least-squares fitting program. K_i values were calculated from IC_{50} values according to the equation of Cheng and Prusoff:[14] $K_i = IC_{50}/(1 + (C/K_D))$, where C is the concentration and K_D the equilibrium dissociation constant of the radioligand.

Association velocity constants were derived from the slope of a plot according to $\ln [B_{max}(L_0 - B_t)/L_0(B_{max} - B_t)] = k_1[1/(L_0 - B_{max})]t$, where L_0 is the radioligand concentration, B_{max} the maximal stereospecific binding, and B_t the stereospecific binding at time t.[15] Dissociation velocity constants were derived according to the equation $\ln B_t = \ln (B_{max} - k_{-1}t)$.

P_{app}, the octanol/water partition coefficient at any given pH, was calculated from $P_{app} = P/(1 + 10^{pK_a - pH})$, where P is the partition coefficient of the uncharged molecule and pK_a the acid dissociation constant.

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Influence of Alkyl Chain Ramification on Estradiol Receptor Binding Affinity and Intrinsic Activity of 1,2-Dialkylated 1,2-Bis(4- or 3-hydroxyphenyl)ethane Estrogens and Antiestrogens

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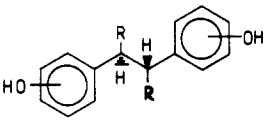
The influence of a symmetrical introduction of CH_3 substitutents in the α or β positions of the 1,2-dialkyl-1,2-bis(4-hydroxyphenyl)ethane estrogens hexestrol (ethyl, HES) and octestrol (*n*-propyl, OCES) [isopropyl (**1**), *tert*-butyl (**2**), *sec*-butyl (**3**), isobutyl (**4**)] and the 1,2-dialkyl-1,2-bis(3-hydroxyphenyl)ethane antiestrogens metahexestrol (ethyl, MetaHES) and metaoctestrol (*n*-propyl, MetaOCES) [isopropyl (**5**), *tert*-butyl (**6**), *sec*-butyl (**7**), isobutyl (**8**)] on estradiol receptor (E_2R) binding affinity and intrinsic activity is described. The synthesis of compounds **1**–**8** was accomplished by reductive coupling of (a) the corresponding α-alkylbenzyl alcohols with $TiCl_3/LiAlH_4$ and separation of the meso diastereoisomers (compounds **2**–**4**, **7**, and **8**), (b) α-*tert*-butyl-3-methoxybenzyl chloride with $CoCl_2/EtMgCl$ and isolation of the meso configurated isomer (**6**), and (c) the isopropyl phenyl ketones with $TiCl_4/Zn$ and subsequent hydrogenation of the corresponding *cis*-hex-3-enes (compounds **1** and **5**). The binding affinity of **1**–**8** to the calf uterine E_2R was measured relative to that of [^{3}H]E_2 by a competitive binding assay. Compounds **1** and **5** showed relative binding affinity (RBA) values exceeding those of HES and MetaHES, respectively. All other derivatives showed RBA values smaller than the corresponding parent compounds. The intrinsic activity was monitored in terms of uterotrophic and antiuterotrophic activity. It is striking that the introduction of a CH_3 group in the α positions of MetaHES and MetaOCES led to compounds with full intrinsic activity (**5**–**7**), i.e., estrogens without antiestrogenic properties. No correlation between E_2R binding affinity and intrinsic activity was found.

We have recently shown that displacement of the phenolic hydroxy groups of the synthetic estrogen hexestrol (HES, Chart I) to the 3,3′ positions only led to a moderate reduction of the binding affinity for the estradiol receptor (E_2R).[1,2] Interestingly metahexestrol [*meso*-3,4-bis(3-hydroxyphenyl)hexane, MetaHES] turned out to be a partial antiestrogen, i.e., it exhibited strongly decreased uterotrophic properties compared to HES and showed a strong inhibition of the estrone-stimulated uterine growth of the immature mouse.[1-3] This compound is of great interest for the treatment of hormone-dependent human breast cancer, since it shows strong inhibitory activity on several rodent mammary tumors.[1-3]

Structural modifications of MetaHES have been performed in order to increase the binding affinity for the E_2R and thus enhance tumor inhibiting properties,[1,4,5] since antitumor activity appears to be correlated with the E_2R binding affinity.[1] Symmetrical substitution in the 4,4′, 5,5′

Chart I



4 OH compd	R	3 OH compd
HES	CH_2CH_3	Meta HES
1	$CH(CH_3)$	5
2	$C(CH_3)_3$	6
OCES	$CH_2CH_2CH_3$	Meta OCES
3	$CHCH_2CH_3$	7
4	$CH_2CH(CH_3)$	8

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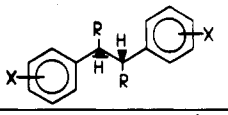
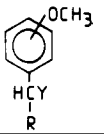
Table I. Methoxy- and Hydroxy-Substituted *meso*-1,2-Dialkyl-1,2-diphenylethanes



