Ring-Substituted 1,2-Dialkylated 1,2-Bis(hydroxyphenyl)ethanes. 2. Synthesis and Estrogen Receptor Binding Affinity of 4,4′-, 5,5′-, and 6,6′-Disubstituted Metahexestrols

Rolf W. Hartmann, Alexander Heindl, and Helmut Schönenberger*

Institute of Pharmacy, Lehrstuhl Pharmazeutische Chemie II, University of Regensburg, Universitätsstraße 31, 8400 Regensburg, Federal Republic of Germany. *Received August 8, 1983*

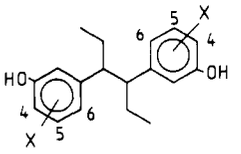
The syntheses of symmetrically 4,4′-, 5,5′-, and 6,6′-disubstituted derivatives of the mammary tumor inhibiting antiestrogen metahexestrol [*meso*-3,4-bis(3-hydroxyphenyl)hexane] (1) are described [4,4′-substituents: F (2), Cl (3), Br (4), I (5), $CH_2N(CH_3)_2$ (6), CH_3 (7), CH_2OCH_3 (8), $CH_2OC_2H_5$ (9), CH_2OH (10), NO_2 (11), NH_2 (12), $N(CH_3)_2$ (13), $COCH_3$ (14), and C_2H_5 (15); 5,5′-substituents: OH (16) and Cl (17); 6,6′-substituents: OH (18), F (19), Cl (20), and CH_3 (21)]. The synthesis of 1–3, 16, and 19 was accomplished by reductive coupling of the propiophenones with $TiCl_4/Zn$ and subsequent hydrogenation of the *cis*-3,4-diphenylhex-3-enes. Compounds 17, 18, 20, and 21 were synthesized by coupling the 1-phenyl-1-propanols with $TiCl_3/LiAlH_4$ and separation of the meso diastereomers, while 4–15 were obtained by substitution of metahexestrol. The binding affinity of these compounds to the calf uterine estrogen receptor was measured relative to that of [^{3}H]estradiol by a competitive binding assay. The test compounds showed relative binding affinity (RBA) values between 15 and <0.01% that of estradiol. Only compound 21 showed an estrogen receptor binding affinity exceeding that of metahexestrol (15 and 10%, respectively). Compounds exhibiting RBA values of >0.5% were evaluated in the mouse uterine weight test. They showed a similar (2 and 12), slightly increased (19 and 21), or strongly enhanced (7 and 20) estrogenicity compared to that of metahexestrol. Compounds 1, 2, 7, 12, and 21 exhibited antiestrogenic activity inhibiting the estrone-stimulated uterine growth (24 to 60% inhibition).

We have recently shown that displacement of the phenolic hydroxy groups of the synthetic estrogen hexestrol to the 3,3′-positions provided the partial antiestrogen metahexestrol [*meso*-3,4-bis(3-hydroxyphenyl)hexane] (1).[1] This compound is of great interest for the treatment of hormone-dependent human breast cancer, since it shows a marked inhibitory activity of the established DMBA-induced mammary carcinoma of the Sprague-Dawley rat,[1-3] which has many similarities with the human breast cancer.[4]

Accounts of experiments elucidating the mode of action of metahexestrol have already appeared in the literature. It was found that the in vivo antiestrogenic and tumor inhibitory activities of 1 are not due to its possible catechol metabolite 3,4-bis(3,4-dihydroxyphenyl)hexane.[2] This hypothesis had been discussed because endogenous catechol estrogens were found to antagonize estrogen effects and exhibit antitumor activity.[5,6] Further experiments addressed the question of whether it is the estrogenic or the antiestrogenic activity of metahexestrol that is responsible for the antitumor effect of this compound,[7-9] since it is well known that estrogens also inhibit the growth of hormone-dependent human and experimental mammary tumors.[10] This question is of general interest, since all hitherto known antiestrogens exhibit some residual estrogenicity, depending on the test model used. Metahexestrol, as well as the partial antiestrogen tamoxifen (Nolvadex), were found to increase the tumor growth stimulating and inhibiting effects of the synthetic estrogen diethylstilbestrol on ovariectomized, DMBA tumor bearing Sprague-Dawley rats.[9,11] This result may indicate that 1, as well as tamoxifen, possibly unfold their antitumor activity by means of their estrogenic potency.

A further increase of the antitumor activity of 1 (i.e., the same antitumor effect obtained by smaller doses) could be realized by synthesizing derivatives with a higher affinity for the estradiol receptor (E_2R), since the antitumor activity of this class of antiestrogens appears to be correlated with their E_2R association constants.[1] In the first part of an extensive structure–activity study in the class of 1,2-di- or tetraalkylated 1,2-bis(hydroxyphenyl)ethanes, we have shown that the E_2R affinity of the synthetic estrogen hexestrol could be enhanced by symmetrical substitution of the aromatic rings in 1 of 21 synthesized analogues.[12]

This paper describes the syntheses and the testing for the E_2R affinity of 4,4′-, 5,5′-, and 6,6′-disubstituted derivatives of metahexestrol (1). The mouse uterine weight test is used to determine the estrogenicity and antiestrogenicity of the most active E_2R interaction inhibiting compounds.[13]



	X		X		X
1	H	8	$4\text{-}CH_2OCH_3$	15	$4\text{-}C_2H_5$
2	4-F	9	$4\text{-}CH_2OC_2H_5$	16	5-OH
3	4-Cl	10	$4\text{-}CH_2OH$	17	5-Cl
4	4-Br	11	$4\text{-}NO_2$	18	6-OH
5	4-I	12	$4\text{-}NH_2$	19	6-F
6	$4\text{-}CH_2N(CH_3)_2$	13	$4\text{-}N(CH_3)_2$	20	6-Cl
7	$4\text{-}CH_3$	14	$4\text{-}COCH_3$	21	$6\text{-}CH_3$

(1) Hartmann, R. W.; Buchborn, H.; Kranzfelder, G.; Schönenberger, H.; Bogden, A. E. *J. Med. Chem.* **1981**, *24*, 1192.
(2) Kranzfelder, G.; Hartmann, R. W.; von Angerer, E.; Schönenberger, H.; Bogden, A. E. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 165.
(3) For recent reviews of the pharmacology of metahexestrol, see: (a) Engel, J.; Hartmann, R. W.; Schönenberger, H. *Drugs Future* **1983**, *8*, 413. (b) Hartmann, R. W. *Cancer Treat. Rev.*, in press.
(4) Fiebig, H. H.; Schmähl, D. *Recent Res. Cancer Res.* **1980**, *71*, 80.
(5) Paul, S. M.; Skolnick, P. *Nature (London)* **1977**, *266*, 559.
(6) Abul-Hajj, Y. J. *Cancer Res.* **1979**, *39*, 4882.
(7) Hartmann, R. W.; Schönenberger, H.; Wrobel, K. H. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 241.
(8) Hartmann, R. W. *Verh. Dtsch. Krebs. Ges.* **1983**, *4*, 156.
(9) Hartmann, R. W. *Eur. J. Cancer Clin. Oncol.* **1983**, *19*, 959.
(10) Huggins, C. *Cancer Res.* **1965**, *25*, 1163.
(11) Ovariectomy causes complete remission of DMBA-induced tumors of Sprague-Dawley rats. This effect can be overcome by small doses of diethylstilbestrol. The maximum stimulation (no difference to the intact control) was observed at a dose of 1 μg/(kg day). Higher doses, however, inhibited tumor growth (ref 9).
(12) Hartmann, R. W.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.* **1983**, *26*, 1137.

