crystallization, since those two compounds were described in the literature;[11,12] therefore, they were used by us to compare their NMR data with the data of the other compounds.

Compounds **16–27** were then converted to the hydroxy derivatives by ether cleavage with BBr_3 and acetylated with acetic anhydride and pyridine to give the acetoxy-substituted 1,1,2-triphenyl-but-1-enes **28–39** (Table III).

The mixtures of *E* and *Z* isomers were separated by fractional crystallization or by column chromatography on silica gel with mixtures of CH_2Cl_2 and ligroin as eluent (see also Table III). In the case of compound **28**, which isomerized during ether cleavage to yield a mixture of the *E* and *Z* isomer, and compound **29**, only one isomer was isolated pure. For the pharmacological tests, a mixture of the *E* and *Z* isomers in a proportion of 1:1 was used in addition to the pure isomer (Table III). In the case of compound **26**, only one isomer was formed in several experiments. During ether cleavage to give **38**, no isomerization took place, either. This fact was confirmed by

Scheme I

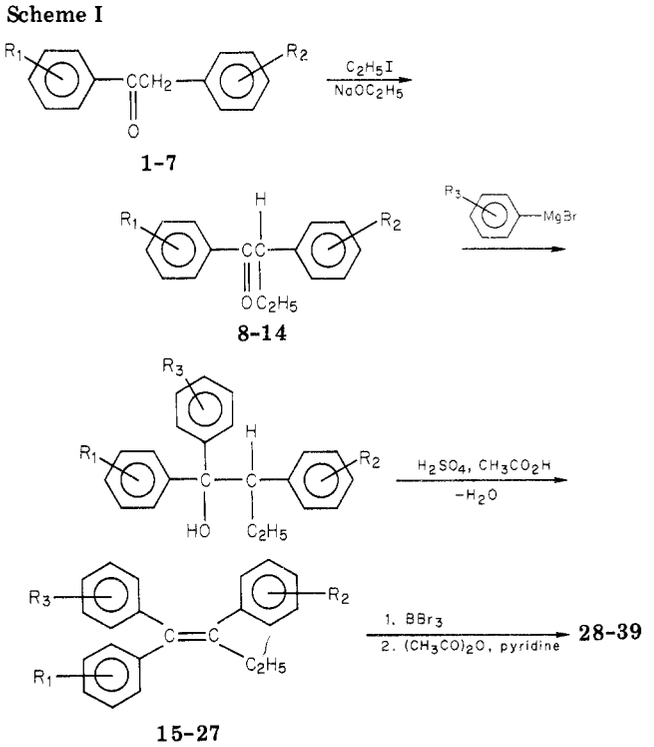


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Table IV. ^{1}H NMR Spectra of Compounds **15-27**

compd	aromatic H	OCH_3	CH_2[a]	CH_3[b]
15	7.31, 7.15, 6.97 (3 s, 15 H)		2.50	0.93
(*Z*)-**16**	7.30, 7.15 (2 s, 10 H), 6.79, 6.51 (AB, *c*, 4 H)	3.65 (s, 3 H)	2.46	0.92
(*E*)-**16**	7.14, 6.95 (2 s, 10 H), 7.14, 6.82 (AB, *c*, 4 H)	3.79 (s, 3 H)	2.50	0.93
(*Z,E*)-**17**	7.30, 7.17, 6.97 (3 s), 7.28-6.43 (m, 14 H)	3.75, 3.46 (2 s, 6 H)	2.49, 2.47	0.92
18	7.30, 6.99 (2 s, 10 H), 7.06, 6.69 (AB, *c*, 4 H)	3.77 (s, 3 H)	2.46	0.93
19	7.29, 6.97 (2 s, 10 H), 7.27-6.50 (m, 4 H)	3.58 (s, 3 H)	2.48	0.94
20	7.15 (s, 5 H), 7.17, 6.86 (AB, *c*, 4 H), 6.80, 6.51 (AB, *c*, 4 H)	3.80, 3.65 (2 s, 6 H)	2.50	0.93
21	7.15 (s, 5 H), 7.23-6.42 (m, 8 H)	3.80, 3.50 (2 s, 6 H)	2.48	0.93
(*Z,E*)-**22**	7.33, 6.98 (2 s), 7.16, 6.89 (AB, *c*), 7.00, 6.72 (AB, *c*), 6.84, 6.55 (AB, *c*, 13 H)	3.80, 3.76, 3.67 (3 s, 6 H)	2.48, 2.45	0.92
(*Z,E*)-**23**	7.30, 6.98 (2 s), 7.23-6.46 (m, 13 H)	3.75, 3.60, 3.49 (3 s, 6 H)	2.47, 2.45	0.94
24	7.17, 6.89 (AB, *c*, 4 H), 6.99, 6.71 (AB, *c*, 4 H), 6.82, 6.51 (AB, *c*, 4 H)	3.82, 3.76, 3.69 (3 s, 9 H)	2.47	0.93
25	7.26-6.46 (m, 12 H)	3.79, 3.63, 3.50 (3 s, 9 H)	2.47	0.93
(*Z*)-**26**	7.17, 6.90 (AB, *c*, 4 H), 7.00, 6.69 (AB, *c*, 4 H), 7.10-6.45 (m, 4 H)	3.82, 3.75, 3.51 (3 s, 9 H)	2.49	0.93
(*Z,E*)-**27**	7.62-6.43 (m, 12 H)	3.80, 3.77, 3.67, 3.61, 3.50 (5 s, 9 H)	2.50, 2.47	0.93

