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Crystal Structure and Hormonal Activity of 1,1-Bis(4-hydroxyphenyl)-2-phenylethene

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Summary. A complete three-dimensional X-ray crystal structure analysis of 1,1-bis(4-hydroxyphenyl)-2-phenylethene (*BHPE*) has been carried out. Reflexes were collected at room temperature. After isotropic refinement of *F*-values by least-squares, *R* is 0.163. *BHPE* crystallizes with 8 molecules in a unit cell of monoclinic symmetry, space group C2/c and cell dimensions $a = 20.851$, $b = 15.505$, $c = 10.536$ Å, $\beta = 107.54°$. The molecule of *BHPE* is not flat, the aromatic rings are twisted out of the ethene plane with angles of $-30.16°$ (ring B), $-51.45°$ (ring C) and $-33.49°$ (ring A). The bond angle between the 1,1-standing, 4-hydroxy-substituted phenyl rings amounts to 115.3° resulting in a distance between the hydroxy groups of 9.636 Å. *BHPE* proved to be a weak "impeded" estrogen with minor antiestrogenic potency, though its estrogen receptor affinity is very high (29%, estradiol 100%). A discussion of the influence of the spatial structure of *BHPE* and related substances on its estrogenic/antiestrogenic and mammary tumor-inhibiting potency is given.

Keywords. 1,1-Bis(4-hydroxyphenyl)-2-phenylethene; X-ray analysis; Estrogenic and antiestrogenic activity; Estrogen receptor affinity.

Kristallstruktur und hormonelle Wirkung von 1,1-Bis(4-hydroxyphenyl)-2-phenylethen

Zusammenfassung. Es wurde eine komplette dreidimensionale Röntgenstrukturanalyse von 1,1-Bis(4-hydroxyphenyl)-2-phenylethen (*BHPE*) durchgeführt, wobei die Reflexe bei Raumtemperatur gesammelt wurden. Nach isotroper Verfeinerung der *F*-Werte ergab sich ein *R*-Wert von 0.163. *BHPE* kristallisiert mit 8 Molekülen in einer Einheitszelle von monokliner Symmetrie mit Raumgruppe C2/c und den Zelldimensionen $a = 20.851$, $b = 15.505$, $c = 10.536$ Å, $\beta = 107.54°$. Das *BHPE*-Molekül ist nicht flach. Die aromatischen Ringe sind aus der Ethen-Ebene mit Winkeln von $-30.16°$ (Ring B), $-51.45°$ (Ring C) und $-33.49°$ (Ring A) herausgedreht. Der Bindungswinkel zwischen den 1,1-ständigen, 4-hydroxysubstituierten Phenylringen beträgt 115.3°, wobei dies einen Abstand von 9.636 Å für die Hydroxylgruppen ergibt. Es stellte sich heraus, daß *BHPE* ein „impeded" Estrogen mit geringer antiestrogener Wirkung ist, obwohl seine Estrogen-Receptor-Affinität sehr hoch ist (29%, Estradiol 100%). Es wird der Einfluß der räumlichen Gegebenheiten in *BHPE* und verwandten Substanzen im Hinblick auf ihre estrogene/antiestrogene und Brustkrebs-hemmende Wirkung diskutiert.

