in this study. The rat stomach fundus preparation employed was essentially that of Vane,[12] with the previously described modifications.[1,2] Two strips were cut from the same tissue and were used in parallel 8-mL muscle baths. The relative sensitivity of the two strips was determined, after a 1-h equilibration period in Tyrodes solution at 37 °C, by the use of 5-HT oxalate doses giving submaximal contractions. Only one compound was tested per preparation. Dose–response curves were obtained for 5-HT, first in the absence of the agent in question and then in the presence of each of usually four different, increasing concentrations thereof. ED_{50} values for half-maximal contraction were determined, and apparent affinities were calculated as pA_2 values by the method of Arunlakshana and Schild.[13] Linear regression analysis gave not only the pA_2 values but also the slopes of the Schild plots.

Behavioral Pharmacology. The discriminative stimulus assay was performed in a manner identical with that which we have previously reported;[9,10] 5-methoxy-*N*,*N*-dimethyltryptamine hydrogen oxalate (1.5 mg/kg) was used as the training drug. Each dose of challenge drug was evaluated in three to six rats; the number and range of doses tested for each of the compounds are as follows: **5e**, three doses from 0.5 to 1.5 mg/kg; **5f**, six doses from 1.0 to 5.0 mg/kg; **5g**, six doses from 0.25 to 5.0 mg/kg; 2,5-DMDMA, five doses from 1.0 to 8.0 mg/kg. Generalization was considered to be greater than 70% responding on the 5-OMe-DMT lever, while partial generalization refers to 50–69% responding.

Acknowledgment. This work was supported, in part, by U.S. Public Health Service Grant DA-01642. We also express our appreciation to Dr. G. M. Anderson III for his gift of (±)-1-[4-(benzyloxy)-3-methoxyphenyl]-2-aminopropane hydrochloride, to Dr. N. Castagnoli for his gift of **5g**, to Dr. A. Manian, Psychopharmacology Branch/NIMH, for his gifts of compounds **7** and **11**, and to R. Young for his assistance in the behaviorial studies.

(12) Vane, J. R.; *Br. J. Pharmacol.* **1959**, *14*, 87.
(13) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
(14) Funek, A.; Paulson, A.; Gombert, R. *Bull. Soc. Chim. Fr.* **1960**, 1644.
(15) Sommers, A. H.; Weston, A. W. *J. Am. Chem. Soc.* **1951**, *73*, 5749.
(16) Brossi, A.; Teitel, S. *Helv. Chim. Acta* **1970**, *53*, 1779.

Estrogen Receptor Based Imaging Agents. 1. Synthesis and Receptor Binding Affinity of Some Aromatic and D-Ring Halogenated Estrogens

Daniel F. Heiman, Stephen G. Senderoff, John A. Katzenellenbogen,* and Richard J. Neeley

The Roger Adams Laboratory, School of Chemical Sciences, Urbana, Illinois 61801. *Received June 29, 1979*

Steroidal and nonsteroidal estrogens substituted with halogens ortho to the phenolic hydroxyl group and in the D ring at C-16 have been prepared as potential estrogen receptor-based imaging agents for human breast tumors. Estrogens bearing an aromatic fluorine ortho to a phenolic hydroxyl group were prepared by the Schiemann reaction on the corresponding methyl esters; other ortho-halogenated estrogens were prepared by direct halogenation. Steroidal estrogens substituted at the 16α position were prepared by halogenation of estrone 3-acetate (17-enol acetate) followed by hydride reduction, and those substituted at the 16β position were prepared by epimerization prior to reduction. The binding affinity of these halogenated estrogens to the uterine estrogen receptor was measured relative to that of [^{3}H]estradiol by a competitive binding assay. All of the monosubstituted ortho-fluorinated estrogens show very high binding affinity for the receptor (64–250% that of estradiol). The monosubstituted and symmetrically disubstituted bromo- and iodohexestrols and 2- and 4-substituted estradiols have binding affinities considerably lower than those of the fluoro compounds, the 4-substituted estradiols having affinities greater than the corresponding 2-substituted isomers. Introduction of a halogen (Cl, Br, I) at the 16α position of 17β-estradiol results in compounds with receptor affinities comparable to that of 17β-estradiol itself; the 16β-epimers and the estrone derivatives are bound less well. Thus, provided that they can be labeled with suitable γ-emitting radioisotopes at sufficiently high specific activity, it appears that the A-ring fluoroestrogens and 16α-bromo- and 16α-iodoestradiol-17β are excellent candidates for receptor-based imaging of human breast tumors.

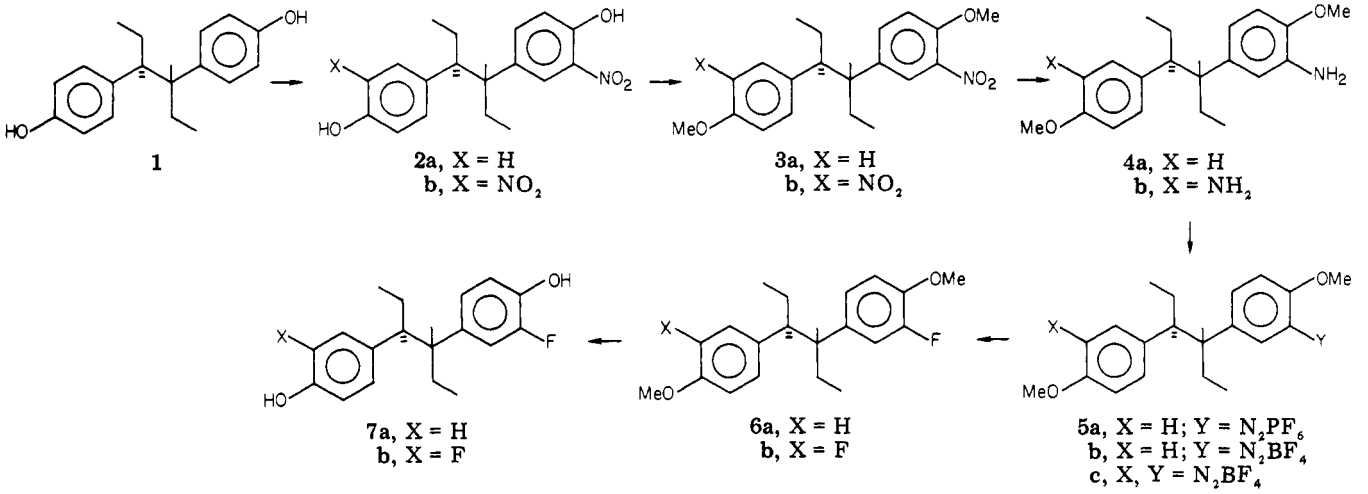
A sizeable fraction of human breast tumors contain significant levels of estrogen receptor,[1] and the measurement of the levels of these receptors by in vitro assays on surgical tumor samples has become an important diagnostic approach to determine the hormone responsiveness of the tumor, information that is essential for the selection of the most appropriate therapy for the breast cancer patient (hormonal therapy vs. chemotherapy or radiation).[2] There has been considerable interest in the development of γ-emitting estrogen analogues that might be concentrated in estrogen receptor-containing breast tumors and thus act as tumor imaging agents (see Discussion). In vivo radiopharmaceuticals of this kind could provide, noninvasively, valuable diagnostic information about both primary and metastatic breast tumors.

We have taken a systematic approach to the development of estrogen receptor based imaging agents, paying particular attention to the binding selectivity of these agents,[3a] that is, the affinity they show for the estrogen receptor relative to their binding affinity for nonreceptor sites. In this paper, we describe the synthesis of several

(1) For reviews, see: McGuire, W. L.; Horwitz, K. B.; Pearson, O. H.; Segaloff, A. *Cancer* **1977**, *39*, 2934. McGuire, W. L.; Horwitz, K. B.; Zava, D. T.; Garola, R. E.; Chamness, G. C. *Metabolism* **1978**, *27*, 487. McGuire, W. L.; Carbone, P. P.; Vollmer, E. P. "Estrogen Receptors in Human Breast Cancer"; Raven Press: New York, 1975.
(2) Allegra, J. C.; Lippman, M. E.; Thompson, E. B.; Simon, R.; Barlock, A.; Green, L.; Huff, K. K.; Do, H. M. T.; Aitken, S. C. *Cancer Res.* **1979**, *39*, 1447. Leclerq, G.; Heuson, J. C. *Curr. Probl. Cancer* **1976**, *1*, 1. Garola, R. E.; McGuire, W. L. *Cancer Res.* **1977**, *37*, 3333. Horwitz, K. B.; McGuire, W. L.; Pearson, O. H.; Segaloff, A. *Science* **1975**, *189*, 726.
(3) (a) Katzenellenbogen, J. A.; Heiman, D. F.; Carlson, K. E.; Lloyd, J. E. In "Receptor Binding Radiotracers" (Unisciences Series "Radiotracers in Biology and Medicine"; L. G. Colombetti, Series Editor); Eckelman, W. C., Ed.; CRC Press, Boca Raton, FL; in press. (b) Goswami, R.; Harsy, S. G.; Heiman, D. F.; Katzenellenbogen, J. A. *J. Med. Chem.* following paper in this issue. (c) Allison, K. J.; Katzenellenbogen, J. A. in preparation. (d) Katzenellenbogen, J. A.; Carlson, K. E.; Heiman, D. F.; Goswami, R. *J. Nucl. Med.* **1980**, *21*, 550. (e) Katzenellenbogen, J. A.; Senderoff, S. G.; Welch, M. J.; McElvaney, K. D. in preparation.