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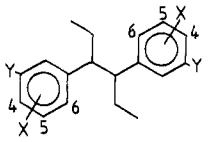
Table I. Substituted *meso*-3,4-Diphenylhexanes



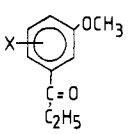
compd	X	Y	synth method[a]	yield,[b] %	mp, °C	recrystn solvent[c]	formula[d]
1a[e]	H	OCH_3	B	95	88	A	$C_{20}H_{26}O_2$
1[e]	H	OH	C	90	182	A	$C_{18}H_{22}O_2$
2a[f]	4-F	OCH_3	B	96	108	A	$C_{20}H_{24}F_2O_2$
2[f]	4-F	OH	C	87	176	A	$C_{18}H_{20}F_2O_2$
3a	4-Cl	OCH_3	B[g]	59	139-140	A	$C_{20}H_{24}Cl_2O_2$
3	4-Cl	OH	C	88	147	A	$C_{18}H_{20}Cl_2O_2$
4	4-Br	OH	U	45	158	A	$C_{18}H_{20}Br_2O_2$
5	4-I	OH	V	49	245	B	$C_{18}H_{20}I_2O_2$
6	4-$CH_2N(CH_3)_2$	OH	F	59	166-167	A	$C_{24}H_{36}N_2O_2$
7	4-CH_3	OH	G	77	206-207	A	$C_{20}H_{26}O_2$
8a	4-CH_2OAc	OAc	H	80	114-115	C	$C_{28}H_{34}O_8$
8	4-CH_2OCH_3	OH	J	81	136-137	A	$C_{22}H_{30}O_4$
9	4-$CH_2OC_2H_5$	OH	J	78	132-133	A	$C_{24}H_{34}O_4$
10	4-CH_2OH	OH	C[h]	99	>240		$C_{20}H_{26}O_4$
11	4-NO_2	OH	K	76	179-180	D	$C_{18}H_{20}N_2O_6$
12	4-NH_2	OH	B	98	258-260	E	$C_{18}H_{24}N_2O_2$
13	4-$N(CH_3)_2$	OH	L	73	131-132	C	$C_{22}H_{32}N_2O_2$
14a	4-$COCH_3$	OCH_3	M	70	212-213	D	$C_{24}H_{30}O_4$
14	4-$COCH_3$	OH	C[i]	89	208-209	F	$C_{22}H_{26}O_4$
15a	4-C_2H_5	OCH_3	N	90	96-97	G	$C_{24}H_{34}O_2$
15	4-C_2H_5	OH	C	91	168-169	H	$C_{22}H_{30}O_2$
16a	5-OCH_3	OCH_3	B	93	126	B	$C_{22}H_{30}O_4$
16	5-OH	OH	C	77	230	G	$C_{18}H_{22}O_4$
17a	5-Cl	OCH_3	E	25	124	A	$C_{20}H_{24}Cl_2O_2$
17	5-Cl	OH	C	80	187	J	$C_{18}H_{20}Cl_2O_2$
18a	6-OCH_3	OCH_3	E	21	114	A	$C_{22}H_{30}O_4$
18	6-OH	OH	C[j]	70	223-224	J	$C_{18}H_{22}O_4$
19a	6-F	OCH_3	B	89	134	A	$C_{20}H_{24}F_2O_2$
19	6-F	OH	C	77	195	J	$C_{18}H_{20}F_2O_2$
20a	6-Cl	OCH_3	E	19	143	A	$C_{20}H_{24}Cl_2O_2$
20	6-Cl	OH	C	91	248	A	$C_{18}H_{20}Cl_2O_2$
21a	6-CH_3	OCH_3	E	15	138	C	$C_{22}H_{30}O_2$
21	6-CH_3	OH	C	69	267	A	$C_{20}H_{26}O_2$

[a] Capital letters refer to synthetic methods B, C, E-N, U, and V under Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] A = EtOH/H_2O; B = MeOH/H_2O; C = EtOH; D = acetone; E = acetone/H_2O; F = CH_2Cl_2/ligroin; G = MeOH/benzene; H = ligroin; J = benzene. [d] All compounds were analyzed for C, H, N, Br, and Cl within ±0.4% of the calculated values. [e] See ref 1. [f] See ref 2. [g] See ref 19. [h] After the addition of MeOH, the solvent was evaporated. The resulting pure (TLC, ^1H NMR) solid was not further recrystallized. [i] The ether cleavage was accomplished with BCl_3, not with BBr_3. [j] The product was not extracted with NaOH solution.

Chemistry. The synthesis of compound **1** and its disubstituted derivatives **2**, **3**, and **16–21** (Scheme I, Table I) was accomplished by reductive coupling of either the corresponding 3-methoxypropiophenones with $TiCl_4/Zn$ (route 1: **1–3**, **16**, and **19**) or the corresponding 1-(3-methoxyphenyl)-1-propanols with $TiCl_3/LiAlH_4$ (route 2: **17**, **18**, **20**, and **21**).

Compound **1c** was obtained by the reactions of 3-methoxybenzaldehyde with EtMgBr and oxidation of the resulting 1-(3-methoxyphenyl)-1-propanol with $Na_2Cr_2O_7/H_2SO_4$. The synthesis of the halogenated 3′-methoxypropiophenones **2c**, **3c**, and **19c**, as well as 3′,5′-dimethoxypropiophenone **16c**, was accomplished by converting the 3-methoxybenzoic acids with $SOCl_2$ into the corresponding acid chlorides and reaction of the latter compounds with $(Et)_2Cd$ (method O, Table II; compounds **2c**, **3c**, and **16c**) or with EtMgBr at −78 °C according to Sato et al.[14] (method P, Table II, **19c**).

Table II. Substituted 3′-Methoxypropiophenones



compd	X	synth method[a]	yield,[b] %	mp, °C	formula
1c	H	c	70	oil	$C_{10}H_{12}O_2$
2c	4-F	O	68	180-181	$C_{10}H_{11}FO_2$
3c	4-Cl	O	59	oil	$C_{10}H_{11}ClO_2$
16c	5-OCH_3	O	71	27	$C_{11}H_{14}O_3$
17c	5-Cl	P	83	oil	$C_{10}H_{11}ClO_2$
19c	6-F	P	67	oil	$C_{10}H_{11}FO_2$
21c	6-CH_3	P	73	oil	$C_{11}H_{14}O_2$

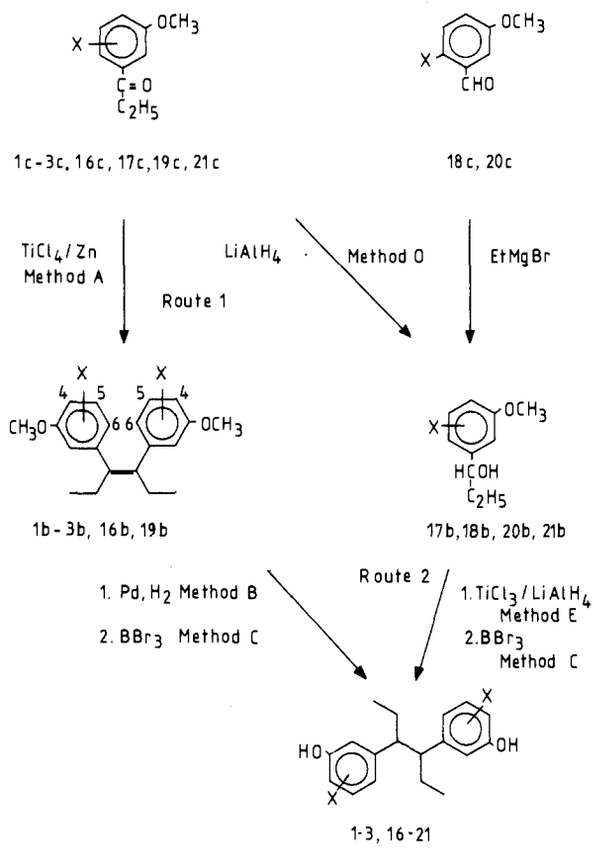
[a] Capital letters refer to synthetic methods O and P under Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] Obtained by Grignard reaction of 3-methoxybenzaldehyde and subsequent oxidation of the resulting compound according to standard procedures.

The synthesis of 4-fluoro-3-methoxybenzoic acid has been described.[2,15] 4-Chloro-3-methoxybenzoic acid was

(13) These experiments are part of our screening procedure for the development of mammary tumor inhibiting antiestrogens: see ref 3b.

(14) Sato, F.; Inoue, M.; Oguro, K.; Sato, M. *Tetrahedron Lett.* **1979**, 4303.

Scheme I[a]



[a] 1c, **1b**, 1, X = H; 2c, **2b**, 2, X = 4-F; 3c, **3b**, 3, X = 4-Cl; 16c, **16b**, X = 5-OCH_3; 16, X = 5-OH; 17c, **17b**, 17, X = 5-Cl; 18c, **18b**, X = 6-OCH_3; 18, X = 6-OH; 19c, **19b**, 19, X = 6-F; 20c, **20b**, 20, X = 6-Cl; 21c, **21b**, 21, X = 6-CH_3.

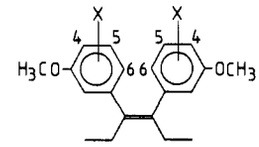
obtained by Sandmeyer reaction of 4-amino-3-methoxybenzoic acid. The synthesis of 6-fluoro-3-methoxybenzoic acid started from 6-nitro-3-methoxytoluene, which was obtained by the procedure of Koelsch.[16] Catalytic reduction of the nitro group with PtO_2 and hydrogen gave 6-amino-3-methoxytoluene. The latter compound was converted with $NaNO_2$ and HBF_4 into the corresponding diazonium tetrafluoroborate, which was decomposed at 150 °C in 1,2,4-trichlorobenzene (method Q). The resulting 6-fluoro-3-methoxytoluene was oxidized with $KMnO_4$ to give the corresponding benzoic acid (method R).