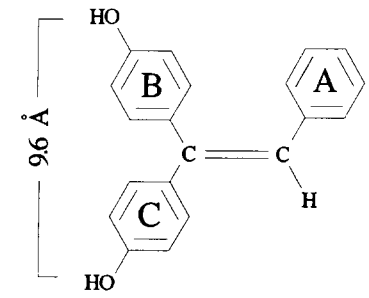
Introduction

Tamoxifen, a non-steroidal antiestrogen of the 1,1,2-triphenylethene type, is presently used in the therapy of the hormone-sensitive breast cancer [1]. However, the good clinical results, especially with postmenopausal patients, are limited in time. Therefore, many groups search for new antiestrogens which produce longer persisting remissions in breast cancer patients. Schneider et al. [2], for instance, investigated the mammary tumor-inhibiting properties of 1,1,2-triphenylbut-1-enes which are substituted with acetoxy groups in *para* or *meta* positions, on one, on two, or on three aromatic rings. In this study 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene (*BAPB*, see formula scheme) proved to be one of the most active compounds of the test series. *BAPB* strongly inhibited the growth of a postmenopausal, human mammary carcinoma implanted in nude mice [2] as well as that of the MXT-M 3.2 mammary carcinoma of the mouse (MXT-MC,ER^+) [3, 4]. Both breast cancer models are hormone-sensitive and can be influenced by endocrine therapeutic measurements like ovariectomy and administration of tamoxifen [2, 4]. The other two 4-acetoxy-substituted 1,1,2-triphenylbut-1-enes (*E*- and *Z*-1,2-bis(4-acetoxyphenyl)-1-phenylbut-1-ene: *E* and *Z-BAPB*, see formula scheme) were also very active on the MXT-MC,ER^+. However, the strong estrogenic side effects of *E*-, and *Z-BAPB*, obvious from markedly increased uterine weights of the test animals in the MXT-MC,ER^+-experiment, are unfavorable to their clinical application. *BAPB* itself caused no significant elevation of the uterine weights in this experiment [4]. In the more sensitive immature mouse uterine weight test, however, *BAPB* showed weak estrogenic potency. The shape of the dose-activity-plot corresponds to that of an "impeded" estrogen. The shift of one of the two 4-acetoxy groups in *BAPB* into the 3-position was a success in reducing the estrogenic side effects further [5]. Both resulting compounds show also antiestrogenic properties ("partial" antiestrogens). However, their mammary tumor-inhibiting effects on the MXT-MC,ER^+-model are markedly weaker than that of *BAPB*. A very interesting result was obtained by the attempt to optimize the antitumor activity of *BAPB* by exchange of the ethyl residue at C-atom 2 by somewhat smaller or bulkier alkyl groups [6]: The CH_3-derivative proved to be comparably strongly active on the MXT-MC,ER^+. The *n*-C_3H_7-derivative, however, produced no significant inhibition of this tumor model. On the other hand the ethyl residue in *BAPB* can be exchanged by an *i*-C_3H_7 group without any alteration in its antitumor activity. Also another minor change in the structure of *BAPB*, the transformation of the double bond between the C-atoms 1 and 2 into a single bond, led virtually to a loss of the mammary tumor-inhibiting activity [6]. Interestingly, this structural variation did not entail a change in estrogenic potency (assessed in the immature mouse uterine weight test) [6]. The results of these studies [2, 4–6] reveal a high structural specifity of the activity on the hormone-sensitive mammary carcinoma in the class of 1,1,2-triphenylethenes. The three-dimensional structure of the 1,1-bis(4-acetoxyphenyl)-2-phenyl-ethene-residue, especially the distance between the two phenolic oxygens*, is decisive for a high

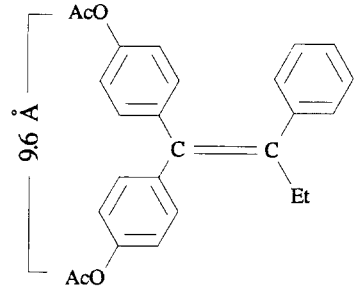
* The 4-acetoxy-derivatives of the 1,1,2-triphenylbut-1-enes (*BAPB*, *E*- and *Z-BAPB*) are presumably prodrugs which are transformed enzymatically into their active hydroxy form (*BHPB*) under in vivo conditions (compare [7])

antitumor activity of *BAPB*. In addition to these already minor structural changes on the ethyl residue of *BAPB* at C-atom 2 have great consequences on the extent of the antitumor activity, presumably due to steric and/or hydrophobic effects [6].

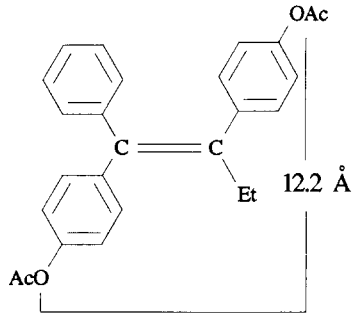
For these reasons we elucidated the three-dimensional structure of the 1,1-bis(4-hydroxyphenyl)-2-phenylalk-2-enes on the model of the parent compound 1,1-bis(4-hydroxyphenyl)-2-phenylethene (*BHPE*) by X-ray analysis as a basis for further theoretical and experimental efforts to develop optimally active drugs for the therapy of breast and prostate** cancer. Additionally a discussion of the influence of the spatial structure of *BHPE* on its estrogenic/antiestrogenic and mammary tumor-inhibiting potential is given.



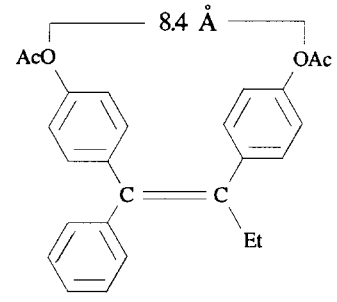
BHPE



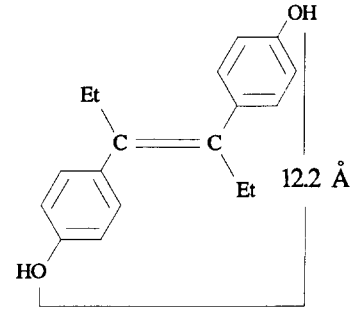
BAPB



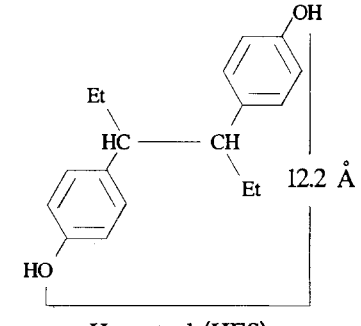
E-BAPB



Z-BAPB



Diethylstilbestrol (DES)



Hexestrol (HES)

** As to the prostatic tumor-inhibiting properties of "impeded" estrogens, especially of *BHPB* compare Ref. [4, 17]

Experimental Section

Synthesis

The synthesis of 1,1-bis(4-hydroxyphenyl)-2-phenylethene was carried out according to the method described by Schneider et al. [4]. Colorless crystals of *BHPE* were grown by slow evaporation of its $CHCl_3$ solution at room temperature.

