MOLECULAR PHARMACOLOGY, 20:43–51

Stereochemical Considerations in the Binding of Nonsteroidal Estrogens to the Estrogen Receptor

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Received December 12, 1980; Accepted February 24, 1981

SUMMARY

LANDVATTER, S. W., AND J. A. KATZENELLENBOGEN. Stereochemical considerations in the binding of nonsteroidal estrogens to the estrogen receptor. *Mol. Pharmacol.* **20**:43–51 (1981).

Derivatives of nonsteroidal estrogens, such as hexestrol, can interact with the estrogen receptor in four possible binding modes—two per enantiomer. Several side chain-functionalized hexestrol and norhexestrol derivatives have been synthesized and resolved into pure enantiomers. The absolute configuration of these enantiomers has been determined in order to assess which of the four binding modes is preferred. Binding studies with lamb uterine estrogen receptor have indicated that there is no appreciable difference in binding between enantiomers in the hexestrol series. Enantiomers in the norhexestrol series, on the other hand, do show significant differences in binding. The (−)-(2*R*,3*S*)-pentyl ester **26** binds to receptor with twice the affinity of racemic material and 14 times the affinity of the (+)-(2*S*,3*R*)-antipode. Similar though less dramatic differences are observed with methyl esters **18–20** and alcohols **30–32.** It is concluded that the norhexestrols prefer one of the four possible binding modes while the hexestrols can adopt two of the four modes equally. Furthermore, comparisons between the binding affinities of corresponding hexestrol and norhexestrol derivatives suggest that the source of chiral recognition is a specific interaction between the carbonyl group in the 2*R*, 3*S* enantiomer of the norhexestrol derivatives that elevates affinity, this interaction not being attainable in the other enantiomer and in the derivatives in the hexestrol series.

INTRODUCTION

The biological activity of steroidal hormones, such as estrogens, depends upon their interaction with certain high-affinity binding proteins, called receptors, that are found in the cells of target tissues. The interaction between a steroid and its receptor is of high affinity (K_d = 0.1–1 nM) and is characterized by a high degree of stereospecificity. Therefore, it is not surprising that small alterations in the structure of certain estrogens often affect the receptor-binding affinity of these compounds and their biological potency.

We have been interested in developing affinity labels for the estrogen receptor and receptor-based agents for imaging breast tumors (1–3). In this endeavor, we have used steroidal as well as certain nonsteroidal estrogens, such as hexestrol.[1] Hexestrol offers several distinct advantages over steroidal estrogen (estradiol) derivatives. The chemistry of hexestrol is simpler; hexestrol also has a lower binding affinity to the specific estrogen-binding proteins in serum, but has a binding affinity for the estrogen receptor that is 3-fold greater than estradiol. Furthermore, substituents on the aromatic rings of hexestrol often have a smaller influence on its affinity for the estrogen receptor than do the substituents at corresponding positions in the A-ring of estradiol (3).

We have undertaken a systematic study of various racemic and resolved hexestrol and norhexestrol derivatives in order to determine how alterations in absolute configuration affect binding to the estrogen receptor. In this paper, we describe the synthesis and resolution of several side chain-functionalized hexestrols and norhexestrols. The binding affinity of these compounds for the estrogen receptor was measured, and the results are discussed here in terms of the stereochemistry of binding to the estrogen receptor.

MATERIALS AND METHODS

Quinine monohydrate, *l*-ephedrine, and tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (III) [Eu(hfc)$_3$] were purchased from Aldrich Chemical Company (Milwaukee, Wisc.) and boron tri-

This work was supported by National Institutes of Health Grants AM15556 and CA25836.

[1] The common names and abbreviations used are: hexestrol, 3,4-bis(4-hydroxyphenyl)-hexane; norhexestrol, 2,3-bis(4-hydroxyphenyl)pentane; THF, tetrahydrofuran; ee, enantiomeric excess; diethylstilbestrol, (E)-3,4-bis(4-hydroxyphenyl)-3-hexene.

0026-895X/81/010043-09$02.00/0

bromide (99.9%) from Apache Chemicals, Inc. (Seward, Ill.). Other reagents and solvents employed were of analytical reagent grade or better. Ethanol-free chloroform was prepared by distillation from phosphorous pentoxide.

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were measured on a Beckman IR-12 spectrophotometer. Proton magnetic resonance spectra (^{1}H-NMR) were obtained at 90 MHz on a Varian EM-390 spectrometer, or where noted, at 220 MHz on a Varian HR-220 spectrometer; proton chemical shifts are reported in ppm downfield from tetramethylsilane as an internal standard (δ scale). Optical rotations were taken on a Rudolf Research Autopol III. Mass spectral data were obtained on a Varian Model CH-5 mass spectrometer. High-resolution mass spectra were obtained on a Varian 731 high-resolution mass spectrometer and were obtained in the Mass Spectrometry Laboratory,[2] School of Chemical Sciences, University of Illinois. Microanalytical data were provided by the Microanalytical Service Laboratory of the University of Illinois. Measurement of binding affinity to the estrogen receptor was performed by a competitive binding assay that has been described previously; binding data have been corrected so that they represent the ratio of association constants (3).

(±)-Erythro-3,4-bis(4-methoxyphenyl) hexanoic acid (2). Methyl (±)-*erythro*-3,4-bis(4-methoxyphenyl)-hexanoate (2) (**1**, 1.30 g, 3.83 mmoles), dissolved in 50 ml of THF, was mixed with 8 ml of 3 N NaOH and sufficient methanol to effect a homogeneous phase and stirred at reflux for 4 hr. The organic solvents were removed under reduced pressure and the residue was taken up in 200 ml of water. Extraction with ethyl acetate (EtOAc) followed by acidification of the aqueous phase with 6 N HCl afforded the crude acid which was recrystallized from EtOH/H_2O to give 1.87 g (97%) of white, crystalline **2**: m.p., 185–186°; IR (KBr), 3400 cm^{-1} (OH), 1720 cm^{-1} (C═O); ^{1}H-NMR ($CDCl_3$, 220 MHz) δ0.53 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.14–1.50 (*m*, 2, $CHCH_2CH_3$), 2.34 (*d*, 2, J = 5 Hz, CH_2COOH), 2.47 (*dt*, 1, J = 4, 11 Hz, $CHCH_2CH_3$), 3.00–3.19 (*m*, 1, $CHCH_2COOH$), 3.78 (*s*, 6, $ArOCH_3$), 6.81 (*d*, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 6.84 (*d*, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 7.07 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.11 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.71 (*s*, 1, COO*H*) mass spectrum (70 eV) *m/e* (relative intensity) 328 (3, M$^+$), 150 (11), 149 (100), 121 (19). Analysis of $C_{20}H_{24}O_4$: calculated: C, 73.15; H, 7.37; found: C, 72.80; H, 7.41.