The *cis*-stilbenes **1b–3b**, **16b**, and **19b** were obtained in good yields by reductive coupling of the propiophenones **1c–3c**, **16c**, and **19c** with the $TiCl_4$/Zn reaction of Mukaiyama et al.[17] (method A, Scheme I, Table III).[18]

Catalytic hydrogenation of **1b–3b**, **16b**, and **19b** with palladium on carbon (method B, Scheme I) gave the *meso*-3,4-diphenylhexane derivatives **1a–3a**, **16a**, and **19a** (Table I).[19] The ether cleavage of **1a–3a**, **16a**, and **19a** to **1–3**, **16**, and **19** was accomplished with BBr_3 (method C, Scheme I, Table I).

The 5,5′- and 6,6′-disubstituted derivatives of metahexestrol (**17**, **18**, **20**, and **21**) were synthesized by a different reductive coupling (route 2, Scheme I, Table I),

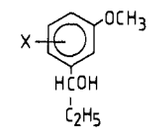
Table III. Substituted *cis*-3,4-Bis(3-methoxyphenyl)hex-3-enes



compd	X	synth method[a]	yield,[b] %	mp, °C	formula
1b	H	A	78	oil	$C_{20}H_{24}O_2$
2b	4-F	A	75	85–86	$C_{20}H_{22}F_2O_2$
3b	4-Cl	A	60	oil	$C_{20}H_{22}Cl_2O_2$
16b	5-OCH_3	A	72	oil	$C_{22}H_{28}O_4$
19b	6-F	A	57	oil	$C_{20}H_{22}F_2O_2$

[a] A refers to synthetic method A under Experimental Section. [b] Yield of analytically pure (TLC) product; no effort was made to optimize yields.

Table IV. Substituted 1-(3-Methoxyphenyl)-1-propanols



compd	X	synth method[a]	yield,[b] %	mp, °C	formula
17b	5-Cl	D	90	oil	$C_{10}H_{13}ClO_2$
18b	6-OCH_3	*c*	75	oil	$C_{11}H_{16}O_3$
20b	6-Cl	*c*	85	oil	$C_{10}H_{13}ClO_2$
21b	6-CH_3	D	96	oil	$C_{11}H_{16}O_2$

[a] D refers to synthetic method D under Experimental Section. [b] Yield of analytically pure (TLC) product; no effort was made to optimize yields. [c] Obtained by Grignard reaction of the corresponding benzaldehydes according to the standard procedure.

which started from the corresponding 1-(5- or 6-substituted-3-methoxyphenyl)-1-propanols **17b**, **18b**, **20b**, and **21b**.

The secondary alcohols **17b** and **21b** were obtained by reduction of the corresponding propiophenones **17c** and **21c** using $LiAlH_4$ (method D, Scheme I, Table IV). The synthesis of **17c** and **21c** was accomplished by converting the corresponding benzoic acids with $SOCl_2$ into the acid chlorides and reaction of the latter compounds with EtMgBr at −78 °C (method P, Table II).

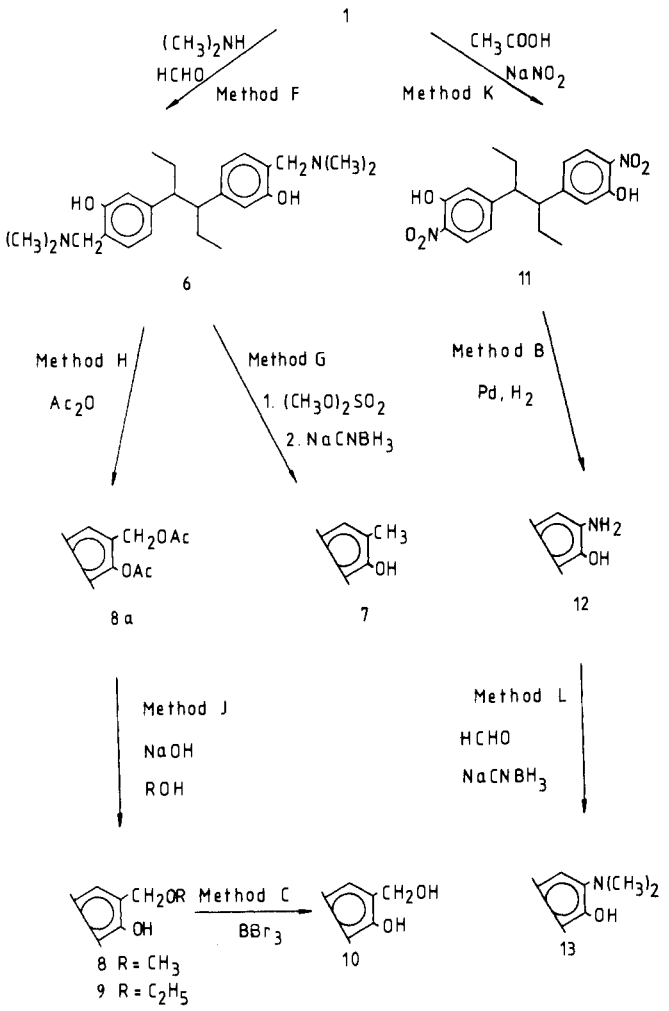
The 5-chloro-3-methoxybenzoic acid was obtained by preparing 5-chloro-3-methoxyphenylmagnesium chloride from 3,5-dichloroanisole and then reaction of the Grignard compound with CO_2 (method S). The synthesis of the 3-methoxy-6-methylbenzoic acid started from *o*-toluic acid, which was converted into 6-methyl-3-sulfobenzoic acid with concentrated H_2SO_4.[20,21] The sodium salt of the latter compound was converted into 3-hydroxy-6-methylbenzoic acid by fusion with KOH (method T). Methylation with $(CH_3O)_2SO_2$ gave the 3-methoxy-6-methylbenzoic acid.

The secondary alcohols **18b** and **20b** were obtained by Grignard reaction of the corresponding benzaldehydes **18c** and **20c** with EtMgBr (Scheme I, Table IV).

The synthesis of **20c** was accomplished by converting 6-chloro-3-methoxytoluene with NBS to the corresponding benzyl halogenide and subsequent treatment of the latter compound with hexamethylenetetramine according to the procedure of Sommelet.

(15) Hartmann, R. W.; Kranzfelder, G.; von Angerer, E.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 841.
(16) Koelsch, C. F. *J. Am. Chem. Soc.* **1944**, *66*, 2019.
(17) Mukaiyama, T.; Sato, T.; Hanna, J. *Chem. Lett.* **1973**, 1041.
(18) For further details, see ref 12.
(19) In the case of **3a**, dehalogenated side products were separated by column chromatography.
(20) Jacobsen, O.; Wierss, F. *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 1959.
(21) Baudisch, O.; Perkin, W. H. *J. Chem. Soc.* **1909**, *95*, 1883.

Scheme II

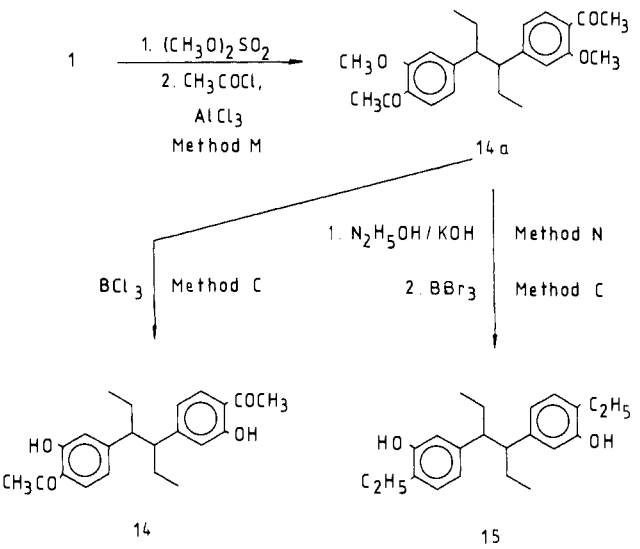


Reductive coupling of the secondary alcohols **17b**, **18b**, **20b**, and **21b** with $TiCl_3/LiAlH_4$ according to the method of McMurry and Silvestri[22] (method E, Scheme I) gave a mixture of the corresponding *meso*- and *d,l*-dibenzyl derivatives.[23,24] The meso diastereomers **17a**, **18a**, **20a**, and **21a** (Table I) were separated by column chromatography. The ether cleavage of the methoxy derivatives to yield **17**, **18**, **20**, and **21** was accomplished with BBr_3 (method C, Scheme I, Table I).