X-Ray Crystallography

The crystal structure of *BHPE* was solved by direct methods. Full matrix-refinement of *F*-values was performed by use of the program SDP [8]. Experimental data are listed in Table 1.

Estrogen Receptor Binding Assay

The applied method was previously described by Hartmann et al. [9]. The relative binding affinity (RBA) of the test compounds to the estrogen receptor (ER) is determined by the displacement of 17β-[^{3}H]estradiol (^{3}H-E_2) from ER. At 4 °C the test compound and ^{3}H-E_2 are shaken with calf uterine

Table 1. Experimental data for the X-ray diffraction study

Formula	$C_{20}H_{16}O_2$
Formula weight	288.4
Crystal system	monoclinic
Space group	C2/c
Data collection temperature	24 °C
Radiation	Cu, graphite
a, Å	20.851 (3)
b, Å	15.505 (1)
c, Å	10.536 (2)
β, deg	107.54 (2)
V, Å^3	3247.8
Z	8
D_{calc}, g·cm^{-3}	1.18
F(000)	1216
Crystal dimension, µm	90 × 120 × 240
Linear absorption coefficient, cm^{-1}	5.6
Max-min transmission factor	0.70–1.0
Diffractometer	Enraf-Nonius CAD4
Scan type	$\omega/2\theta$
Max time per reflex, sec	60
Scan width, deg	$0.75 + 0.14 \operatorname{tg} \theta$
θ range, deg	2–57.5°
No. of reflexes measured	4145
No. of unique data	2194
No. of unique observed data [$I > 2\sigma(I)$]	2978
R	0.163
R_w	0.061
w	$4I/\sigma^2(I)$

cytosol for 16 h. To stop the drug-receptor-interaction dextran-coated charcoal is added and after centrifugation the radioactivity of 200 µl supernatant aliquots (i.e. ^{3}H-E_2-ER-complex) is counted. On a semilog plot the percentage of bound ^{3}H-E_2 vs. concentration of the competitors (i.e. E_2 and *BHPE*) is plotted. Six concentrations of each compound are used. From the resulting linear graph the molar concentrations of the competitors (E_2 and *BHPE*) which reduce the binding of the radioligand by 50% are obtained.

Estrogen and Antiestrogen Assays

Estrogenic and antiestrogenic effects are determined by stimulation of the uterine growth or by inhibition of the uterine growth stimulated by estrone, respectively, as described previously [2]. On three consecutive days the compounds, dissolved in polyethylene glycol 400/H_2O, 1:1 (0.1 µl/mouse), are daily administered sc to female, immature NMRI mice (age: 20 days at test beginning; body weight: 10–12 g; 6 mice/group). The uteri are excised 24 h after the last injection, fixed with Bouin's solution, dried and weighed.

Results

X-Ray Analysis

The atomic positional parameters of *BHPE* are listed in Table 2. Table 3 presents important bond and torsion angles. For numbering of the atoms see Fig. 1.

BHPE crystallizes with 8 molecules in the unit cell of space group C2/c. A single molecule of *BHPE* is shown in Fig. 1. The bond angles on the sp^2-hybridized C-atoms of the ethene-bridge of *BHPE* are comparable to those of the unsubstituted ethene and are only marginally influenced by the aromatic rings. The two 4-hydroxyphenyl rings at C(1) as well as the phenyl ring and the olefinic proton H1 at C(2) take angles of about 116° (see Table 3). Due to the steric repulsion the aromatic rings are twisted out of the ethene plane, so the molecule is not as flat as the chemical drawing might suggest. Ring A is arranged $-33.49 \pm 0.88°$, ring B $-30.16 \pm 0.74°$ and ring C $-51.45 \pm 0.70°$ out of the plane. Thereby the molecule exists in a propeller like conformation in which the double bond works as the hub and the phenyl rings as the three blades (dihedral angles between ring A and B = $117.70 \pm 0.17°$, A and C = $57.15 \pm 0.18°$ and B and C = $108.87 \pm 0.15°$). The O–O-distance between the phenolic hydroxy groups amounts to 9.636Å independent of the conformation of the molecule. Under physiological conditions *BHPE* presumably assumes a conformation by rotation around the aryl-C-axis which allows an optimal fit to the receptor. In such rotamers the positions of the hydroxy groups and therefore also the O–O-distance remain unchanged since the OH-groups are located along the rotational axes. A similar three-dimensional structure was described for triphenylacrylonitrile derivatives by Pons et al. [10].

Biological Activity

The affinity of *BHPE* to the ER was determined in vitro by use of the dextran-coated charcoal method and of cytosol from calf uteri as receptor source. *BHPE* possesses a high affinity to the ER with an RBA-value of 29.1 and a binding plot parallel to that of estradiol (RBA = 100), so a competitive inhibition of the ^{3}H-E_2-ER-interaction by *BHPE* can be assumed. These findings are contrary to the results of Ruenitz et al.