0022-2623/80/1823-0994$01.00/0

Scheme I



estrogens bearing a halogen (that can potentially be labeled with a γ-emitting isotope) at an aromatic position ortho to the phenolic hydroxyl group or at C-16 in the D ring, and we describe the binding affinity of these analogues to the estrogen receptor from uterine tissue. In related papers, we describe the synthesis of other potential estrogen receptor based imaging agents,[3b,c] the preparation of some of them in tritium- and γ-labeled form,[3d,e] studies on their binding selectivity in vitro,[3d] and their target tissue and tumor selective uptake in vivo.[3d,e]

Results

Chemical Synthesis. Synthesis of Estrogens Bearing a Halogen Ortho to the Phenolic Hydroxyl Group. The oldest and most widely used method for the selective introduction of fluorine into an aromatic ring is the Balz–Schiemann reaction.[4a] The extensive literature on this reaction was surveyed to determine which variation seemed most suited for the production of ortho-fluorinated phenols.[4] Although one report claimed that the parent 2-fluorophenol could be prepared from 2-aminophenol in 35% overall yield by diazotization in concentrated tetrafluoroboric acid followed by pyrolysis,[4f] other workers have reported that unprotected phenols gave unsatisfactory results, either because the phenol diazonium salts were too soluble to be recovered in good yields[4a] or because of very low yields in the pyrolysis step.[4b-d] Comparison of these results with those obtained for phenol methyl ethers, and the relative simplicity of the methylation[5] and demethylation[6] reactions, led us to carry out the introduction of fluorine into the aromatic rings of hexestrol protected as the dimethyl ether (Scheme I).

Hexestrol (1) was nitrated with 0.5 molar equiv of copper(II) nitrate/acetic anhydride to give a 57% yield of nitrohexestrol (**2a**), a considerably better result than had been achieved using nitric acid in acetic acid (30% yield).[7] A small amount of dinitrohexestrol (**2b**) was removed by crystallization and chromatography. The nitrophenol **2a** was then methylated with potassium carbonate and methyl iodide in dimethylformamide[5] and hydrogenated over 5% palladium on charcoal to give 3-aminohexestrol dimethyl ether (**4a**) in nearly quantitative yield.

It has been reported[4b] that the yield of the Schiemann reaction is improved if the diazonium hexafluorophosphate is decomposed, rather than the more commonly used fluoroborate salt. Diazotization of the aminohexestrol methyl ether (**4a**) with sodium nitrite and hydrochloric acid in aqueous tetrahydrofuran, followed by the addition of concentrated hexafluorophosphoric acid,[4b] gave hexestrol 3-diazonium hexafluorophosphate dimethyl ether (**5a**). This material was a dark red oil rather than a solid precipitate, making the crude product difficult to purify. Pyrolysis of the oil in xylene[8] at reflux temperature gave a mixture of products, with the major one resulting from the addition of xylene rather than fluorination. A low yield of the desired product, 3-fluorohexestrol dimethyl ether **6a** (18.5% based on amine **4a**), was obtained when the hexafluorophosphate salt **5a** was pyrolized in the presence of nonane. The diazonium salt is completely insoluble in this solvent, so the nonpolar products, which are soluble, could be protected from thermal degradation by periodically withdrawing and replacing the nonane.

Addition of tetrafluoroboric acid instead of hexafluorophosphoric acid after the diazotization[4a] again failed to produce a crystalline solid, but the oily fluoroborate salt thus obtained was much less colored, and when it was pyrolyzed in nonane it gave a much higher yield of 3-fluorohexestrol dimethyl ether (35%, based on amine). The cleavage of the methyl ethers with boron tribromide gave the fluorinated estrogen 3-fluorohexestrol (**7a**). The literature procedure for this reaction[6] was modified by carrying it out at 4 °C, rather than allowing the temperature to rise to 25 °C, and by quenching at −78 °C with methanol, rather than by addition of water at room temperature. These modifications improved the yield somewhat.

The 3,3′-difluorohexestrol (**7b**) was prepared similarly. Nitration of hexestrol with 1.1 molar equiv of copper(II) nitrate gave an 88% yield of 3,3′-dinitrohexestrol (**2b**), which was methylated and reduced in the same manner as the mononitro compound to give 3,3′-diaminohexestrol dimethyl ether (**4b**). After diazotization of the amine with

(4) (a) Schiemann, G.; Winkelmüller, W.; Basler, E.; Ley, E. *J. Prakt. Chem.* **1935**, *143*, 18. (b) Rutherford, K. G.; Redmond, W.; Rigamonti, J. *J. Org. Chem.* **1961**, *26*, 5149. (c) Sellers, C.; Suschintzky, H. *J. Chem. Soc.* **1968**, 2317. (d) Ferm, R. L.; VanderWerf, C. A. *J. Am. Chem. Soc.* **1950**, *72*, 4809. (e) Beatty, R. D.; Musgrave, W. *J. Chem. Soc.* **1952**, 875. (f) Danek, O.; Snobl, D.; Knizek, I.; Nouzova, S. *Collect. Czech. Chem. Commun.* **1964**, *29*, 730.

(5) Williams, J.; Longcope, C.; Williams, K. *Steroids* **1974**, *24*, 687.

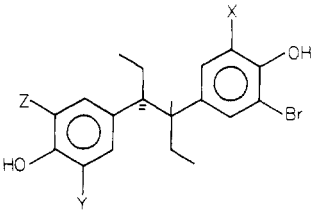
(6) McOmie, J.; Watts, M.; West, D. *Tetrahedron* **1968**, *24*, 2289.

(7) Katzenellenbogen, J. A.; Myers, H. N.; Johnson, H. J. *J. Org. Chem.* **1973**, *38*, 3525.

(8) Neeman, M.; Osawa, Y. *Tetrahedron Lett.* **1963**, 1987. Neeman, N.; Osawa, Y.; Mukai, T. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2297. Neeman, M.; Mukai, T.; O'Grodnick, J.; Rendall, A. L. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2300.

hydrochloric acid and sodium nitrite, addition of tetrafluoroboric acid precipitated the hexestrol bis(diazonium tetrafluoroborate) dimethyl ether (**5c**) in 50% yield as a well-behaved solid. Decane was used as the medium for the pyrolysis of this compound, since the melting/decomposition point of the salt was above the boiling point of nonane. It gave a 53% yield of 3,3′-difluorohexestrol dimethyl ether (**6b**, 26.5%, based on amine **4b**). The methyl ether groups were cleaved using the procedure applied to the monofluoro compound to give a 75% yield of the bisfluorinated estrogen 3,3′-difluorohexestrol (**7b**).

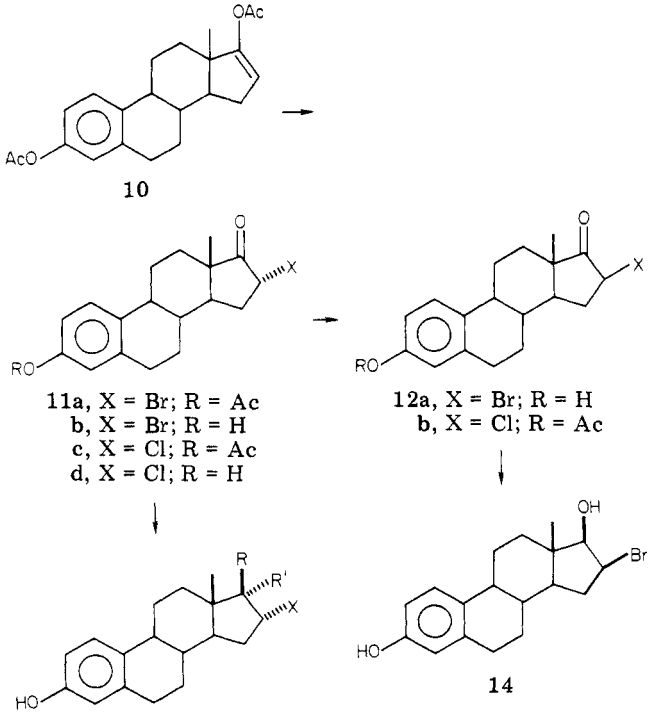
The bromohexestrols (**9a–d**) were prepared by direct



9a, X, Y, Z = H
b, X, Y = H; Z = Br
c, X = H; Y, Z = Br
d, X, Y, Z = Br

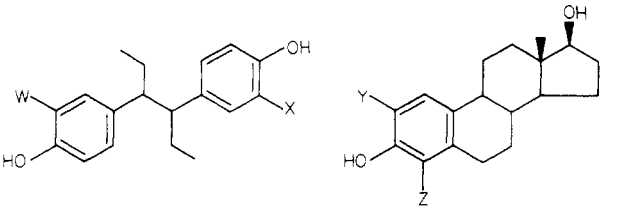
bromination, using bromine in acetic acid buffered with potassium acetate. The mixture of mono-, di-, tri- and tetrabromo compounds thus obtained was separated by medium pressure liquid chromatography, employing gradient elution.

Synthesis of Steroidal Estrogens Bearing Halogen at C-16. The 16-haloestrones were prepared by an adaptation of the procedure of Johnson and Johns.[9a] Estrone 3-acetate (17-enol acetate) (**10**) was brominated with



(9) (a) Johnson, W. S.; Johns, W. F. *J. Am. Chem. Soc.* **1957**, *79*, 2005. (b) Mueller, G. P.; Johns, W. F. *J. Org. Chem.* **1961**, *26*, 2403. (c) Gensler, W. J.; Johnson, F. A.; Sloan, A. D. B. *J. Am. Chem. Soc.* **1960**, *82*, 6078.

Table I. Binding Affinity of Aromatic Ring Halogenated Hexestrols and Steroids for the Uterine Estrogen Receptor[a]



compd	W	X	RAC × 100[b]
1 (hexestrol)	H	H	300
7a	F	H	205
7b	F	F	64
9a	Br	H	16[c]
9b	Br	Br	2.6[c]
	I	H	14[d]
	I	I	3.2[d]
	Y	**Z**	
estradiol	H	H	100
	F	H	86
	H	F	128
	Br	H	1.2
	H	Br	10
	I	H	<0.1[e]
	H	I	*f*

[a] Various concentrations of unlabeled compounds (10^{-4} to 10^{-10} M) were incubated with 10^{-8} M [^{3}H]estradiol and lamb uterine cytosol (ca. 2.5 nM receptor site concentration) for 16 h at 0 °C. Free ligands were removed by a brief treatment with dextran-coated charcoal. For details, see ref 10. [b] The affinity relative to that of estradiol is expressed by RAC × 100, which is the ratio of association constants ($K_a^{compd}/K_a^{estradiol}$) × 100. [c] RAC × 100 values for other bromohexestrols are: 3,5,3′-tribromohexestrol, 0.72; 3,5,3′,5′-tetrabromohexestrol, <0.17. [d] RAC × 100 values are taken from ref 11. RAC × 100 values reported therein for the other iodinated hexestrols are: 3,5-diiodohexestrol, 1.9; 3,5,3′-triiodohexestrol, 0.14; 3,5,3′,5′-tetraiodohexestrol, 0.002. [e] Data taken from ref 16 and 13. [f] The receptor binding affinity of 4-iodoestradiol is reported to be somewhat less than that of 2-iodoestradiol, although data were not presented in quantitative form (see ref 21b). On the other hand, 4-iodo-Δ^6-estradiol is reported to have a RAC × 100 of 5 (ref 13).