[a] q, J = 7 Hz, 2 H. [b] t, J = 7 Hz, 3 H. [c] J = 9 Hz. [d] *Z,E* = mixture of *Z* and *E* isomers; AB = AA'BB'.

Table V. ^{1}H NMR Spectra of Compounds **28-39**

no.	aromatic H	CH_2[a]	$OCOCH_3$	CH_3[b]
(*Z,E*)-**28**[a]	7.30, 7.14, 6.96 (3 s), 7.29, 6.99 (AB, *c*), 6.89, 6.69 (AB, *c*, 14 H)	2.48, 2.45	2.28, 2.18 (2 s, 3 H)	0.92
(*E*)-**28**	7.14, 6.96 (2 s, 10 H), 7.31, 6.99 (AB, *c*, 4 H)	2.48	2.28 (s, 3 H)	0.92
(*Z*)-**29**	7.30, 7.17 (2 s, 10 H), 7.27-6.62 (m, 4 H)	2.46	2.15 (s, 3 H)	0.92
(*Z,E*)-**29**	7.30, 7.16, 6.96 (3 s), 7.27-6.61 (m, 14 H)	2.48, 2.46	2.26, 2.14 (2 s, 3 H)	0.93
30	7.30, 6.97 (2 s, 10 H), 7.25, 6.94 (AB, *c*, 4 H)	2.47	2.23 (s, 3 H)	0.94
31	7.30, 6.97 (2 s, 10 H), 7.23-6.82 (m, 4 H)	2.48	2.24 (s, 3 H)	0.91
32	7.16 (s, 5 H), 7.28, 7.02 (AB, *c*, 4 H), 6.91, 6.72 (AB, *c*, 4 H)	2.50	2.29, 2.18 (2 s, 6 H)	0.92
33	7.15 (s, 5 H), 7.18-6.40 (m, 8 H)	2.48	2.25, 2.13 (2 s, 6 H)	0.92
(*Z*)-**34**	7.30 (s, 5 H), 7.19, 6.89 (AB, *c*, 4 H), 6.91, 6.72 (AB, *c*, 4 H)	2.47	2.25, 2.19 (2 s, 6 H)	0.92
(*E*)-**34**	6.96 (s, 5 H), 7.29, 6.99 (AB, *c*, 4 H), 7.23, 6.92 (AB, *c*, 4 H)	2.50	2.30, 2.25 (2 s, 6 H)	0.94
(*Z*)-**35**	7.30 (s, 5 H), 7.25-6.64 (m, 8 H)	2.45	2.23, 2.17 (2 s, 6 H)	0.92
(*E*)-**35**	6.99 (s, 5 H), 7.23-6.83 (m, 8 H)	2.49	2.27, 2.23 (2 s, 6 H)	0.93
36	7.27, 6.99 (AB, *c*, 4 H), 7.23, 6.91 (AB, *c*, 4 H), 6.91, 6.72 (AB, *c*, 4 H)	2.47	2.29, 2.24, 2.20 (3 s, 9 H)	0.92
37	7.42-6.60 (m, 12 H)	2.48	2.28, 2.24, 2.18 (3 s, 9 H)	0.94
(*Z*)-**38**	7.30, 6.99 (AB, *c*, 4 H), 7.23, 6.92 (AB, *c*, 4 H), 7.23-6.65 (m, 4 H)	2.49	2.30, 2.25, 2.18 (3 s, 9 H)	0.93
(*Z*)-**39**	7.29, 7.07 (AB, *c*, 4 H), 7.33-6.67 (m, 8 H)	2.47	2.28, 2.22, 2.16 (3 s, 9 H)	0.92
(*Z*)-**39**	6.96, 6.76 (AB, *c*, 4 H), 7.33-6.86 (m, 8 H)	2.48	2.29, 2.24, 2.23 (3 s, 9 H)	0.92

[a] q, J = 7 Hz, 2 H. [b] t, J = 7 Hz, 3 H. [c] J = 9 Hz. [d] *Z,E* = mixture of *Z* and *E* isomer (1:1); AB = AA'BB'.