compd	X	R	synth method[a]	yield,[b] %	mp, °C	recrystn solv[c]	formula[d]
1a	4-OCH_3	$CH(CH_3)_2$	H	90	114–116	K	$C_{22}H_{30}O_2$
1	4-OH	$CH(CH_3)_2$	C	80	228–229	K	$C_{20}H_{26}O_2$
2a	4-OCH_3	$C(CH_3)_3$	B	32	165–166	K	$C_{24}H_{34}O_2$
2	4-OH	$C(CH_3)_3$	C	75	241–242 dec	K	$C_{22}H_{30}O_2$
3a	4-OCH_3	$CHCH_3C_2H_5$	B	52	126–127	K	$C_{24}H_{34}O_2$
3	4-OH	$CHCH_3C_2H_5$	C	80	194–195	K	$C_{22}H_{30}O_2$
4a	4-OCH_3	$CH_2CH(CH_3)_2$	B	18	127–128	K	$C_{24}H_{34}O_2$
4	4-OH	$CH_2CH(CH_3)_2$	C	92	208–209	K	$C_{22}H_{30}O_2$
5a	3-OCH_3	$CH(CH_3)_2$	H	76	167–168	K	$C_{22}H_{30}O_2$
5	3-OH	$CH(CH_3)_2$	C	86	225–226	N	$C_{20}H_{26}O_2$
6a	3-OCH_3	$C(CH_3)_3$	E	18	140–141	L	$C_{24}H_{34}O_2$
6	3-OH	$C(CH_3)_3$	C	81	230–232	M	$C_{22}H_{30}O_2$
7a	3-OCH_3	$CHCH_3C_2H_5$	B	35	106–107	L	$C_{24}H_{34}O_2$
7	3-OH	$CHCH_3C_2H_5$	C	85	194–195	M	$C_{22}H_{30}O_2$
8a	3-OCH_3	$CH_2CH(CH_3)_2$	B	26	95	K	$C_{24}H_{34}O_2$
8	3-OH	$CH_2CH(CH_3)_2$	C	88	189	N	$C_{22}H_{30}O_2$

[a] Capital letters refer to synthetic methods B, C, E, and H under the Experimental Section. [b] Yield of analytically pure product; no effort made to optimize yields. [c] K = EtOH, L = EtOH/H_2O, M = acetic acid/H_2O, N = benzene/EtOH. [d] All compounds were analyzed for C and H within ±0.4% of the calculated values.

Table II. 1-Hydroxy- and 1-Chloro-Substituted 1-(Methoxyphenyl)alkanes



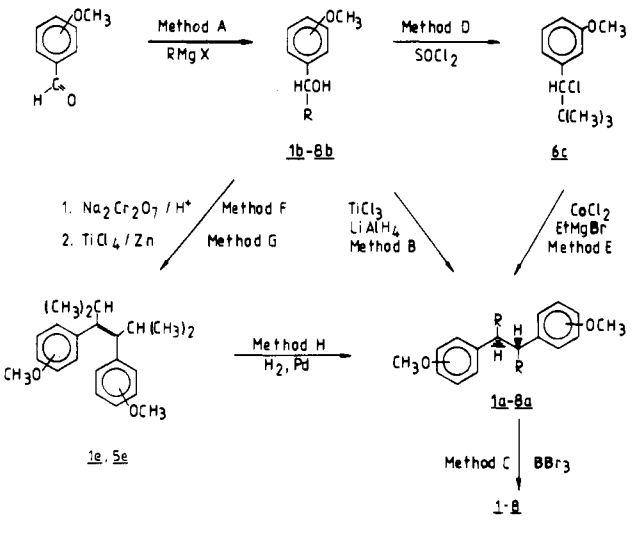
compd		Y	R	synth method[a]	yield,[b] %	mp, °C	formula
1b	4-OCH_3	OH	$CH(CH_3)_2$	A	85	oil	$C_{11}H_{16}O_2$
2b	4-OCH_3	OH	$C(CH_3)_3$	A[c]	72	oil	$C_{12}H_{18}O_2$
3b	4-OCH_3	OH	$CHCH_3C_2H_5$	A	92	oil	$C_{12}H_{18}O_2$
4b	4-OCH_3	OH	$CH_2CH(CH_3)_2$	A	91	oil	$C_{12}H_{18}O_2$
5b	3-OCH_3	OH	$CH(CH_3)_2$	A	79	oil	$C_{11}H_{16}O_2$
6b	3-OCH_3	OH	$C(CH_3)_3$	A[c]	65	oil	$C_{12}H_{18}O_2$
6c	3-OCH_3	Cl	$C(CH_3)_3$	D	74	oil	$C_{12}H_{17}ClO$
7b	3-OCH_3	OH	$CHCH_3C_2H_5$	A	52	oil	$C_{12}H_{18}O_2$
8b	3-OCH_3	OH	$CH_2CH(CH_3)_2$	A	68	oil	$C_{12}H_{18}O_2$

[a] A and D refer to synthetic methods A and D under the Experimental Section. [b] Yield of analytically pure (TLC) product; no effort made to optimize yields. [c] 2-Chloro-2-methylpropane and a mixture of toluene and THF were used.

or 6,6′ positions only led to an increase of the binding affinity in the case of 3,4-bis(3-hydroxy-6-methylphenyl)hexane.[4] But this compound showed also enhanced estrogenicity.[4] Substitution of the Et groups of MetaHES by CF_3 groups changed the pharmacological properties only slightly,[5] whereas the exchange of the Et substituents by CH_3 or *n*-alkyl groups reduced E_2R binding affinity, not very strongly, however, in the case of the *n*-propyl derivative metaoctestrol (MetaOCES).[1] While these derivatives of MetaHES exhibited antiestrogenic properties,[1,4,5] it has been observed that replacement of the 1,2-diethyl groups of MetaHES by isopropyl groups destroys the antiestrogenic activity and generates a true estrogen.[6]

The aim of the present study is the investigation of the latter phenomenon. In the following the syntheses of symmetrical α or β CH_3-substituted derivatives of HES, OCES, MetaHES, and MetaOCES will be described (compounds **1–8**, Chart I), and the effects of these structural modifications on E_2R binding affinity as well as intrinsic activity—monitored in terms of uterotrophic and antiuterotrophic activity—will be examined.

Chart II



Chemistry. The syntheses of compounds **1–8** (Table I) started from 4- or 3-methoxybenzaldehyde (Chart II). The latter compounds were converted with alkylmagnesium halide into the corresponding 1-(4- and 3-methoxyphenyl)alkan-1-ols **1b–8b** (method A, Table II).