Table 2. Positional parameters[a] and equivalent isotropic displacement parameters (Å^2) of 1,1-bis(4-hydroxyphenyl)-2-phenylethene

Atom	*x*	*y*	*z*	*B*
O(1)	0.9595 (2)	0.0678 (3)	0.9933 (4)	7.1 (1)
O(2)	0.5112 (2)	0.2941 (2)	0.6331 (3)	5.48 (9)
C(1)	0.7319 (2)	0.0908 (3)	0.4223 (5)	4.2 (1)
C(2)	0.7303 (2)	0.1122 (3)	0.5435 (5)	3.7 (1)
C(11)	0.6813 (2)	0.0961 (3)	0.2935 (5)	4.0 (1)
C(12)	0.7000 (3)	0.1083 (4)	0.1788 (5)	5.6 (2)
C(13)	0.6528 (3)	0.1111 (4)	0.0530 (6)	6.4 (2)
C(14)	0.5841 (3)	0.1015 (4)	0.0339 (6)	7.2 (2)
C(15)	0.5655 (3)	0.0891 (4)	0.1481 (6)	6.7 (2)
C(16)	0.6122 (3)	0.0846 (4)	0.2768 (5)	5.4 (1)
C(21)	0.7884 (2)	0.0996 (3)	0.6640 (5)	3.9 (1)
C(22)	0.8345 (3)	0.0308 (4)	0.6697 (5)	5.2 (1)
C(23)	0.8919 (2)	0.0211 (4)	0.7802 (5)	5.1 (1)
C(24)	0.9028 (3)	0.0771 (4)	0.8815 (5)	5.3 (1)
C(25)	0.8593 (3)	0.1437 (4)	0.8818 (5)	5.5 (2)
C(26)	0.8003 (2)	0.1548 (3)	0.7728 (5)	4.4 (1)
C(31)	0.6718 (2)	0.1586 (3)	0.5654 (5)	3.5 (1)
C(32)	0.6467 (2)	0.2339 (3)	0.4962 (5)	4.0 (1)
C(33)	0.5938 (2)	0.2802 (3)	0.5181 (5)	4.4 (1)
C(34)	0.5646 (2)	0.2491 (3)	0.6118 (5)	4.3 (1)
C(35)	0.5878 (2)	0.1753 (3)	0.6825 (5)	4.8 (1)
C(36)	0.6409 (2)	0.1313 (4)	0.6592 (5)	4.8 (1)
H(1)	0.773 (2)	0.068 (3)	0.422 (4)	3 (1)

[a] E.s.d.s. in the least significant digits are shown in parentheses

Table 3. Important bond angles (°), torsion angles (°) and distances (Å)

C(1)–C(2)–C(21)	122.7 (3)	C(11)–C(1)–C(2)–C(21)	178.88 (0.51)
C(1)–C(2)–C(31)	121.8 (3)	C(1)–C(2)–C(21)–C(22)	−30.16 (0.74)
C(2)–C(1)–C(11)	131.7 (3)	C(1)–C(2)–C(31)–C(32)	−51.45 (0.70)
C(2)–C(1)–H(1)	113 (2)	C(2)–C(1)–C(11)–C(16)	−33.49 (0.88)
H(1)–C(1)–C(11)	116 (2)		
C(21)–C(2)–C(31)	115.3 (3)	O–O	9.636

[11], who found an RBA-value of 178. On the other hand De Sombre et al. [12] determined a receptor affinity (RBA = 21) comparable to our results, but they could not find any estrogenic potency of *BHPE*. Presumably the used dose of 3 × 20 µg/animal/day was too low to bring about estrogenic effects in the immature rat uterine weight test. *BHPE* acts as an "impeded" estrogen as we could demonstrate in the immature mouse uterine weight test. The maximum effect of estrone (E_1)

Table 4. Estrogenic and antiestrogenic effects of 1,1-bis(4-hydroxyphenyl)-2-phenylethene

RBA[a]	Dose[b] [nmol]	Estrogenic effect[c]	Antiestrogenic effect[d]
29.1	1	4	–
	10	12	25[e]
	100	60	25[f]
	1000	100	−23

[a] 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 radioactivity by 50%; E_2 = 17β-estradiol

[b] Dose per animal and day

[c] Estrogenic effect = $[(E_T - E_V)/(E_S - E_V)] \times 100$. Effect = uterus dry weight (mg)/body weight (g) × 100. E_T = effect of test compound; E_V = effect of vehicle; E_S = estrone standard (0.4 µg)

[d] Antiestrogenic effect = % inhibition = $100 - [(E_S - E_{S,T})]/(E_S - E_V)] \times 100$; E_S = estrone standard (0.1 µg); $E_{S,T}$ = effect of standard under simultaneous administration of test compound

[e] Significant $p < 0.025$. The U-test according to Wilcoxon, Mann and Whitney was used

[f] Significant $p < 0.01$

is only reached at the very high dose of 1000 nmol *BHPE*/animal/day (see Table 4). Furtheron at the dosages of 1 and 10 nmol/animal/day *BHPE* produced weak antiestrogenic effects. A testing of *BHPE* on the MXT-MC, ER^+ was not performed. The acetyl derivative of *BHPE* (i.e. *BAPE*), however, proved to be moderately active on this tumor model [6]. It must be assumed that *BAPE* is transformed into its hydroxy derivative under in vivo conditions.