HPLC analyses and NMR spectroscopy.

The purity of the *E* and *Z* isomers was proved by HPLC analyses. The identity of the occurring *E* and *Z* isomers was established by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of **15**, the compound with unsubstituted aromatic rings, shows one singlet for each phenyl group (Table IV).

By means of the molecular model, it is assumed that the aromatic ring C (Table III) is in plane with the ethylene system. Therefore, the resonance for ring C appears at higher field compared to the resonances of A and B, because of the double shielding effect of the ring currents of A and B. Furthermore, ring B is nearly vertical to the plane of the double bond, and ring A is at an angle of at least 40° to it. For these sterical reasons, the protons of A are closer to ring C and, therefore, are paramagnetically more influenced than the protons of B. From the molecular model, a difference of 0.15 ppm between the resonances of ring A and B was estimated; the more shielded ring B should show the lower δ values. Consistent with these considerations, we observed a decrease in the δ values for the aromatic protons in the order A (7.31) > B (7.15) > C (6.97).

This sterical interpretation of the NMR spectra was supported by the UV spectrum of **15** [λ 268 nm (ϵ 20 000), 223 (25 000), in MeOH], which resembles the spectra of *cis*-stilbene [λ 277 nm (ϵ 10 400), 224 (22 000)] and α-methyl-*cis*-stilbene (λ 267 nm). From the UV spectrum of the latter, an interplanar angle of 40° was calculated,[17] which is mainly due to the rotation around the Ph-α-C bond.[18] In the NMR spectrum, compounds (*Z*)- and (*E*)-**16**, the structures of which were already confirmed by Bedford and Richardson,[12] show, beside the AA'BB' resonance of the para-substituted phenyl group, the singlets of the unsubstituted aromatic rings at the δ values estimated above. The agreement with the postulated order of the aromatic resonances is confirmed by the ^{1}H NMR spectra of **20** and **21**, the compounds with an unsubstituted ring B, and of **18** and **19**, the compounds with a substituent in ring C.

In this way, the identity of the *E* and *Z* isomers of the compounds with at least one unsubstituted aromatic ring was established.

The AA'BB' resonance groups of the para-substituted rings show the same order as those of the unsubstituted ones, both in the case of the methoxy- and acetoxy-substituted compounds. Thus, it was possible to relate the *E* and *Z* structure to the isomers with three substituted aromatic rings.

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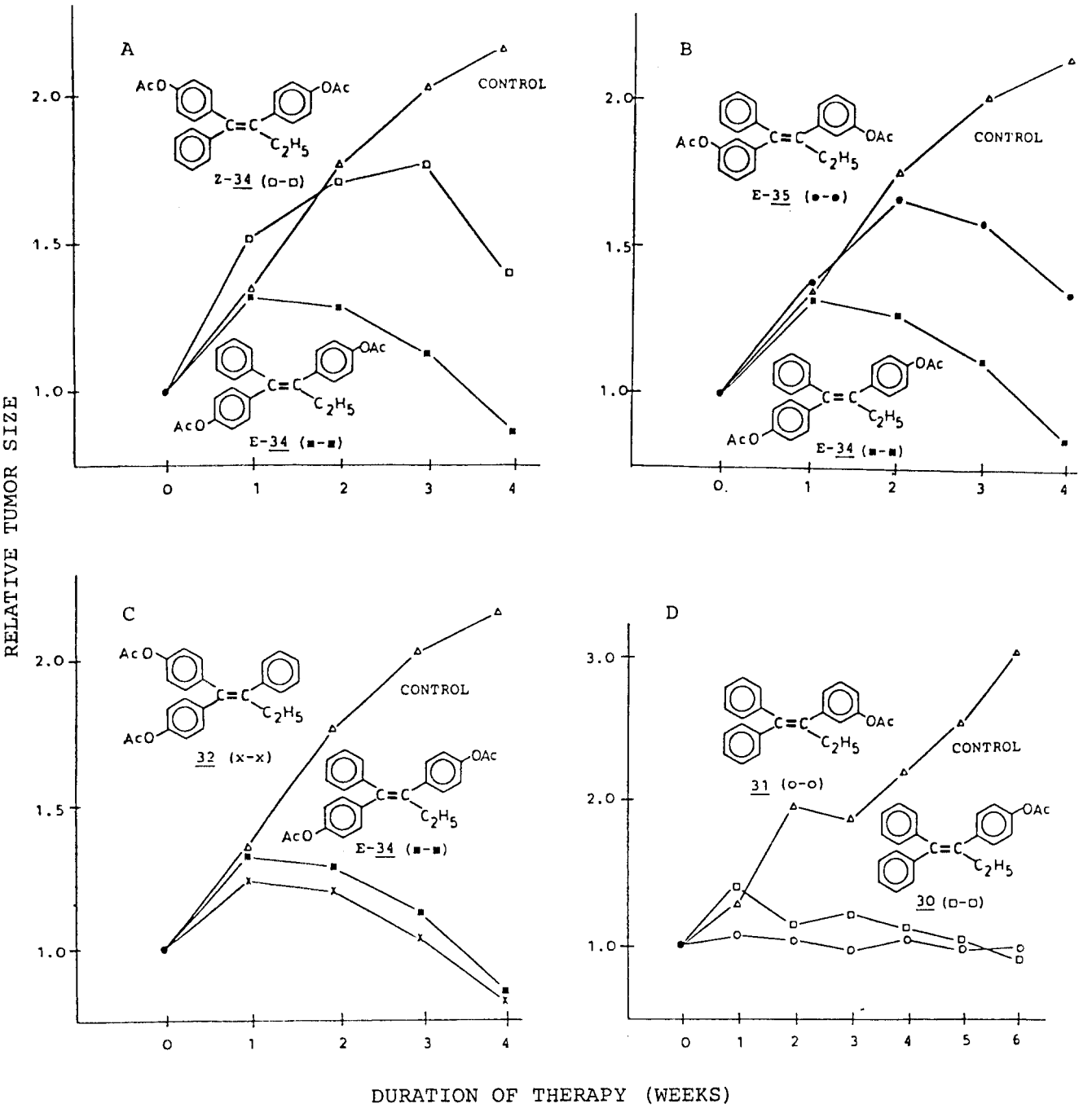