Compounds **4–15** were obtained by substitution of metahexestrol. The bromo and iodo derivatives **4** and **5** were synthesized by direct halogenation (method U and V, respectively). In the case of compound **4** mono-, di-, tri-, and tetrasubstituted side products were separated by column chromatography.[25] The 4,4′-disubstituted iodo compound (**5**) was separated from the 4-monosubstituted derivative by column chromatography as well.

The dimethylaminomethyl compound **6** was obtained by reaction of metahexestrol with dimethylamine and formaldehyde (method F, Scheme II, Table I). The separation of the monosubstituted side product was accomplished by repeated fractional crystallization.

Scheme III



The methyl derivative **7** was synthesized in a "one-pot" reaction, converting compound **6** to the quaternary ammonium salt with dimethyl sulfate and reducing the latter compound with $NaCNBH_3$ in hexamethylphosphoramide (method G, Scheme II, Table I).

In order to synthesize the hydroxymethyl derivative **10**, compound **6** was converted to the acetoxy compound **8a** by reaction with Ac_2O (method H, Scheme II, Table I). Experiments of directly converting the acetic acid ester **8a** to compound **10** were not successful. The benzyl methyl ether (**8**) and the benzyl ethyl ether (**9**) were obtained by alkaline saponification of **8a** with NaOH in 80% methanol and ethanol, respectively (method J, Scheme II, Table I). The cleavage of the ethers of **8** and **9** was accomplished with BBr_3 (method C, Scheme II). In both cases, the hydroxymethyl derivative **10** was obtained, which was found to be relatively unstable (see Table I).

The nitro compound **11** was obtained in good yields by reaction of compound **1** with $NaNO_2$ in glacial acetic acid (method K, Scheme II, Table I). Acetic acid as a solvent is described to favor substitution in the ortho position of the OH group.[26] Catalytic hydrogenation of **11** by using palladium on carbon gave compound **12** (method B, Scheme II, Table I). The latter compound was converted with formaldehyde and $NaCNBH_3$ to the dimethylamino derivative **13** (method L, Scheme II, Table I).

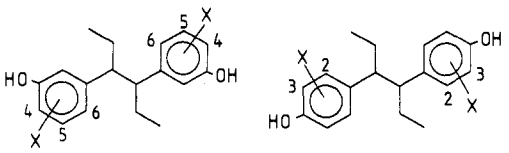
The acetyl compound **14** was obtained by Friedel–Crafts acylation of the dimethyl ether of metahexestrol with acetyl chloride and $AlCl_3$ (method M, Scheme III, , Table I) and subsequent cleavage of the methyl ethers of **14a** with BCl_3 (method C, modified, Scheme III, Table I). BCl_3 is selective for the cleavage of methoxy groups ortho to a carbonyl group.[27]

The synthesis of the ethyl derivative **15** started from compound **14a** (Scheme III). The reduction of the acetyl groups was carried out with KOH and hydrazine hydrate (method N, Scheme III, Table I). The ether cleavage of the methoxy ethyl derivative **15a** to **15** was accomplished with BBr_3 (method C, Scheme III, Table I).

Biological Properties. The biological experiments presented in the following paragraphs are part of our

(22) McMurry, J. E.; Silvestri, M. *J. Org. Chem.* **1975**, *40*, 2687.
(23) In the case of compounds **17** and **20**, this method was used to avoid partial dehalogenation as a consequence of catalytic hydrogenation of the *cis*-stilbenes (route 1).
(24) In the case of compound **21**, catalytic hydrogenation of the *cis*-stilbene according to method B was not possible.
(25) This compound was also synthesized starting from 4′-bromo-3′-methoxypropiophenone according to route 1. However, dehalogenation in the step of catalytical hydrogenation made this procedure inferior to method U regarding overall yield.
(26) Seidenfaden, W.; Pawellek, D. *Methoden Org. Chem.* (*Houben-Weyl*), *4th Ed.* **1971**, *10*(1), 793.
(27) Dean, F. M.; Goodchild, J.; Houghton, L. E.; Martin, J. A.; Morton, R. B.; Parton, B.; Price, A. W.; Somvichien, N. *Tetrahedron Lett.* **1966**, 4153.

Table V. Relative Binding Affinity (RBA) of Metahexestrol, Hexestrol,[a] and Their Disubstituted Derivatives for Calf Uterine Estrogen Receptor



compd	X	RBA value[b]	X	RBA value[b]
1	H	10	H	27
2	4-F	1.1	3-F	16
3	4-Cl	0.07	3-Cl	1.6
4	4-Br	0.03	3-Br	0.25
5	4-I	<0.01	3-I	0.17
6	$4\text{-}CH_2N(CH_3)_2$	<0.01	$3\text{-}CH_2N(CH_3)_2$	0.04
7	$4\text{-}CH_3$	0.73	$3\text{-}CH_3$	8.1
8	$4\text{-}CH_2OCH_3$	0.03	$3\text{-}CH_2OCH_3$	0.55
9	$4\text{-}CH_2OC_2H_5$	<0.01	$3\text{-}CH_2OC_2H_5$	0.18
10	$4\text{-}CH_2OH$	0.03	$3\text{-}CH_2OH$	0.71
11	$4\text{-}NO_2$	0.02	$3\text{-}NO_2$	0.14
12	$4\text{-}NH_2$	0.52	$3\text{-}NH_2$	5.6
13	$4\text{-}N(CH_3)_2$	<0.01	$3\text{-}N(CH_3)_2$	<0.01
14	$4\text{-}COCH_3$	<0.01	$3\text{-}COCH_3$	<0.01
15	$4\text{-}C_2H_5$	0.04	$3\text{-}C_2H_5$	1.2
16	5-OH	0.01		
17	5-Cl	0.03		
18	6-OH	<0.01	2-OH	32
19	6-F	7	2-F	6.5
20	6-Cl	4.2	2-Cl	2.2
21	$6\text{-}CH_3$	15	$2\text{-}CH_3$	8.5

[a] The data of hexestrol and its disubstituted derivatives (taken from ref 12) are given for comparative purposes. [b] 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.

standard screening procedure for the development of mammary tumor inhibiting antiestrogens.[3b]

The affinities of compounds 1–21 for the E_2R were determined by a competitive binding assay with 17β-[^{3}H]-estradiol and by the Dextran-coated charcoal method.[15] The test compounds show relative binding affinity (RBA) values between 15 and <0.01% that of estradiol (Table V).

All 4,4′-disubstituted derivatives (2–15) show a decreased binding affinity compared to 1 (Table V).[28] A decrease caused by substituents ortho to the OH group has already been observed in the class of 3,3′-disubstituted hexestrols (Table V, ref 12). This diminution of binding affinity in both series seems to be primarily caused by an increase of the size of the substituents.

When the substituents are arranged according to an increasing ability to decrease the E_2R binding affinity, almost identical sequences are obtained [metahexestrol: $H < F < CH_3 < NH_2 < Cl < C_2H_5 < CH_2OH$, CH_2OCH_3, $Br < NO_2 < CH_2OC_2H_5$, I, $CH_2N(CH_3)_2$, $COCH_3$, $N(CH_3)_2$; hexestrol: $H < F < CH_3 < NH_2 < Cl < C_2H_5 < CH_2OH < CH_2OCH_3 < Br < CH_2OC_2H_5 < I < NO_2 < CH_2N(CH_3)_2 < COCH_3$, $N(CH_3)_2$]. However, this decreasing effect of the E_2R binding affinity is stronger in the metahexestrol class than in the hexestrol series. While the unsubstituted parent compounds differ in their RBA values only by a factor of 2.7, the 4,4′-disubstituted derivatives of 1 exhibit a stronger decrease of the E_2R binding affinity compared to the correspondingly 3,3′-disubstituted hexestrols. However, not only steric effects seem to influence the E_2R interaction. Though the $COCH_3$ and C_2H_5 groups have about the same volume, the binding affinities of the acetyl compounds are much smaller compared to those of the ethyl compounds. This might be due to an intramolecular hydrogen bond, thus reducing the hydrogen-bond interaction with the receptor area.