Discussion

The weak estrogenic and antiestrogenic potencies of *BHPE* are in contrast to its high RBA-value of 29.1. From compounds which possess such a high affinity to the ER, either strong estrogenic or strong antiestrogenic properties should be expected. A possible explanation for this deviating behavior of *BHPE* is a fast in vivo transformation into a metabolite which is inactive or which is rapidly excreted. If this is true the derivatisation of the OH groups in *BHPE* is supposed to give compounds with improved activity.

In a recent publication Schuderer and Schneider [7] investigated the influence of the derivatisation of the OH groups in the structurally comparable compound *BHPB* (leading to esters of various chain lengths and degrees of halogen substitution, carbamates, imidoesters and ethers) on the ER-affinity and hydrolytic stability of the new drugs. The carbamate of *BHPB* proved to be the only stable compound

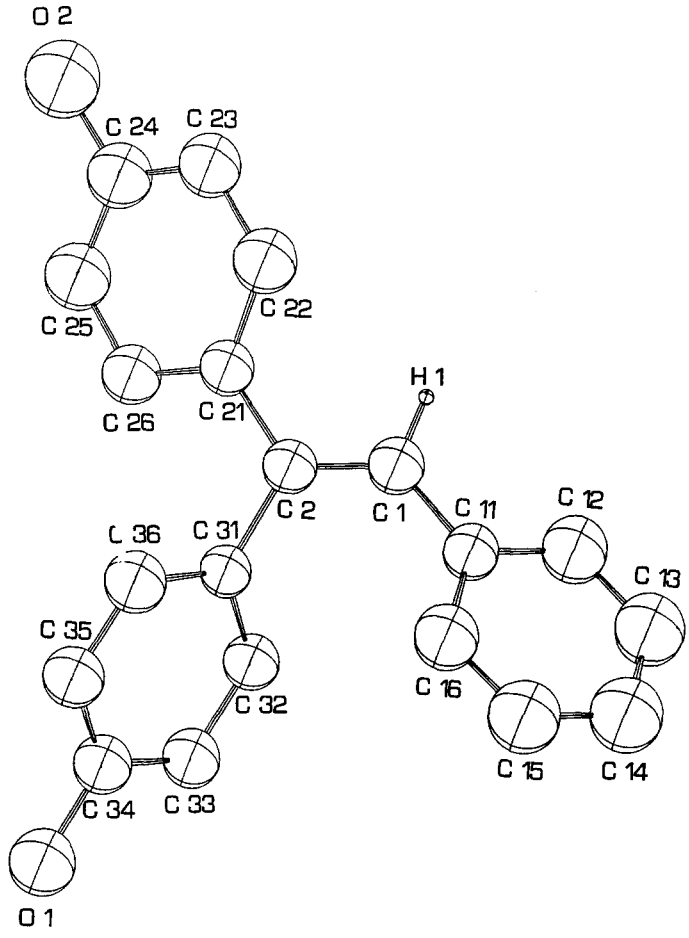


Fig. 1. ORTEP draw of 1,1-Bis(4-hydroxyphenyl)-2-phenylethene (*BHPE*)

with an RBA-value comparable to that of the parent compound. It is also equipotent to *BHPB* in the experiment on the MXT-MC,ER$^+$[7]. The ER-affinity of several ester derivatives (e.g. the acetate: *BAPB*) was also similar or even stronger than that of *BHPB*. In contrast to the carbamate the ester derivatives are relatively quickly hydrolyzed. Interestingly the growth-inhibiting effect of *BAPB* (% T/C = 0.4 at a dose of 8 mg/kg sc, 3 times a week, duration of therapy 6 weeks) on the MXT-MC, ER$^+$ was much better than that of *BHPB*. This may be due to improved pharmacokinetic properties: On the one hand derivatisation probably causes a protracted release of *BHPB* leading to a retarded phase II metabolism and thereby to an increased plasma level of the compound. On the other hand, the lipophilic character of *BAPB* can result in a fast resorption and therefore in a higher level.

Some years ago Schneider [4] also investigated the estrogen receptor affinity and the estrogenic as well as the antiestrogenic properties of the acetoxy derivative of *BHPE* (i.e. *BAPE*). Compared to *BHPE* the acetoxy derivative showed a reduced affinity to the ER (RBA = 6.3), but increased estrogenic activity in the immature mouse uterine weight test (compare Table 4 with Fig. 2). The maximum effect of estrone was already reached at a dose of 67 nmol *BAPE*/animal/day, while for this