In the case of compounds **2b–4b**, **7b**, and **8b** reductive

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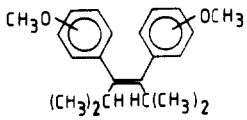
Table III. *cis*-2,5-Dimethyl-3,4-bis(methoxyphenyl)hex-3-enes



compd		synth method[a]	yield,[b] %	mp, °C	recrystn solv	formula[c]
1e	4-OCH_3	G	45	133–135	EtOH	$C_{22}H_{28}O_2$
5e	3-OCH_3	G	80	164–165	EtOH	$C_{22}H_{28}O_2$

[a] G refers to synthetic method G under the Experimental Section. [b] Yield of analytically pure (TLC) product; no effort made to optimize yields. [c] All compounds were analyzed for C and H within ±0.4% of the calculated values.

coupling was performed with $TiCl_3/LiAlH_4$ (method B).[7] The meso diastereoisomers **2a–4a**, **7a**, and **8a** (Table I) were separated from the mixture of the *meso*- and *d,l*-dibenzyl derivatives by column chromatography. *meso*-3,4-Bis(3-methoxyphenyl)-2,2,5,5-tetramethylhexane (**6a**)[8] (Table I) was obtained by converting **6b** with $SOCl_2$ into 1-chloro-2,2-dimethyl-1-(3-methoxyphenyl)propane (**6c**) (method D, Table II), reductive coupling of the latter compound with $CoCl_2$ and EtMgBr (method E)[9] and column chromatography of the resulting mixture of the meso and *d,l* diastereoisomers.

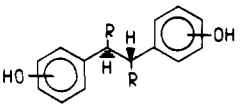
In accordance with the previously described hexestrol,[10] metahexestrol,[4] butestrol,[11] and metabutestrol[11] the ^1H NMR spectra of the *meso*-1,2-dialkyl-1,2-bis(4- and 3-methoxyphenyl)ethanes generally showed the OCH_3 signals shifted downfield compared to the signals of the corresponding *d,l* compounds. The *meso*-2,5-dimethyl-3,4-bis(4- and 3-methoxyphenyl)hexanes (**1a** and **5a**) (Table I) were obtained by catalytic hydrogenation of the corresponding *cis*-3,4-bis(methoxyphenyl)-2,5-dimethylhex-3-enes (**1e** and **5e**) (Table III) using palladium on carbon (method H).

The *cis*-hex-3-ene derivatives **1e** and **5e** were obtained—in the case of the 3-methoxyphenyl derivative **5e** in very good yield—by reductive coupling of the 1-(4- and 3-methoxyphenyl)-2-methylpropan-1-ones (**1d** and **5d**) with $TiCl_4$ and Zn (method G)[12] and separation from the trans isomers by column chromatography. The cis configuration of **1e** and **5e** was proved by their ^1H NMR spectra: the signal of the OCH_3 protons is shifted upfield compared to the signal of the trans isomers, as it has been observed in the case of the previously described 3,4-bis(methoxyphenyl)hex-3-enes.[2,13] Compounds **1d** and **5d** were obtained by oxidation of **1b** and **5b** with $Na_2Cr_2O_7/H_2SO_4$ (method F).[14]

The ether cleavage of the methoxy derivatives **1a–8a** to yield 1–8 (Table I) was accomplished with BBr_3 (method C).

Biological Properties. The compounds were tested for their relative binding affinity (RBA) for the E_2R by a competitive binding assay with calf uterine cytosol, 17β-[^{3}H]estradiol, and the Dextran-coated charcoal technique.[3b]

Table IV. Relative Binding Affinity (RBA) of Hexestrol, Metahexestrol, Octestrol, Metaoctestrol, and Compounds 1–8 for Calf Uterine Estrogen Receptor



4-OH compd	RBA value[a]	R	RBA value[a]	3-OH compd
HES[b]	25	C_2H_5	11[d]	MetaHES
1	30	$CH(CH_3)_2$	15	5
2	1.5	$C(CH_3)_3$	0.7	6
OCES[c]	8	$CH_2CH_2CH_3$	4[e]	MetaOCES
3	5	$CHCH_3C_2H_5$	3	7
4	1.5	$CH_2CH(CH_3)_2$	0.5[f]	8

[a] Relative binding affinity for the calf uterine estrogen receptor is the ratio of molar concentrations of 17β-estradiol (E_2) and inhibitor required to decrease the amount of bound [^{3}H]E_2 by 50% × 100. The mean value of four determinations is given. In each determination RBA 1 > RBA HES, RBA OCES > RBA 3, and RBA 5 > RBA MetaHES. [b] Compound was commercially obtained. RBA value of ref 10: 27. [c] See ref 15. Preparation was performed by using the $TiCl_3/LiAlH_4$ coupling. [d] Prepared as described, see ref 4. RBA value of ref 4: 10. [e] Prepared as described, see ref 1. K_a value of ref 1: 0.91 × 10^8 M^{-1}. [f] K_a value of ref 1: 0.11 × 10^6 M^{-1}.

The introduction of a CH_3 group into the α-positions of HES and MetaHES leads to a significant increase of the affinity for the E_2R (compounds **1** and **5**; Table IV). The introduction of a further CH_3 group on the other hand results in a dramatic decrease of the RBA-values (compounds **2** and **6**).

The elongation of the alkyl chains of HES and MetaHES also leads to a decrease of the affinity for the E_2R (OCES, MetaOCES). In contrast to the α,α′-dimethyl-substituted 3,4-bis(hydroxyphenyl)hexanes (compounds **1** and **5**) the correspondingly substituted octane derivatives (compounds **3** and **7**) show reduced RBA values compared to their parent compounds. In the case of the CH_3 groups standing in the β-positions the affinity for the E_2R is even more reduced (compounds **4** and **8**).