Figure 1. Effect of (*Z*)-**34**, (*E*)-**34**, (*E*)-**35**, and **32** (2.5 mg/kg) and **30** and **31** (2.0 mg/kg) on the growth of an estrogen and progesterone receptor positive, postmenopausal human carcinoma serially implanted in nude mice. Compounds were administered 6 times a week sc.

The identity of the *E* and *Z* isomers determined above was also confirmed by the resonances of the OCH_3 and $OCOCH_3$ protons (e.g., 4-OCH_3: ring A, δ 3.79 [(*E*)-**16**]; ring B, δ 3.77 (**18**); ring C, δ 3.65 [(*Z*)-**16**].

Biological Properties. The RBA (relative binding affinity) values of compounds **15** and **28–39**, which were determined using calf uterine cytosol,[6] are listed in Table III. To evaluate the tumor-inhibiting effect, we used an estrogen and progesterone receptor positive postmenopausal human mammary carcinoma serially implanted in nude mice.[19] This tumor model is similar to the DMBA-induced mammary carcinoma of the rat with regard to its response to the administration of estrogens and antiestrogens.[20,21]

In the competitive binding assay, all compounds, except **15**, had a binding curve parallel to that of estradiol; therefore, it can be stated that these compounds exhibit a competitive inhibition of the interaction of estradiol with its receptor. Compound **15**, with unsubstituted aromatic rings, showed a smaller slope which gives rise to the assumption that the inhibition type is not competitive. To our surprise, **15** exerted a rather good, but not significant, antitumor effect (Table VI), which might probably be caused by a metabolic transformation to a hydroxylized

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Table VI. Effect of **15** and **28–39** on the Growth of an Estrogen and Progesterone Receptor Positive, Hormone-Dependent, Postmenopausal Human Mammary Carcinoma Implanted in Nude Mice

compd	dose,[a] (mg/kg)/day	tumor size[b]	tumor size of related control
15	2.2	1.61[d]	2.17
(*Z,E*)-**28**	2.5	1.30[c]	2.21
(*E*)-**28**	2.5	1.24[c]	2.21
(*Z*)-**29**	2.5	2.41[d]	2.60
(*Z,E*)-**29**	2.5	2.12[d]	2.60
30	2.5	1.12[c]	2.21
31	2.5	1.04[c]	2.21
32	3.0	0.81[c]	2.17
33	3.0	2.40[d]	2.60
(*Z*)-**34**	3.0	1.39[c,e]	2.17
(*E*)-**34**	3.0	0.83[c,e]	2.17
(*Z*)-**35**	3.0	2.36[d,e]	2.17
(*E*)-**35**	3.0	1.35[c,e]	2.17
36	3.4	1.52[c]	2.17
37	3.4	2.12[c]	3.02
(*Z*)-**38**	3.4	2.04[d]	2.17
(*Z*)-**39**	3.4	1.69[d]	2.60
(*E*)-**39**	3.4	2.44[d]	2.60

[a] Compounds were administered 6 times/week sc. [b] Average after 4 weeks of therapy; for definition of tumor size, see Experimental Section. [c] Significant ($\alpha \leq 0.05$) vs. control. [d] Not significant ($\alpha > 0.05$) vs. control. [e] Significant difference ($\alpha \leq 0.05$) between *E* and *Z* isomer.

compound as already seen in the case of tamoxifen.[1]

The para-substituted compounds showed an affinity to the estrogen receptor that is about 5 times higher than that of the corresponding meta-substituted ones (Table III). That does not hold true in the case of the compounds substituted on ring C, since **30** has an affinity only about twofold the affinity of **31**. Moreover, the antitumor effect of the 4-$OCOCH_3$ compounds was better than that of the respective 3-$OCOCH_3$ compounds (Table VI, Figure 1B), with the exception of **30** and **31**, which exhibited nearly the same effect (Figure 1D).