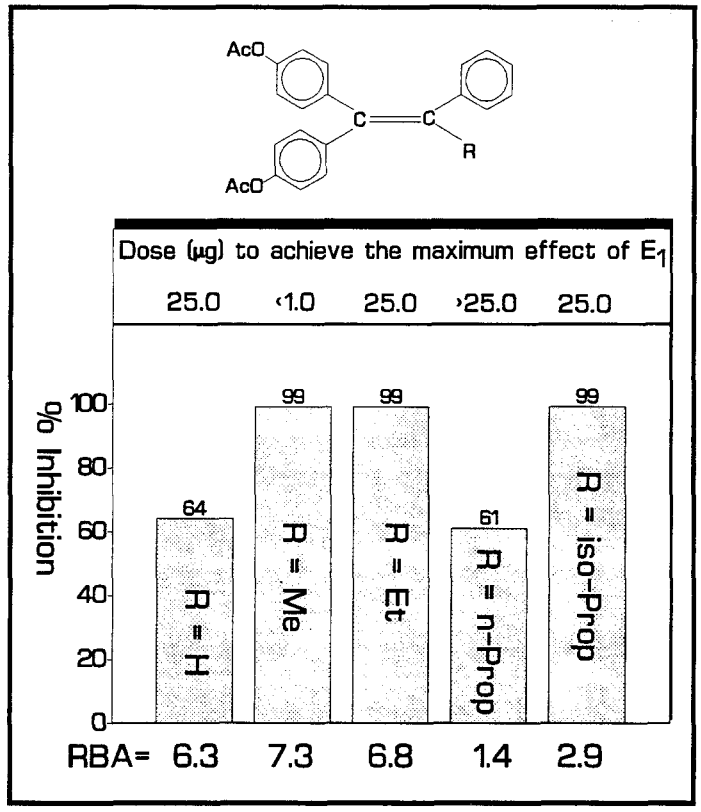


Fig. 2. Estrogen receptor affinity, estrogenic and mammary tumor-inhibiting potency of 2-alkyl-substituted 1,1-bis(4-acetoxyphenyl)-2-phenylethenes [6] (RBA: estrogen receptor affinity; RBA (E_2) = 100; estrogenic potency: immature mouse uterine weight test; MXT-M 3.2 mammary tumor of the mouse)

effect a dose of 1000 nmol of *BHPE* is required. On the other hand, *BHPE* and *BAPE* showed comparable antiestrogenic properties. However, the effect of *BAPE* on the MXT-MC,ER$^+$ was moderate (% T/C = 54.8) in comparison to *BAPB* (% T/C = 0.4) when administered in equimolar dosage, which indicates the importance of the 2-standing ethyl residue for the extent of the antitumor activity (compare the remarks in "Introduction" and Ref. [3, 6]. Schneider [6] showed in this study also that already minimal structural changes in position 2 of *BAPE* like the substitution of the H atom by short alkyl chains can cause a drastic alteration of the pharmacological properties (compare Fig. 2). Thus the exchange of the olefinic hydrogen in the weak "impeded" estrogen *BAPE* by CH_3 led to a highly active "true" estrogen (*BAPP*). However, a C_2H_5-, *n*-C_3H_7- or *i*-C_3H_7-residue did not cause a stronger alteration of the estrogenic activity of *BAPE*. The low activity of *BAPE* on the MXT-MC,ER$^+$ is also considerably increased if its H atom at position 2 is exchanged by C_2H_5 or *i*-C_3H_7 (compare Fig. 2). However, by use of *n*-C_3H_7 instead of *i*-C_3H_7 no alteration in the antitumor activity takes place.

The results of the discussed studies [2, 3, 6, 7] give rise to the assumption that by minor structural variation in position 2 and by appropriate derivatisation of the two phenolic OH groups compounds can be obtained from *BHPE* which are highly

active on the hormone-sensitive breast cancer but which possess only marginal estrogenic side effects. Apparently the presence of an 1,1-bis(4-hydroxyphenyl)-2-phenylethene-fragment is a prerequisite in such compounds so that properties of an "impeded" estrogen can be develop, as shown by experiments in the class of the 1,1,2-triphenylbut-1-enes [3]. By translocation of one of the two acetoxy groups in 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene (*BAPB*) into the 2-standing phenyl ring (which results in the diastereomeric compounds *E*- and *Z*-1,2-bis(4-acetoxyphenyl)--1-phenylbut-1-ene (i.e. *E-BAPB* and *Z-BAPB*) an alteration of the activity profile into that of a strongly active "true" estrogen takes place*. Of the two geometric isomers *E-BAPB* is an only 4 times more active "true" estrogen than *Z-BAPB***. This is surprising since the O–O-distances of their hydroxy derivatives *E*- and *Z-BHPB* (which are considered as the real active compounds) are quite different***. The distance of the two oxygen atoms in *E-BHPB* amounts to 12.2 Å, a value which corresponds to those of the therapeutically used non-steroidal estrogens *DES* and *HES* (see formula scheme). In the similarly active *Z-BHPB*, however, the two oxygens are only 8.4 Å apart from each other (estimation by molecular modeling, program; Alchemy, Fa. Tripos).