It is striking that the 4-hydroxyphenyl derivatives (HES, OCES, compounds 1–4) show an about twofold higher RBA value compared to the corresponding 3-hydroxyphenyl analogues (MetaHES, MetaOCES, compounds 5–8).

All compounds were tested for their uterotrophic activity in the immature mouse as a measure of their estrogenicity (Figures 1 and 2).

It can be seen from Figure 1 that the introduction of a CH_3 group in the α-positions of HES leads to a slight decrease of uterotrophic activity, though compound **1** had shown a stronger affinity for the E_2R than the parent compound. Nevertheless compound **1** just like HES is a strong estrogen. The strongly reduced uterotrophic activity shown as the result of the introduction of a further CH_3 group (compound **2**) is fully compatible with the drastically reduced RBA value of this compound.

OCES shows a strongly diminished uterotrophic activity compared to HES. Although the introduction of a CH_3 group in the α-positions has led to a slight reduction of E_2R affinity, compound **3** exhibits a stronger uterotrophic activity compared to the parent compound. In the case of the CH_3 substituents standing in the β-positions on the other hand the uterotrophic activity is strongly reduced (compound **4**).

It is striking that HES and the α,α′-CH_3-substituted compounds **1–3** show a steep dose–response curve. Me-

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(8) This compound could not be obtained by reductive coupling of **6b** with $TiCl_3/LiAlH_4$.
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(12) Mukaiyama, T.; Sato, T.; Hanna, J. *Chem. Lett.* **1973**, 1041.
(13) Schneider, M.; von Angerer, E.; Kranzfelder, G.; Schönenberger, H. *Arch. Pharm. (Weinheim, Ger.)* **1980**, *313*, 919.
(14) The overall yield for compounds **1a** and **5a** on this route was considerably higher—especially for **5a**—than using the $TiCl_3/LiAlH_4$ coupling.

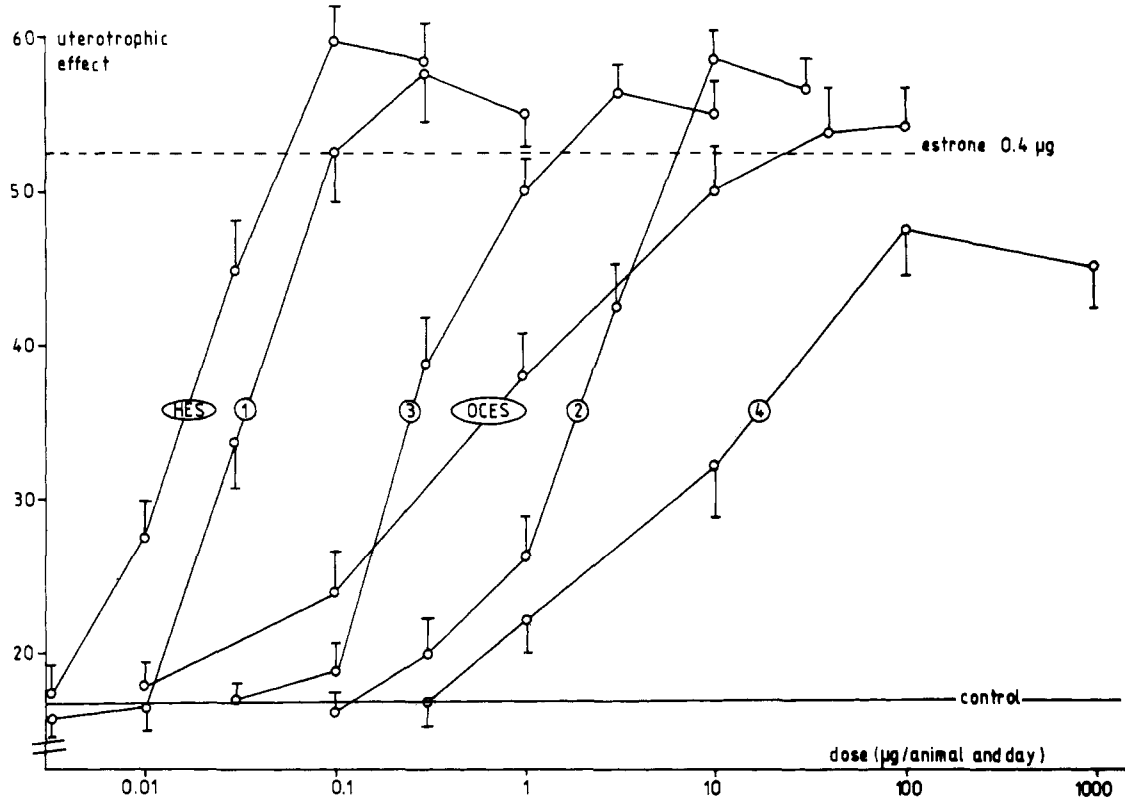


Figure 1. Estrogenic activity of the *meso*-1,2-dialkyl-1,2-bis(4-hydroxyphenyl)ethanes HES, OCES, and compounds 1–4 in the uterine weight test of the immature mouse. Compounds were administered at three consecutive days sc; the uteri were removed 24 h after the last injection. Uterotrophic effect = uterus dry weight (mg)/body weight (g) × 100; the values are means of 10 animals/group ± SD.

