Acetoxy-Substituted 1,1,2-Triphenylbut-1-enes with Antiestrogenic and Mammary Tumor Inhibiting Properties

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1,1,2-Triphenylbut-1-enes (*E*- and *Z*-10–12), which are substituted with one *p*- and one *m*-acetoxy group in two different aromatic rings, were synthesized. The *E* and *Z* isomers were isolated, and their identity was established by ^{1}H NMR spectroscopy. A study of the structure–activity relationship was carried out with regard to estradiol receptor affinity in vitro, estrogenic and antiestrogenic properties (mouse), inhibition of the hormone-dependent human MCF7 breast cancer cell line in vitro, and the hormone-dependent MXT mammary tumor of the mouse in vivo. Among the tested compounds, (*E*)- and (*Z*)-1-(3-acetoxyphenyl)-1-(4-acetoxyphenyl)-2-phenylbut-1-enes (*E*-10 and *Z*-10) and (*Z*)-1-(3-acetoxyphenyl)-1-phenyl-2-(4-acetoxyphenyl)-but-1-ene (*Z*-12) proved to be partial antiestrogens, which lead to an inhibition of the MCF7 cell line. They exert a growth-inhibiting activity on the hormone-dependent MXT mammary carcinoma of the mouse. In the case of *E*-10 and *Z*-10, this effect is only slightly weaker than that of 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene (13) and tamoxifen. Under the applied experimental conditions, there were no significant changes of uterine weight as an indicator of estrogenic side effects.

Many nonsteroidal antiestrogens of the triarylethylene type have been tested concerning their mammary tumor inhibiting activities.[1-3] One of these compounds, tamoxifen, is now routinely used for the treatment of the hormone-dependent breast cancer.[1] The antitumor activity of these substances is explained by an inhibition of the interaction of estrogens with the estrogen receptor (ER).[3] However, most antiestrogens also display weak estrogenic activity, which may stimulate mammary tumor growth in the presence of a low estrogen level as is found after ovariectomy.[2,4,5] The pharmacological activity pattern of these triarylethylene drugs concerning their ER affinities, estrogenic and antiestrogenic properties, and mammary tumor inhibiting activities strongly depends on the kind and position of substituents in the aromatic rings and on the steric configuration (i.e., *E,Z* isomerism).[1-3,6] A (dialkylamino)ethoxy residue attached at one of the aromatic rings was thought to be essential for a strong antiestrogenic activity.[1,2] However, in recent publications of Rochefort et al.[3] and of our group,[7] it was shown that compounds lacking this side chain can still have antiestrogenic and mammary tumor inhibiting activity.

In the first of several parts of a structure–activity study in the class of 1,1,2-triphenylbut-1-enes, we have evaluated the effect of a substitution with either *p*-acetoxy or *m*-acetoxy groups in one, two, or three aromatic rings.[7] A good correlation between the ER affinity and the growth-inhibiting effect on the hormone-dependent postmenopausal human mammary carcinoma implanted in nude mice was observed. The tumor-inhibiting effect of mono- and disubstituted compounds was better than that of trisubstituted ones.[7] Compounds with *p*-acetoxy substituents had a higher receptor affinity and antitumor activity than their metasubstituted isomers.[7]

On the other hand, we found metastilbestrol[8-10] and metahexestrol (and their acetoxy derivatives),[11-13] the 3,3′-hydroxyisomers derived from the synthetic estrogens diethylstilbestrol (DES) and hexestrol (HES), to be very active on several hormone-dependent experimental breast cancers. Both compounds are antiestrogens with only weak estrogenic side effects. Extensive structure–activity studies were performed with metastilbestrol[8-10,14] and metahexestrol.[13,15-17] The same strategy of shifting the hydroxy groups was applied to indenestrol A (6-hydroxy-2-(4-hydroxyphenyl)-3-ethyl-1-methyl-1*H*-indene), a compound with potent estrogenic but no antiestrogenic properties to give the new mammary tumor inhibiting antiestrogen 5-acetoxy-2-(3-acetoxyphenyl)-3-ethyl-1-methyl-1*H*-indene.[18]

In the class of 6-acetoxy-3-(4-acetoxyphenyl)indoles, related to indenestrol A, highly active antiestrogens were achieved by introducing appropriate alkyl residues into positions 1 and 3 and by shifting the acetoxy group from 6- to 5-position.[19] In contrast to indenestrol A, the additional shift of the 4-hydroxy group to the 3-position causes a severe loss of antiestrogenic potency.[19] Among a great number of tested 2-phenylindoles, 5-acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) proved to be very effective in vitro and in vivo on several hormone-dependent mammary tumor models.[20]