Table II. Binding Affinity of Steroidal Estrogens with Halogens in the D Ring for the Uterine Estrogen Receptor[a]

	RAC × 100[b]					
	estrone		estradiol-17β		estradiol-17α	
	16α	16β	16α	16β	16α	16β
H	12		100[c]		10	
Cl	0.48		100		9.2	
Br	3.5	0.14	127	4.7	4.8	
I			100[d]	57[e]		

[a] See Table I, footnote *a*. [b] See Table I, footnote *b*. [c] By definition. [d] Datum is from ref 12. [e] Datum is from ref 13.

bromine in acetic acid to give 16α-bromoestrone 3-acetate (**11a**) or was chlorinated with *tert*-butyl hypochlorite to give a 3:1 mixture of 16α- and 16β-chloroestrone 3-acetates (**11c** plus **12b**) from which the pure 16α-chloro epimer (**11c**) was separated by fractional crystallization. The free phenols **11b** and **11d** were obtained from **11a** and **11c**, respectively, by hydrolysis with sulfuric acid under conditions that avoided epimerization at C-16. The 16α-halo ketone acetates **11a** and **11c** were reduced with lithium aluminum hydride to a mixture of 16α-haloestradiols ep-

imeric at C-17 (**13a,b** from **11a** and **13c,d** from **11c**).[9b] In both cases, the epimeric halohydrins could be separated readily by chromatography on silica gel. The bromo ketone **11a** was epimerized further to a 1:1.7 mixture of **11b** and **12a** with sulfuric acid in ethanol. While these epimeric bromo ketones (**11b** and **12a**) could be separated with some difficulty by high-pressure LC on silica gel, 16β-bromoestradiol-17β (**14**) was obtained most conveniently by zinc borohydride[9c] reduction of the mixture of **11b** and **12a**, followed by chromatographic separation of **14** from **13a,b** and other byproducts. The use of this mild, neutral reducing agent minimized debromination (especially of **12a**) and epoxide formation. Data on the other D-ring halogenated estrogens shown in Table II were taken from the literature.

Binding Affinity of Halogenated Estrogens for the Uterine Estrogen Receptor. The binding affinity of nonradiolabeled estrogen analogues for the estrogen receptor can be measured readily by a competitive binding assay.[10] The affinities are obtained relative to that of the tracer compound, [^{3}H]estradiol, and are conveniently expressed on a percent scale, where the binding of estradiol is defined as 100%. The binding affinities of the aromatic ortho-halogenated estrogens are shown in Table I and that of the C-16 halogenated estrogens in Table II.

o-Fluorohexestrol (**7a**) has a binding affinity nearly the same as that of hexestrol; this compound has the highest affinity for any halogenated estrogen that we have ever prepared. While a single *o*-fluorine substituent decreases the receptor affinity of hexestrol only slightly, a second fluorine at the ortho position of the other ring (compound **7b**) causes a substantial (three- to fourfold) decrease.

We have previously discussed the ambiguities associated with making relative affinity measurements on racemic mixtures of monosubstituted hexestrols in terms of inferring which region of the receptor is interacting with the substituent.[10] Hence, in this case, from the relative affinities of hexestrol, *o*-fluorohexestrol, and *o,o'*-difluorohexestrol, it is apparent only that of the two regions of the receptor that normally bind the A ring and the D ring of a steroidal estrogen, one can accommodate an *o*-fluoro group more readily than the other; it is not clear though which is the more accomodating region. However, the fact that both 2- and 4-fluoroestradiol bind with very high affinity suggests that it is the A-ring binding site which is more tolerant of the fluorine substituent than the D-ring site.

The monosubstituted and symmetrically disubstituted bromo- and iodohexestrols have substantially lower affinities than the corresponding fluorohexestrols. This suggests that the receptor has only limited tolerance for bulky substituents that project into the regions that bind the A and D rings of estradiol. The 2- and 4-substituted bromo- and iodoestradiols also have lower affinities than the corresponding fluoro compounds. The higher affinity of the 4-substituted isomers becomes more apparent as the steric size of the halogen increases, the ratio of relative affinities, 4-substituted/2-substituted, going from 1.5 for the fluoro to 8.3 for the bromo compounds.

The relative binding affinities of steroidal estrogens bearing halogens at C-16 are shown in Table II. The highest affinity compounds are those bearing the halogen at a 16α position in estradiol-17β, their binding being comparable to or greater than that of estradiol-17β itself. 16α-Iodoestradiol-17β has been prepared in γ-emitting form by Hochberg[12] and 16α-bromoestradiol-17β has been labeled with bromine-77 by ourselves (see Discussion). In the estradiol-17β series, the halogen is less well tolerated as a a 16β substituent, although the binding reported for 16β-iodoestradiol-17β by Arunachalam et al.[13] is quite high. Not unexpectedly, the binding of 16α-halogenated estradiol-17β is lower than those of the 17β epimers, but it is of note that the halogen does not appear to lower the affinity of the parent compound very significantly. The D-ring haloestrones also have lower affinity, which reflects to a large degree the lower affinity of the parent ligand (estrone) as compared to estradiol-17β.

Discussion

We have described the preparation of several steroidal and nonsteroidal estrogens (hexestrols) bearing halogen substituents at aromatic positions ortho to the phenolic hydroxyl group or in the D ring at C-16 as potential estrogen receptor based breast tumor imaging agents. We have measured the estrogen receptor binding affinities of these compounds and have compared these with the affinities of related compounds which we have synthesized previously or which have been prepared by others. These data have enabled us to make a preliminary assessment of the potential selectivity with which these compounds will interact with target sites.

Among the aromatic halogenated derivatives that we have studied (Table I), we have found the monofluorinated hexestrol and estradiols to have the highest receptor binding affinity. The promise of these compounds as breast tumor imaging agents depends upon the suitability of methods for introducing fluorine-18 at an aromatic position without a dilution of specific activity that would be too great to make these compounds useful imaging agents. While the Balz–Schiemann reaction is unsuitable in this respect,[14] the recently described aryltriazene decomposition approach may prove to be applicable.[15] Some of the aromatic brominated and iodinated estrogens have moderate binding affinity for the receptor; nevertheless, our previous experience[11] with *o*-iodohexestrol as well as that of others[16] indicates that the level of nonreceptor binding of these compounds is quite high because of the lipophilizing effect of the large halogen as an aromatic substituent.[3a]

Estrogens bearing halogen substituents at aromatic ring positions have been described in the literature in numerous instances: 2- and 4-iodoestradiol and 2,4-diiodoestradiols have been prepared in γ-emitting form,[17,18] and although

(10) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. *Biochemistry* **1973**, *12*, 4085.

(11) Katzenellenbogen, J. A.; Hsiung, H. M.; Carlson, K. E.; McGuire, W. L.; Kraay, R. J.; Katzenellenbogen, B. S. *Biochemistry* **1975**, *14*, 1742.

(12) (a) Hochberg, R. B. *Science* **1979**, *205*, 1138. (b) Hochberg, R. B.; Rosner, W. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 328.

(13) Arunachalam, T.; Longcope, C.; Caspi, E. *J. Biol. Chem.* **1979**, *254*, 5900.

(14) Palmer, A. J.; Clark, J. C.; Goulding, R. W. *Int. J. Appl. Radiat. Isot.* **1977**, *28*, 53.

(15) (a) Rosenfeld, M. N.; Widdowson, D. A. *J. Chem. Soc., Chem. Commun.* **1979**, 914. (b) Tewson, T. J.; Welch, M. J. *Ibid.* **1979**, 1149.

(16) (a) Komai, T.; Eckelman, W. C.; Johnsonbaugh, R. E.; Mazaitis, A.; Kubota, H.; Reba, R. C. *J. Nucl. Med.* **1977**, *18*, 360. (b) Mazaitis, J. K.; Gibson, R. E.; Komai, T.; Eckelman, W. C.; Francis, B.; Reba, R. C. *J. Nucl. Med.* **1980**, *21*, 142.

these compounds show negligible uterotrophic activity,[17c,d] some attempts have been made to utilize them as imaging agents for the prostate.[18] Estrogens labeled in the A ring with bromine have also been prepared in γ-emitting form;[19] these compounds have relatively low estrogen receptor affinity, and in most cases they were prepared with a specific activity that was too low for their uptake by an estrogen receptor mechanism to be detectable.[14] 4-Fluoroestradiol-17β labeled to low specific activity with fluorine-18 has been prepared by the Balz–Schiemann reaction.[20]

Extensive studies have also been done on the chemistry of iodination of diethylstilbestrol and the corresponding diphosphate (stilphosterol) with the view of preparing breast tumor imaging agents,[21] but these, as well as other iodinated stilbestrol analogues and antiestrogens,[22] appear to have low uptake selectivity. We[11] and Komai et al.[16] have carefully evaluated the binding selectivity of *o*-[^{125}I]iodohexestrol and have concluded that the level of its interaction with nonreceptor proteins was so great as to preclude its successful use as a breast tumor imaging agent. Other *o*-fluoro- and *o*-chlorohexestrols have been prepared,[23] but their biological activity has not been well characterized.