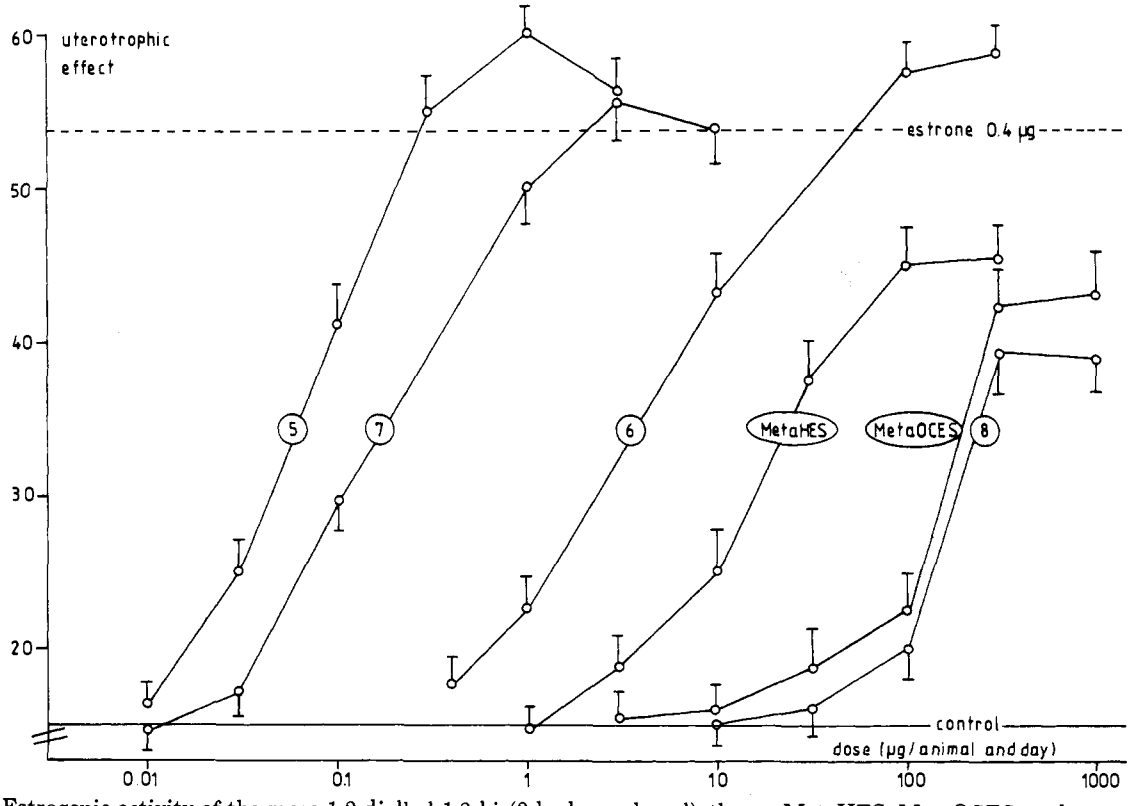


Figure 2. Estrogenic activity of the *meso*-1,2-dialkyl-1,2-bis(3-hydroxyphenyl)ethanes MetaHES, MetaOCES, and compounds 5–8 in the uterine weight test of the immature mouse. See Figure 1 legend.

tahexestrol is a partial agonist (Figure 2). In spite of a relatively high RBA value, uterotrophic activity is only seen in high doses. The maximum effect of 0.4 µg of estrone is not reached. The introduction of a CH_3 group into the α-positions leads to a very strong increase of uterotrophic activity (compound 5), which cannot only be explained by the higher RBA value. The introduction of further CH_3 groups into the α-positions (compound 6) decreases uterotrophic activity. This effect corresponds to the decrease in E_2R affinity. But it is remarkable that

Table V. Antiestrogenic Activity of MetaHES, MetaOCES, and 8 in the Mouse Uterine Weight Test

	antiuterotrophic test		
compd	dose,[a] μg	effect,[b] means ± SD	% inhibn[c,d]
MetaHES	0	14.5 ± 1.2	
	3	40.1 ± 2.4	20[f]
	10	34.1 ± 2.6	39[e]
	30	30.2 ± 2.9	51[e]
	100	32.7 ± 2.9	43[e]
	300	30.9 ± 2.4	49[e]
estrone	0.1	46.5 ± 2.5	
MetaOCES	0	14.5 ± 1.2	
	10	42.1 ± 2.8	14
	30	40.8 ± 2.7	18
	100	34.7 ± 3.1	37[e]
	300	33.0 ± 2.0	42[e]
estrone	0.1	46.5 ± 2.5	
8	0	14.5 ± 1.2	
	30	40.3 ± 2.9	19
	100	37.9 ± 2.4	27[f]
	300	38.2 ± 1.9	26[f]
estrone	0.1	46.5 ± 2.5	

[a] Dose per animal and day. [b] Uterus dry weight (milligrams)/body weight (g) × 100. [c] Percent inhibition = $100 - (E_{S,T} - E_V)/(E_S - E_V) \times 100$; E_S = effect of estrone standard; $E_{S,T}$ = effect of standard under simultaneous application of test substance, E_V = effect of vehicle. [d] The U test according to Wilcoxon, Mann, and Whitney was used. [e] Significant ($\alpha = 0.01$). [f] Significant ($\alpha = 0.05$).

the uterotrophic activity of **6** is still stronger than that effect seen with MetaHES, though the latter compound had shown a much higher RBA value.

MetaOCES exhibits a reduced binding affinity for the E_2R compared to MetaHES and compatible with this a diminished uterotrophic activity. As seen with MetaHES, the maximum effect of 0.4 μg of estrone is not reached. The introduction of CH_3 groups into the α-positions also leads to a strong increase of uterotrophic activity (compound **7**). This phenomenon is even more remarkable considering the fact that the RBA value of compound **7** is smaller than that of MetaOCES. In the case of the CH_3 groups standing in the β-positions (compound **8**) no increase of uterotrophic activity can be observed. The dose–response curve of compound **8** is not significantly different from that of MetaOCES.