These results induced us to synthesze 1,1,2-triphenyl-

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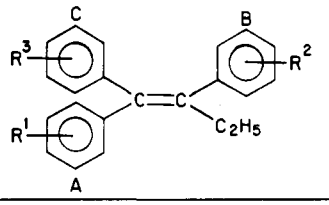
Table I. Acetoxy-Substituted 1,1,2-Triphenylbut-1-enes[a]



compd	R^1	R^2	R^3	yield, %	mp,[b] °C	formula[c]	RBA,[d] %
Z-10[e]	4-$OCOCH_3$	H	3-$OCOCH_3$	45	143	$C_{26}H_{24}O_4$	8.7
E-10	3-$OCOCH_3$	H	4-$OCOCH_3$	30	120	$C_{26}H_{24}O_4$	3.5
Z-11[e]	H	3-$OCOCH_3$	4-$OCOCH_3$	42	103	$C_{26}H_{24}O_4$	6.1
E-11	4-$OCOCH_3$	3-$OCOCH_3$	H	50	116	$C_{26}H_{24}O_4$	11.1
Z-12[f]	H	4-$OCOCH_3$	3-$OCOCH_3$	38	118	$C_{26}H_{24}O_4$	1.8
E-12	3-$OCOCH_3$	4-$OCOCH_3$	H	42	102	$C_{26}H_{24}O_4$	4.2

[a] Synthetic method D under the Experimental Section. [b] All compounds were crystallized from EtOH. [c] All compounds were analyzed for C and H within 0.40% of the calculated values. [d] RBA = $[E_2]/[I] \times 100$; $[E_2]$ and [I] are the molar concentrations of nonradioactive E_2 and inhibitor required to decrease the bound [^{3}H]-E_2 by 50%, E_2 = 17β-estradiol. [e] The mixture of *E* and *Z* isomers was separated by fractional crystallization from EtOH. [f] The mixture of *E* and *Z* isomers was separated by column chromatography on silica gel with CH_2Cl_2 as eluent.

Table II. ^{1}H NMR Spectra of Compounds 10–12[a]

compd	δ values: aromatic H	CH_2[b]	$OCOCH_3$[c]	CH_3[d]
Z-10	7.15 (s, 5 H), 7.29, 7.05 (AB, 4 H), 7.05–6.83 (m, 4 H)	2.43	2.27, 2.16	0.96
E-10	7.16 (s, 5 H), 6.93, 6.76 (AB, e, 4 H), 7.50–7.02 (m, 4 H)	2.48	2.28, 2.21	0.96
Z-11	7.31 (s, 5 H), 6.89, 6, 71 (AB, e, 4 H), 7.23–6.82 (m, 4 H)	2.43	2.21, 2.17	0.96
E-11	6.94 (s, 5 H), 7.31, 6.99 (AB, e, 4 H), 7.23–6.82 (m, 4 H)	2.48	2.27, 2.21	0.96
Z-12	7.31 (s, 5 H), 7.17, 6.87 (AB, e, 4 H), 7.07–6.57 (m, 4 H)	2.46	2.23, 2.16	0.96
E-12	6.97 (s, 5 H), 7.16, 6.89 (AB, e, 4 H), 7.47–7.03 (m, 4 H)	2.50	2.28, 2.23	0.96
TRI[f]	7.31, 7.15, 6.97 (3 s, 15 H)	2.50		0.93

[a] Solvent: $CDCl_3$. [b] q, J = 7 Hz, 2 H. [c] 2 s, 6 H. [d] t, *J* = 7 Hz, 3 H. [e] *J* = 9 Hz. [f] TRI = 1,1,2-triphenylbut-1-ene; AB = AA'BB'.

but-1-enes with one *p*- and one *m*-acetoxy group in two different aromatic rings and to evaluate their ER affinity as well as their estrogenic and antiestrogenic potency and their mammary tumor inhibiting property.

Chemistry. The acetoxy-substituted 1,1,2-triphenylbut-1-enes (**10–12**) were synthesized according to the method of Dodds et al.[21] as previously described by us,[7] starting with 1-(4-methoxyphenyl)-2-phenylethanone (**1**),[7] 1-(4-methoxyphenyl)-2-(3-methoxyphenyl)ethanone (**2**), and 1-phenyl-2-(4-methoxyphenyl)ethanone (**3**).[7] Compound **2** was prepared by Friedel–Crafts acylation of anisole with phenylacetyl chloride.

The 1,2-diphenylethanones **1–3** were alkylated with ethyl iodide to give the corresponding 1,2-diphenylbutan-1-ones **4–6** (Scheme I, method A). Compounds **4–6** were converted with the respective phenylmagnesium bromide into the corresponding tertiary carbinols, which were dehydrated with a mixture of sulfuric acid and acetic acid to give the methoxy-substituted 1,1,2-triphenylbut-1-enes **7–9** (Scheme I, methods B and C) as mixtures of the corresponding *E* and *Z* isomers. These mixtures were not separated at this point, since isomerization can occur during the subsequent ether cleavage.[7]

Compounds **7–9** 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-triphenylbut-1-enes **10–12** (Scheme I, method D; Table I). The mixtures of *E* and *Z* isomers were separated by fractional crystallization or by column chromatography on silica gel with CH_2Cl_2 as eluent (Table I).