The results show that the concept concerning the binding of triphenylethenes to the ER and the following formation of the "activated" receptor (which initiates the physiological response) is much more complicated than has been discussed by Pons et al. [10]. The authors studied the influence of *para*-standing OH-groups on the affinity of the parent compound triphenylacrylonitrile to the ER. They claimed that a 4-hydroxyphenyl ring in position C (compare formula scheme and Fig. 3) is of importance for the recognition step in the binding of triphenylethene derivatives to the ER as well as for their correct orientation towards the receptor area. In case of estradiol this function is performed by the phenolic OH-group on C_3 of the A-ring. This OH-group forms a hydrogen bridge with the acceptor site S_1 in the ER (compare Fig. 3). The energetic contribution which arises from the interaction between ring C and S_1 is regarded as an essential for the ER-affinity. Therefore ring C is considered as the main anchorage point of triphenylethenes. The *para*-hydroxylation of two aromatic rings in triphenylacrylonitrile can lead to an increase (substitution of the rings A/C and B/C) as well as to a decrease (substitution of the rings A/B) of the ER-affinity, which in the two former examples should be accompanied by an elevated estrogenic potency. Pons et al. [10] interpret these differences in the affinities of the isomers to the ER as follows: In the case of the B/C-substitution-pattern, the *para*-OH-group in ring C is directed toward S_1 and that in ring B toward S_2 (i.e. an orientation in C_7 or C_{11} direction of E_2). On the other hand an A/C-substitution-pattern leads to a direction of ring C towards S_1 and of ring A towards S_3 (close to the zone of interaction of the 17β-OH-group of E_2). The latter mode of interaction with the ER is most equivalent to that of E_2 and should therefore

* However, the strong mammary tumor-inhibiting effect of *BAPB* does not change by this structural variation (compare Ref. [3])

** RBA and dose for the achievement of the 100% effect of E_1 in x µg/animal/day 1–3, immature mouse uterine weight test; *E-BAPB*: RBA = 5.9, $x = 0.25$; *Z-BAPB*: RBA = 1.0, $x = 1.0$

*** Under in vivo conditions the prodrugs *E*- and *Z-BAPB* are transformed into the active drugs under participation of esterases (compare Ref. [7])

lead to a better stabilisation of the "activated" form of the ER than that which is achieved by triphenylethenes bearing B/C-substitution-pattern. Schneider [3], who used the estrogenic potency (estimated in the immature mouse uterine weight test)

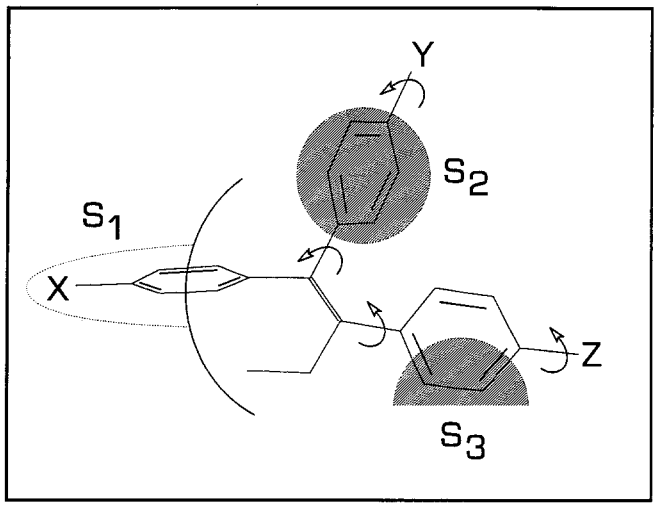
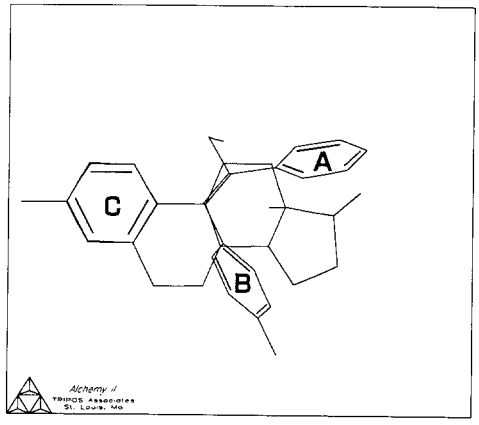


Fig. 3. Model for binding of 1,1,2-triphenylbut-1-ens (*BHPB*: X, Y = OH, Z = H; *E-BHPE*: X, Z = OH, Y = H; *Z-BHPB*: Y, Z = OH, X = H) to the estrogen receptor