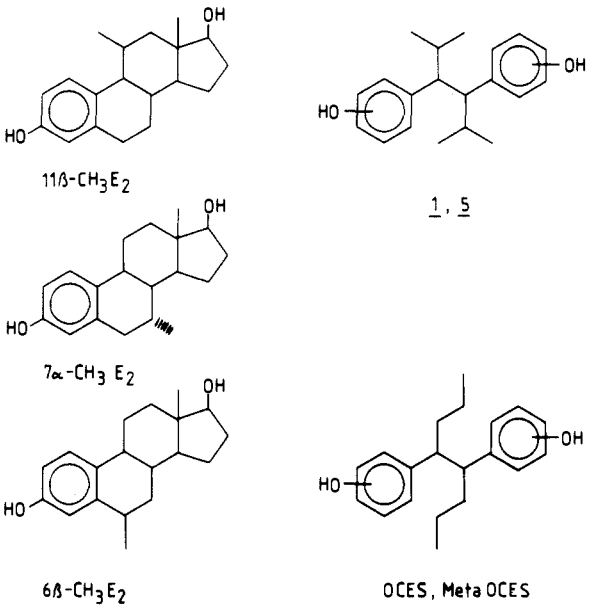
It is striking that the α-CH_3-substituted compounds **5**–**7** show a steep dose–response curve reaching the maximum effect of estrone (0.4 μg), whereas the two parent compounds as well as the β-CH_3-substituted compound **8** do not reach this effect.

Regardless of their differing estrogenic properties, the test compounds were evaluated in doses of 0.1–100 μg for their antiuterotrophic activity by determining their inhibiting effects on the uterine growth stimulated by estrone. In accordance with the properties shown in the uterotrophic test, some compounds (HES, **1**–**3**, **5**, and **6**) led to an enhancement of the uterotrophic activity of estrone (data not given). The latter effect was only inhibited by compound **4** (not significant, data not given), MetaHES, MetaOCES, and compound **8** (Table V).

Discussion

The finding that the E_2R binding affinity of HES and MetaHES can be increased by substitution of the ethyl groups by isopropyl groups is suggestive of the observation that 11β-CH_3 E_2 and 7α-CH_3 E_2 also show a higher binding affinity for the E_2R than E_2 (in the case of 7α-CH_3 E_2 this might not be significant).[16] This correlation might indicate

Chart III



that the flexible 1,2-dialkyl-1,2-diphenylethane molecules show a conformation at the E_2R binding site mimicking the structure of the E_2 derivatives (Chart III). These phenomena are probably due to the increased lipophilicity of the CH_3-substituted derivatives. In the case of the 1,2-diphenylethane compounds the π-values of the corresponding substituents are the following: $\pi_{C_2H_5}$ 1.02, $\pi_{i\text{-}C_3H_7}$ 1.53.[17]

It becomes apparent from the fact that substitution of the isopropyl groups of **1** and **5** by *n*-propyl groups results in a strong decrease of the RBA values (OCES and MetaOCES)—though both substituents show very similar π-values ($\pi_{n\text{-}C_3H_7}$ 1.55[17])—that lipophilicity in the center of the molecule is not the only important parameter for E_2R binding affinity. Steric requirements are also to be met for maximum RBA values. Again the analogy with E_2 becomes obvious: 6β-CH_3 E_2 also shows a reduced E_2R binding affinity compared with E_2.[18]

In spite of a further increased lipophilicity and only slightly increased molecular volume the *tert*-butyl compounds **2** and **6** show a drastically reduced binding affinity. This is probably due to the compact structure, hindering the rotation on the benzylic C *tert*-butyl C bonds. For this reason the molecule cannot adjust to the E_2R binding site in the way necessary to an optimum binding affinity.

The finding that introduction of CH_3 groups into the α-positions of OCES and MetaOCES decreased E_2R binding affinity, though the same structural manipulation increased the binding affinity of HES and MetaHES, is an indication that the 4,5-diphenyloctane derivatives might be differently adjusted to the E_2R binding site.

The further reduced E_2R binding affinity of the β-CH_3-substituted compounds **4** and **8** is certainly due to an increased steric hindrance of the interaction with the receptor.

The most important finding is that there is no correlation between the binding affinity for the E_2R and the intrinsic activity. The introduction of CH_3 groups in the

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α-positions of the partial antiestrogens MetaHES and MetaOCES did influence E_2R binding affinity differently but in every case led to compounds with full intrinsic activity (**5–7**). The same structural manipulation did not lead to a further increase of estrogenic activity in the series of the 4-OH diphenylethane estrogens. But all these compounds also showed strong agonistic properties.

Which structural requirements are essential for full intrinsic activity? As observed in the case of E_2R binding affinity, the distance between the phenolic OH groups seems to be important. A location in the 4,4′ positions leads to full intrinsic activity only in case of a certain lipophilicity in the center of the molecule. A shortening of the alkyl chains of HES for example causes a loss of intrinsic activity (butestrol[11]). That this loss was due to a reduction of lipophilicity is shown by the fact that the exchange of the CH_3 groups by CF_3 substituents completely restores agonistic activity.[5]

In order to obtain full intrinsic activity the OH groups need not necessarily be placed in the 4,4′ positions. The loss of agonistic properties induced by displacement of the phenolic OH groups of HES into the 3,3′ positions (MetaHES) can easily be neutralized by the introduction of CH_3 groups in the α-positions.