The purity of the *E* and *Z* isomers was proven by HPLC analyses. The identity of the occurring *E* and *Z* isomers

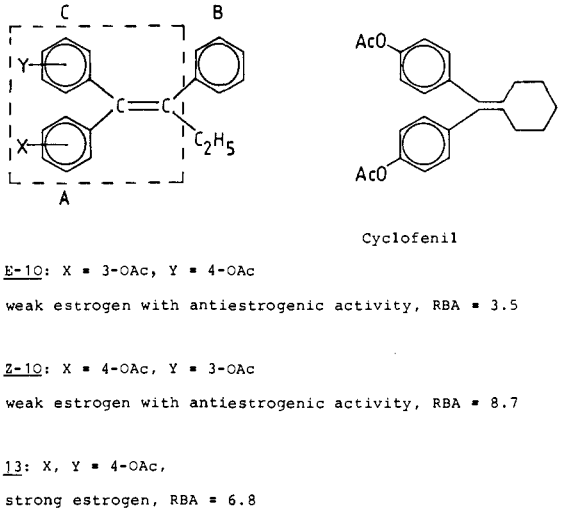


Figure 1. Cyclofenil fragment (1,1-bis(acetoxyphenyl)ethene moiety) containing compounds.

was established by ^{1}H NMR spectroscopy as we have previously described in detail.[7] Briefly, the ^{1}H NMR spectrum of the unsubstituted 1,1,2-triphenylbut-1-ene (TRI) shows one singlet for each phenyl group in the following order: ring in position A (δ 7.31) > B (δ 7.15) > C (δ 6.97) (Table II).[22] This order of the aromatic signals remains unchanged after substitution.[7] The arrangement of the 3- and 4-acetoxy-substituted phenyl rings to their respective positions (A, B, or C) can be achieved by means of their typical substitution-dependent pattern.

Biological Properties. For a discussion of structure–activity relationships, compounds *E*- and *Z*-**10–12** will be

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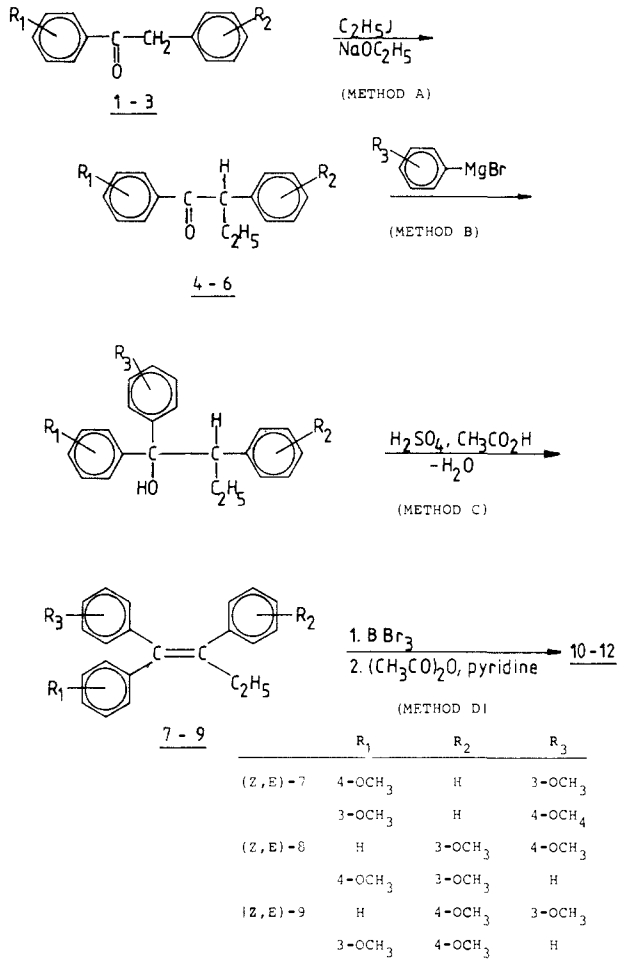
(22) Concerning the definition of rings A, B, and C, see the formula in Table IV and Figures 1 and 2.

Scheme I



	R_1	R_2	R_3
(Z,E)-7	4-OCH_3	H	3-OCH_3
	3-OCH_3	H	4-OCH_4
(Z,E)-8	H	3-OCH_3	4-OCH_3
	4-OCH_3	3-OCH_3	H
(Z,E)-9	H	4-OCH_3	3-OCH_3
	3-OCH_3	4-OCH_3	H

arranged in groups comprising a 1,1-bis(acetoxyphenyl)-ethene moiety (*E*- and *Z*-10; cyclofenil fragment containing compounds; Figure 1) or a 1,2-bis(acetoxyphenyl)ethene moiety (*E*- and *Z*-11 and 12; diethylstilbestrol fragment containing compounds, Figure 2).