Erythro-2,3-bis(4-methoxyphenyl)pentane nitrile (4). 2,3-Dimethoxy-α-cyanostilbene (4) (**3**, 3.0 g, 15.1 mmoles) was dissolved in 160 ml of dry THF, and cuprous iodide (288 mg, 1.51 mmoles) was added. The resulting suspension was placed under a nitrogen atmosphere and cooled to −20° in a Dry Ice/CCl_4 bath, and ethyl magnesium chloride in ether (26.6 ml, 45.3 mmoles) was added dropwise. After 2.5 hr, the reaction was poured over ice-dilute HCl with rapid stirring, and the THF removed under reduced pressure. The residue was extracted (EtOAc), washed (saturated aqueous NH_4Cl), and dried ($MgSO_4$). Solvent removal gave crude **4** which was recrystallized from EtOH giving 3.61 g of **4** (81%) as white needles: m.p., 131–132° [lit (5) 130–131°; IR (Nujol), 2230 cm^{-1} (CN); ^{1}H-NMR ($CDCl_3$) δ0.74 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.70 (quintet, 2, J = 5 Hz, CH_2CH_3), 2.67 (quartet, 1, J = 7 Hz, $CHCH_2$), 3.60 (*s*, 6, $ArOCH_3$), 3.77 (*d*, 1, J = 7 Hz, CHCN), 6.52 (*d*, 4, J = 8 Hz, Ar*H ortho* to OCH_3), 6.66 (*d*, 4, J = 8 Hz, Ar*H ortho* to alkyl); mass spectrum (70 eV) *m/e* (relative intensity) 295 (1, M$^+$), 150 (11), 149 (100), 121 (42), 91 (9)]. Analysis of $C_{19}H_{21}NO_2$: calculated: C, 77.26; H, 7.17; N, 4.74; found: C, 77.50; H, 7.15; N, 5.03.

(±)-Erythro-2,3-bis(4-methoxyphenyl)pentanoic acid (5). (±)-*Erythro*-2,3-bis(4-methoxyphenyl)pentane nitrile (**4**, 3.39 g, 11.5 mmoles) was dissolved in 20 ml of ethylene glycol. Sodium hydroxide (1.2 g, 30 mmoles) and 2.2 ml of water were added, and the mixture was heated to 160° for 36 hr. An equal volume of water was added, and the solution was filtered to remove a small amount of unreacted intermediate amide. Acidification with 1 N HCl gave a white precipitate which was recrystallized from EtOH giving 1.18 g (33%) of acid **5**: m.p., 178–180°; [lit (5) 177.5–179°] IR (KBr), 3190 cm^{-1} (OH), 1760 cm^{-1} (C═O); ^{1}H-NMR ($CDCl_3$, 220 MHz) δ0.55 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.15–1.49 (*m*, 2, CH_2CH_3), 3.04 (*dt*, 1, J = 4, 11 Hz, $CHCH_2$) 3.64 (*d*, 1, J = 11 Hz, CHCOOH), 3.77 (*s*, 3, $ArOCH_3$), 3.80 (*s*, 3, $ArOCH_3$), 6.79 (*d*, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 6.88 (*d*, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 7.12 (*d*, 2 J = 9 Hz, Ar*H ortho* to alkyl), 7.31 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl); mass spectrum (70 eV) *m/e* (relative intensity) 314 (4, M$^+$), 149 (100), 73 (58), 44 (57), 43 (83). Analysis of $C_{20}H_{24}O_4$: calculated: C, 72.59; H, 7.05; found: C, 72.28; H, 6.82.

(+)-(3S,4R)-(6) and (−)-(3R,4S)-Erythro-3,4-bis(4-methoxyphenyl)hexanoic acid (7). (±)-*Erythro*-3,4-bis(4-methoxyphenyl)hexanoic acid (**2**, 1.55 g, 3.52 mmoles) and quinine monohydrate (1.20 g, 3.52 mmoles) were dissolved in hot aqueous EtOH and allowed to crystallize slowly. After a total of seven recrystallizations, 760 mg of salt (32%) were recovered with >95% ee according to NMR (see Fig. 1). The free acid was recovered by dissolving the salt in aqueous EtOH and acidifying with 4 N HCl, extracting with EtOAc and drying ($MgSO_4$). Removal of solvent *in vacuo* gave 340 mg (58% based on pure enantiomer) of white crystalline **6**, which shows spectral characteristics identical with those of racemic acid **2**. (+)-Acid **6**: m.p., 181–182°; $[\alpha]_D^{21}$ = +1.7° (*c* = 0.377, dioxane); ^{1}H-NMR (resolved (+)-quinine salt (CD_3OD) δ3.58 (*s*, 3, acid $ArOCH_3$), 3.71 (*s*, 3, quinine $ArOCH_3$), 3.82 (*s*, 3, acid $ArOCH_3$). Analysis of $C_{20}H_{24}O_4$: calculated: C, 73.15; H, 7.37; found: C, 72.83; H, 7.17.

The mother liquors from the recrystallizations of acid **6** were concentrated, and the acid was recovered in the usual manner. The 485 mg (1.48 mmoles) thus obtained were combined with an equimolar amount of *l*-ephedrine (244 mg, 1.48 mmoles), dissolved in hot aqueous EtOH, and allowed to crystallize slowly. The *l*-ephedrine salt was recrystallized to a constant melting point (3 recrystallizations, m.p., 165–167°). The acid was recovered by the usual procedure and gave 293 mg (51% based on pure entatiomer) of **7** as white needles. The product shows identical spectral characteristics with racemic acid **2**. A small portion of resolved acid **7** was combined with an equimolar portion of quinine; ^{1}H-NMR showed the acid

[2] Supported in part by Grant GM 27029 from the National Institute of General Medical Sciences.

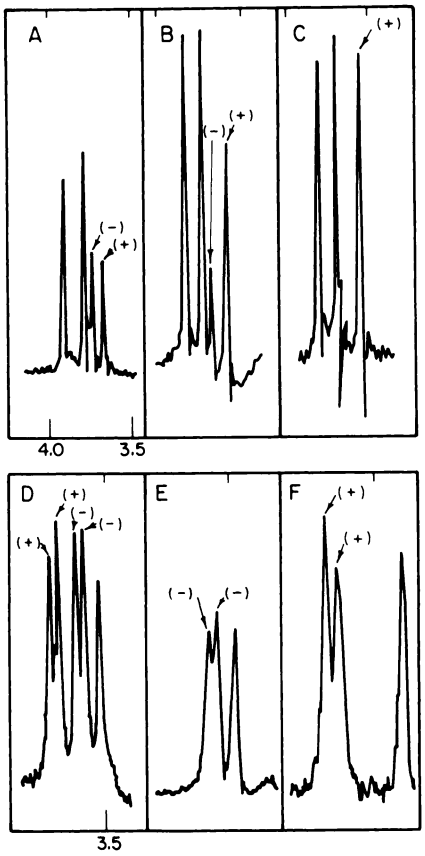


FIG. 1. *^{1}H-NMR (at 90 MHz) of hexestrol acid-quinine salt (A–C) and norhexestrol acid-quinine salt (D–F)*