The displacement of the substituents from the 4,4′-positions into the 5,5′-positions leads to a significant decrease of the E_2R binding affinity. This is demonstrated with the Cl and OH derivatives (3, 16, and 17; see ref 28). However, the binding affinity of 1 is even more reduced by OH substituents in the 6,6′-positions (18). This is a surprise, since the 2,2′-dihydroxyhexestrol in the hexestrol series had shown a RBA value exceeding that of the parent compound (Table V).[29] Compounds with substituents that increase the lipophilicity in the center of the molecule [F (19), Cl (20), and CH_3 (21)] exhibit a much stronger binding affinity compared to the correspondingly 4,4′-disubstituted analogues (2, 3, and 7). In the case of compound 21, the receptor affinity is even significantly increased compared to the unsubstituted compound.

The most active inhibitors of the E_2R interaction (RBA > 0.5), compounds 1, 2, 7, 12, and 19–21, were tested for their uterotrophic and antiuterotrophic activity in the immature mouse as a measure of their estrogenicity and antiestrogenicity.

The ortho to the OH group F or NH_2 disubstituted compounds 2 and 12 show, like metahexestrol, a moderate uterotrophic activity in small doses, and in high doses, a stronger uterine growth stimulating effect without reaching the maximum stimulation of estrone is shown (Table VI) Disubstitution of 1 in the 6,6′-positions with F or CH_3 (19 and 21) increases the estrogenicity slightly. The 4,4′-disubstituted CH_3 compound (7) and the 6,6′-disubstituted Cl compound (20), however, exhibit strongly enhanced estrogenic activity compared to 1.

Regardless of their differing estrogenic properties, the test compounds were evaluated for their antiuterotrophic activity by determining their inhibiting effects on the uterine growth stimulated by estrone. The fluorine derivative 2 shows an antiuterotrophic activity comparable to 1, reaching a 60% inhibition (Table VII). In small doses, compound 12 slightly reduces the estrone-stimulated uterine growth. Inhibitory effects disappear completely by increasing the dose. Compound 19 exhibits no antiuterotrophic activity, probably due to its increased estrogenicity. Compound 21, however, shows an inhibitory effect, which is diminished by increasing the dose. In accordance with their strong estrogenic activity, compound 20 shows no antiuterotrophic effect, and compound 7 inhibits the estrone-stimulated uterine growth only at the low dose of 1 μg per animal per day.

Discussion

The differences in the RBA values of the newly synthesized metahexestrol derivatives are caused by the steric and hydrophobic properties of the substituents. In the case of the 4,4′-disubstituted compounds, intramolecular hydrogen bonds are also of importance for the receptor affinity.

While the metahexestrol derivatives bearing the substituents in the ortho positions to the OH groups show a graduation of receptor affinity very similar to the hexestrol derivatives, it is striking that substituents standing in the

(28) An exception to that rule is the OH derivative of 1 [*meso*-3,4-bis(3,4-dihydroxyphenyl)hexane; RBA = 20]. Since this compound is a 3,3′-dihydroxylated derivative of hexestrol as well, its synthesis and biological data have been described in the hexestrol publication (ref 12).

(29) Further investigations to elucidate this phenomenon are presently being planned.

Table VI. Estrogenic Activity of Compounds 1, 2, 7, 12, and 19-21 in the Mouse Uterine Weight Test

	uterotrophic test	
compd	dose,[a] μg	effect,[b] means ± SD
1[c]	0	10.7 ± 1.8
	0.8	11.8 ± 2.1
	8	14.4 ± 1.1
	24	18.5 ± 3.5
	80	30.5 ± 4.0
	800	30.8 ± 4.9
estrone	0.4	39.5 ± 4.1
2[c]	0	8.7 ± 3.1
	2	8.9 ± 1.8
	8	18.5 ± 2.9
	16	25.4 ± 2.8
	80	31.8 ± 3.5
	240	32.8 ± 3.5
	800	32.6 ± 5.5
estrone	0.4	47.1 ± 2.7
7	0	13.9 ± 2.1
	0.1	14.3 ± 1.5
	1	21.7 ± 2.0
	10	45.0 ± 3.0
	100	46.2 ± 2.6
	1000	43.5 ± 5.2
estrone	0.4	48.9 ± 4.1
12	0	13.9 ± 2.1
	10	19.9 ± 3.7
	100	19.5 ± 2.9
	1000	36.6 ± 2.0
estrone	0.4	48.9 ± 4.1
19	0	13.1 ± 1.3
	1	15.5 ± 2.5
	10	23.2 ± 3.0
	100	39.0 ± 4.6
	1000	34.6 ± 3.1
estrone	0.4	47.4 ± 4.7
20	0	13.7 ± 2.2
	0.1	16.7 ± 1.8
	1	25.1 ± 2.6
	10	47.8 ± 3.3
	100	41.6 ± 3.9
	1000	38.5 ± 3.6
estrone	0.4	47.5 ± 3.9
21	0	13.7 ± 2.2
	0.5	13.3 ± 2.6
	5	19.8 ± 2.6
	10	37.5 ± 2.0
	50	41.9 ± 2.8
	100	52.1 ± 1.8
	1000	50.5 ± 2.6
estrone	0.4	47.5 ± 3.9

[a] Dose per animal per day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Data taken from ref 2.

Table VII. Antiestrogenic Activity of Compounds 1, 2, 7, 12, and 19-21 in the Mouse Uterine Weight Test

		antiuterotrophic test	
compd	dose,[a] μg	effect,[b] means ± SD	% inhibn[c,d]
1[g]	0	12.5 ± 2.6	
	2.5	29.5 ± 3.0	27[e]
	5	23.5 ± 2.8	53[e]
estrone	0.1	35.7 ± 3.1	
	0	10.0 ± 0.9	
	25	28.1 ± 0.8	48[e]
	50	28.3 ± 1.5	47[e]
	500	30.9 ± 3.2	40[e]
estrone	0.1	44.8 ± 6.8	
2[g]	0	13.5 ± 1.8	
	1	34.2 ± 3.0	7
	5	30.2 ± 3.1	25[e]
	25	28.6 ± 3.1	32[e]
	50	24.7 ± 3.1	50[e]
	100	22.4 ± 2.2	60[e]
	250	24.4 ± 3.9	51[e]
estrone	0.1	35.7 ± 3.4	
7	0	14.8 ± 2.4	
	1	34.3 ± 2.7	24[f]
	10	39.6 ± 3.5	3
	100	41.8 ± 3.2	
estrone	0.1	40.3 ± 2.9	
12	0	14.8 ± 2.4	
	1	33.0 ± 2.7	29[e]
	10	41.7 ± 3.1	
	100	42.3 ± 3.4	
estrone	0.1	40.3 ± 2.9	
19	0	13.7 ± 2.2	
	1	37.8 ± 4.2	
	10	38.9 ± 5.7	
	100	41.2 ± 3.5	
estrone	0.1	35.8 ± 3.1	
20	0	13.7 ± 2.2	
	1	40.6 ± 3.7	
	10	41.0 ± 3.1	
	100	41.5 ± 3.8	
estrone	0.1	35.8 ± 3.1	
21	0	13.7 ± 2.2	
	1	27.2 ± 3.6	39[e]
	10	25.4 ± 3.0	47[e]
	100	31.0 ± 2.8	22[f]
estrone	0.1	35.8 ± 3.1	

[a] Dose per animal and day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Percent inhibition = $100 - (E_{S,T} - E_V)/(E_S - E_V) \times 100$; E_S = effect of estrone standard; $E_{S,T}$ = effect of standard under simultaneous application of test substance; E_V = effect of vehicle. [d] The U test according to Wilcoxon, Mann, and Whitney was used. [e] Significant ($\alpha = 0.01$). [f] Significant ($\alpha = 0.05$). [g] Data taken from ref 2.

ortho positions to the ethane bridge (substituents standing in the 6,6′-positions in the metahexestrol series and those standing in the 2,2′-positions in the hexestrol series) do not change receptor affinities correspondingly in the two series. The OH groups lead to an increase of the binding affinity in the hexestrol class, whereas the same substituents show a strong decrease in the metahexestrol series. Reverse effects are obtained with the CH_3 groups in both classes. Our explanation for this phenomenon is based on the ability of diphenylethanes to form conformation isomers due to the sp^3-hybridized C–C bond between the benzylic carbons. Interaction with the binding site of the receptor leads to a fixation of a conformation that is specific for the receptor. The displacement of the OH groups of hexestrol into the meta positions to form metahexestrol may be accompanied by an altered binding conformation.[30] This is why the same ring substituents in the corresponding ortho positions to the ethane bridge produce derivatives with such significantly different changes in the binding affinities of the parent compounds.