The estrogenic activity of the new 1,1,2-triphenylbut-1-enes was determined in the immature mouse uterine weight test.[8] Compound *E*-11 with a *p*-acetoxy group in ring A and a *m*-acetoxy group in ring B showed the highest estrogenic activity among the new 1,1,2-triphenylbut-1-enes (Table III; Figure 2). Its uterotrophic effect is comparable to that of HES (*meso*-3,4-bis(4-hydroxyphenyl)ethane), DES (diethylstilbestrol), and estrone.[8] Thus, this type of substitution (*trans*-1,2-bis(acetoxyphenyl)ethene moiety) may be apt to create a strong estrogen. The corresponding *Z* isomer *Z*-11 causes the same type of effect at a 10-fold dosage. In case of stilbestrol and metastilbestrol (3,3′-dihydroxy-α,β-diethylstilbene) the *Z* isomers also display a lower estrogenic activity than their corresponding *E* isomers.[8,9] Compound *E*-12 also containing the *trans*-1,2-bis(acetoxyphenyl)ethene moiety (*E*-12: ring A, 3-OAc; ring B, 4-OAc) is an estrogen, which is less active than *E*-11. Apparently, the substitution pattern 4-OAc in ring A and 3-OAc in ring B is most favorable for the estrogenic potency (compare *E*-11 with *E*-12). The geometric isomer of *E*-12, i.e., *Z*-12, is a weak estrogen (*cis*-1,2-bis(acetoxyphenyl)ethene moiety) that does not reach the maximum effect of estrone. Compounds *Z*-10 and *E*-10, which are substituted in rings A and C with 4- or 3-OAc groups (1,1-bis(acetoxyphenyl)ethene moiety, Figure 1), are weak estrogens as well. Among these types of compounds, the isomer with a 4-OAc group in ring A (*Z*-10) is slightly more estrogenic than *E*-10. In contrast, compound 13 (Figure 1)[7] also containing the 1,1-bis(acetoxyphenyl)ethene moiety, but with two 4-OAc groups is a strong estrogen comparable to *E*-12 in its potency (Table II). Thus, it is shown that a low uterotrophic activity depends not only on the existence of the cyclofenil fragment but also on the substitution pattern. A 3-OAc substituent in rings A or

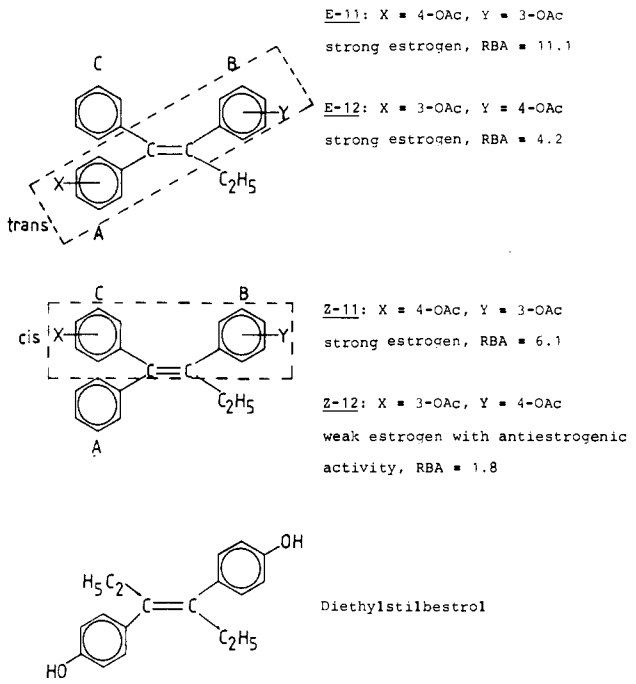


Figure 2. Diethylstilbestrol fragment (*trans*- or *cis*-1,2-bis-(acetoxyphenyl)ethene moiety) containing compounds.

Table III. Estrogenic and Antiestrogenic Effect of Compounds 10–12 in the Immature Mouse Uterine Weight Test

compd	dose,[a] μg	estrogenic effect[b]	dose,[a] μg	antiestrogenic effect:[c,d] % inhibn
Z-10	1	23	5	41[e]
	5	35	25	32[e]
	50	73	100	3
	250	85		
E-10	1	8	5	28[e]
	5	15	25	33[e]
	50	66	100	9
	250	66		
Z-11	1	21	1	0
	5	94	5	0
	50	82	25	0
E-11	0.5	124		
	1	114		not tested
	5	81		
Z-12	1	0	5	45[e]
	5	18	25	16[f]
	50	61	100	9
	500	62		
E-12	1	18	1	12
	5	80	5	0
	50	92	25	0
13	1	37	1	0
	5	72	5	0
	10	85		
	25	98		

[a] Dose per animal and day. [b] Estrogenic effect = $[(E_T - E_V)/(E_S - E_V)] \times 100$. Effect = uterus dry weight (milligrams)/body weight (grams) × 100. E_T = effect of test compound; E_V = effect of vehicle; E_S = effect of estrone standard (0.4 μg). [c] Antiestrogenic effect = % inhibn = $[(E_S - E_{S,T})/(E_S - E_V)] \times 100$. E_S = effect of estrone standard (0.1 μg); $E_{S,T}$ = effect of standard under simultaneous application of test compound. [d] The U-test according to Wilcoxon, Mann, and Whitney was used. [e] Significant ($\alpha \leq 0.01$). [f] Significant ($\alpha \leq 0.05$).