Spectra were run in CD_3OD. Racemic material is shown in A (**2**) and D (**5**); partially resolved salt is shown in B and resolved material is shown in C [(+)-**6**], E [(−)-**9**], and F [(+)-**8**]. The two downfield singlets in A–C are from one of the two methyl ether groups on the hexestrol acid and from the methyl ether group on quinine. The two upfield singlets are from one methyl ether group on the acid in the diastereomeric salts with hexestrol acid enantiomers indicated. The upfield singlet in D–F is from the quinine methyl ether. The four downfield singlets are from the methyl ether groups on the norhexestrol acid. A–C are seen at a sweep width of 10 ppm and D–F at 2 ppm.

to have >95% ee. (−)-Acid **7**: m.p., 180–182°; $[\alpha]_D^{21} = -1.8°$ (c = 0.195, dioxane); ^{1}H-NMR (resolved (−)-quinine salt, CD_3OH) δ3.66 (s, 3, acid ArOCH_3), 3.71 (s, 3, quinine ArOCH_3), 3.82 (s, 3, acid ArOCH_3). Analysis of $C_{20}H_{24}O_4$: calculated: C, 73.15; H, 7.37; found: C, 72.93; H, 7.25.

*(+)-(2S,3R) (**8**) and (−)-(2R,3S)-erythro-2,3-bis(4-methoxyphenyl)pentanoic acid (**9**).* Resolved norhexestrol acids **8** and **9** were obtained in >95% ee and 87% ee respectively, according to the method of Collins and Hobbs (6). The resolutions could be followed by ^{1}H-NMR (see Fig. 1). The products display the same spectral characteristics as racemic acid **5**. (+)-Acid **8**: m.p., 173–174°; $[\alpha]_D^{25} = +24.7°$ (c = 1.50, dioxane; lit (6) = +22.7°, dioxane). Analysis of $C_{19}H_{22}O_4$: calculated: C, 72.59; H, 7.05; found: C, 72.29; H, 6.83. (−)-Acid **9**: m.p., 172–173°; $[\alpha]_D^{23} = -21.2°$ (c = 0.210, dioxane; lit (6) = −22.4°, dioxane). Analysis of $C_{19}H_{22}O_4$: calculated: C, 72.59; H, 7.05; found: C, 72.74; H, 7.06.

*Methyl (±)-erythro-3,4-bis(4-hydroxyphenyl)hexanoate (**15**).* Methyl (±)-*erythro*-3,4-bis(4-methoxyphenyl)-hexanoate (**1**, 510 mg, 1.49 mmoles) was dissolved in 5 ml of CH_2Cl_2 and cooled to −78°, and a 1 M solution of BBr_3 in CH_2Cl_2 (6 ml, 6 mmoles) was added dropwise. Stirring was continued for 1 hr at −78°, followed by storage at +6° for 4 hr. The solution was recooled to −78° and quenched by dropwise addition of anhydrous MeOH. The residue obtained after concentration *in vacuo* was taken up in EtOAc, filtered through ~1 g of neutral alumina and again taken to dryness. Recrystallization from EtOAc-cyclohexane gave 428 mg (91%) of white, crystalline bisphenolic methyl ester **15**: m.p., 166–168°; IR (KBr), 3430 cm^{-1} (OH), 1700 cm^{-1} (C=O); ^{1}H-NMR (acetone-d_6) δ0.50 (t, 3, J = 7 Hz, CH_2CH_3), 1.10–1.61 (m, 2, CH_2CH_3), 2.26 (d, 2, J = 7 Hz, CH_2COOCH_3), 2.38–2.82 (m, 1, $CHCH_2$), 2.87–3.14 (m, 1, $CHCH_2COO$—), 6.67 (d, 2, J = 9 Hz, Ar*H ortho* to OH), 6.71 (d, 2, J = 9 Hz, Ar*H ortho* to OH), 6.98 (d, 4, J = 9 Hz, Ar*H, ortho* to alkyl), 7.97 (s, 2, ArO*H*); mass spectrum (70 eV) *m/e* (relative intensity) 314 (1, M^+), 137 (24), 135 (100), 107 (45). Analysis (high resolution mass spectrum) of $C_{19}H_{22}O_4$: calculated: 314.1518; found 314.1522.

*Methyl (+)-(3R,4S)-erythro-3,4-bis(4-hydroxyphenyl)-hexanoate (**16**).* (−)-(3*R*,4*S*)-*Erythro*-3,4-bis(4-methoxyphenyl)hexanoic acid (**7**, 50 mg, 0.15 mmol) was dissolved in 5 ml of THF and cooled to 0°. A diazomethane solution in Et_2O was added dropwise until a yellow color persisted. A stream of dry N_2 was passed over the solution until it was again colorless. The mixture was filtered through glass wool and taken to dryness *in vacuo*, giving 52 mg (100%) of white crystalline methyl (−)-(3*R*,4*S*)-*erythro*-3,4-bis(4-methoxyphenyl)hexanoate **11**: m.p., 101–104°, ^{1}H-NMR (CCl_4) δ0.53 (t, 3, J = 7 Hz, CH_2CH_3), 1.04–1.59 (m, 2, CH_2CH_3), 2.10–2.57 (m, 3, $CHCH_2CH_3$ + CH_2COO—), 2.90–3.20 (m, 1, $CHCH_2COO$—), 3.29 (s, 3, $COOCH_3$), 3.75 (s, 6, ArOCH_3), 6,70 (d, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 6.73 (d, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 6.99 (d, 4, J = 9 Hz, Ar*H ortho* to alkyl).

Ester **11** was used directly in the next step without further purification or characterization. Demethylation with BBr_3, as previously described, gave (+)-(3*R*,4*S*)-bisphenolic methyl ester **16** (37 mg, 80%) as white needles. This compound displays spectral characteristics identical with those of ester **15**. Ester **16**: m.p., 167–170°; $[\alpha]_D^{22} = +8.1°$ (c = 0.210, dioxane). Analysis (high resolution mass spectrum) of $C_{19}H_{22}O_4$: calculated: 314.1518; found: 314.1521.

*Methyl (−)-(3S,4R)-erythro-3,4-bis(4-hydroxyphenyl)-hexanoate (**17**).* This compound was prepared in 92% yield from (+)-acid **6** according to the preceding preparation of the corresponding (+)-methyl ester **16**. The product shows spectral characterisitics identical with those of racemic ester **15**. (−)-Methyl ester **17**: m.p., 172.5–174°; $[\alpha]_D^{22} = -8.0°$ (c = 0.150, dioxane). Analysis (high resolution mass spectrum) of $C_{19}H_{22}O_4$: calculated: 314.1518; found: 314.1521.