While the 4,4′- and 5,5′-positions are unsuitable for an enhancement of the receptor affinity of metahexestrol, it

(30) This hypothesis is supported by the finding that the displacement of the phenolic OH groups of hexestrol and diethylstilbestrol (DES) leads to a smaller decrease in the hexestrol series. (K_D [M]: hexestrol, 1.6×10^{-9}; 1, 5.0×10^{-9}; orthohexestrol, 6.6×10^{-7}; DES, 1.2×10^{-9}; meta-DES, 6.4×10^{-9}; ortho-DES, 2.7×10^{-6}). Kranzfelder, G. Ph.D. Thesis, University of Munich, 1977.

is possible to increase the binding affinity by symmetrical disubstitution in the 6,6'-positions. One of four synthesized compounds showed a significantly higher RBA value than compound 1 (CH_3; 21).

In the case of this derivative, the increased binding affinity leads to an increase of the estrogenicity as well. This is no real surprise, since from the discovery that the replacement of the 1,2-diethyl groups by isopropyl groups in metahexestrol destroys the antiestrogenic activity and generates a true estrogen,[31] one could have expected that the introduction of CH_3 substituents in the 6,6'-positions of metahexestrol would mimic isopropyl groups at the benzylic C atoms.

The opposite observation has been made as well: An increase in estrogenicity following a reduced binding affinity. This effect is very striking with **7** and **20**. Halogen substitution seems to increase the estrogenicity in general: compound **2** shows similar estrogenic effects compared to metahexestrol in spite of a markedly reduced binding affinity, **19** exhibits a slightly increased estrogenicity in spite of a moderately reduced RBA value, and compound **20** strongly enhances the uterine weight in spite of a further reduced binding affinity. This "halogen effect" has also been observed in the butestrol- and metabutestrol class,[32] as well as with other antiestrogens (to be published), and cannot be explained at the present time.

While **1** and **2** show strong antiuterotrophic effects over a wide dose range, the other tested compounds exhibit no potency (**19** and **20**) or an antagonistic potency only in small doses. This effect is probably due to their increased estrogenicity. It is remarkable that in most cases inhibitory effects are obtained in doses in which these compounds have only weak uterotrophic activity. The uterotrophic and antiuterotrophic effects of the N-isosteric catechol compound **12** are very similar to those described for the correspondingly substituted hexestrol derivative.[12]

Generally, it must be noticed that it was not possible to reduce the estrogenic activity of **1** by disubstitution. On the contrary, the uterotrophic activities of the different compounds in relation to their RBA values show a more or less pronounced stimulation of the estrogenicity of metahexestrol. Thus, in some cases, the type of action of the parent compound changed from a partial antiestrogen to a true estrogen (**19** and **20**). This means that in contrast to the true estrogen hexestrol,[12,33] the partial antiestrogen metahexestrol responds very sensitively to the introduction of additional ring substituents. These variations may lead to a decrease or even a loss of the antiestrogenic potency of the parent compound.

Further experiments evaluating the antitumor activity of the most active E_2R interaction inhibiting compounds and elucidating their mode of action (especially addressing the question of whether it is the estrogenic or the antiestrogenic activity that is responsible for the antitumor effects) are presently being carried out and will be published elsewhere.

Experimental Section

General Procedures. TLC of each compound was performed on Merck F 254 silica gel plates. Melting points were determined on a 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 360 A, 60 MHz). HPLC was accomplished with an Altex 110A pump and a Kontron Uvikon 720 LC spectrometer. A LiChrosorb Si 60, 5-μm (Merck) column and a RP 18, 10-μm (Altex) column were used [solvent systems CH_2Cl_2–hexane (1:1) and MeOH–H_2O (3:1), respectively].

Method A. *cis*-3,4-Bis(4-fluoro-3-methoxyphenyl)hex-3-ene (2b). Zinc powder (1.96 g, 0.03 mol) was added slowly under N_2 into a mixture of 4'-fluoro-3'-methoxypropiophenone (**2c**; 1.82 g, 0.01 mol) and $TiCl_4$ (2.85 g, 0.015 mol) in 20 mL of dry dioxane at 15 °C. The color of the solution changed from yellow to purple and then turned dark brown when heated to reflux. After 4 h at 101 °C, the reaction mixture was cooled, and alkaline hydrolysis was performed with 10% K_2CO_3 solution. After extraction with ether and removal of the ether, the crude product was purified by chromatography [silica gel column eluted with CH_2Cl_2–hexane (1:1)] and recrystallized from EtOH/H_2O to give 1.25 g (75%) of **2b**.

Method B. *meso*-3,4-Bis(4-fluoro-3-methoxyphenyl)hexane (2a). Palladium on charcoal (10%, 0.1 g) was added to a solution of **2b** (3.32 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/H_2O to give 3.21 g of **2a**.

Method C. *meso*-3,4-Bis(4-fluoro-3-hydroxyphenyl)hexane (2). A solution of **2a** (3.34 g, 0.01 mol) in 250 mL of dry CH_2Cl_2 was cooled to −60 °C. BBr_3 (7.52 g, 0.03 mol) was added under nitrogen with stirring. In the case of compound **14**, BCl_3 (3.52 g, 0.03 mol) was used. After 0.5 h, the freezing mixture was removed, and the reaction mixture was kept at room temperature for 4 h. Fifty milliliters of MeOH was added, and the mixture was shaken with 2 N NaOH. After neutralization of the aqueous layer with 3 N H_2SO_4, the solution was extracted with ether. After removal of the ether, the crude product was recrystallized from EtOH/H_2O to give 2.67 g of **2**.

Method D. 1-(5-Chloro-3-methoxyphenyl)-1-propanol (17b). A solution of 5'-chloro-3'-methoxypropiophenone (**17c**; 1.99 g, 0.01 mol) in 10 mL of ether was added dropwise to a stirred suspension of $LiAlH_4$ (0.10 g, 2.8 mmol) in 20 mL of ether. After stirring for 3 h at room temperature, the mixture was heated to reflux for 1 h. The mixture was cooled and decomposed by the dropwise addition of ice–water, followed by 10% H_2SO_4 to give two clear phases. The ethereal layer was separated, washed (H_2O), and dried ($MgSO_4$). The solvent was removed, and 1.8 g of **17b** was obtained.

Method E. *meso*-3,4-Bis(5-chloro-3-methoxyphenyl)hexane (17a). $TiCl_3$ (4.60 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 **17b** (2.01 g, 0.01 mol) was dissolved in 10 mL of dry glyme and added dropwise with stirring. The mixture was heated to reflux and kept there for 16 h. After cooling, the reaction mixture was quenched by the addition of 2 N HCl, diluted with water, and extracted with ether. The ether extract was washed ($NaHCO_3$ and H_2O) and dried ($MgSO_4$). The solvent was removed, and the resulting crude product was fractionally crystallized from EtOH/H_2O to give 0.46 g of **17a**.

Method F. *meso*-3,4-Bis[4-[(dimethylamino)methyl]-3-hydroxyphenyl]hexane (6). Metahexestrol (2.70 g, 0.01 mol) was dissolved in 100 mL of EtOH. Solutions of dimethylamine (2.25 mL of a 40% aqueous solution, 0.02 mol) and formaldehyde (1.60 mL of a 37% aqueous solution, 0.02 mol) were added dropwise with stirring. The reaction mixture was then refluxed for 8 h. After the solvent had been removed, the crude product was fractionally crystallized from EtOH/H_2O. The yield of **6** was 2.27 g.

Method G. *meso*-3,4-Bis(3-hydroxy-4-methylphenyl)hexane (7). A solution of Me_2SO_4 (25.2 g, 0.2 mol) in 50 mL of ether was added dropwise at room temperature to a solution of **6** (3.85 g, 0.01 mol) in 800 mL of ether. After the solution was stirred for 1 h, the ether was removed. A solution of $NaCNBH_3$ (2.50 g, 0.04 mol) in 200 mL of hexamethylphosphoramide was added to the quaternary ammonium salt. The reaction mixture was kept

(31) Schmitt-Wallenborn, H. Ph.D. Thesis, University of Munich, 1978. The RBA value of 3,4-bis(3-hydroxyphenyl)-2,5-dimethylhexane is 14.

(32) Hartmann, R. W.; Heindl, A.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.*, in press.

(33) With the exceptions of the catechol compound and the N-isosteric catechol derivative, all other tested compounds (RBA > 5) were true estrogens.

at 120 °C for 16 h. After the reaction mixture was cooled and after the addition of H_2O, the reaction mixture was extracted with ether. The ether was removed, and the crude product was recrystallized from EtOH/H_2O to yield 2.30 g of **7**.