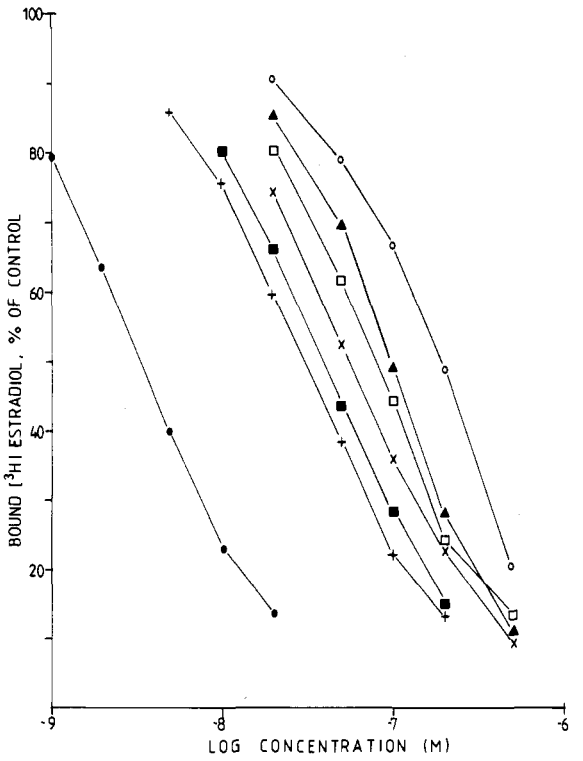


Figure 3. Competitive binding assay of **10**–**12**. Calf uterine cytosol was incubated for 16 h at 4 °C with the stated concentration of competitor and 1×10^{-9} M [^{3}H]estradiol. After incubation, dextran-coated charcoal was added to absorb unbound ligand (90 min, 4 °C), and radioactivity was determined in the supernatant. Competing ligands: 17β-estradiol (●); *Z*-**10** (■); *E*-**10** (▲); *Z*-**11** (×); *E*-**11** (+); *Z*-**12** (○); *E*-**12** (□).

C generally lowers the estrogenic potency.

As expected, the weak estrogens *Z*-**10**, *E*-**10**, and *Z*-**12** show comparable antiestrogenic activities (Table III). This property is due to the already mentioned 1,1-bis(acetoxyphenyl)ethene (*Z*-**10** and *E*-**10**) or *cis*-1,2-bis(acetoxyphenyl)ethene moieties (*Z*-**12**). However, the occurrence of an estrogen antagonistic potency in *Z*-**12** depends not only on the existence of a *cis*-DES fragment but also on the substitution pattern (ring B, 4-OAc; ring C, 3-OAc). The related compound *Z*-**11** with interchanged OAc positions (ring B, 3-OAc; ring C, 4-OAc) does not exert any antiuterotrophic activity. The importance of the kind of substitution is also demonstrated with **13** (two 4-OAc groups) as this compound has no antiestrogenic potency in contrast to *Z*-**10** and *E*-**10** (one 4-OAc and one 3-OAc group).

As was to be expected, compounds *E*- and *Z*-**10**–**12** show a marked affinity to the estrogen receptor (Table I; Figure 3). The parallelism of the binding curves to that of unlabeled estradiol indicates a competitive inhibition of the estrogen receptor interaction (Figure 3). Concerning the strong estrogens of the 1,1,2-triphenylbut-1-ene type (*E*-**11**, *Z*-**11**, *E*-**12**), which did not show antiestrogenic properties, a good correlation between ER affinity and estrogenic activity is observed (Table I; *E*-**11** > *Z*-**11** > *E*-**12**). The most active estrogen *E*-**11** also displays the highest ER affinity. The interconversion to the less active *Z* isomer (*Z*-**11**) is accompanied by a decreased ER affinity. The same phenomenon appears in the cases of *E*-**12** and *Z*-**12**. Furthermore, the slightly stronger estrogenic activity of the *Z* isomer of *E*,*Z*-**10** corresponds to a higher ER affinity. Surprisingly, compound *E*-**11** (ring A, 4-OAc; ring B, 3-OAc) shows an ER affinity that is higher than that of (*E*)-1,2-bis(4-acetoxyphenyl)-1-phenylbut-1-ene (RBA = 5.9).[7] Thus, a 3-OAc substituent in phenyl ring B seems to fit the ER better than a 4-OAc substituent in the 1,1,2-triphenylbut-1-ene series. The ER affinity of *Z*-**10** (ring A, 4-OAc; ring C, 3-OAc), however, is similar to that of compound **13** with two 4-OAc groups in rings A and C (Figure 1). This shows that the position of the acetoxy substituent in ring C is less important for the receptor binding than that in ring B.

These results confirm and complete our former study on the structure–activity relationship in the class of acetoxy-substituted 1,1,2-triphenylbut-1-enes.[7] In that paper, we have shown that a 4-OAc group in ring A is essential for a high ER affinity, that a second 4-OAc group in ring B or C slightly increases the affinity, whereas a third 4-OAc group diminishes the binding to the ER. The 1,1,2-triphenyl-but-1-enes with one, two, or three 3-OAc groups have considerably lower ER affinities. Similar observations were recently made by Pons et al.[23] in the class of triphenyl acrylonitriles substituted with a 4-OH group in one, two, or three phenyl rings.

Whereas a correlation between ER affinity and estrogenic activity (in the case of estrogens already described by Korenman[24]) is obvious with the strong estrogens in this study, there is evidently no similar correspondence between ER affinity and antiestrogenic activity in these types of compounds. A mechanism of action of *E*-**10**, *Z*-**10**, and *Z*-**12** due to a binding to the postulated "antiestrogen receptor" is improbable since it was shown that triphenylethylenes lacking the aminoalkoxy chain do not bind to this receptor[25] and that the affinity of antiestrogens to this receptor does not correlate with their antiestrogenic potency on the rat uterus or on the growth inhibition of the hormone-dependent MCF7 breast cancer cell line.[25,26]

The aminoalkoxy residue in ring C of 1,1,2-triphenylbut-1-enes, as in tamoxifen, is thought to be essential for the antiestrogenic properties.[27] Some of our new 1,1,2-triphenylbut-1-enes (*E*-**10**, *Z*-**10**, *Z*-**12**), however, exert antiestrogenic activity in the mouse uterine weight assay. The antiestrogen *E*-**10** and its related β-(dimethylamino)ethyl ether (3-hydroxytamoxifen).[28] show nearly identical ER affinities. Therefore, it can be assumed that a basic ether chain at ring C does not lead to an increased ER affinity compared to an acetoxy group in the same position.