*Methyl (±)-(**18**), (+)-(2S,3R)-(**19**), and (−)-(2R,3S)-erythro-2,3-bis(4-hydroxyphenyl)pentanoate (**20**).* Methyl esters **18**, **19**, and **20** were prepared in 98% (279 mg), 82% (123 mg), and 72% (159 mg) yield from racemic acid **5**, (+)-acid **8** and (−)-acid **9**, respectively, in the same manner as hexestrol methyl esters **16** and **17**.

Methyl (±)-*erythro*-2,3-bis(4-methoxyphenyl)pentanoate **12**: m.p., 123–125°; ^{1}H-NMR($CDCl_3$) δ0.57 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.00–1.62 (*m*, 2, CH_2CH_3), 2.82–3.56 (*m*, 1, $CHCH_2$), 3.41 (*s*, 3, $COOCH_3$), 3.79 (*d*, 1, J = 12 Hz, $CHCOO$—), 3.90 (*s*, 6, $ArOCH_3$), 6.99 (*d*, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 7.04 (*d*, 2, J = 9 Hz, Ar*H ortho* to OCH_3), 7.32 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.52 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl). This compound was used directly without further purification or characterization.

Methyl (±)-*erythro*-2,3-bis(4-hydroxyphenyl)pentanoate **18**: m.p., 180–181°; IR (KBr), 3440 cm^{-1} (OH), 1730 cm^{-1} (C═O); ^{1}H-NMR (acetone-d_6) δ0.57 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.17–1.57 (*m*, 2, CH_2CH_3), 2.90–3.27 (*m*, 1, $CHCH_2$), 3.39 (*s*, 3, $COOCH_3$), 3.79 (*d*, 1, J = 12 Hz, $CHCOO$—), 6.93 (*d*, 2, J = 9 Hz, Ar*H ortho* to ArOH), 7.00 (*d*, 2, J = 9 Hz, Ar*H ortho* to ArOH), 7.29 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.48 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 8.20 (*s*, 1, ArO*H*), 8.40 (*s*, 1, ArO*H*); mass spectrum (70 eV) *m/e* (relative intensity) 300 (2, M$^+$), 135 (100), 107 (60), 41 (14). Analysis of $C_{18}H_{20}O_4$: calculated: C, 71.97; H, 6.71; found: C, 71.86; H, 6.61.

Methyl (+)-(2*S*,3*R*)-*erythro*-2,3-bis(4-hydroxyphenyl)-pentanoate **19**: m.p., 241–243°; $[\alpha]_D^{22}$ = +25.5° (*c* = 0.243, dioxane). Analysis of $C_{18}H_{20}O_4$: calculated: C, 71.97; H, 6.71; found: C, 71.76; H, 6.97.

Methyl (−)-(2*R*,3*S*)-*erythro*-2,3-bis(4-hydroxyphenyl)-pentanoate **20**: m.p., 183–185°; $[\alpha]_D^{21}$ = −22.3° (*c* = 0.260, dioxane). Analysis of $C_{18}H_{20}O_4$: calculated: C, 71.97; H, 6.71; found: C, 71.80; H, 6.72.

*Pentyl (±)-erythro-3,4-bis(4-hydroxyphenyl)hexanoate (**21**).* Racemic bisphenolic methyl ester **15** (20 mg, 0.064 mmol) was dissolved in 1.1 ml of *n*-pentanol and saturated with anhydrous HCl. After stirring for 1 hr at room temperature, the solution was resaturated with HCl and heated to 90° for 7 hr with resaturation again at 1.5 hr. The solvent was removed *in vacuo*, the residue was taken up in acetone, filtered through ~1 g of neutral alumina, and again taken to dryness, giving pentyl ester **21.** Recrystallization from EtOAc-cyclohexane gave 7.4 mg (37%) of product. This yield can be increased to >70% with additional rinsings of the alumina column. Pentyl ester **21**: m.p., 177–177.5°; IR (KBr), 3430 cm^{-1} (OH), 1695 cm^{-1} (C═O); ^{1}H-NMR (acetone-d_6) δ0.51 (*t*, 3, J = 7 Hz, CH_2CH_3), 0.80 (*t*, 3, J = 6 Hz, *n*-$C_4H_8CH_3$), 0.97–1.63 [*m*, 8, $CHCH_2$— + $OCH_2(CH_2)_3CH_3$], 2.26 (*d*, 2, J = 8 Hz, CH_2COO—), 2.30–2.80 (*m*, 1, $CHCH_2$), 2.92–3.36 (*m*, 1, $CHCH_2COO$—), 3.68 (*t*, 2, J = 6 Hz, OCH_2—), 6.69 (*d*, 2, J = 9 Hz, Ar*H ortho* to ArOH), 6.73 (*d*, 2, J = 9 Hz, Ar*H ortho* to ArOH), 7.00 (*d*, 4, J = 9 Hz, Ar*H ortho* to alkyl), 7.97 (*s*, 2, ArO*H*); mass spectrum (70 eV) *m/e* (relative intensity) 370 (1, M$^+$), 135 (100), 107 (30), 43 (23). Analysis (high resolution mass spectrum) of $C_{23}H_{30}O_4$: calculated: 370.2144; found: 370.2154.

*Pentyl (+)-(3R,4S)-(**22**) and (−)-(3S,4R)-erythro-3,4-bis(4-hydroxyphenyl)hexanoate (**23**).* Pentyl esters **22** and **23** were prepared in 72% (8 mg) and 77% (10 mg) yield from bisphenolic methyl esters **16** and **17**, respectively, in the same manner as racemic pentyl ester **21.** These products show identical spectral characteristics with racemic ester **21.** (+)-Pentyl ester **22**: m.p., 177–179°; $[\alpha]_D^{22}$ = +10.8° (*c* = 0.030, dioxane). Analysis (high resolution mass spectrum) of $C_{23}H_{30}O_4$: calculated: 370.2144; found: 370.2150.

(−)-Pentyl ester **23**: m.p., 184–185°; $[\alpha]_D^{22}$ = −10.8° (*c* = 0.210, dioxane). Analysis (high resolution mass spectrum) of $C_{23}H_{30}O_4$: calculated: 370.2144; found: 370.2139.

*Pentyl (±)-(**24**), (±)-(2S,3R)-(**25**), and (−)-(2R,3S)-erythro-2,3-bis(4-hydroxyphenyl)pentanoate (**26**).* Pentyl esters **24–26** were prepared in 64% (47 mg), 76% (88 mg), and 70% (12 mg) yield from bisphenolic methyl esters **18–20**, respectively, in the same way as hexestrol pentyl esters **21–23**, except that the reaction was heated at 90° for 21 hr.