The effect of the α-CH_3 substitution on the intrinsic activity cannot only be explained by the increased lipophilicity. It seems much more that the CH_3 groups exert a special sterical effect. The high receptor affinity of 7α-CH_3 E_2 and a similar substituent influence observed with the C-2 methyl groups of 3-phenyl-4-aryl chromans led to the suggestion that these CH_3 groups interact with a special hydrophobic pocket on the receptor.[19] This pocket might also be of importance for the intrinsic activity of the α-CH_3-substituted 1,2-dialkyl-1,2-diphenylethanes. The α-CH_3 ramification might cause a good anchoring on this receptor area and thus lead to the same full conformational change observed with HES. In this context it is of interest that 7α-CH_3 E_2 shows 150% of the estrogenic activity of E_2.[16]

Experimental Section

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

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

Method A. 1-(4-Methoxyphenyl)-2-methylbutan-1-ol (3b). 2-Iodobutane (2.21 g, 12 mmol) was dissolved in ether and added dropwise, with stirring, to magnesium turnings (0.29 g, 0.012 mol) in 20 mL of dry ether. The mixture was heated at reflux for 0.5 h. A solution of 4-methoxybenzaldehyde (1.36 g, 0.01 mol) in ether was added dropwise with stirring. After heating at reflux for 2 h, the mixture was cooled and poured onto ice. The resulting precipitate was dissolved by the addition of a NH_4Cl solution. The ethereal layer was separated, washed ($NaHSO_3$, $NaHCO_3$, and H_2O), and dried ($MgSO_4$). The solvent was removed, and the resulting oil was distilled under high vacuum to give 1.78 g of **3b** (colorless oil).

Method B. *meso*-4,5-Bis(4-methoxyphenyl)-3,6-dimethyloctane (3a). $TiCl_3$ (4.63 g, 0.03 mol) was placed under N_2 in a flask with 150 mL of dry glyme. $LiAlH_4$ (0.38 g, 0.01 mol) was quickly added to the stirred $TiCl_3$ slurry. The resulting black suspension was stirred for 10 min. Compound **3b** (1.94 g, 0.01 mol) was dissolved in 10 mL of dry glyme, and the resultant mixture was added dropwise with stirring. The mixture was heated at reflux for 16 h. After cooling, the reaction mixture was quenched by the addition of 2 N HCl, diluted with H_2O, and extracted with ether. The ether extract was washed ($NaHCO_3$ and H_2O) and dried ($MgSO_4$). The solvent was removed, and the meso isomer **3a** was isolated by chromatography [silica gel column eluted with CH_2Cl_2/ligroin, 1:1]. Recrystallization from EtOH gave 0.92 g of **3a**.

Method C. *meso*-4,5-Bis(4-hydroxyphenyl)-3,6-dimethyloctane (3). A solution of **3a** (3.55 g, 0.01 mol) in 250 mL of dry CH_2Cl_2 was cooled to −60 °C. Under N_2, BBr_3 (7.52 g, 0.03 mol) was added with stirring. After 0.5 h the freezing mixture was removed, and the reaction mixture was kept at room temperature for 4 h. MeOH (50 mL) was added, and the mixture was shaken with 2 N NaOH. After neutralization of the aqueous layer with 3 N HCl, the solution was extracted with ether. After removal of the ether, the crude product was crystallized from EtOH to give 2.61 g of **3**.

Method D. 1-Chloro-2,2-dimethyl-1-(3-methoxyphenyl)-propane (6c). Compound **6b** (1.94 g, 0.01 mol) was dissolved in 25 mL of $SOCl_2$ and heated at reflux for 3 h. After cooling the solvent was removed, and the resulting oil was distilled under high vacuum to give 1.57 g of **6c** (colorless oil).

Method E. *meso*-3,4-Bis(3-methoxyphenyl)-2,2,5,5-tetramethylhexane (6a). Anhydrous $CoCl_2$ (0.10 g, 0.8 mmol) was added at room temperature with stirring to a solution of ethylmagnesium bromide (2.00 g, 0.015 mol) in 10 mL of ether. A solution of compound **6c** (2.13 g, 0.01 mol) in 10 mL of dry ether was added dropwise. After the vigorous evolution of gas had stopped, the black mixture was poured onto ice and HCl and extracted with ether. The ethereal extracts were washed and dried, the solvent was removed, and the meso isomer **6a** was isolated by chromatography [silica gel column eluted with CH_2Cl_2/ligroin, 1:1]. Recrystallization from EtOH/H_2O gave 0.32 g of **6a**.

Method F. 1-(4-Methoxyphenyl)-2-methylpropan-1-one (1d). A solution of $Na_2Cr_2O_7$ (2.62 g, 0.01 mol) in 25 mL of H_2SO_4 (10%) was added dropwise at 0 °C to a stirred solution of compound **1b** (1.80 g, 0.01 mol) in 50 mL of ether. Stirring was continued for 4 h. After separation, the ethereal layer was washed ($NaHCO_3$ solution, H_2O) and dried ($MgSO_4$). The solvent was removed, and the resulting oil was distilled under reduced pressure to yield 1.51 g (85%) of **1d** (colorless oil), bp 174 °C (14 mmHg).

1-(3-Methoxyphenyl)-2-methylpropan-1-one (5d). After removal of the solvent, the resulting oil was distilled under reduced pressure to give 1.43 g (80%) of **5d** (colorless oil), bp 137–138 °C (14 mmHg).

Method G. *cis*-3,4-Bis(4-methoxyphenyl)-2,5-dimethylhex-3-ene (1e). $TiCl_4$ (2.85 g, 0.015 mol) was added dropwise to 100 mL of cooled, dry dioxane under N_2 with stirring. A suspension of Zn powder (1.96 g, 0.03 mol) in 20 mL of dry dioxane was added in small portions over a period of 10 min. After the addition of a solution of **1d** (1.78 g, 0.01 mol) in 20 mL of dry dioxane the reaction mixture was heated at reflux for 4 h. After cooling, alkaline hydrolysis was performed with 10% K_2CO_3 solution. After extraction with ether and removal of the solvent, the cis isomer **1e** was isolated by chromatography [silica gel column eluted with CH_2Cl_2/ligroin, 1:1]. Recrystallization from EtOH gave 0.73 g of **1e**.