To determine the mammary tumor inhibiting effect of the new 1,1,2-triphenylbut-1-enes in vitro, we used the human estrogen receptor positive MCF7 breast cancer cell line.[29,30] Its growth is inhibited by antiestrogens like tamoxifen.[29]

For the evaluation of the inhibitory effect, we determined the drug-mediated decrease in DNA content compared to that of the control. This parameter resembles the growth rate of the cells during the whole experiment and not only that of a short period as with the determination of the [^{3}H]thymidine incorporation. At a concentration of 1×10^{-7} M, the antiestrogenic compounds *E*-**10**,

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Table IV. Effect of Compounds 10–12 on the Growth of the Hormone-Dependent MXT Mouse Mammary Carcinoma and of the Uterus

compd	dose,[a] mg/kg	tumor[b] T/C, %	uterus[c] T/C, %
Z-10	8.0	25[d]	116
E-10	8.0	30[d]	82
Z-11	8.0	7[d]	193[d]
E-11	8.0	1[d]	205[d]
Z-12	8.0	62	96
E-12	8.0	2[d]	175[d]
13	8.0	7[d]	110
tamoxifen (citrate)	11.3	4[d]	79
DES	5.4	3[d]	200[d]
ovariectomy		2[d]	39[d]
E-10	12.0	8[d]	110

[a] All compounds were administered in equimolar doses three times a week sc. [b] T/C, %: tumor weight of the treated animals/tumor weight of the solvent control × 100 after 6 weeks of therapy (mean of 10 animals). [c] T/C, %: uterotrophic effect of the treated animals/uterotrophic effect of the solvent control × 100 after 6 weeks of therapy (mean of 10 animals). [d] Significant ($\alpha \leq 0.05$).

Z-10, and *Z*-12 significantly inhibited the growth of this cell line (percent inhibition of DNA content compared to the control: *E*-10, 46; *Z*-10, 32; *Z*-12, 32; Tam, 22; α, <0.05). The estrogens *E*-11, *Z*-11, and *E*-12 did not exert any significant inhibition on the MCF7 cell line.

For the evaluation of the tumor-inhibiting effect in vivo, we used the transplantable, hormone-dependent MXT mammary tumor of the BDF_1 mouse.[31,32] Its growth is inhibited by ovariectomy and also by the administration of antiestrogens like tamoxifen as well as of estrogens like DES.[32] In our experiments, ovariectomy caused a strong inhibition of tumor growth (Table IV). DES was also inhibitory to nearly the same extent. However, the estrogenic side effect, determined by the uterine dry weight at the end of therapy, was very high. On the other hand, tamoxifen caused a strong inhibition of tumor growth without increasing the uterine weight above the control value. The simultaneous evaluation of tumor and uterine weight in the MXT mammary carcinoma bearing mouse is a suitable test model for determining the antitumor activity and the estrogenic side effects of newly developed antiestrogens in one experiment. Compounds that strongly reduce the tumor weight without affecting the uterine weight might be of great interest for the treatment of the hormone-dependent mammary carcinoma.

The 1,1,2-triphenylbut-1-enes *E*- and *Z*-10–12 were administered in a dosage of 3 × 8 mg/kg per week. Tamoxifen and DES were used in equimolar doses. Similar to DES, the estrogens *E*-11, *Z*-11, and *E*-12 caused a strong inhibition of tumor growth associated with an about 2-fold elevation of the uterine weight. However, the antiestrogens *E*-10 and *Z*-10, containing the 1,1-bis(acetoxyphenyl)-ethene moiety, caused a marked and significant tumor inhibition without estrogenic side effects. The antitumor activity of *E*-10 and *Z*-10 is slightly weaker than that of tamoxifen. Yet, by elevating the dose to the 1.5-fold, approximately the same antitumor effect was obtained. Moreover, the uterine weight was not significantly changed under these experimental conditions (see Table IV, *E*-10).

Compared to *E*-10 (ring A, 3-OAc; ring C, 4-OAc) and *Z*-10 (ring A, 4-OAc; ring C, 3-OAc), compound **13** (ring A, 4-OAc; ring C, 4-OAc; Figure 1) is slightly more effective in the MXT tumor system (Table IV). Surprisingly, the uterine weight is not signficantly changed by **13** during therapy, though it has a similar uterotrophic potency as *E*-12 in the immature mouse uterine weight test. This difference in estrogenic side effects on the mature mouse during the MXT tumor assay may be due to the 1,1-bis-(acetoxyphenyl)ethene moiety compared to the *trans*-1,2-bis(acetoxyphenyl)ethene skeleton of *E*-12 (Tables III and IV). The antiestrogen *Z*-12, which contains the *cis*-1,2-bis(acetoxyphenyl)ethene moiety, shows a distinctly weaker and not significant inhibition of the MXT mammary tumor compared to *E*-10 and *Z*-10, but no estrogenic side effects, either.