(±)-Pentyl ester **24**: m.p., 140–142°; IR (KBr), 3430 cm^{-1} (OH), 1720 cm^{-1} (C═O); ^{1}H-NMR (acetone-d_6) δ0.52 (*t*, 3, J = 7 Hz, CH_2CH_3), 0.76 (*t*, 3, J = 6 Hz, *n* − $C_4H_8CH_3$), 0.91–1.53 [*m*, 8, CH_2CH_3 + $OCH_2(CH_2)_3CH_3$], 2.72–3.20 (*m*, 1, $CHCH_2$), 3.42–3.72 (*m*, 3, $CHCOO$— + OCH_2—), 6.50 (*d*, 2, J = 9 Hz, Ar*H ortho* to ArOH), 6.56 (*d*, 2, J = 9 Hz, Ar*H ortho* to ArOH), 6.82 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.02 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.83 (*s*, 2, ArO*H*); mass spectrum (70 eV) *m/e* (relative intensity) 356 (1, M$^+$), 136 (11), 135 (100), 107 (36). Analysis (high resolution mass spectrum) of $C_{22}H_{28}O_4$: calculated: 356.1988; found: 356.1991.

(+)-Pentyl ester **25**: m.p., 165–167°; $[\alpha]_D^{24}$ = +10.2° (*c* = 0.133, dioxane). Analysis (high resolution mass spectrum) of $C_{22}H_{28}O_4$: calculated: 356.1988; found: 356.1992. Other spectral characteristics were identical with racemic pentyl ester **24.**

(−)-Pentyl ester **26**: m.p., 155–157°; $[\alpha]_D^{24}$ = −8.9° (*c* = 0.090, dioxane). Analysis (high resolution mass spectrum) of $C_{22}H_{28}O_4$: calculated: 356.1988; found: 356.2006. Other spectral characteristics were identical with those of racemic ester **24**.

*(±)-Erythro-3,4-bis(4-hydroxyphenyl)-1-hexanol (**27**).* Lithium aluminum hydride (3.5 ml of a 0.16 M solution in THF, 0.56 mmol) was added slowly, with stirring, to 174 mg of bisphenolic methyl ester **15** (0.056 mmol) in 5 ml of THF. After 15 min, the reaction was quenched by cautious addition of anhydrous MeOH followed by removal of solvent *in vacuo*. The residue was partitioned between 5% HCl and EtOAc. The combined organic layers were washed (5% $NaHCO_3$) and dried ($MgSO_4$). Removal of solvent gave 141 mg (88%) of white crystalline **27**. An analytical sample was obtained by recrystallization from THF-hexane. Alcohol **27**: m.p., 222–223°; IR (KBr), 3430 cm^{-1} (OH); ^{1}H-NMR (acetone-d_6) δ0.53 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.09–1.82 (*m*, 4, CH_2CH_3 + CH_2CH_2OH), 2.30–2.84 (*m*, 2, benzylic *H* atoms), 2.78 (*s*, 1, O*H*), 3.09 (*t*, 2, J = 7 Hz, CH_2OH), 6.73 (*d*, 4, J = 9 Hz, Ar*H ortho* to ArOH), 6.99 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.02 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.96 (*s*, 2, ArO*H*); mass spectrum (70 eV) *m/e* (relative intensity) 286 (1, M$^+$), 151 (31), 135 (100), 121 (26), 107 (70). Analysis of $C_{18}H_{23}O_3$: calculated: C, 75.50; H, 7.74; found: C, 75.20; H, 7.59.

*(+)-(3R,4S)-(**28**) and (−)-(3S,4R)-Erythro-3,4-bis(4-hydroxyphenyl)-1-hexanol (**29**).* Alcohols **28** and **29** were obtained from methyl esters **16** and **17** in 93% (117 mg) and 87% (134 mg) yield, respectively, by the method

described above for preparation of alcohol **27**. Both compounds display identical spectral characteristics with racemic **27**.

(+)-Hexanol **28**: m.p., 228–230°; $[\alpha]_D^{24}$ = +7.5° (*c* = 0.080, dioxane). Analysis (high resolution mass spectrum) of $C_{18}H_{22}O_3$: calculated: 286.1567, found: 286.1565.

(−)-Hexanol **29**: m.p., 228–230°; $[\alpha]_D^{24}$ = −7.5° (*c* = 0.100, dioxane). Analysis of $C_{18}H_{22}O_3$: calculated: C, 75.50; H, 7.74; found: C, 75.53; H, 7.64.

*(±)-(**30**), (+)-(2S,3R)-(**31**) and (−)-(2R,3S)-erythro-2,3-bis(4-hydroxyphenyl)-1-pentanol (**32**).* Pentanols **30**, **31**, and **32** were obtained from methyl esters **18–20** in 96% (261 mg), 94% (85 mg), and 96% (39 mg) yield, respectively, via lithium aluminum hydride reduction as described for alcohols **27–29.**

(±)-Pentanol **30**: m.p., 220–221°; IR (KBr), 3450 cm^{-1} (OH); ^{1}H-NMR (acetone-d_6) δ0.52 (*t*, 3, J = 7 Hz, CH_2CH_3), 1.06–1.48 (*m*, 2, CH_2CH_3), 2.57–2.83 (*m*, 2, $CHCH_2CH_3$ + CH_2OH), 3.41 (*d*, 2, J = 6 Hz, CH_2OH), 6.77 (*d*, 4, J = 9 Hz, Ar*H ortho* to ArOH), 7.04 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.08 (*d*, 2, J = 9 Hz, Ar*H ortho* to alkyl), 7.99 (*s*, 2, ArO*H*); mass spectrum (70 eV) *m/e* (relative intensity) 272 (1, M^+), 135 (100), 134 (16), 107 (48). Analysis of $C_{17}H_{20}O_3$: calculated: C, 74.97; H, 7.40; found: C, 74.68; H, 7.58.

(+)-Pentanol **31**: m.p., 225°; $[\alpha]_D^{22}$ = +5.0° (*c* = 0.190, dioxane). Analysis (high resolution mass spectrum) of $C_{17}H_{20}O_3$: calculated: 272.1412; found: 272.1410.

(−)-Pentanol **32**: m.p., 226°; $[\alpha]_D^{22}$ = −4.2° (*c* = 0.130, dioxane). Analysis (high resolution mass spectrum) of $C_{17}H_{20}O_3$: calculated: 272.1412; found: 272.1411. Other spectral characteristics are the same as **30.**

*Absolute configuration of (+)-(3S,4R)-(**6**) and (−)-(3R,4S)-erythro-3,4-bis(4-methoxyphenyl)hexanoic acid (**7**).* (+)-(2*S*,3*R*)-*Erythro*-2,3-bis(4-methoxyphenyl) pentanoic acid (**8**, 100 mg, 0.32 mmol) was dissolved in 2 ml of EtOH-free $CHCl_3$. Thionyl chloride (0.240 ml, 3.30 mmoles) was added, and the solution was heated to 50° for 24 hr. The reaction mixture was taken to dryness; 1 ml of EtOH-free $CHCl_3$ was added, and solvents were reevaporated. This procedure was repeated twice. The residue was dissolved in 2 ml of EtOH-free $CHCl_3$, and 5 ml of CH_2N_2 (3.2 mmoles, in Et_2O) added at 0° in the dark. After 3 hr at 0° and 21 hr at room temperature, preparative thin-layer chromatography ($CHCl_3/Et_2O$, 5:2) of the concentrated reaction mixture gave 41 mg (38%) of (+)-(3*S*,4*R*)-*erythro*-1-diazo-3,4-bis(4-methoxyphenyl)-2-hexanone. This diazoketone (36 mg, 0.11 mmol) was dissolved in dioxane-H_2O in a quartz tube and irradiated with a sunlamp for 30 hr at room temperature. Removal of solvent *in vacuo* and two recrystallizations from aqueous EtOH gave 19 mg (55%) of white, crystalline (+)-(3*S*,4*R*)-*erythro*-3,4-bis(4-methoxyphenyl)hexanoic acid (**6**): m.p., 180–181°; $[\alpha]_D^{21}$ = +1.8° (*c* = 0.180, dioxane). Analysis (high resolution mass spectrum) of $C_{20}H_{24}O_4$: calculated: 328.1674; found: 328.1681.