Comparison of the corresponding *E* and *Z* isomers (**28**, **29**, **34**, **35**) revealed the superiority of the *E* isomers in receptor affinity and mammary tumor inhibiting activity (Table VI, Figure 1A) to the *Z* isomers, which is statisfically significant.

No difference, either in receptor affinity or in antitumor activity, can be seen between the 1,1- and 1,2-disubstituted compounds [**32** vs. (*E*)-**34**, **33** vs. (*E*)-**35**] (Tables III and VI, Figure 1C).

Concerning the estradiol receptor affinity of the compounds substituted in ring A, A and B, A and C, or A, B, and C, there exist only slight differences between the mono- [e.g., (*E*)-**28**], di- [e.g., **32** and (*E*)-**34**], and trisubstituted (e.g., **36**) ones (Table III). The tumor-inhibiting effect of the disubstituted compounds [e.g., **32** and (*E*)-**34**] seemed to be a little stronger than that of the compounds with only one $OCOCH_3$ group [e.g., (*E*)-**28**]. The trisubstituted compounds **36**, **37**, (*Z*)-**38**, (*Z*)-**39**, and (*E*)-**39**, however, showed, compared to the corresponding mono- and disubstituted ones, a weaker antitumor effect (Table VI). Comparison of the RBA values of the compounds with three acetoxy groups reveals an interesting graduation. The decrease in receptor affinity is accompanied by a decrease in the number of *p*-$OCOCH_3$ groups and by an increase in the number of the *m*-$OCOCH_3$ groups [Table III; % RBA: **36** > (*Z*)-**38** > (*Z*)-**39** > (*E*)-**39** > **37**].

Since **37** is a structural analogue of 3,3′-dihydroxy-α,β-diethylstilbene,[6] in which one of the ethyl side chains is replaced by a 3-$OCOCH_3$-C_6H_4 moiety, we also tested the tumor-inhibiting effect of **37** on the DMBA-induced, hormone-dependent mammary carcinoma of the rat.

Table VII. Effect of **37** on the Growth of the DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat

compd (dose,[a] (mg/kg)/day)	no. of tumors		% of tumors with				% change of	
	B	NT	CR	PR	NC	P	tumor area[b]	body wt
control	23	24	0	0	11	89	483	3.6
37 (0.85)	20	25	2	14	44	40	102[d]	5.2[e]
37 (1.71)	16	16	3	16	37	44	78[d]	3.2[e]
37 (6.83)	22	15	5	30	30	35	54[d]	3.6[e]
OV[f]	24	0	79	21	0	0	−93[d]	0.9[e]

[a] Compounds were administered 6 times/week sc. [b] Average on the 28th day of therapy. [c] Average on the 7th day of therapy. [d] Significant ($\alpha \leq 0.01$). [e] Not significant ($\alpha > 0.01$). [f] OV = ovariectomy. B = at test beginning; NT = occurring during the test; CR = complete remission, tumor not palpable; PR = partial remission, tumor size ≤50% of initial size; NC = no change, tumor size 51–150% of initial size; P = progression, tumor size >150% of initial size.

Compound **37** showed a significant, dose-dependent inhibition of the growth of this tumor, although not to the same extent as 3,3′-dihydroxy-α,β-diethylstilbene[6] or ovariectomy (Table VII).

Discussion

The results given above demonstrate that the biological properties of these 1,1,2-triphenylbut-1-enes depend on the number and position of the ring substituents.

As we have already shown in the class of 3,3′-diacetoxy-α,β-dialkylstilbenes and of related stilbene oxides,[21] acetylation of the phenolic hydroxy groups only caused a slight decrease in receptor affinity. In the case of similar para-substituted compounds, a reduction to about one-fifth of the affinity of the corresponding phenolic compounds was determined (unpublished results). We prepared the acetoxy-substituted compounds instead of their phenolic analogues, since an *E,Z* isomerization is more likely in the case of the OH compounds. Winkler et al.[22] showed that the *E,Z* isomerization of diethylstilbestrol takes place through a quinoid intermediate, the formation of which is possible only via free phenolic hydroxy groups. In the case of (*E*)- and (*Z*)-**34**, for example, we observed no *E,Z* isomerization in ethanolic solution. In previous experiments (unpublished results) we were able to show that enzymatic cleavage of phenolic acetates does not occur under the conditions of the receptor binding assay (15 h, 4 °C). In vivo, a cleavage of the acetoxy groups is likely; therefore, the *E,Z* isomerization may influence the results obtained in the tumor assay to some extent.