An aspect that should be kept in mind in the interpretation of structure–activity relationships of *E* and *Z* isomers is the possible isomerization. It is described in the literature that *cis*- and *trans*-hydroxytamoxifen readily undergo isomeric interconversion at 37 °C as determined in vitro and therefore show quite similar biological properties in vitro as well as in vivo.[33,34] An *E,Z* isomerization, thought to be possible through a quinoid intermediate,[35] is also seen with diethylstilbestrol. With 3,3′-dihydroxy-α,β-diethylstilbene, however, we have not found any isomerization in vitro as determined by ^{1}H NMR spectroscopy and TLC after incubation at 37 °C.[9] Concerning the compounds of this study, an interconversion of *E*-11 and *Z*-11 and of *E*-12 and *Z*-12 in vivo is unlikely or takes place very slowly as there are big differences in the biological properties, e.g. in the uterotrophic assay between the *E* and *Z* isomers (Table III). Even in the 6-week MXT tumor assay, the tumor-inhibiting activity and the estrogenic side effect of *E*-12 are very different from those of its *Z* isomer *Z*-12. In the case of *Z*-10 and *E*-10, an *E,Z* isomerization would not have important consequences since both compounds are similar in estrogenic, antiestrogenic, and tumor-inhibiting properties.

In conclusion, it can be stated that 1,1-bis(acetoxyphenyl)-2-phenylbut-1-enes with 4-OAc groups in rings A and C (**13**) and with a 4-OAc and a 3-OAc group in rings A and C (*Z*-10) or vice versa (*E*-10) are very active mammary tumor inhibiting antiestrogens without estrogenic side effects on the hormone-dependent MXT mammary carcinoma of the mouse and are therefore of great interest for further evaluation.

Experimental Section

General Procedures. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg. ^{1}H NMR spectra were obtained with a Varian EM 360 A (60 MHz) and an EM 390 A (90 MHz) (internal standard Me_4Si, chemical shifts in δ). TLC of each compound was accomplished on Merck F 254 silica gel plates. HPLC was performed by using an Altex 110 A pump in a Kontron Uvikon 720 LC spectrophotometer [column Lichrosorb Si 60 (5 μm), Merck, West Germany].

Syntheses. Synthetic methods A–D are representatives for compounds reported in Table I.

1-(4-Methoxyphenyl)-2-(3-methoxyphenyl)ethanone (2). Compound **2** was synthesized as described in ref 7: yield 60%; mp 44 °C.

Method A. 1-(4-Methoxyphenyl)-2-phenylbutanone (4). A hot solution of sodium (2.3 g, 0.10 mol in 44 mL of EtOH was

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added to a mixture of 1-(4-methoxyphenyl)-2-phenylethanone (22.6 g, 0.10 mol) and ethyl iodide (15.6 g, 0.10 mol). The solution was refluxed for 10 min. A further quantity of sodium (1.15 g, 0.05 mol) 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 dried over Na_2SO_4. The solvent was removed. After crystallization from EtOH, 24.1 g of **4** was obtained.

Method B. 1-(3-Methoxyphenyl)-1-(4-methoxyphenyl)-2-phenylbutan-1-ol. A solution of 1-(4-methoxyphenyl)-2-phenylbutanone (25.4 g, 0.10 mol) in 60 mL of ether was added dropwise to a solution of (3-methoxyphenyl)magnesium bromide (63.39 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-(3-methoxyphenyl)-1-(4-methoxyphenyl)-2-phenylbutan-1-ol. No efforts were made to purify the crude product because spontaneous elimination of water occurred.

Method C. 1-(3-Methoxyphenyl)-1-(4-methoxyphenyl)-2-phenylbut-1-ene (*Z*,*E*-7). To the crude product of 1-(3-methoxyphenyl)-1-(4-methoxyphenyl)-2-phenylbutan-1-ol was added 30 mL of a mixture of sulfuric acid and acetic acid (8:2 (v/v)). The whole was heated at 70 °C 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 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) to give a mixture of the *Z* and *E* isomers of **7**.

Method D. 1-(3-Acetoxyphenyl)-1-(4-acetoxyphenyl)-2-phenylbut-1-ene (*Z*-10, *E*-10). A solution of *Z*,*E*-7 (3.44 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. After 15 min, the freezing mixture was removed, and the reaction mixture was stirred for 4 h at room temperature. After that, 50 mL of MeOH was added with cooling, and the solvents were removed under reduced pressure. Acetic anhydride (3.06 g, 0.03 mol) and pyridine (3.17 g, 0.04 mol) were added. The mixture was refluxed for 1 h. After cooling, 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 crystallized from EtOH to give a mixture of *Z*- and *E*-10.

Biological Methods. Estradiol Receptor Binding Assay. The method described in ref 7 was used with some modifications. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^{3}H]estradiol. 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 200-μL supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabeled test compounds. Five or 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 properties were determined by stimulation of the uterine growth or by inhibition of the uterine growth stimulated by estrone, respectively, on immature NMRI mice as described previously.[7,8] Female mice (body weight 10–12 g; age 20 days at test beginning; 10 mice per group) were injected sc daily for 3 consecutive days with 0.1 mL of olive oil solutions containing the test compounds. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, dried, and weighed.