In similar fashion, (−)-(3*R*,4*S*)-hexestrol acid **7** was obtained in 20% yield (23 mg) from (−)-(2*R*,3*S*)-acid **9**. (−)-Hexestrol acid **7**: m.p., 180–181°; $[\alpha]_D^{22}$ = −1.6° (*c* = 0.237, dioxane). Analysis (high resolution mass spectrum) of $C_{20}H_{24}O_4$: calculated: 328.1674; found: 328.1677.

Both acids **6** and **7**, prepared from acids **8** and **9**, respectively, by the Arndt-Eistert homologation procedure above, were identical to the acids **6** and **7** obtained by resolutions of the racemic acid **2**, except that **7** prepared in this manner had a slightly lower rotation, because its precursor **9** was only 87% ee.

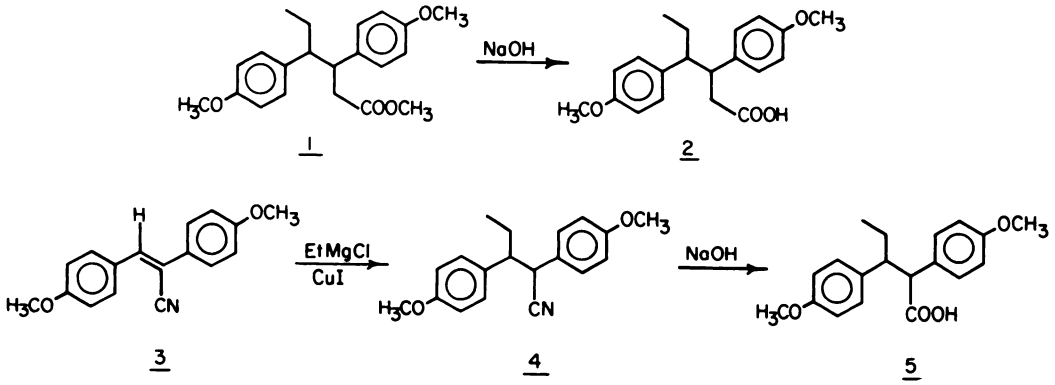
RESULTS

Chemical synthesis. Resolution of hexestrol and norhexestrol acids. Racemic hexestrol acid **2** and norhexestrol acid **5** were obtained as outlined in Scheme 1. Base-catalyzed ester hydrolysis of methyl ester **1**, previously prepared in our laboratory (2), afforded acid **2** in nearly quantitative yield. Noracid **5** was obtained in two steps starting from 4,4′-dimethoxy-α-cyanostilbene **3** (4). Copper-catalyzed Grignard addition to **3** gives up to a 9:1 mixture of *erythro* and *threo* isomers in 90% overall yield. This is a distinct improvement over the previously reported procedure (5) which gives a 0.77:1 mixture of *erythro* and *threo* isomers in the Grignard addition without copper catalysis. Unfortunately, base-catalyzed nitrile hydrolysis gives noracid **5** in only 33% yield. Other modes of hydrolysis (base and acid catalysis with varying reaction conditions; activated manganese dioxide) proved unsuccessful.

With the acids in hand, resolution was successfully carried out via quinine and *l*-ephedrine diastereomeric salt formation and fractional crystallization, according to the method of Collins and Hobbs (6). As shown in Fig. 1, progress of the resolution of the (+)-antipodes was conveniently monitored by ^{1}H-NMR of the quinine salts. In methanol-d_4, the quinine-hexestrol acid **2** salt shows one set of nonequivalent acid methyl ether protons. These appear as two singlets for racemic salt (Fig. 1A) with the high-field signal corresponding to the (+)-(3*S*,4*R*)-enantiomer (**6**) and the low field to the (−)-(3*R*,4*S*)-enantiomer (**7**). Progressive resolution by recrystallization of the less soluble quinine (+)-**6** salt is evident by the decrease in the low-field signal (Fig. 1, B and C). In contrast, the quinine-norhexestrol acid **5** salt shows nonequivalence in both sets of methyl ether protons of the acid; thus, these appear as four singlets for racemic material (Fig. 1D). Resolved (−)-(2*R*,3*S*)-noracid (**9**)-quinine salt displays only two singlets (Fig. 1E), as does (+)-(2*S*,3*R*)-noracid (**8**)-quinine salt (Fig. 1F).

In both cases, the (+)-antipode was easily obtained by recrystallization of the quinine salt. The (−)-antipodes, especially in the case of the norhexestrol acid, were obtained only with great difficulty. The optical purity of both enantiomers of hexestrol acid **2** are >95% enantiomeric excess as determined by the previously described ^{1}H-NMR technique and chiral lanthanide shift studies on the (+)-(**10**) and (−)-(**11**) methyl esters with Eu(hfc)$_3$. The (+)-noracid **8** is also obtained in >95% enantiomeric excess and was determined in the same manner as hexestrol acid antipodes. The (−)-noracid **9** is obtained with 87% enantiomeric excess, as determined by optical rotation.

*Absolute configuration of (+)-(**6**) and (−)-hexestrol acid (**7**).* Absolute configurations of (+)-(**6**) and (−)-hex-