Of the steroidal estrogens bearing a halogen in the D ring at C-16 (cf. Table II), we have found 16α-bromoestradiol-17β to have the highest affinity for the estrogen receptor; the affinity of this compound is greater than both that of estradiol and that of 16α-iodoestradiol-17β, a compound recently prepared in γ-labeled form by Hochberg (see below).[12]

Other workers have been interested in estrogens labeled with γ-emitting radionuclides in the D ring. Counsell[24] prepared iodine-125 labeled 16α-iodoestrone acetate but found this material to be unstable in vivo. Mazaitis et al.[16b] have prepared 17α-iodoethynylestradiol; this compound appeared to have high affinity for receptor, but it suffers loss of iodine in the presence of thiol functions. Arunachalam et al.[13] have described the synthesis and receptor binding of several iodinated estrogens substituted at the 16, 17, and 6 positions. Recently, Hochberg and Rosner[12] have described the preparation of 16α-iodoestradiol-17β labeled with iodine-125 in nearly carrier-free form. This material has a binding affinity for the estrogen receptor comparable to that of estradiol-17β, shows similar (or lower) levels of nonspecific binding, and is selectively taken up into estrogen target tissues in vivo. We have prepared 16α-bromoestradiol-17β labeled with bromine-77 in high specific activity. As expected from the high receptor binding affinity of this compound, it demonstrates receptor-specific uptake into the uterus of immature and mature rats and into DMBA-induced mammary adenocarcinoma in mature rats. Details of this work will be described elsewhere.[3e] Numerous other steroidal estrogens bearing halogen substituents in the D ring have been prepared and have been investigated not only as hormonal agents but as hypochlolesteremic agents[9b] and as irreversible enzyme inhibitors.[25]

In conclusion, on the basis of binding studies with the estrogen receptor done with the compounds described in this paper, it appears that at aromatic positions only fluorine substitution is consistent with high receptor binding affinity; in contrast, both bromine and iodine are well tolerated at the 16α position of estradiol-17β. Thus, provided that they can be prepared with a suitable radioisotope in high specific activity form, these compounds appear to be excellent candidates as estrogen receptor based agents for imaging breast tumors. Further studies on the behavior of these compounds in vivo will appear in future publications.[3d,e]

Experimental Section

Materials. Hexestrol was purchased from Sigma Chemical Co., estrone from Searle Chemicals, Inc., 65% hexafluorophosphoric acid (practical grade) from Matheson, Coleman and Bell, and 48% tetrafluoroboric acid from Alfa (Ventron).

2-Fluoroestradiol and 2-bromo- and 4-bromoestradiol were prepared by the method of Utne[26,27] and exhibited spectroscopic properties identical with those reported by Utne. A sample of 4-fluoroestradiol prepared by Utne[27] was obtained through the National Institutes of Health. Estrone 3-acetate (17-enol acetate) (**10**) was prepared by the method of Leeds et al.[28] The preparation of monoiodo- and symmetrical diiodohexestrol has been described by us.[11] A clarified solution of lithium aluminum hydride was prepared by stirring $LiAlH_4$ (1.25 g) in 25 mL of dry tetrahydrofuran for 3 h at room temperature under a nitrogen at-

(17) (a) Varvarigou, A.; Villa, M.; Rovano, S. *Eur. J. Nucl. Med.* **1978**, *3*, 191. (b) Thakur, M. L.; Waters, S. L. *Int. J. Appl. Radiat. Isot.* **1976**, *27*, 585. (c) Albert, S.; Heard, R. D. H.; Leblond, C. P.; Saffron, J. *J. Biol. Chem.* **1949**, *177*, 247. (d) Hillmann-Elies, A.; Hillmann, G.; Schiedt, U. *Z. Naturforsch., B* **1953**, *8*, 436. (e) Matkovics, B.; Rakonczay, Z.; Rajki, S. E.; Balaspiri, *Steroidologia* **1971**, *2*, 77. (f) Nambara, T.; Akiyama, S.; Honma, S. *Chem. Pharm. Bull.* **1971**, *19*, 1727. (g) Tarle, M.; Padovan, R.; Spaventi, S. *J. Labelled Compd. Radiopharm.* **1978**, *15*, 7. (h) Tarle, M.; Padovan, R.; Spaventi, S. *Ibid.* **1978**, *15*, 665. (i) Moore, D. H.; Wolf, W. *Ibid.* **1978**, *15*, 443. (j) Sweet, F.; Patrick, T. B.; Mudd, J. M. *J. Org. Chem.* **1979**, *44*, 2296.

(18) (a) Ghanadian, R.; Waters, S. L.; Thakur, M. L.; Chisholm, G. D. *Int. J. Appl. Radiat. Isot.* **1975**, *26*, 343. (b) Ghanadian, R.; Waters, S. L.; Williams, G.; Chisholm, G. D. *Eur. J. Nucl. Med.* **1976**, *1*, 159. (c) Szendröi, Z.; Kocsár, L.; Karika, Z.; Eckhardt, S. *Lancet* **1973**, *1*, 1252. (d) Shida, K.; Shimazaki, J.; Kurihara, H.; Ito, Y.; Furuya, N. *Recent Adv. Nucl. Med., Proc. World Congr. Nucl. Med., 1st*, **1974**, 674. Ghanadian, R.; Waters, S. L.; Williams, G.; Chisholm, G. D. *Eur. J. Nucl. Med.* **1976**, *1*, 159.

(19) (a) Twombly, G. H.; McClintock, L.; Engelman, M. *Am. J. Obstet. Gynecol.* **1948**, *56*. (b) Twombly, G. H.; Schoenewaldt, E. F. *Cancer* **1950**, *3*, 601. (c) Spicer, J. A.; Preston, D. F.; Robinson, R. G.; Bradshaw, D. L.; Stern, S. H.; Dean, R. D.; Martin, N. L.; Rhodes, B. A. *Int. J. Appl. Radiat. Isot.* **1977**, *28*, 163. (d) Spicer, J. A.; Preston, D. F.; Baranczuk, R. J.; Harvey, E.; Guffey, M. M.; Bradshaw, D. L.; Robinson, R. G. *J. Nucl. Med.* **1979**, *20*, 761.

(20) (a) Palmer, A. J.; Widdowson, D. A. *J. Labelled Compd. Radiopharm.* **1979**, *16*, 14. (b) Eakins, M. N.; Palmer, A. J.; Waters, S. L. *Int. J. Appl. Radiat. Isot.* **1979**, *30*, 695.

(21) (a) Maysinger, D.; Marcus, C. S.; Wolf, W.; Tarle, M.; Casanova, J. *J. Chromatogr.* **1977**, *130*, 129. (b) Maysinger, D. Ph.D. Thesis, University of Southern California, 1976.

(22) Counsell, R. E.; Buswink, A.; Korn, N.; Johnson, M.; Ranade, V.; Yu, T. In "Steroid Hormone Action and Cancer"; Menow, K. M. J.; Reel, J. R., Eds.; Plenum: New York, 1976; p 107.

(23) Chan, R. P.; Dekanski, J. B.; Gottfried, S.; Parke, D. V.; Pope, D. J. *Br. J. Pharmacol.* **1978**, *63*, 350P. Sisdo, K.; Odo, Y.; Nozaki, H.; Jensen, E. V. *J. Org. Chem.* **1961**, *26*, 1227. McLachlan, J. A.; Baucom, K.; Korach, K. S.; Levy, L.; Metzler, M. *Steroids* **1979**, *33*, 543.

(24) Counsell, R. E.; Buswink, A.; Korn, N.; Johnson, M.; Ranade, V.; Yu, T. In "Steroid Hormone Action and Cancer", Menon, K. M. J.; Reel, J. R., Eds.; Plenum Press: New York, 1976; p 107.

(25) For example: Pons, M.; Nicolas, J. C.; Boussioux, A. M.; Descomps, B.; Crastes de Paulet, A. *FEBS Lett.* **1973**, *31*, 256.

(26) Utne, T.; Jobson, R. B.; Landgraf, F. W. *J. Org. Chem.* **1968**, *33*, 1654.

(27) Utne, T.; Jobson, R. B.; Babson, R. D. *J. Org. Chem.* **1968**, *33*, 2469.

(28) Leeds, N. F.; Fukushima, D. K.; Gallagher, T. F. *J. Am. Chem. Soc.* **1953**, *76*, 2943.

mosphere. The suspension was filtered in a Schlenk-type funnel under positive nitrogen pressure into a 50-mL Erlenmeyer flask equipped with a three-way stopcock. Standardization with a gas burette to measure hydrogen evolution gave a concentration of 1.20 M.

Methods. Analytical thin-layer chromatography was performed using 0.25-mm silica gel glass-backed plates with F-254 indicator (precoated TLC plates, silica gel 60 F-254, Merck), and compounds were visualized by ultraviolet light (254 nm), iodine vapor, or by using a copper(II) acetate–phosphoric acid spray reagent. Preparative thin-layer chromatography was carried out using 2-mm glass-backed silica gel plates with F-254 indicator (precoated TLC plates, silica gel F-254, Merck). Silica gel column chromatography was performed using 0.05–0.2 mm silica gel (Brinkman). Medium-pressure liquid chromatography (MPLC) was performed with a system composed of a Milton Roy Series D reciprocating pump with 0.25-in. piston (0–4800 mL/h capacity), an ISCO Model UA-5 ultraviolet absorbance monitor, and columns constructed of thick-walled glass tubing by the University of Illinois School of Chemical Sciences Glassworking Shop or of thin-wall stainless-steel tubing by the University of Illinois School of Chemical Sciences Metalworking Shop. The plumbing was done with 0.125-in. Teflon tubing and Gyrolok compression fittings. Teflon tubing and Gyrolok compression fittings. The columns were hand packed with silica gel purchased from Alfa (Ventron) and mechanically sieved to give particle size ranges of 45–63 (column diameter 2 cm or less) or 63–88 μm (column diameter greater than 2 cm).

Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. Infrared spectra were taken as KBr pellets or chloroform solutions on a Beckman Model IR-12 spectrophotometer. Data are presented in reciprocal centimeters, and only the important diagnostic bands are reported. Proton magnetic resonance spectra (^{1}H NMR) were obtained at 90 MHz on a Varian Model EM-390 spectrometer, and chemical shifts are reported in δ relative to tetramethylsilane (Me_4Si) as an internal standard. Fluorine magnetic resonance spectra (^{19}F NMR) were obtained at 84.6 MHz on a Varian EM-390 spectrometer, and chemical shifts are reported in parts per million downfield from internal fluorotrichloromethane (−50 ppm). Mass spectral data were obtained on a Varian MAT Model CH-5 mass spectrometer. Microanalytical data were provided by the Microanalytical Service Laboratory of the University of Illinois School of Chemical Sciences.