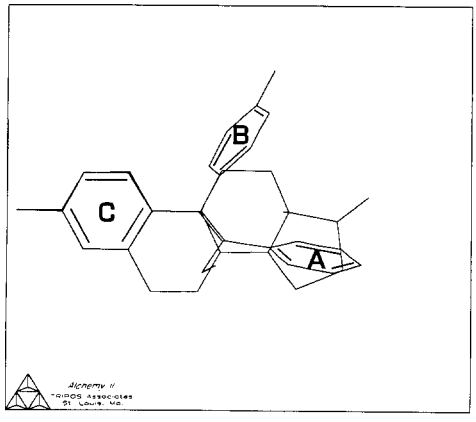


Fig. 4. Comparable fit of *E-BHPB* and E_2 to the ER detectable by superposition of both molecules. If ring C covers the A-ring of E_2 and ring B is close to position C_{11} of E_2 the ring A approximates also optimally to the D-ring of E_2. In this conformation the hydroxy groups in the ring A and C can form strong hydrogen bridges to the acceptor sites S_1 and S_3 in ER (compare Fig. 3). The 6, 7-positions of E_2 is also superimposed namely by the 2-standing ethyl group which presumably contributes to the hydrophobic binding to the receptor

instead of the ER-affinity, like Pons et al. [10] did, as pharmacological parameter, could not observe such a relation between activity and position of the two *para*-standing OH-groups in the class of 1,1,2-triphenylbut-1-enes. Not only the A/C-substitution but also, unexpectedly, the A/B-substitution led to strongly active "true" estrogens [3]. For these experiments the acetoxy derivatives of *E-BHPB* and *Z-BHPB* were used. With the model, discussed by Pons et al. [10], an interaction of *E-BHPB* (but not of *Z-BHPB*) with the ER can be described, which is in accordance with that of known steroidal and non-steroidal estrogens like E_2, *DES* and *HES* (compare Fig. 3). Superposition experiments on E_2 achieve the same result. It shows that only *E-BHPB* is capable of forming strong hydrogen bridges to the acceptor sites S_1 and S_3 in the ER, as E_2, *DES* and *HES* do (compare Fig. 4). Duax et al. [13], however, remark that the B-ring of triphenylethenes, e.g. of 4-hydroxytamoxifen, (which is directed to the C_{11} region of E_2) lies outside the molecular envelop of E_2 and therefore may interfere with a conformational change in the receptor needed for hormonal response. If this is true, *para*-hydroxy-substituted triphenylethenes should elicit little or no hormonal response, a prediction which was disproved by Schneider [3]. Only *BHPB*, a compound with B/C-substitution-pattern, showed weak estrogenic properties. However, the exchange of the ethyl group in *BHPB* by the methyl group increases drastically its estrogenic potency, indicating that a further sterically sensitive binding site, which is of importance for the triggering of an agonistic effect exists in ER. Apparently the methyl derivative stabilizes the "active" conformation of ER better than the ethyl derivative.

The contradictory structure-activity-data show that the concept on the estrogen receptor-interaction of triphenylethenes must be revised. Presumably several binding areas for estrogens are present in ER, whose activation by the binding of a drug triggers the physiological effect. According to this concept estrogens with structures quite different from that of E_2 bind to areas in ER not identical with the binding area of E_2. However, several groups [10, 14–16] discuss a single binding area for steroidal and non-steroidal estrogens, which is located in the vicinity of Cis 530 in the ER. The investigations will be continued with the aim of the development of new triphenylethenes for the therapy of breast and prostate cancer.

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References

[1] Westerberg H. (1980) Cancer Treat. Rep. **64**: 117
[2] Schneider M. R., von Angerer E., Schönenberger H., Michel R. T., Fortmeyer H. P. (1982) J. Med. Chem. **25**: 1070
[3] Schneider M. R. (1986) J. Cancer Res. Clin. Oncol. **112**: 119
[4] Schneider M. R., Hartmann R. W., Sinowatz F., Amselgruber W. (1986) J. Cancer Res. Clin. Oncol. **112**: 258
[5] Schneider M. R., Ball H., Schönenberger H. (1985) J. Med. Chem. **28**: 1880

[6] Schneider M. R. (1986) J. Med. Chem. **29**: 1494
[7] Schuderer M. L., Schneider M. R. (1987) Arch. Pharm. (Weinheim) **320**: 635
[8] Enraf-Nonius (1988) Structure Determination Package, Version 3.1. Enraf-Nonius, Delft, The Netherlands
[9] Hartmann R., Kranzfelder G., von Angerer E., Schönenberger H. (1980) J. Med. Chem. **23**: 841
[10] Pons M., Bignon E., Crastes De Paulet A., Gilbert J., Ojasoo T., Raynand J. P. (1990) J. Steroid Biochem. **36**: 391
[11] Ruenitz R. C., Bagley J. R., Nanavati N. T. (1988) J. Med. Chem. **31**: 1471
[12] De Sombre E. R., Mease R. C., Sanghavie J., Singh T., Seevers R. H., Hughes A. (1988) J. Steroid Biochem. **29**: 583
[13] Duax W. L., Griffin J. F., Weeks C. M., Wawrzak Z. (1988) J. Steroid Biochem. **31**: 481
[14] Butler I. S., Vessières A., Jonen G. (1989) Comments Inorg. Chem. **8**: 269
[15] Vessières A., Vaillant C., Salmain M., Jaouen G. (1989) J. Steroid Biochem. **34**: 301
[16] Harlow K. W., Smith D. N., Katzenellenbogen J. A., Greene G. L., Katzenellenbogen B. S. (1989) J. Biol. Chem. **264**: 17476
[17] Gust R., Schönenberger H., Klement U., Range K. J. (in press) Arch. Pharm. (Weinheim)

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