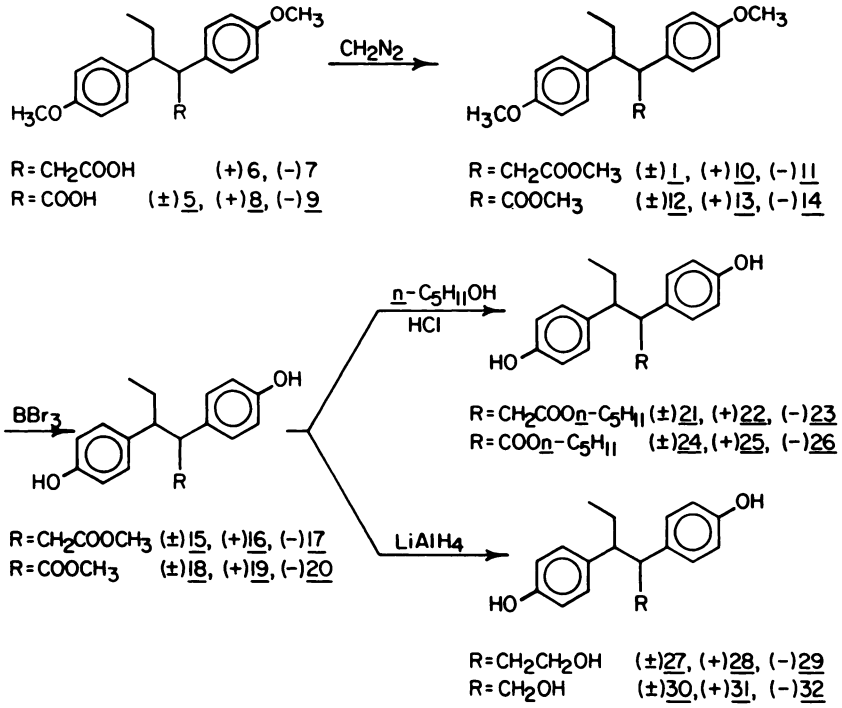
SCHEME 1. *Preparation of hexestrol and norhexestrol acids*

estrol acid (**7**) were determined by employing an Arndt-Eistert reaction sequence on resolved noracids **8** and **9**, as the absolute configurations of these acids has previously been determined (6, 7). Furthermore, the initial Wolff rearrangement is known to proceed with complete, or nearly complete, retention of configuration (8). Thus, (+)-(**8**) and (−)-*erythro*-2,3-bis(4-methoxyphenyl)pentanoic acid (**9**) are converted to their acid chlorides via treatment with thionyl chloride. Reaction with diazomethane followed by photolysis of the diazoketone in dioxane-water affords the homologated acids. The (+)-noracid **8** is converted to the (+)-hexestrol acid **6**. Since the absolute configuration of **8** is 2*S*,3*R*, (+)-hexestrol acid **6** must have the 3*S*,4*R* configuration. Similarly, (−)-(2*R*,3*S*)-noracid **9** is converted to hexestrol acid **7**, which must therefore be 3*R*,4*S*.

Side-chain-functionalized hexestrols and norhexestrols. The approach to side-chain-functionalized hexestrols and norhexestrols is illustrated in Scheme 2. Esterification with diazomethane on the appropriate acid is quantitative. Methyl ether cleavage with boron tribromide in methylene chloride followed by methanol quench affords bisphenolic methyl esters **15–20**. The yields are variable (72–98%) and seem to be dependent upon boron tribromide purity and reaction temperature during addition and quenching (−78°). This modification of the literature procedure (9), which involves a water quench at room temperature, gives improved yields and products of high purity after passage through neutral alumina.

Bisphenolic pentyl esters **21–26** were prepared by hydrogen chloride-catalyzed ester interchange on the appropriate methyl ester. Triols **27–32** were obtained in nearly quantitative yield via reduction of the appropriate methyl ester with lithium aluminum hydride.

Binding affinity of hexestrols and norhexestrols for the uterine estrogen receptor. The binding affinity of nonradiolabeled estrogen analogues for estrogen receptor can be measured readily by a competitive binding assay (3). The affinities are obtained relative to that of the tracer compound [^{3}H]estradiol, and are conveniently ex-



SCHEME 2. *Preparation of hexestrol and norhexestrol derivatives*

pressed as a ratio of association constants on a percentage scale, in which the binding of estradiol is defined at 100%. The binding affinities are shown in Table 1.

Two striking contrasts are immediately evident between the hexestrol and norhexestrol series. First, although there is no significant difference in binding between any of the racemic and optically pure hexestrol derivatives, there is a dramatic difference apparent in the norhexestrol series: the (−)-(2*R*,3*S*)-pentyl norester **26** binds 14.7 times as well as its enantiomer **25**, and the (−)-(2*R*,3*S*)-methyl norester **20** binds 2.1 times as well as its enantiomer **19**; the (−)-(2*R*,3*S*)-noralcohol **32** has only 1.6 times the affinity for the estrogen receptor as its (+)-(2*S*,3*R*)-antipode **31**, however. Second, within each stereochemical series (i.e., +, −, and racemic), the norhexestrol compounds generally show a higher affinity for the estrogen receptor than do their hexestrol counterparts: racemic methyl norester **18** has an affinity 3.5 times that of the racemic hexestrol methyl ester **15**, whereas the racemic pentyl norester **24** binds over 9 times as well as its hexestrol analogue. Only the nortriols **30–32** bind less well than their hexestrol homologues.

DISCUSSION

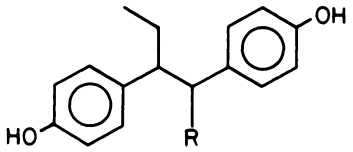
The steroidal estrogens are relatively rigid, polycyclic molecules with pronounced asymmetry, and their binding to the estrogen receptor generally reflects a high degree of stereospecificity. Thus, steroidal estrogens are considered to be bound to receptor in a unique manner, and, as such, substituents on steroidal estrogens should be positioned within the receptor binding site in an unambiguous fashion. In contrast, the nonsteroidal estrogens such as *meso*-hexestrol and its derivatives have elements of symmetry and conformational flexibility so that one cannot be certain in what way their substituents will be positioned within the binding site of the receptor. Thus, there appear to be four orientations that an unsymmetrical, side-chain-substituted hexestrol derivative might reasonably adopt when bound to the estrogen receptor, two per enantiomer (3, 10) (Scheme 3). These can best be defined if one considers that carbon atoms 3 and 4 in the hexane chain of hexestrol (2 and 3 in the pentane chain of norhexestrol) are roughly analogous to carbon atoms 8 and 9 of estradiol, which have an 8β, 9α configuration. If the side-chain-functionalized hexestrols and norhexestrols bind in the "normal" mode, in which the 8β, 9α stereochemical congruency is maintained, one enantiomer will project its substituent into the "upper left" region of the receptor and the other into the "lower right". However, if they bind in the "reversed" mode (8α, 9β), this is reversed: the first enantiomer projects its group into the lower right and the second into the upper left.