Except where mentioned otherwise, a standard procedure was used for product isolations; this involved quenching by addition to water, exhaustive extractions with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, filtration, and evaporation of solvent under reduced pressure. The particular solvents, aqueous washes (if used), and drying agents are mentioned in parentheses after the phrase "product isolation".

***erythro*-3-Nitrohexestrol (2a).** Acetic anhydride (17 mL, 18.4 g, 180 mmol) was added gradually, with periodic cooling in an ice bath, to 5.45 g of finely powdered copper(II) nitrate trihydrate (22.5 mmol), and the mixture was swirled until all of the dark blue solid had turned a deep turquoise color (~10–15 min). This mixture was then added in small portions over a 10-min period to an ice-cooled solution of 10.8 g of hexestrol (1; 40 mmol) in 75 mL of 1:1 glacial acetic acid/tetrahydrofuran. After the addition was complete, the mixture was stirred for an additional 10 min without cooling and poured into 600 mL of cold water. The solution was brought to pH 6 with 6 M sodium hydroxide, and the semisolid product, collected by filtration, was washed with water until the washings were colorless. The organic residue was eluted with THF and dried ($MgSO_4$), and the solvent was removed, giving a crude yield of 15.83 g. A partial separation of the products was effected by triturating the residue with acetone and filtering, leaving 1.21 g of pure 3,3-dinitrohexestrol (**2b**), very sparingly soluble in common organic solvents. The filtrate was chromatographed (SiO_2, benzene/hexane) to give first 820 mg of dinitrohexestrol (**2b**, 14% including the solid recovered by trituration), 120 mg of an unidentified yellow byproduct, and then 7.20 g of bright yellow crystalline *erythro*-3-nitrohexestrol (**2a**; 57%): mp 139–142 °C from ethanol (lit.[7] 139–142 °C). Anal. ($C_{18}H_{21}NO_4$) C, H, N.

***erythro*-3-Nitrohexestrol Dimethyl Ether (3a).** *erythro*-3-Nitrohexestrol (**2a**; 655 mg, 2.1 mmol) and 2.84 g of methyl iodide (20 mmol) were dissolved in 4 mL of dimethylformamide and stirred with 2.76 g of anhydrous potassium carbonate (20 mmol) at room temperature under a nitrogen atmosphere for 15 h. The mixture was diluted with water and filtered to collect the precipitated solid. The product was washed through the filter with benzene, dried ($MgSO_4$) and the solvent removed, giving 710 mg of pale yellow solid (99.5%). Recrystallization from chloroform–ethanol afforded a nearly colorless solid: mp 116–117 °C; IR (KBr) 1532 and 1362 (NO_2), 1267, 1250 and 1027 cm^{-1} (Ar-O-Me); ^{1}H NMR (CCl_4) δ 0.55 (t, 6, J = 6.5 Hz, CH_3), 1.06–1.77 (m, 4, CH_2), 2.20–2.76 (m, 2, CH), 3.62 (s, 3, $ArOCH_3$), 3.96 (s, 3, nitro-$ArOCH_3$), 6.80 (d, 2, J = 9 Hz, ArH ortho to hydroxyl), 6.98 (m, 5, ArH), 7.25 (dd, 1, J = 9 Hz, 2.5 Hz, ArH meta to nitro), 7.54 (d, 1, J = 2.5 Hz, ArH ortho to nitro); mass spectrum (10 eV) m/e (rel intensity) 343 (0.6, M$^+$), 194 (1.0), 149 (100), 121 (4). Anal. ($C_{20}H_{25}NO_4$) C, H, N.

***erythro*-3-Aminohexestrol 4,4′-Dimethyl Ether (4a).** *erythro*-3-Nitrohexestrol 4,4′-dimethyl ether (**3a**; 3.13 g, 9.15 mmol) was dissolved in 15 mL of tetrahydrofuran, 315 mg of 5% palladium on charcoal was added, and the mixture was stirred under a hydrogen atmosphere at ambient temperature and pressure for 36 h. (At this point uptake of hydrogen ceased, a total of 615 mL (27.4 mmol) having been consumed.) The solution was filtered to remove the catalyst, and the solvent was removed, yielding 3.00 g (88%) of *erythro*-3-aminohexestrol 4,4′-dimethyl ether (**4a**), which, after recrystallization from 90% ethanol, had mp 159.5–163.5 °C: IR (KBr) 3485, 3396 cm^{-1} (NH_2); ^{1}H NMR (acetone-d_6) δ 0.50 (t, 6, J = 7 Hz, CH_3), 1.02–1.60 (m, 4, CH_2), 2.21–2.64 (m, 2, CH), 3.76 (s, 3, amino-$ArOCH_3$), 3.80 (s, 3, $ArOCH_3$), 4.21 (br s, 2, NH_2), 6.37–6.78 (m, 3, amino-ArH), 6.86 (d, 2, ArH ortho to methoxyl), 7.10 (d, 2, ArH ortho to alkyl); mass spectrum (70 eV), m/e (relative intensity) 313 (13.5, M$^+$), 165 (29), 164 (100), 149 (32), 121 (13). Elemental analysis was not attempted, since the compound remained discolored even after repeated recrystallization.

***erythro*-3-Fluorohexestrol 4,4′-Dimethyl Ether (6a).** *erythro*-3-Aminohexestrol 4,4′-dimethyl ether (**4a**; 186 mg, 0.6 mmol) was dissolved in a mixture of 5 mL of water, 5 mL of acetone, and 0.4 mL of concentrated hydrochloric acid (4.8 mmol) and diazotized at ice-bath temperature by the dropwise addition of 42 mg of sodium nitrite (0.6 mmol) dissolved in 5 mL of H_2O. An additional 4 mg of sodium nitrite was required before potassium iodide–starch paper indicated an excess of nitrous acid. Urea (2 mg) was then added to destroy the excess, and 0.48 mL of 48% tetrafluoroboric acid was added all at once. A yellow oil separated which refused to crystallize on scratching. It was extracted with ethyl acetate, washed with water, and dried ($MgSO_4$), and the solvent was removed, yielding 340 mg of orange-yellow oil. This material was pyrolyzed as a thin film on the inside of a flask containing 10 mL of nonane. The flask was heated in an oil bath at 120 to 140 °C for 3 h, pipetting off and replacing the nonane every 20 to 30 min. Removal of the nonane in vacuo left 130 mg of a semicrystalline yellowish solid. Recrystallization of the crude product twice from ethanol/water afforded 50 mg of white crystalline solid. Chromatography of the mother liquors (MPLC, 10% CH_2Cl_2 in hexane) yielded a further 16 mg of the fluorinated compound **6a** as the first major peak: total yield 66 mg (35%, based on the amine); mp 137–138 °C; IR (KBr) 1276 (ArOMe), 1258 (ArF); ^{1}H NMR ($CDCl_3$) δ 0.53 (t, 6, J = 6.5 Hz, CH_3), 1.17–1.67 (m, 4, CH_2), 2.13–2.60 (m, 2, CH), 3.77 (s, 3, $ArOCH_3$), 3.84 (s, 3, fluoro-$ArOCH_3$), 6.60–7.11 ppm (m, 7, ArH); ^{19}F NMR ($CDCl_3$) δ 132.8 (dd, J = 13 Hz, 6 Hz); mass spectrum (70 eV), m/e (relative intensity) 316 (1.6, M$^+$), 167 (6.4), 149 (100), 139 (6.6), 121 (24). Anal. ($C_{20}H_{25}FO_2$) C, H, F.

***erythro*-3-Fluorohexestrol (7a).** 3-Fluorohexestrol 4,4′-dimethyl ether (**6a**; 56 mg, 0.18 mmol) was dissolved in 2 mL of dry methylene chloride (4 Å molecular sieves), cooled in an ice–salt bath under a dry nitrogen atmosphere, and 0.55 mL of a 1 M solution of boron tribromide in methylene chloride (0.55 mmol) was added dropwise via syringe. After addition was complete, the mixture was stirred for a further 30 min in the ice–salt bath, stoppered, and stored in a refrigerator (+4 °C) for 12 h. The reaction was quenched, after cooling in a dry ice–2-propanol bath, by the dropwise addition of absolute methanol followed by con-

centrated aqueous ammonia. After removal of the solvents under nitrogen stream, the brown residue was partitioned between ethyl acetate and water, washed once with water, and dried ($MgSO_4$), and the solvent was removed. The crude product was chromatographed (MPLC, SiO_2, 3% ethyl acetate in methylene chloride) to give 41 mg (80%) of 3-fluorohexestrol **7a**. Recrystallization from THF/cyclohexane gave white crystals: mp 200.5–201.5 °C; IR (KBr) 3370 (OH), 1295 cm^{-1} (ArF); ^1H NMR (acetone-d_6) δ 0.54 (t, 6, J = 7.5 Hz, CH_3), 0.97–1.63 (m, 4, CH_2), 2.27–2.68 (m, 2 H, CH), 6.67–7.07 (m, 7, ArH), 8.09 (br s, 2, OH), mass spectrum (10 eV), m/e (relative intensity) 288 (1.7, M^+), 153 (6), 135 (100). Anal. ($C_{18}H_{21}FO_2$) C, H, F.

***meso*-3,3′-Dinitrohexestrol (2b).** Finely powdered copper(II) nitrate trihydrate (10.9 g, 45 mmol) was treated with 34 mL of acetic anhydride (36.8 g, 360 mmol) and stirred under dry nitrogen for 10 min with occasional cooling in an ice bath. This mixture was then added in small portions to a cooled solution of 10.8 g of hexestrol (40 mmol) in 120 mL of 5:1 diethyl ether/acetic acid. After the addition of the nitrating reagent was complete, the mixture was stirred for an hour and the precipitated product was collected by suction filtration on a sintered glass funnel. The solid was washed with water until the washings no longer showed a blue color, then once with 1:1 diethyl ether/acetic acid, and once with diethyl ether and dried in vacuo overnight. Yield of crude greenish yellow 3,3′-dinitrohexestrol (**2b**) was 15.2 g. Recrystallization from THF/ethanol afforded 12.7 g of bright yellow crystalline **2b** (88%), mp 240 °C (lit.[7] 240 °C dec). Anal. ($C_{18}H_{20}N_2O_6$) C, H, N.