The RBA values given in Table III clearly show that a substitution on ring A, especially in the para position (corresponding to the C_3 OH of estradiol), is necessary for a high binding affinity to the estrogen receptor, as has already been pointed out by other authors.[23] The introduction of a *p*-OH group onto the C_1 phenyl of tamoxifen (hydroxytamoxifen) also produces a marked increase in receptor affinity.[4]

Surprisingly, in the case of the disubstituted compounds, the affinity to the estrogen receptor was only slightly increased by the introduction of an acetoxy group into ring B, but a substitution of ring C (**32**) caused an increase in affinity in comparison with (*E*)-**34**.

Most of the antiestrogenic triphenylethylenes, e.g., ta-

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moxifen, are in this ring C, substituted with a basic side chain. It was postulated[23,24] that this basic side chain interacts with a region of the receptor different from those with which steroidal estrogens or diethylstilbestrol normally interact. It was shown that the receptor affinity of certain chromans with a basic side chain is much higher than the affinity of the corresponding phenols.[24] Furthermore, (*E,Z*)-**28** (Table III) has a lower affinity than tamoxifen, for which we evaluated a RBA of 2.8 in our binding assay. In some recent publications,[25,26] the existence of a receptor distinct from the classical estrogen receptor site, called "antiestrogen receptor", was postulated. In a competitive binding assay, estradiol[20] was not able to compete with [^{3}H]tamoxifen for this binding site, whereas CI 628 and nafoxidin[26] displaced [^{3}H]tamoxifen. The ability of our triphenylbut-1-enes to compete with [^{3}H]tamoxifen for this "site" will be examined in future studies.

This basic side chain is thought to be essential in evoking antiuterotrophic properties. Lednicer et al.,[27] however, showed that a 1-aryl-6-methoxy-2-phenyl-3,4-dihydronaphthalene with a glyceryl ether side chain also exhibits antiuterotrophic activity. Preliminary investigations also demonstrate that compound **37**, for example, possesses antiuterotrophic activity [54% inhibition at a dose of 5 (μg/animal)/day] in the immature mouse uterine weight test. Thus, it is shown that compounds of the triphenylethylene type without a basic side chain exert antiuterotrophic effects, too.

Concerning the antitumor activity of our compounds, it can be stated that a good correlation between estradiol receptor affinity and tumor inhibiting properties exists (see Tables III and VI), except for the compounds with three acetoxy groups. This fact can be explained by a more rapid clearance from the body, as also seen with hydroxytamoxifen, which exerted a weaker tumor-inhibiting effect than tamoxifen itself.[5]

In our tumor model, **32** and (*E*)-**34** [6 × 2.0 (mg/kg)/week] did not only cause a delay in tumor growth, but the tumor size was even smaller than at the beginning of the test. When tamoxifen [2 × 40 (mg/kg)/week; 14 animals] was administered, however, the tumor size was the same at the end of therapy as it had been at the start of treatment.[19]

We cannot say yet whether the antitumor activity of our compounds is due to estrogenic or antiestrogenic properties; therefore, we intend to carry out further studies on the mode of action of those triphenylbut-1-enes.

In conclusion, it can be stated that (1) substitution in ring A with an acetoxy group in this class of 1,1,2-triphenylbut-1-enes causes a high estradiol receptor affinity and a good antitumor effect; (2) substitution with an additional acetoxy group increases those two effects only slightly, whereas introduction of a third acetoxy group diminishes the antitumor activity; (3) para-substituted compounds are generally superior to the corresponding meta-substituted ones in receptor affinity and tumor-inhibiting properties; and (4) with the exception of the compounds with three acetoxy groups, a good correlation between receptor affinity and antitumor activity was observed.

Experimental Section

General Procedures. Melting points, which are uncorrected, were taken on a Büchi 510 melting point apparatus. ^{1}H NMR spectra were obtained with a Varian EM 360 A, 60-MHz spectrometer (recorded in $CDCl_3$; internal standard Me_4Si; chemical shifts in δ (parts per million)]. TLC of each compound was performed on Merck F 254 silica gel plates. HPLC was performed using an Altex Model 110 A pump and a Kontron Uvikon 720 LC spetrophotometer [column LiChrosorb Si 60 (5 μm), Merck, West Germany]. Elemental analyses were accomplished by the microlaboratory of the University of Regensburg.

Syntheses. Synthetic methods A–H are representatives for compounds reported in Tables I–III.