Growth Inhibition of Hormone-Dependent Human MCF-7 Breast Cancer Cells.[29,30] The MCF-7 cell line was provided by Dr. M. E. Lippman, NCI, Bethesda, MD. Cells were grown in 5% CO_2 at 37 °C in Richter's improved minimal essential medium (MEM, Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/L), gentamycin (40 mg/L), and 5% newborn calf serum (NCS, Gibco) or charcoal-treated NCS (CCS). CCS was prepared by incubation of 500 mL of NCS with a dextran-coated charcoal pellet at 0–4 °C for 3 h. After removal of the charcoal by centrifugation, the incubation was repeated at 56 °C for 1 h with a fresh pellet. After centrifugation, the serum was filtered through a 0.2-μm filter (Sartorius, Göttingen, FRG). At least 1 week before we started the experiment, cells were switched from NCS to 5% CCS. After an additional change of the medium, cells were harvested with 0.05% trypsin–0.02 % EDTA in 0.15 M NaCl. They were syringed gently to prevent clumping, and 2 mL of the cell suspension ((5–8) × 10^3 cells/mL) was plated in six well dishes (Costar). On the following day, the cells were switched to 2% CCS containing the test compound and 0.1% EtOH, in which the compounds had been dissolved. The medium of the control contained an equal volume of EtOH. After 5 days, media were changed. At the eighth day, cells were washed with cold PBS and harvested with PBS containing 0.02% EDTA. After centrifugation, the pellets were washed with PBS. DNA buffer (1 mL) (100 mg of $CaCl_2$, 200 mg of KCl, 100 mg of $MgCl_2 \cdot 6H_2O$, 8 g of NaCl, 115 mg of $NaH_2PO_4 \cdot H_2O_m$ in 1 L of H_2O) was added and the cells were destroyed by sonification. The amount of total DNA was measured by use of the ethidium bromide technique:[36] % inhib = $[(C - T)/C] \times 100$, where C = DNA content of control and T = DNA content in the presence of test compound.

Hormone-Dependent, Transplantable MXT Mammary Tumor of the BDF1 Mouse.[31,32] The MXT tumor used in these studies was the MXT line 3.2 provided by Dr. Bogden, Laboratory of Experimental Oncology, EG Bogden Laboratories, Worcester, MA, in a frozen state. The tumor was transplanted in pieces of about 2 mm^3 subcutaneously in female, 8-week-old BDF1 mice (body weight 20 ± 1.6 g, Charles River Wiga, West Germany). After the tumor had reached a diameter of about 1 cm, it was transplanted to 20 mice to determine the hormone dependence. After transplantation the animals were randomly distributed in two groups of 10. The animals of one group were ovariectomized. The tumor grew well in control animals but only very slowly in the ovariectomized mice. Take rate of control animals was >95%. In an experiment to determine the tumor-inhibiting activity of new compounds, transplantation was carried out as above (1 tumor piece/animal). After transplantation, the animals were randomly distributed into groups of 10. Starting with the first day after transplantation, the test compounds were injected sc 3 times a week (Monday, Wednesday, Friday) as olive oil solutions (0.1 mL/mouse). The duration of treatment was 6 weeks. At the end of treatment, the animals were killed by cervical dislocation and weighed. The tumors were removed, washed in 0.9% sodium chloride solution, blotted dry, and weighed, and the average tumor weight was calculated. The uteri were also removed and prepared as described in ref 8 to serve as an indicator of the estrogenic side effects of the compounds.

Acknowledgment. The authors thank Dr. A. E. Bogden and Dr. G. Leclercq for providing us with the MXT tumor and for helpful discussions in setting up the MXT tumor model, M. Beer, K. Röhrl, and G. Tondl for skilful technical assistance, and the Deutsche Krebshilfe e.V., Dr. Mildred Scheel-Stiftung, the Deutsche Forschungsgemeinschaft (SFB 234), and the Verband der Chemischen Industrie, Fonds der Chemischen Industrie, for financial support.

Registry No. **2**, 98540-26-2; **4**, 78423-10-6; (Z)-**7**, 98633-97-7; (E)-**7**, 98540-28-4; (Z)-**8**, 98540-35-3; (E)-**8**, 98540-36-4; (Z)-**9**, 98540-37-5; (E)-**9**, 13732-58-6; (Z)-**10**, 98540-29-5; (E)-**10**, 98540-30-8; (Z)-11, 98540-31-9; (E)-11, 98540-32-0; (Z)-**12**, 98540-33-1; (E)-**12**, 98540-34-2; 1-(4-methoxyphenyl)-2-phenylethanone, 1023-17-2; ethyl iodide, 75-03-6; 1-bromo-3-methoxyphenyl, 2398-37-0; 1-(3-methoxyphenyl)-1-(4-methoxyphenyl)-2-phenylbutan-1-ol, 98540-27-3; acetic anhydride, 108-24-7.

Supplementary Material Available: Tables V–X giving ^{1}H NMR spectral data (6 pages). Ordering information is given on any current masthead page.