***meso*-3,3′-Dinitrohexestrol Dimethyl Ether (3b).** *meso*-3,3′-Dinitrohexestrol (**2b**; 540 mg, 1.5 mmol), 2.13 g of methyl iodide (15 mmol), and 2.10 g of anhydrous potassium carbonate (15 mmol) were stirred in 15 mL of dimethylformamide for 48 h at room temperature under nitrogen and poured into water. The precipitated solid was collected by suction filtration, dried on the filter overnight, and washed through with CH_2Cl_2. Yield of the crude yellowish-tan solid dinitrohexestrol dimethyl ether (**3b**) was 602 mg (theoretical: 582 mg). Though nearly pure, it proved difficult to recrystallize because of extreme insolubility in all common organic solvents. A small portion was purified by chromatography (silica gel column eluted with benzene). After recrystallization from THF/hexane and then from acetonitrile, it gave pale yellow crystals: mp 207.5–210.5 °C; IR (KBr) 1547, 1362 (NO_2), 1270 (ArOMe), 738 ($ArNO_2$); ^1H NMR ($CDCl_3$) δ 0.61 (t, 6, J = 7 Hz, CH_3), 1.17–1.62 (m, 4, CH_2), 2.38–2.77 (m, 2, CH), 3.98 (s, 6, $ArOCH_3$), 7.02 (d, 2, J = 8.5 Hz, ArH ortho to methoxyl), 7.25 (dd, 2, J = 8.5 and 3 Hz, ArH para to nitro), 7.60 (d, 2, J = 3 Hz, ArH ortho to nitro); mass spectrum (10 eV), m/e (relative intensity) 338 (0.13, M^+), 194 (100), 193 (25), 166 (16), 148 (6). Anal. ($C_{20}H_{24}N_2O_6$) C, H, N.

***meso*-3,3′-Diaminohexestrol 4,4′-Dimethyl Ether (4b).** 3,3′-Dinitrohexestrol 4,4′-dimethyl ether (**3b**; 454 mg, 1.17 mmol) was dissolved in 50 mL of tetrahydrofuran, 100 mg of 5% palladium on charcoal was added, and the mixture was stirred under hydrogen at atmospheric pressure for 12 h. After filtration (with the aid of Celite), the solvent was removed in vacuo to give 376 mg (97%) of diaminohexestrol dimethyl ether (**4b**). Repeated recrystallizations from THF/methanol and ethanol/water failed to improve the purity of the product: IR (KBr) 3490 and 3395 (NH), 1292 and 1236 (NH_2), 1250 and 1033 cm^{-1} ($ArOCH_3$); ^1H NMR (acetone-d_6) δ 0.50 (t, 6, J = 7 Hz, CH_3), 0.97–1.55 (m, 4, CH_2) 2.13–2.49 (m, 2, CH), 3.74 (s, 6, $ArOCH_3$), 4.18 (br s, 4, NH_2), 6.30–6.77 (m, 6, ArH); mass spectrum (70 eV), m/e (relative intensity) 328 (11, $M^{+\cdot}$), 165 (11), 164 (100), 136 (6).

***meso*-Hexestrol 4,4′-Dimethyl Ether 3,3′-Bis(diazonium tetrafluoroborate) (5c).** 3,3′-Diaminohexestrol 4,4′-dimethyl ether (**4b**; 286 mg, 0.87 mmol) was dissolved in 0.4 mL of concentrated hydrochloric acid (4.8 mmol) and 5 mL of water and diazotized by the dropwise addition of 125 mg of sodium nitrate (1.8 mmol) dissolved in 5 mL of water. The slight excess of nitrous acid was destroyed by the addition of a few milligrams of urea, and 0.8 mL of 48% tetrafluoroboric acid (5.5 mmol) was added all at once. The yellow precipitate which appeared immediately was collected by filtration, washed with water, ethanol, and ether, and dried in vacuo, yielding 228 mg (50%) of hexestrol dimethyl ether bis(diazonium tetrafluoroborate) (**5c**): mp 155 °C dec, with darkening above 130 °C; IR (mineral oil mull) 2265 cm^{-1} (ArN_2^+).

***meso*-3,3′-Difluorohexestrol 4,4′-Dimethyl Ether (6b).** Hexestrol 4,4′-dimethyl ether 3,3′-bis(diazonium tetrafluoroborate) (**5c**; 200 mg, 0.38 mmol) was suspended in 10 mL of *n*-decane and heated at 160 °C for 30 min, by which time evolution of gas had ceased. The decane was removed by heating under a nitrogen stream, the solid residue was taken up in CH_2Cl_2 and filtered through ca. 1 g of basic alumina, and the solvent was removed. The crude solid (114 mg) was purified by preparative layer chromatography (CH_2Cl_2), affording 61 mg (53%) of 3,3′-difluorohexestrol 4,4′-dimethyl ether (**6b**): mp 161–165.5 °C (from benzene/hexane); ^1H NMR ($CDCl_3$) δ 0.53 (t, 6, J = 7 Hz, CH_3), 1.09–1.56 (m, 4, CH_2), 2.27–2.48 (m, 2, CH), 3.78 (s, 6, $ArOCH_3$), 6.63–6.93 (m, 6, ArH); ^{19}F NMR (CCl_4) δ 134.5 (dd, J = 13 Hz, 7 Hz); mass spectrum (70 eV), m/e (relative intensity) 334 (1.5, M^+), 168 (13), 167 (100), 139 (38).

***meso*-3,3′-Difluorohexestrol (7b).** *meso*-3,3′-Difluorohexestrol 4,4′-dimethyl ether (**6b**; 60 mg, 0.18 mmol) was dissolved in 1.5 mL of dry (4 Å molecular sieves) methylene chloride and cooled in an ice–salt bath under a dry nitrogen atmosphere. Boron tribromide (0.55 mL of a 1 M solution in CH_2Cl_2, 0.55 mmol) was added slowly, and the reaction mixture was stoppered and stored in a refrigerator (+4 °C) for 12 h. After being cooled in a dry ice–2-propanol bath, it was quenched by the dropwise addition of ca. 1 mL of absolute methanol. The solvents were removed under a nitrogen stream, and the residue was purified by MPLC (3% ethyl acetate in methylene chloride) to give, as the most mobile fraction, 41 mg (75%) of white crystals of 3,3′-difluorohexestrol (**7b**). The analytical sample was obtained by semipreparative chromatography on a Varian 4100 high-pressure LC (SiO_2, hexane/dichloromethane/2-propanol): mp 206–209 °C, with sublimation above 170 °C; IR (KBr) 3400 (OH), 1293 and 1205 cm^{-1} (ArF); ^1H NMR (acetone-d_6) δ 0.54 (t, 6, J = 7 Hz, CH_3), 1.09–1.55 (m, 4, CH_2), 2.41–2.70 (m, 2, CH), 6.74–7.07 (m, 6, ArH), 8.29 (br s, 2, OH); mass spectrum (10 eV), m/e (relative intensity) 306 (3.7, M^+), 153 (100), 152 (20), 125 (8). Anal. ($C_{18}H_{20}F_2O_2$) C, H.

***meso*-3,3′,5,5′-Tetrabromohexestrol (9d), *erythro*-3,3′,5-Tribromohexestrol (9c), *meso*-3,3′-Dibromohexestrol (9b), and *erythro*-2-Bromohexestrol (9a).** A solution of 1 g of anhydrous potassium acetate (10 mmol) in 15 mL of glacial acetic acid was added all at once to a solution of 540 mg of hexestrol (1; 2 mmol) in 5 mL of THF. The mixture was cooled in an ice bath, and 5 mL of a freshly prepared 1 M solution of bromine in acetic acid (5 mmol) was added dropwise. After 5 min the products were isolated by partitioning between EtOAc and water, washing (H_2O), drying ($MgSO_4$), and removal of solvent. The crude product (1.15 g) was chromatographed (MPLC, SiO_2, 0 to 20% EtOAc in 2:1 hexane/CH_2Cl_2 gradient elution) to give successively 306 mg of tetrabromohexestrol (**9d**; 26%), 205 mg of tribromohexestrol (**9c**; 20%), 251 mg of dibromohexestrol (**9b**; 29%), and 74 mg of bromohexestrol (**9a**; 6%).

The tetrabromo compound (**9d**) had mp 235.5–236.5 °C after recrystallizations from EtOH/H_2O, THF/hexane, and EtOH/HOAc/H_2O: IR (KBr) 3495 cm^{-1} (OH); ^1H NMR ($CDCl_3$) δ 0.56 (t, 6, J = 7 Hz, CH_3), 1.27 (m, 4, CH_2), 2.34 (m, 2, CH), 5.70 (s, 2, OH), 7.17 (s, 4, ArH); mass spectrum (10 eV), m/e (relative intensity) 590 (2), 588 (5), 586 (9), 584 (7), 582 (2, all M^+), 295 (26), 294 (59), 293 (58), 292 (100), 291 (30), 290 (68). Anal. ($C_{18}H_{18}Br_4O_2$) C, H, Br.

Since repeated recrystallizations from a variety of solvents failed to remove traces of the di- and tetrabromo compounds, a portion of the tribromohexestrol (**9c**) was rechromatographed (MPLC, 2% EtOAc in 3:1 hexane/CH_2Cl_2) and recrystallized from THF/EtOH/H_2O to give a colorless solid: mp 140.5–141.5 °C; IR (KBr) 3445 cm^{-1} (OH); ^1H NMR ($CDCl_3$) δ 0.57 (t, 6, J = 7 Hz, CH_3), 1.31 (m, 4, CH_2), 2.37 (m, 2, CH), 5.52 (br s, 2, ArOH), 6.92 (s, 2, ArH meta and para to bromine), 7.16 (s, 3, ArH ortho to bromine); mass spectrum (10 eV), m/e (relative intensity) 510 (3), 508 (16), 506 (15), 504 (5, all M^+), 294 (22), 292 (41), 290 (24), 215 (100), 214 (99), 213 (99), 212 (94). Anal. ($C_{18}H_{19}Br_3O_2$) C, H.