Evidence for the importance of the stereochemical configuration at these carbon atoms in the binding of ligands to the estrogen receptor appears to be, in part, contradictory. For example, compared to *meso*-hexestrol, (+)-(3*R*,4*R*) and (−)-(3*S*,4*S*)-hexestrol have an affinity only a few per cent of that of *meso*-hexestrol,[3] and the

[3] Ellis has determined the binding affinity of 140 steroid derivatives for a partially purified estrogen-binding protein from mature rat uterus by using a competitive binding assay with [^{3}H]estradiol and charcoal

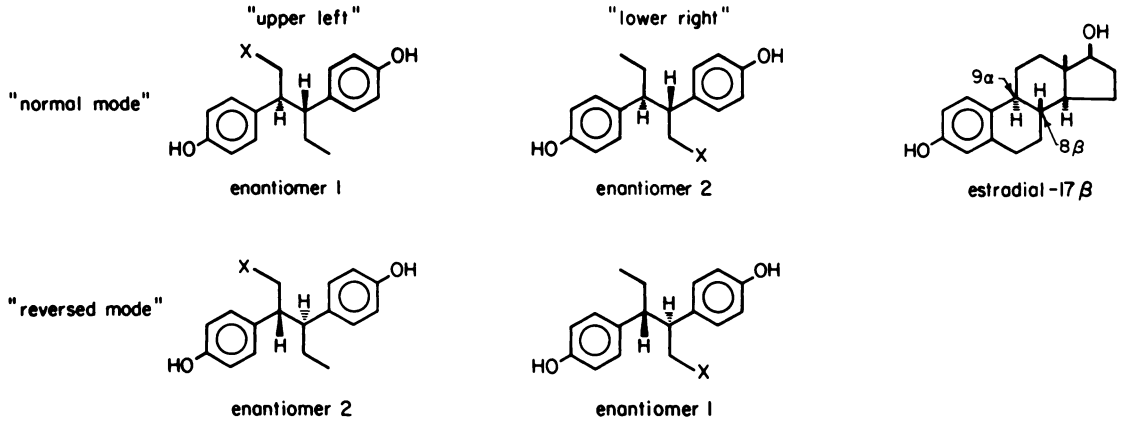
TABLE 1

Estrogen receptor binding affinities of hexestrol and norhexestrol derivatives



R	Racemic	Ratio of association constants × 100[a] (estradiol-17β = 100) (*R*,*S*) (3*R*,4*S*)	(*S*,*R*) (3*S*,4*R*)	(*R*,*S*)/(*S*,*R*)
Hexestrol series				
$CH_2CO_2CH_3$	20.0	19.1	20.5	0.93
CH_2CO_2n–C_5H_{11}	4.2	4.5	4.7	0.96
CH_2CH_2OH	16.2	17.8	15.5	1.15
		(2*S*,3*R*)	(2*R*,3*S*)	
Norhexestrol series				
CO_2CH_3	69.2	37.2	77.0	2.1
CO_2n–C_5H_{11}	38.9	5.3	78.0	14.7
CH_2OH	8.9	6.3	10.2	1.6

[a] The ratio of association constants ($K_a^{compound}/K_a^{estradiol}$) were determined in a competitive radioreceptor binding assay by using lamb uterine cytosol as a source of estrogen receptor, [^{3}H]estradiol as a tracer, and charcoal-dextran adsorption to separate free and bound tracer. Affinities are measured relative to that of estradiol (=100). Data represent duplicate or triplicate determinations, and in each case, enantiomeric pairs have been compared within the same experiment. Within each experiment, relative affinities are reproducable within 15% and within 30% between different assays. For details, see ref. 3.



SCHEME 3. *Modes of binding of hexestrol and norhexestrol side chain derivatives with the estrogen receptor*

enantiomer of estradiol has an affinity only 1% that of estradiol.[4] In the first case, however, alteration of the configuration of a single center in hexestrol causes a profound change in conformational preferences, shifting from preferred antiperiplanar in the *meso* diastereomer to preferred synclinal in the *dl*-series (11), and in the case of *ent*-estradiol, configuration at three additional centers have been altered.

There are cases in which changes in configuration at these centers seem to have little effect on receptor binding affinity: 8α-estradiol, which is epimeric to estradiol at C-8, binds with an affinity 57% that of estradiol (12); there is only a 1.6-fold difference in the binding affinity of (+)-(3*R*,4*R*)- and (−)-(3*S*,4S)-hexestrol, and diethylstilbestrol, which has both of these centers sp^2 hybridized, has an affinity very close to that of *meso*-hexestrol. Furthermore, from examination of space-filling models, it is difficult to perceive differences in steric contour between *meso*-hexestrol oriented in the normal versus reversed orientation (cf. Scheme 3).

The racemic modifications and resolved enantiomers in the hexestrol series have nearly identical affinities for the estrogen receptor (cf. Table 1). This implies either that the receptor does discriminate between the configurations at carbon atoms 3 and 4 but is capable of accommodating the various side-chain substituents in the lower right or upper left region of the receptor with equal facility, or that the receptor can tolerate the substituents at one of these regions but binds the enantiomers equally because it cannot distinguish between the normal and reversed mode of ligand presentation. There are some binding data in the literature that suggest that the latter situation is more likely. For example, by using estrogenic activity as a measure of receptor binding, it was found that *erythro*-5,6-bis(4-hydroxyphenyl)decane is 0.2% as active as hexestrol and that *erythro*-4,5-bis(4-hydroxyphenyl)-2,7-dimethyloctane is only 0.02% as active as hexestrol (ref. 13 and references therein). Thus, hexestrol derivatives that present two bulky side-chain substituents simultaneously in the upper left and lower right regions of the receptor, are bound very poorly.

The situation with the norhexestrols is clearly different, as here large differences in the binding affinity of certain enantiomers indicate that the receptor is capable of configurational differentiation at these stereocenters. It is of note that the preferred binding of one enantiomer is most markedly apparent in the two esters, which suggest that one enantiomer [(−)-(2*R*,3*S*)-antipode] may be engaging in a productive interaction (perhaps by accepting a hydrogen bond) that enhances its affinity, whereas the stereochemical disposition of the other enantiomer does not permit this interaction to take place; this lower affinity, (+)-(2*S*,3*R*)-enantiomer may be bound to the receptor so as to project its substituent into the same general region of the receptor, but since it has its stereocenters reversed, the carbonyl group is not disposed in the precise manner needed to engage in the productive interaction. This argument also suggests that because of the extra methylene unit, the topographic presentation of the carbonyl group of the derivatives in the hexestrol series also differs from that of the (−)-(2*R*,3*S*)-norhexestrol derivatives so that this productive interaction cannot take place with either enantiomer in the hexestrol series.

Since the absolute configuration of all the chiral derivatives we have prepared are known, we can say that if the "high-binding" enantiomers in the norhexestrol series, which have the 2*R*,3*S* configuration, are bound by the receptor in the normal mode, then they project their functional group into the lower right region; if they are bound in the reversed mode, the group is projected into the upper left region (cf. Scheme 3, Enantiomer 1).

In conclusion, it is apparent that the interaction between nonsteroidal estrogens of the hexestrol type with the estrogen receptor is a complex process, and that unambiguous stereochemical probes cannot always be prepared, nor can the affinity of enantiomeric pairs be interpreted in a unique manner. Nevertheless, this study provides evidence that under appropriate circumstances (e.g., in the norhexestrol series), the interaction between the estrogen receptor and nonsteroidal ligands can display chiral recognition.

dextran adsorption. These data are available from Dr. David J. Ellis, Institute of Biological Sciences, Syntex Research Center, Stanford Industrial Park, Palo Alto, California 94304.

[4] D. F. Heiman, K. E. Carlson, and J. A. Katzenellenbogen, unpublished observations.

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