Method A. 1-Phenyl-2-(4-methoxyphenyl)ethanone (2). A solution of phenylmagnesium bromide (45.3 g, 0.25 mol) in 200 mL of THF was added dropwise at −78 °C to a solution of 4-methoxyphenylacetyl chloride (42.1 g, 0.25 mol) in 150 mL of THF. The reaction mixture was brought to room temperature over 1 h. After 300 mL of water was added to the mixture, the aqueous layer was extracted with ether. The extract was washed with 1 N NaOH and dried over Na_2SO_4, and the solvent was removed. The crude product was recrystallized from EtOH to give 28.8 g of **2**.

Method B. 1-(4-Methoxyphenyl)-2-phenylethanone (4). To a solution of phenylacetyl chloride (38.5 g, 0.25 mol) in 160 mL of 1,2-dichloroethane were added anisole (54.0 g, 0.5 mol) and then $AlCl_3$ (73.4 g, 0.55 mol). The whole was refluxed for 2 h, allowed to stand for an additional 2 h, and then treated with water. The 1,2-dichloroethane layer was separated, and the aqueous layer was extracted with 1,2-dichloroethane. The organic extracts were washed with water and dried over Na_2SO_4, and the solvent was removed. The crude product was recrystallized from EtOH to give 34.4 g of **4**.

Method C. 1,2-Bis(3-methoxyphenyl)ethanone (7). 3-Methoxybenzamide (37.7 g, 0.25 mol) was added in portions to a solution of 3-methoxybenzylmagnesium chloride (151.0 g, 1.0 mol). The mixture was refluxed for 48 h. After ice–water was added and the mixture was acidified with 3 N sulfuric acid, the ether layer was separated. The water layer was extracted with ether. The ether extracts were washed with a saturated $NaHCO_3$ solution and water and then dried over Na_2SO_4, and the solvent was removed. The crude product was purified by column chromatography on silical gel (CH_2Cl_2 as the eluent) to give 22.3 g of **7**.

Method D. 1,2-Bis(4-methoxyphenyl)butanone (13). A hot solution of sodium (2.3 g, 0.10 g-atom) in 44 mL of EtOH was added to a mixture of 1,2-bis(4-methoxyphenyl)ethanone (25.6 g, 0.10 mol) and ethyl iodide (15.6 g, 0.10 mol) under mechanical stirring. The solution was refluxed for 10 min. A further quantity of sodium (1.15 g, 0.05 g-atom) in 22 mL of EtOH and ethyl iodide (7.8 g, 0.05 mol) was added, and the whole was refluxed for 4 h. EtOH was removed under reduced pressure, and water was added. The water layer was extracted with ether. The organic extracts were washed with 0.5 N sodium thiosulfate solution and water and then dried over Na_2SO_4, and the solvent was removed to give 37.2 g of **13**. The completeness of alkylation was proved by TLC and ^{1}H NMR spectroscopy. No further efforts were made to purify the product.

Method E. 1,1,2-Tris(4-methoxyphenyl)butan-1-ol. A solution of 1,2-bis(4-methoxyphenyl)butanone (28.4 g, 0.10 mol) in 60 mL of ether was added dropwise to a solution of 4-methoxyphenylmagnesium bromide (63.3 g, 0.30 mol) in 300 mL of ether. The mixture was refluxed for 2 h and then decomposed with ice and 3 N sulfuric acid. The ethereal layer was separated, the water layer was extracted with ether, and the organic extracts were washed with saturated $NaHCO_3$ solution and water. After the extracts were dried over Na_2SO_4, the solvent was removed to give 1,1,2-tris(4-methoxyphenyl)butan-1-ol as crude product. No efforts were made to purify the crude product because spontaneous decomposition of water occurred.

Method F. 1,1,2-Tris(4-methoxyphenyl)but-1-ene (17). To the crude product of 1,1,2-tris(4-methoxyphenyl)butan-1-ol was added a mixture of sulfuric acid and acetic acid (8:2 v/v). The

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whole was heated on a water bath for 20 min. After the mixture was cooled and water was added, the aqueous layer was extracted with ether. The ethereal extracts were washed with 1 N NaOH and water and then dried over Na_2SO_4, and the solvent was removed. The crude product was purified by column chromatography on silica gel (CH_2Cl_2 as the eluent). After recrystallization from EtOH, 12.7 g of **17** was obtained.