The analytical sample of the dibromohexestrol (**9b**) was rechromatographed (MPLC, 5% EtOAc in 1:1 hexane/CH_2Cl_2) and recrystallized from acetone/chloroform to give a colorless solid: mp 145–147 °C; IR (KBr) 3410 cm^{-1} (OH); ^1H NMR (acetone-d_6) δ 0.56 (t, 6, J = 6.5 Hz, CH_3), 1.34 (m, 4, CH_2), 2.50 (m, 2, CH),

6.96 (m, 4, ArH meta and para to bromine), 7.32 (m, 2, ArH ortho to bromine), ca. 7.5 (br, OH); mass spectrum (10 eV), *m/e* (relative intensity) 430 (7), 428 (12), 426 (5, all M^+), 215 (94), 214 (100), 213 (100), 212 (99). Anal. ($C_{18}H_{20}Br_2O_2$) C, H, Br.

The analytical sample of monobromohexestrol (**9a**) was recrystallized from EtOAc/CCl_4/hexane, MeOH/H_2O, and HOAc/H_2O to give colorless crystals; mp 128–129 °C; IR (KBr) 3440 cm^{-1} (ArOH); ^1H NMR (acetone-d_6) δ 0.52 (t, 6, J = 7.5 Hz, CH_3), 1.29 (m, 4, CH_2), 2.42 (m, 2, CH), 6.73 (d, 2, J = 9 Hz, ArH ortho to hydroxyl), 6.93 (m, 4, ArH), 7.23 (s, 1, ArH ortho to bromine), 7.9 (br s, 2, OH); mass spectrum (70 eV), *m/e* (relative intensity) 350 (1), 348 (1, both M^+), 215 (5), 213 (5), 187 (3), 185 (4), 135 (100), 134 (14), 107 (36). Anal. ($C_{18}H_{21}BrO_2$) C, H, Br.

16α-Bromo-3-acetoxyestra-1,3,5(10)-trien-17-one (16α-Bromoestrone Acetate; 11a). Estrone 3-acetate (17-enol acetate)[28] was brominated with bromine and buffered with acetic acid according to the method of Johnson and Johns.[9a] The product was obtained as long white needles from ethanol (84%): mp 170–172 °C (lit.[9a] 168–170 °C); ^1H NMR ($CDCl_3$) δ 0.93 (s, 3, 18-methyl), 2.20 (s, 3, 3-acetate methyl), 4.60 (m, 1, 16β-H), 6.77, 6.83, 7.23 (3); mass spectrum (70 eV), *m/e* (relative intensity) 392 (9), 390 (9, M^+), 350 (99), 348 (100), 214 (40), 172 (19), 159 (24), 157 (16), 146 (28), 133 (20), 55 (20), 43 (23).

16α-Bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (16α-Bromoestrone; 11b). 16α-Bromoestrone acetate (**11a**; 0.30 g, 0.77 mmol) was hydrolyzed by treatment with concentrated sulfuric acid (1.0 mL) in anhydrous ethanol (20 mL) at room temperature. Product isolation (chloroform, sodium sulfate) gave a white solid that was recrystallized from benzene to afford 0.21 g (86%) of 16α-bromoestrone (**11b**): mp 226–228 °C (lit.[29] 225–228 °C); ^1H NMR (Me_2SO-d_6) δ 0.88 (s, 3, 18-methyl), 4.97 (dd, 1, J = 6 and 1.5 Hz, 16β-H), 6.45, 6.87, 7.80 (3 H), 7.3 (s, 1, OH); mass spectrum (70 eV), *m/e* (relative intensity) 350 (97), 348 (100, M^+), 269 (93), 241 (16), 214 (73), 172 (50), 159 (61), 157 (44), 133 (44), 91 (20), 79 (19), 53 (41), 28 (60).

16α-Bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (16α-Bromoestrone; 11b) and 16β-Bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (16β-Bromoestrone; 12a). A solution of 16α-bromoestrone acetate (**11a**; 0.50 g, 1.28 mmol) in 50 mL of ethanol was treated with concentrated sulfuric acid (2.63 mL) and heated at reflux for 20 h. Product isolation (ethyl acetate, sodium sulfate) afforded 0.42 g (93%) of a mixture of the epimeric 16-bromoestrones (**11b**, **12a**) as a white solid. The epimeric ratio, 16α/16β (**11b**/**12a**), was shown to be 1:1.8 by observation of the ^1H NMR resonances of the 16β/α protons, appearing at δ 4.97 and 4.52, respectively. Although fractional crystallization, analytical and preparative TLC, column chromatography, and reverse-phase high-pressure LC (C_{18}-functionalized SiO_2, methanol–water gradients) failed to separate these epimers, they were separated by normal phase high-pressure LC (4.6 mm × 25 cm Waters Porasil column utilizing a gradient of ether–hexane, 20–75% over 10 min). 16β-Bromoestrone (**12a**): mp 233–234 °C; ^1H NMR (Me_2SO-d_6) δ 1.02 (s, 3, 18-methyl), 4.52 (t, 1, J = 9 Hz, 16α-H), 6.45, 6.87, 7.80 (3), 7.3 (s, 1 OH); mass spectrum (70 eV), *m/e* (relative intensity) 350 (98), 348 (100, M^+), 269 (62), 214 (58), 199 (22), 172 (37), 159 (41), 133 (30), 91 (15), 79 (12), 55 (33), 41 (14), 28 (25). Anal. (exact mass, HR-EIMS) Calcd for $C_{18}H_{21}O_2Br$: 348.0723. Found: 348.0724.

16α-Bromoestra-1,3,5(10)-triene-3,17β-diol (16α-Bromoestradiol-17β; 13a) and 16α-Bromoestra-1,3,5(10)-triene-3,17α-diol (16α-Bromoestradiol-17α; 13b). A solution of 16α-bromoestrone acetate (**11a**; 0.50 g, 1.28 mmol) in tetrahydrofuran (3.0 mL) was added in a dropwise fashion to a rapidly stirred suspension of lithium aluminum hydride (0.19 g, 5.12 mmol) in tetrahydrofuran (10 mL) at 0 °C. After 2 h, the reaction was quenched by the careful addition of water, followed by 10% hydrochloric acid until strongly acidic. Product isolation (ethyl acetate, sodium sulfate) gave a solid that was shown to be a three-component mixture by TLC. ^1H NMR analysis indicated the ratio of epimeric 17β/17α alcohols to be ca. 2:1. The solid (0.38 g, 85%) was dissolved in tetrahydrofuran and adsorbed to 1.5 g of silica gel which was layered on top of a silica gel column (30 g). Elution with 5–10% ethyl acetate–benzene gave 0.11 g (25%) of 16α-bromoestradiol-17α (**13b**) and 0.19 g (42%) of 16α-bromoestradiol-17β (**13a**). Analytical samples were obtained by recrystallization from acetone (**13b**) or acetone–petroleum ether (**13a**).

16α-Bromoestradiol-17β (**13a**): mp 217–219 °C (lit.[9b] 217–219 °C); ^1H NMR (Me_2SO-d_6) δ 0.67 (s, 3, 18-methyl), 3.50 (t, 1, J = 5 Hz, 17α-H), 4.72 (m, 1, 16β-H), 5.13 (d, 1, J = 6 Hz, 17β-OH), 6.43, 6.52, 7.03 (3), 9.05 (3 OH); mass spectrum (70 eV), *m/e* (relative intensity) 352 (99), 350 (100, M^+), 271 (22), 253 (25), 251 (26), 185 (31), 159 (88), 145 (32), 133 (88), 107 (42), 81 (24), 57 (98), 44 (34).

16α-Bromoestradiol-17α (**13b**): mp 255–257 °C (lit.[9b] 253–255 °C); ^1H NMR (Me_2SO-d_6) δ 0.72 (s, 3, 18-methyl), 3.50 (t, 1, J = 5 Hz, 17β-H), 4.72 (m, 1, 16β-H), 5.13 (d, 1, J = 6 Hz, 17-OH), 6.43, 6.52, 7.03 (3), 9.05 (3 OH); mass spectrum (70 eV), *m/e* (relative intensity) 352 (20), 350 (20, M^+), 271 (16), 253 (14), 215 (17), 159 (31), 157 (16), 153 (46), 133 (31), 109 (65), 81 (100), 79 (46), 44 (87).

16β-Bromoestra-1,3,5(10)-triene-3,17β-diol (16β-Bromoestradiol-17β; 14). A solution of the mixture of 16α- and 16β-bromoestrones (**11b**, **12a**; 0.42 g, 1.20 mmol) in tetrahydrofuran (3 mL) was added in a dropwise fashion to a rapidly stirred solution of zinc borohydride[9b] in tetrahydrofuran (18 mL of 0.14 M solution, 4.80 mmol) at 0 °C. After 3 h at 0 °C, the reaction was quenched by the careful addition of water, followed by a solution of 5% hydrochloric acid until acidic. Product isolation (ethyl acetate, sodium sulfate) gave a white solid (0.40 g, 90%) that was shown to be a four-component mixture by TLC. The solid was dissolved in tetrahydrofuran and adsorbed to 1.0 g of silica gel which was layered on top of a column of silica gel (50 g). Elution with 5% ethyl acetate–benzene gave as the second component crystalline solid (0.090 g) that was identified as 16β-bromoestradiol-17β (**14**). An analytical sample, obtained by recrystallization from acetone–petroleum ether, melted at 176–178 °C, resolidifying, and remelting at 238–240 °C (lit.[12a] 176–178 °C and 255–256 °C); ^1H NMR (Me_2SO-d_6) δ 0.83 (s, 3, 18-methyl), 3.43 (t, 1, J = 6 Hz, 17α-H), 4.57 (q, 1, J = 7 Hz, 16α-H), 5.15 (d, 1, J = 6 Hz, 17β-OH), 6.42, 6.47, 7.03 (3), 9.01 (s, 1, OH); mass spectrum (10 eV), *m/e* (relative intensity) 352 (70), 350 (100, M^+), 270 (55), 215 (34), 185 (15), 159 (16), 133 (16).