Method H. *meso*-3,4-Bis[3-acetoxy-4-(acetoxymethyl)phenyl]hexane (8a). A solution of **6** (3.85 g, 0.01 mol) in 30 mL of Ac_2O was heated to reflux for 1.5 h. The solvent was removed, and the residual oil was dissolved in $CHCl_3$. This solution was washed ($NaHCO_3$ and H_2O) and dried ($MgSO_4$). The solvent was removed, and the crude product was recrystallized from EtOH to give 3.99 g of **8a**.

Method J. *meso*-3,4-Bis[3-hydroxy-4-(methoxymethyl)phenyl]hexane (8). A solution of **8a** (4.99 g, 0.01 mol) and NaOH (8.00 g, 0.2 mol) in 200 mL of MeOH (80%) was heated to reflux for 4 h. After cooling, the solution was neutralized with glacial acetic acid and extracted with ether. The ether was removed, and the crude product was recrystallized from EtOH/H_2O to yield 2.90 g of **8**.

Method K. *meso*-3,4-Bis(3-hydroxy-4-nitrophenyl)hexane (11). Metahexestrol (2.70 g, 0.01 mol) was dissolved in 200 mL of hot acetic acid. After the solution was cooled, $NaNO_2$ (6.20 g, 0.09 mol) was added in small portions to the stirred solution. After 5 h of stirring at room temperature, the reaction mixture was poured into 400 mL of H_2O. The resulting precipitate was separated and recrystallized from acetone to yield 2.74 g of **11**.

Method L. *meso*-3,4-Bis[4-(dimethylamino)-3-hydroxyphenyl]hexane (13). To a stirred solution of compound **12** (3.00 g, 0.01 mol) and 20 mL of aqueous 37% formaldehyde (0.25 mol) in 150 mL of acetonitrile was added $NaCNBH_3$ (5.00 g, 0.08 mol) in small portions. The reaction mixture was stirred for 15 min, and then glacial acetic acid was added dropwise until the solution tested neutral. Stirring was continued for an additional 45 min, glacial acetic acid being added to maintain the pH near neutrality. The solvent was removed, and H_2O was added to the residue. The resulting mixture was extracted with ether. The solvent was removed, and the crude product was recrystallized from EtOH to give 2.61 g of **13**.

Method M. *meso*-3,4-Bis(4-acetyl-3-methoxyphenyl)hexane (14a). A solution of metahexestrol dimethyl ether (2.98 g, 0.01 mol) and acetyl chloride (3.92 g, 0.05 mol) in 40 mL of nitrobenzene was cooled in an ice bath. Finely powdered $AlCl_3$ (6.66 g, 0.05 mol) was added in small portions with stirring. After 4 h of stirring at room temperature, the reaction mixture was poured onto ice and acidified with HCl. The nitrobenzene was removed by steam distillation. The crude product was extracted with $CHCl_3$. After the product was washed with 1 N NaOH and H_2O, the solvent was removed, and the remaining solid was recrystallized from acetone to give 2.67 g of **14a**.

Method N. *meso*-3,4-Bis(4-ethyl-3-methoxyphenyl)hexane (15a). A mixture of compound **14a** (3.83 g, 0.01 mol), KOH (9.00 g, 0.16 mol), 9 mL of 85% hydrazine hydrate, and 100 mL of diethyleneglycol was heated under reflux for 1.5 h. After removal of the water formed, the mixture was heated at 195 °C for an additional 4 h. The solution was diluted with cold H_2O, poured into HCl, and extracted with ether. The solvent was removed, and the crude product was recrystallized from MeOH/benzene to yield 3.20 g of **15a**.

Method O. 4′-Fluoro-3′-methoxypropiophenone (2c). 4-Fluoro-3-methoxybenzoic acid (1.70 g, 0.01 mol) dissolved in 10 mL of thionyl chloride was heated to reflux for 4 h. The thionyl chloride was removed, and the crude product was distilled under high vacuum to give 1.73 g (92%) of 4-fluoro-3-methoxybenzoyl chloride. Anhydrous $CdCl_2$ (1.23 g, 6.7 mmol) was added under nitrogen to a solution of EtMgBr (12.5 mmol) in 10 mL of ether at −5 °C. The mixture was heated to reflux for 1 h. The ether was distilled, and 10 mL of dry benzene was added to the mixture. 4-Fluoro-3-methoxybenzoyl chloride (1.89 g, 0.01 mol) dissolved in 5 mL of dry benzene was added dropwise. After heating to reflux for 1 h, the reaction mixture was cooled and then decomposed by the addition of ice–water, followed by NH_4Cl solution to give two clear phases. The benzene layer was separated, washed, and dried. After removal of the benzene, the resulting crude product was recrystallized from EtOH/H_2O to give 1.24 g (68%) of **2c**.

Method P. 5′-Chloro-3′-methoxypropiophenone (17c). EtMgBr (0.01 mol) in 100 mL of dry ether was added dropwise under N_2 to a solution of 5-chloro-3-methoxybenzoyl chloride (1.64 g, 0.008 mol) in 100 mL of THF at −78 °C. The reaction mixture was brought to room temperature over about 1 h and then decomposed by the addition of ice–water, followed by NH_4Cl solution. The organic layer was separated, washed (H_2O), and dried ($MgSO_4$). The solvent was removed, and the crude product was purified by column chromatography (SiO_2; eluent: CH_2Cl_2) to give 1.53 g of **17c**.

Method Q. 6-Fluoro-3-methoxytoluene. A solution of $NaNO_2$ (6.90 g, 0.1 mol) in 15 mL of H_2O was added dropwise at 5–10 °C to a stirred solution of 6-amino-3-methoxytoluene (13.70 g, 0.1 mol) in 80 mL of 21% HBF_4. After the solution was cooled to 0 °C, the precipitate was filtered off and washed with 15 mL of 5% HBF_4, 20 mL of MeOH, and ether. The yield of the dried salt was 21.80 g (92.5%). Decomposition was accomplished by the addition of a mixture of the salt (0.1 mol) and NaF (4.00 g) in 60 mL of 1,2,4-trichlorobenzene to 25 mL of trichlorobenzene containing 1.0 g of NaF at 150 °C. After the mixture was cooled to 40 °C, the precipitate was filtered off, and the filtrate was washed with 15% NaOH, dried, and fractionally distilled (bp 23 °C) to yield 9.17 g (65.5%) of a colorless oil.

Method R. 6-Fluoro-3-methoxybenzoic Acid. $KMnO_4$ (5.21 g, 0.033 mol) was added in small portions to a vigorously stirred mixture of 6-fluoro-3-methoxytoluene (1.40 g, 0.01 mol), 5 mL of pyridine, and 15 mL of H_2O at 50 °C. After stirring for 2 h at the same temperature, the mixture was allowed to stand overnight and was then filtered. MnO_2 was suspended in 25 mL diluted H_2SO_4 and again filtered off. The combined filtrates were distilled under reduced pressure until a total of 25 mL remained. After neutralization with K_2CO_3, unchanged 6-fluoro-3-methoxytoluene was extracted with ether. On acidification, precipitated 6-fluoro-3-methoxybenzoic acid was extracted with ether and dried ($MgSO_4$). The solvent was removed, and the crude product was recrystallized from 1,2-dichloroethane to give 0.87 g (51%) of colorless crystals (mp 142–143 °C).

Method S. 5-Chloro-3-methoxybenzoic Acid. 5-Chloro-3-methoxyphenylmagnesium chloride was prepared from 3,5-dichloroanisole (1.77 g, 0.01 mol) and Mg turnings (0.24 g, 0.01 mol) in 5 mL of dry THF. This solution was added dropwise to a slurry of dry ice in ether, while dry CO_2 was bubbling through the reaction mixture. After having stood overnight, the reaction mixture was hydrolyzed by the dropwise addition of ice–water, followed by diluted HCl. After extraction with ether, the organic layer was treated with $KHCO_3$ solution. On acidification, the aqueous layer was again extracted with ether. After the solvent was removed, the crude product was recrystallized from $CHCl_3$ to yield 1.08 g (58.2%) of white–yellow crystals (mp 170–171 °C) of 5-chloro-3-methoxybenzoic acid.