Method G. 1,1,2-Tris(4-methoxyphenyl)but-1-ene (29). A solution of **17** (3.74 g, 0.01 mol) in 250 mL of dry CH_2Cl_2 was cooled to −60 °C. Under nitrogen, BBr_3 (10.02 g, 0.04 mol) was added. After 0.5 h, the freezing mixture was removed, and the reaction mixture was stirred at room temperature for 4 h. After that, 50 mL of MeOH was added with cooling, and the solvents were removed under reduced pressure. Acetic anhydride (4.08 g, 0.04 mol) and pyridine (3.96 g, 0.05 mol) were added. The mixture was refluxed for 1 h. After the mixture was cooled, 300 mL of ice–water was added. The aqueous layer was extracted with ether, and the ethereal extracts were washed with 1 N HCl and saturated $NaHCO_3$ solution and dried over Na_2SO_4. The solvent was removed and the crude product was recrystallized from EtOH to give 3.48 g of **29**.

Biological Methods. Estradiol Receptor Binding Assay. The method described in ref 6 was used with some modifications. 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.

Mammary Tumor Growth Inhibition Tests. (a) Hormone-Dependent Human Mammary Carcinoma Serially Transplanted in Nude Mice.[19] Animals of a random-bred strain (NMRI, nu/nu, castrated female or male mice, 10–12 animals per group) with a body weight of approximately 25 g and aged 6–7 weeks served as recipients. Tumor slices (0.5 cm in diameter) from postmenopausal women were transplanted under sterile conditions behind the shoulder blade into the area of the mammary gland of castrated male or female nude mice. The postmenopausal tumor was estrogen receptor and progesterone receptor positive. The receptors were measured by gel electrophoresis according to ref 28. Tumors were measured once a week by two diameters. Compounds were administered as olive oil solutions 6 times a week sc. Control animals received vehicle for a period of 4–6 weeks. At the beginning of treatment, tumor size was defined as "1".

(b) DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat. A single dose of 20 mg of DMBA (9,10-dimethylbenz[*a*]anthracene) was administered by gastric intubation to female SD (Sprague–Dawley) rats at an age of 50 days. After the appearance of tumors, about 4 weeks later, animals with at least one tumor with an area >140 mm^2 were classified in groups of ten. Compounds were administered in olive oil solution 6 times a week sc. The duration of treatment was 28 days. Measurement of tumor area was made twice weekly. The tumor area was defined by length × width of the tumor.

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Synthesis, 470-MHz ^{1}H NMR Spectra, and Activity of Delactonized Derivatives of the Anticancer Drug Etoposide[1]

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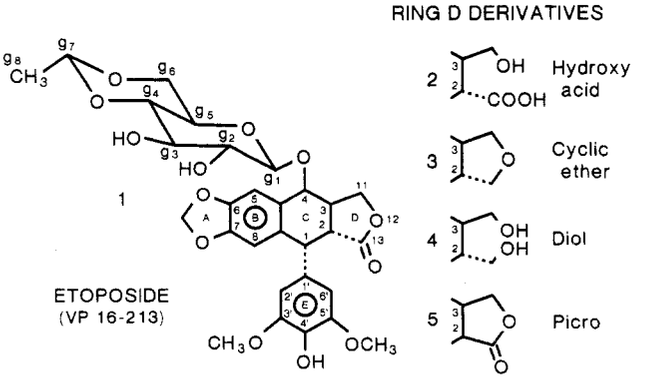
The anticancer drug etoposide (VP 16-213, 1) contains a highly strained trans-fused γ-lactone. This functionality is readily metabolized to an inactive ring-opened hydroxy acid (**2**). To prevent this detoxification of the drug and to investigate whether the lactone is essential for drug activity, we synthesized a cyclic ether analogue of etoposide (**3**) and tested it in the mouse leukemia L1210 system in vitro and in vivo. This ether analogue of etoposide was found to retain activity in the L1210 system, but the activity was reduced relative to the parent drug. A synthetic intermediate, the ring D opened diol of the reduced lactone (**4**), was also tested and found to be inactive in the L1210 system. The complete 470-MHz ^{1}H NMR spectra of etoposide and its derivatives are reported. The usefulness of introducing deuterium at C-13 to determine $J_{2,3}$ is also demonstrated. This coupling constant is characteristic of cis or trans stereochemistry across the C–D ring fusion.

Etoposide (4′-demethylepipodophyllotoxin ethylidene-β-D-glucoside) (**1**) is a highly effective clinical anticancer agent.[2] The drug is a semisynthetic derivative of the potent antimitotic natural product podophyllotoxin.[3,4] Podophyllotoxin is the aglycon of etoposide with a methoxy group at C-4′ and the opposite configuration of the oxygen at C-4. However, whereas podophyllotoxin binds avidly to tubulin to prevent its polymerization, etoposide does not bind to tubulin and does not inhibit microtubule assembly. In fact, etoposide causes an irreversible blocking of the cell cycle in the late S and G_2 phases.[5] While effects on cellular respiration have been postulated to play a role in this regard,[6,7] the precise mechanism of action of etoposide is unknown at the present time. In a comparative study with other oncolytic agents, only X-irradiation



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