16α-Chloro-3-acetoxyestra-1,3,5(10)-trien-17-one (11c). A solution of the enol acetate **10** (0.50 g, 1.4 mmol) in acetone (25 mL), buffered with sodium acetate (0.30 g) and glacial acetic acid (0.28 mL)/water (2.88 mL), was treated with *tert*-butyl hypochlorite (0.17 mL, 1.4 mmol). The clear solution was warmed to 55 °C and stirred for 1.5 h. Product isolation (ether, sodium sulfate) furnished a white solid that was crystallized from ethanol to give 16α-chloroestrone acetate (**11c**; 0.29 g, 61%). A second crop, taken from the mother liquor with ethanol–water, was shown by NMR analysis to be mostly the epimeric chloro ketone, 16β-chloroestrone acetate (**12b**).

16α-Chloroestrone acetate (**11c**): mp 165–167 °C (lit.[9b] 163–166 °C); ^1H NMR ($CDCl_3$) δ 1.00 (s, 3, 18-methyl), 2.30 (s, 3), 4.43 (m, 1, 16β-H), 6.80, 6.93, 7.23 (3); mass spectrum (70 eV), *m/e* (relative intensity) 348 (3), 346 (7, M^+), 306 (36), 304 (100), 214 (35), 71 (24), 57 (81), 43 (30).

16α-Chloro-3-hydroxyestra-1,3,5(10)-trien-17-one (16α-Chloroestrone; 11d). 16α-Chloroestrone acetate (**11c**; 0.085 g, 0.25 mmol) was hydrolyzed by treatment with concentrated sulfuric acid (0.28 mL) in absolute ethanol (5.5 mL) at room temperature for 6.5 h. Product isolation (ethyl acetate, sodium sulfate) gave a white solid which was crystallized from benzene to afford the phenol **11d** as white needles (0.047 g, 61%): mp 236–237 °C (lit.[9b] 239–240 °C); ^1H NMR (Me_2SO-d_6) 0.97 (s, 3, 18-methyl), 4.88 (d, 1, J = 6 Hz, 16β-H), 6.80, 6.92, 7.05 (3), 9.00 (1, s, OH); mass spectrum (70 eV), *m/e* (relative intensity) 306 (33), 304 (100, M^+), 240 (35), 214 (84), 199 (32), 172 (50), 159 (42), 146 (58), 44 (55).

16α-Chloroestra-1,3,5(10)-triene-3,17β-diol (16α-Chloroestradiol-17β; 13c) and 16α-Chloroestra-1,3,5(10)-triene-3,17β-diol (16α-Chloroestradiol-17α; 13d). A solution of 16α-chloroestrone acetate (**11c**; 0.30 g, 0.86 mmol) in tetrahydrofuran (7.5 mL) was chilled to 0 °C and a clarified solution of lithium aluminum hydride in THF (1.20 M, 1.44 mL, 1.73 mmol) (see Methods) was added in a slow, dropwise fashion with rapid magnetic stirring. After 45 min, the reaction was quenched by

(29) Fishman, J.; Biggerstaff, W. R. *J. Org. Chem.* 1958, *23*, 1190.

the dropwise addition of a 1:1 solution of tetrahydrofuran–ethyl acetate, followed by 10% hydrochloric acid until strongly acidic. Product isolation (ethyl acetate, sodium sulfate) gave a solid that was shown to be a three-component mixture by TLC, the most polar component being estradiol-17β. The solid was dissolved in tetrahydrofuran and adsorbed on to 0.80 g of silica gel that was layered on to a column of silica gel (35 g). Elution with 5% ethyl acetate–benzene gave 16α-chloroestradiol-17α (**13d**; 69 mg, 26%) and 16α-chloroestradiol-17β (**13c**; 178 mg, 67%). Analytical samples were obtained by crystallization from benzene.

16α-Chloroestradiol-17β (**13c**): mp 212–214 °C (lit.[30] 213–214 °C); ^{1}H NMR (MeSO-d_6) δ 0.70 (s, 3, 18-methyl), 3.60 (m, 1, 17α-H), 4.10 (m, 1, 16β-H), 5.27 (m, 1, 17β-OH), 6.77, 6.86, 7.10 (3), 8.93 (m, 1, OH); mass spectrum (70 eV), *m/e* (relative intensity) 308 (33), 306 (100, M$^+$), 220 (29), 185 (28), 172 (30), 160 (34), 159 (40), 133 (30), 43 (26), 28 (21).

(30) Nambara, T.; Nokubo, M.; Bae, Y. H. *Chem. Pharm. Bull.* **1971**, *19*, 2096.

16α-Chloroestradiol-17α (**13d**): mp 225–226 °C (lit.[30] 228–229 °C); ^{1}H NMR (MeSO-d_6) δ 0.73 (s, 3 H, 18-methyl), 3.53 (m, 1, 17β-H), 4.60 (m, 1, 16β-H), 4.93 (m, 1, 17α-OH), 6.40, 6.47, 7.00 (3), 8.90 (s, 1, OH); mass spectrum (70 eV) *m/e* (relative intensity) 308 (34), 306 (100, M$^+$), 220 (21), 185 (23), 172 (25), 160 (38), 159 (38), 133 (31), 41 (21).

Acknowledgment. This research was supported by grants from the American Cancer Society (BC-223) and the National Institutes of Health (AM 15556 and CA 25836). J.A.K. is a Camille and Henry Dreyfus Teacher–Scholar, and D.F.H. is a Proctor and Gamble Fellow. Alphonse McMahon, Kurt Raack, and Kenneth J. Christy assisted in the preparation of the A-ring brominated and fluorinated estradiols. The binding affinity measurements to the uterine estrogen receptor were performed by Kathryn E. Carlson. The high resolution mass spectrometry equipment used in this study was provided by a grant from the National Cancer Institute (CA 11388).

Estrogen Receptor Based Imaging Agents. 2. Synthesis and Receptor Binding Affinity of Side-Chain Halogenated Hexestrol Derivatives

Ramanuj Goswami, Stephen G. Harsy, Daniel F. Heiman, and John A. Katzenellenbogen*

The Roger Adams Laboratory, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801.
Received June 29, 1979

We have synthesized as potential imaging agents for human breast tumors a series of hexestrol analogues bearing the halogens fluorine, chlorine, bromine, and iodine at the terminus of the hexane chain. The binding affinity of these compounds for the estrogen receptor from uterine tissues forms a monotonically decreasing series, starting at 129% of that of estradiol for the fluoro analogue and decreasing to 60% for the iodo analogue. Such a modest decrease in binding affinity is thought to reflect the preference of the receptor for lipophilic groups and for substituents of moderate steric size at this site, parameters which change in opposite directions in the halogen sequence going from fluorine to iodine. Three estrogenic bis(trifluoromethyl)diphenylethylenes, prepared by DuPont, also showed substantial binding affinities for the estrogen receptor. In terms of ease of radiolabeling and high receptor binding selectivity, the compound that appears to be the most promising candidate for a breast tumor imaging agent in these series is the chain terminal fluorohexestrol.

γ-Emitting analogues of estrogens have the potential for being concentrated in tissues and tumors that contain estrogen receptors. An application of particular interest would be the use of such agents to provide diagnostic information about human breast tumors that have significant levels of estrogen receptor.[1] Such information would assist in the selection of the most appropriate therapeutic approach.[2]

We have undertaken a systematic investigation of various halogenated estrogen analogues in order to determine what structural features are important in attaining high binding selectivity, that is, high affinity for the estrogen receptor with relatively low affinity for other nonreceptor sites. In this paper, we describe synthesis of a series of hexestrol analogues that bear a halogen at the terminus of the hexane chain. We have also measured the binding affinity of these compounds together with some others that have been prepared elsewhere, for the estrogen receptor from uterine tissue. In related papers,[3] we describe the synthesis and receptor binding affinity of other potential estrogen receptor based imaging agents,[3a,b] the preparation of some of those compounds in tritium- and γ-labeled form,[3c,d] studies on their binding selectivity in vitro,[3c] and their target tissue and selective uptake in vivo.[3c,d]

Results

Chemical Synthesis. *erythro*-3,4-Bis(4-hydroxyphenyl)-1-hexanol (7). The approach to side-chain functionalized hexestrols is shown in Scheme I. Deoxyanisoin (**1**) was ethylated by sequential treatment with sodium hydride and ethyl iodide, giving a good yield (85%) of α-ethyldeoxyanisoin (**2**). The functionalized side chain was introduced by a Reformatsky reaction using methyl bromoacetate and zinc, producing a mixture of diastereomeric β-hydroxy esters (**3**). Although these diastereomers

(1) For reviews, see McGuire, W. L.; Horwitz, K. B.; Pearson, O. H.; Segaloff, A. *Cancer* **1977**, *39*, 2394. McGuire, W. L.; Horwitz, K. B.; Zava, D. T.; Garola, R. E.; Chamness, G. C. *Metabolism* **1978**, *27*, 487. McGuire, W. L.; Carbone, P. P.; Vollmer, G. P. "Estrogen Receptors in Human Breast Cancer"; Raven Press: New York, 1975. Allegra, J. C.; Lippman, M. E.; Thompson, E. B.; Simon, R.; Barlock, A.; Green, L.; Huff, K. K.; Do, H. M. T.; Aitken, S. C. *Cancer Res.* **1979**, *39*, 1447.

(2) LeClerq, G.; Heuson, J. C. *Curr. Probl. Cancer* **1976**, *1*, 1. Garola, R. E.; McGuire, W. L. *Cancer Res.* **1977**, *37*, 3333. Horwitz, K. B.; McGuire, W. L.; Pearson, O. H.; Segaloff, A. *Science* **1975**, *189*, 726.

(3) (a) Heiman, D. F.; Katzenellenbogen, J. A.; Neeley, R. J. *J. Med. Chem.*, preceding paper in this issue. (b) Allison, K. J.; Katzenellenbogen, J. A., in preparation. (c) Katzenellenbogen, J. A.; Carlson, K. E.; Heiman, D. F.; Goswami, R. *J. Nucl. Med.* **1980**, *21*, 550. (d) Katzenellenbogen, J. A.; Senderoff, S. G.; Welch, M. J.; McElvaney, K. D., in preparation. (e) Katzenellenbogen, J. A.; Heiman, D. F.; Carlson, K. E.; Lloyd, J. E. In "Receptor Binding Radiotracers" (Uniscience Series "Radiotracers in Biology and Medicine"; Colombetti, L. G., Ed.); Eckelman, W. C., Ed.; CRC Press, Boca Raton, FL, in press.

0022-2623/80/1823-1002$01.00/0