Method H. *meso*-3,4-Bis(4-methoxyphenyl)-2,5-dimethylhexane (1a). Palladium on charcoal (10%, 0.1 g) was added to a solution of **1e** (3.24 g, 0.01 mol) in 100 mL of EtOH. The suspension was shaken under a hydrogen atmosphere until no more H_2 was accepted. The reaction mixture was filtered. The alcohol was removed, and the crude product was recrystallized from EtOH to give 2.94 g of **1a**.

Biological Methods

Estradiol Receptor Binding Assay. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^3H]estradiol. A previously described procedure[20] was used with modifications. Test compounds were incubated with cytosol from calf uteri and [^3H]estradiol at 4 °C for 16 h.

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Incubation was stopped by adding Dextran-coated charcoal. After centrifugation the radioactivity of a 100-μL supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabeled test compounds. Six concentrations of the competitors were tested. They were chosen to provide a linear portion on a semilog plot crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled estradiol and of test compounds reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activities were determined by stimulation of the uterine growth and the inhibition of the uterine growth stimulated by estrone, respectively, with immature NMRI mice as described previously.[20] Twenty-day-old female mice (weight 15.0 ± 2.5 g, mean ± SD) were randomly distributed into groups of 10 animals. They were subcutaneously (sc) injected daily for 3 days with 0.1 mL of olive oil solutions containing the test compound. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, washed, dried, and weighed.

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Registry No. 1, 102808-03-7; **1a**, 102808-10-6; **1b**, 18228-46-1; **1d**, 2040-20-2; **1e**, 102808-11-7; **2**, 102808-04-8; **2a**, 86439-85-2; **2b**, 6013-92-9; **3**, 102808-05-9; **3a**, 102808-12-8; **3b**, 14248-29-4; **4**, 102808-06-0; **4a**, 102808-13-9; **4b**, 100757-90-2; **5**, 102808-07-1; **5a**, 102808-14-0; **5b**, 61751-33-5; **5d**, 6026-75-1; **5e**, 102808-15-1; **6**, 102808-08-2; **6a**, 102808-16-2; **6b**, 32578-11-3; **6c**, 102808-17-3; **7**, 102808-09-3; **7a**, 102808-18-4; **7b**, 102808-19-5; **8**, 78682-45-8; **8a**, 78682-41-4; **8b**, 78682-47-0; 2-iodobutane, 513-48-4; 4-methoxybenzaldehyde, 123-11-5; 3-methoxybenzaldehyde, 591-31-1; *tert*-butyl bromide, 507-19-7; isobutyl bromide, 78-77-3; 2-bromopropane, 75-26-3; 2-bromobutane, 78-76-2; 2-chloro-2-methylpropane, 507-20-0.

Supplementary Material Available: ^{1}H NMR data (Tables VI and VII) of compounds 1-8, **1a-8a**, **1e**, and **5e** (5 pages). Ordering information is given on any current masthead page.

Potential Tumor- or Organ-Imaging Agents. 26. Polyiodinated 2-Substituted Triacylglycerols as Hepatographic Agents[1]

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A series of ω-(3-amino-2,4,6-triiodophenyl)alkanoic acids and the corresponding 1,3-dipalmitoylglycerol 2-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] were synthesized, radioiodinated with iodine-125, and evaluated for their ability to selectively localize in the liver for potential use as hepatographic imaging agents. Acid analogues **1d** and **1e** afforded relatively high levels of radioactivity in the liver (45 and 49% injected dose) 5 min after intravenous administration to rats. These acids displayed a marked propensity to become bound to plasma albumin. In contrast, triacylglycerol analogues **10a** and **10c** did not become immediately associated with plasma albumin but instead rapidly became associated with plasma lipoproteins and showed a different tissue distribution profile than free acids **1a** and **1c**. Although long-chain triacylglycerol analogues **10d** and **10e** exhibited some capacity to accumulate in the liver at 5 and 30 min, respectively, analysis of the plasma revealed significant in vivo ester hydrolysis. It would thus appear that liver radioactivity following administration of **10d** and **10e** was due to uptake of the free acid and not the intact triacylglycerol. Triacylglycerol analogues **10a** and **10c**, on the other hand, were taken up intact and showed liver accumulations of 25 and 35% of the administered dose at 30 min.

Among the imaging modalities currently in widespread use, radiography and radioscintiscanning owe much of their success to the radiopaques (contrast agents) and radiopharmaceuticals used in conjunction with these procedures. A major pharmacological difference between radiopaques and radiopharmaceuticals is that large doses of the former are required for opacification of soft tissues, whereas radiopharmaceuticals are administered in tracer doses. In each case, the procedure generally depends on the ability of the agent to selectively accumulate in the organ under consideration.

The most widely used liver scintigraphy agent, [^{99m}Tc]sulfur colloid, is removed from the circulation by the phagocytic action of reticuloendothelial (Kupffer) cells. The static anatomic image thus obtained proves useful in the location of primary and secondary lesions as small as 2-3 cm in diameter, but has no utility in assessing hepatocyte function. Hepatocyte function can, however, be studied by monitoring the uptake and clearance of [^{131}I]-rose bengal, a dye which is extracted by hepatocytes and, under normal conditions, is quickly secreted into the bile.[2] Problems with this agent have recently been documented and mainly concern the slow rate of hepatic-to-intestinal output and the poor imaging characteristics and high radiation doses associated with iodine-131.[3] The need for a better hepatobiliary agent eventually led to the development of the ^{99m}Tc-labeled iminodiacetic acid (IDA) analogues. As a group, the [^{99m}Tc]Ida analogues improve hepatobiliary imaging, but they are less useful for the quantitative study of hepatocyte function because of uptake by extrahepatic tissues, variable urinary excretion, and presence of varying amounts of free ^{99m}Tc.[4] Furthermore, plasma clearance of these agents by the hepatocytes occurs via the anionic pathway and thus competes with serum bilirubin for uptake.[5] Therefore, as serum

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