Method T. 3-Hydroxy-6-methylbenzoic Acid. A mixture of *o*-toluic acid (13.60 g, 0.1 mol) and 68 g of concentrated H_2SO_4 was heated at 160 °C for 2.5 h. After the addition of 3.5 mL of H_2O, it was allowed to stand overnight and filtered. The crystals were dissolved in 50 mL of H_2O and poured into 135 mL of a saturated NaCl solution at 100 °C. On addition of 3.4 g of NaCl, this recrystallization procedure was repeated to yield 21.1 g (81%) of colorless needles of sodium 6-methyl-3-sulfobenzoate. The latter compound (26.0 g, 0.1 mol) was dissolved in 10 mL of a concentrated NaOH solution and heated on a steam bath. It was mixed with 5 g of powdered NaOH into a paste, which solidified when allowed to cool. Small pieces of this product were added in portions to fused KOH (26.0 g), the internal temperature being maintained at 210–220 °C during the addition and for an additional 2.5 h. After cooling, the mass was dissolved in 140 mL of H_2O, filtered, and acidified with HCl. The precipitate was recrystallized from H_2O, yielding 14.2 g (93.4%) colorless crystals (mp 183–184 °C) of 3-hydroxy-6-methylbenzoic acid.

Method U. *meso*-3,4-Bis(4-bromo-3-hydroxyphenyl)hexane (4). A solution of anhydrous potassium acetate (5.00 g, 0.05 mol) in 75 mL of glacial acetic acid was added to a solution of metahexestrol (2.70 g, 0.01 mol) in 25 mL of THF. The mixture was cooled in an ice bath, and 25 mL of a freshly prepared solution of bromine (4.00 g, 0.025 mol) in acetic acid was added dropwise. After 5 min, the products were isolated by partitioning between EtOAc and H_2O. After the solution was washed with H_2O, the EtOAc layer was removed, and the mixture of mono-, di-, tri-, and tetrabromo compounds was separated by chromatography

(silica gel column eluted with CH_2Cl_2) to yield 1.93 g of 4.

Method V. ***meso*-3,4-Bis(3-hydroxy-4-iodophenyl)hexane (5).** A solution of iodine (5.08 g, 0.02 mol) in 25 mL of THF was added dropwise to a solution of metahexestrol (2.70 g, 0.01 mol) in a mixture of 75 mL of MeOH and 25 mL of concentrated NH_4OH with stirring. After 0.5 h, glacial acetic acid was added to neutralize NH_3. Water was then added, and reaction products were extracted with EtOAc. After removal of the solvent, **5** (2.55 g) was separated by silica gel chromatography with $CHCl_3$ as eluent.

Biological Methods. Estradiol Receptor Binding Assay. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^{3}H]estradiol. A previously described procedure[15] was used with modifications. Test compounds were incubated with cytosol from calf uteri and [^{3}H]estradiol at 4 °C for 16 h. Incubation was stopped by adding Dextran-coated charcoal. After centrifugation, the radioactivity of a 100-μL supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabeled test compounds. Six concentrations of the competitors were tested. They were chosen to provide a linear portion on a semilog plot crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled estradiol and of test compounds reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activities were determined by stimulation of the uterine growth and by inhibition of the uterine growth stimulated by estrone, respectively, by using immature NMRI mice as described previously.[15] Twenty-day-old female mice (weight 14.5 ± 1.2 g, mean plus or minus SD) were randomly distributed into groups of 10 animals. They were subcutaneously injected daily for 3 days with 0.1 mL of olive oil solutions containing the test compound. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, washed, dried, and weighed.

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Registry No. 1, 71953-72-5; 1 dimethyl ester, 71953-69-0; **1a**, 71953-69-0; **1b**, 76473-05-7; **1c**, 37951-49-8; **2**, 89106-12-7; **2a**, 89106-13-8; **2b**, 89106-14-9; **2c**, 82846-20-6; **3**, 89106-15-0; **3a**, 89106-16-1; **3b**, 89106-17-2; **3c**, 89106-18-3; 4, 89106-19-4; **5**, 89106-20-7; **6**, 89106-21-8; 7, 89106-22-9; **8**, 89106-23-0; **8a**, 89106-24-1; **9**, 89106-25-2; **10**, 89106-26-3; 11, 89106-27-4; **12**, 89106-28-5; **13**, 89106-29-6; **14**, 89106-30-9; **14a**, 89106-31-0; **15**, 89106-32-1; **15a**, 89106-33-2; **16**, 89106-34-3; **16a**, 89106-35-4; **16b**, 89106-36-5; **16c**, 41497-31-8; **17**, 89106-37-6; **17a**, 89106-38-7; **17b**, 89121-16-4; **17c**, 89106-39-8; **18**, 89106-40-1; **18a**, 89106-41-2; **18b**, 89106-42-3; **19**, 89106-43-4; **19a**, 89106-44-5; **19b**, 89106-45-6; **19c**, 89106-46-7; **20**, 89106-47-8; **20a**, 89106-48-9; **20b**, 89106-49-0; 21, 89106-50-3; **21a**, 89106-51-4; **21b**, 89106-52-5; **21c**, 29578-81-2; 4-fluoro-3′-methoxybenzoic acid, 82846-18-2; 4-fluoro-3-methoxybenzoyl chloride, 82846-19-3; 5-chloro-3-methoxybenzoyl chloride, 89106-53-6; 6-fluoro-3-methoxytoluene, 2338-54-7; 2-methyl-*p*-anisidine, 102-50-1; 6-fluoro-3-methoxybenzoic acid, 367-83-9; 5-chloro-3-methoxybenzoic acid, 82477-67-6; 3,5-dichloroanisole, 33719-74-3; 5-hydroxy-2-methylbenzoic acid, 578-39-2; *o*-toluic acid, 118-90-1; 6-methyl-3-sulfobenzoic acid, 89106-54-7; sodium 6-methyl-3-sulfobenzoate, 52238-48-9.

Supplementary Material Available: ^{1}H NMR data (Tables VIII and IX) of the disubstituted metahexestrol derivatives (**1a**–**21a** and 1–21) and the disubstituted *cis*-3,4-bis(3-methoxyphenyl)hex-3-enes (**1b**–**3b**, **16b**, and **19b**) (7 pages). Ordering information is given on any current masthead page.

Ibotenic Acid Analogues. Synthesis and Biological and in Vitro Activity of Conformationally Restricted Agonists at Central Excitatory Amino Acid Receptors

Povl Krogsgaard-Larsen,*[†] Elsebet Ø. Nielsen,[†] and David R. Curtis[‡]

Department of Chemistry, Royal Danish School of Pharmacy, DK-2100 Copenhagen Ø, Denmark, and Department of Pharmacology, The Australian National University, Canberra City, A.C.T. 2601, Australia. *Received October 3, 1983*

A number of analogues of ibotenic acid [(*RS*)-3-hydroxy-5-isoxazoleglycine] were synthesized; they were tested as excitants on neurons in the cat spinal cord, by using microelectrophoretic techniques, and as inhibitors of the binding of kainic acid (KA) in vitro, by using synaptic membranes prepared from rat brains. The excitatory effects of the 3-isoxazolol amino acids (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (**4**, 7-HPCA), (*RS*)-α-amino-3-hydroxy-5,6-dihydro-4*H*-cyclohept[1,2-*d*]isoxazole-8-propionic acid (**8**, 8-AHCP), (*RS*)-α-amino-3-hydroxy-7,8-dihydro-6*H*-cyclohept[1,2-*d*]isoxazole-4-propionic acid (**12**, 4-AHCP), and (*RS*)-α-(methylamino)-3-hydroxy-5-methyl-4-isoxazolepropionic acid (**15**, *N*-Me-AMPA) were shown to be sensitive to (*S*)-glutamic acid diethyl ester (GDEE), an antagonist at quisqualic acid (QUIS) receptors, and insensitive to (*RS*)-2-amino-5-phosphonovaleric acid (2APV), an antagonist at *N*-methyl-(*R*)-aspartic acid (NMDA) receptors. The compounds **4** and **12** proved to be particularly potent agonists at the former class of receptor, assumed to represent physiological glutamic acid receptors. The amino acids (*RS*)-β-(2-carboxyphenyl)alanine (**19**), an analogue of **12**, and (*RS*)-2-(3-carboxyphenyl)glycine were weak GDEE-sensitive excitants with potencies comparable with that of **8**. All of the compounds were tested as inhibitors of KA binding. With the exception of **12** and **19**, which showed very low affinity for the KA binding sites, the compounds studied were inactive in this in vitro test system.

A number of naturally occurring amino acids, including quisqualic acid (QUIS), kainic acid (KA), and ibotenic acid, which are heterocyclic analogues of (*S*)-glutamic acid (Glu) (Chart I),[1-4] excite neurons in the mammalian central nervous system (CNS).[5,6] These amino acids also destroy neurons when injected into the brain,[7,8] and both excitation and neurotoxicity are assumed to be mediated by neuronal receptors normally operated by Glu and (*S*)-aspartic acid (Asp), putative central excitatory neurotransmitters.[2,5,6,11] The possible involvement of these amino acid transmitters

[†] Royal Danish School of Pharmacy.
[‡] The Australian National University.

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