MOLECULAR PHARMACOLOGY, 19:388–398

Conformational Analysis of Nonsteroidal Estrogens

The Effect of Conformer Populations on the Binding Affinity of *meso*- and *dl*-Hexestrol to the Estrogen Receptor

MICHAEL R. KILBOURN, A. J. ARDUENGO, JOON T. PARK, AND JOHN A. KATZENELLENBOGEN
The Roger Adams Laboratory, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801

Received June 12, 1980; Accepted December 10, 1980

SUMMARY

KILBOURN, M. R., A. J. ARDUENGO, J. T. PARK, AND J. A. KATZENELLENBOGEN. Conformational analysis of nonsteroidal estrogens: the effect of conformer populations on the binding affinity of *meso*- and *dl*-hexestrol to the estrogen receptor. *Mol. Pharmacol.* **19**:388–398 (1981).

The conformational preferences of three nonsteroidal estrogens, *meso*-hexestrol, *dl*-hexestrol, and 1,2-bis(4-hydroxyphenyl)butane (isobutestrol), have been studied by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and force field calculations (on model compounds), and have been used to rationalize the strikingly different binding affinities of these compounds for the uterine estrogen receptor. The chemical shifts for the methyl and methylene resonances in *meso*-hexestrol appear at field strengths 0.2 ppm higher than those of the related diastereomer *dl*-hexestrol. This shielding effect suggests that *meso*-hexestrol exists predominantly in the antiperiplanar conformation and *dl*-hexestrol predominantly in synclinal conformations. The lack of a shielding effect in isobutestrol suggests that it too prefers a synclinal conformation. Analysis of the vicinal coupling constants of the benzylic protons in two diastereomeric hexestrol analogues leads to similar predictions of conformer population and, together with the chemical shift data, suggests that the (−)-synclinal conformer may be the major contributor in *dl*-hexestrol. Force field calculations that were done on *meso*- and *dl*-3,4-diphenylhexane and 1,2-diphenylbutane have confirmed the conformational preferences suggested by ^{1}H-NMR: the two synclinal conformation of *meso*-3,4-diphenylhexane lie 1.29 Kcal/mole above the antiperiplanar conformer, and the (+)-synclinal and antiperiplanar conformers lie above the (−)-synclinal conformation of *dl*-3,4-diphenylhexane by 0.79 Kcal/mole and 3.01 Kcal/mole, respectively. The (+)-synclinal conformation is preferred for 1,2-diphenylbutane, with the antiperiplanar and (−)-synclinal being more energetic by 0.73 Kcal/mole and 1.27 Kcal/mole, respectively. The binding affinities of the hexestrol diastereomers for the uterine estrogen receptor, measured relative to that for estradiol (100%), are 300% for the *meso* and 3.2% for the *dl* form. The ratio of receptor binding affinities for the two hexestrol diastereomers is comparable to the ratio of population of the antiperiplanar conformers of the diphenyl hexane diastereomers. This suggests a model in which the receptor binds only the antiperiplanar conformation of each diastereomer and does not distinguish between different configurations at the benzylic centers. The intermediate affinity of isobutestrol (25%) is thought to reflect both its lower lipophilicity and its intermediate preference for the antiperiplanar conformation.

INTRODUCTION

The role of conformational isomerism in the binding of a drug, hormone, or neurotransmitter to its receptor has remained an intriguing topic. In the "lock and key" model, originally proposed by Fischer, a single preferred conformer of the ligand binds with the receptor in a productive fashion; furthermore, this conformer is generally considered to be that of the lowest free energy. More recently, alternative possibilities have been suggested: first, that the species which is biologically active may indeed be a minor (higher free energy) conformer

---

This work was supported by Grants AM 15556 and CA 25836 from the National Institutes of Health, Grant BC-223 (to J. A. K.) from the American Cancer Society, and Grant CHE77-20862 (to A. J. A.) from the National Science Foundation.

0026-895X/81/030388-11$02.00/0

(1, 2), and, second, that the interaction of the ligand with the receptor may involve a sequence of conformational changes in the ligand, the receptor, or both. In either case, the conformation of the molecule bound to the receptor might be significantly different than that of the molecule free in solution (3).

Conformational analysis has been applied in a number of instances where flexible ligands interact with macromolecular receptors in order to illuminate the relationships between the conformational energy of ligands and their receptor binding affinity or biological potency (for recent reviews see refs. 4–7). The role of conformational isomerism in the binding of nonsteroidal estrogens to the estrogen receptor is of particular interest since increasing use is being made of the nonsteroidal estrogens, hexestrol,[1] DES, and dienestrol in the design of functionalized estrogens. Unlike the steroidal estrogens, which are nearly flat and have fairly rigid structures, molecules such as hexestrol, DES, and dienestrol are conformationally quite flexible. Conformational analysis of the nonsteroidal estrogens has been largely ignored until recently, when X-ray crystallographic studies of DES (8), dienestrol (9), and *dl*-hexestrol (10) (Fig. 1) were reported. However, despite the various conformations of these compounds suggested by X-ray crystallography, the applications of these data to describe biological activities (estrogenic potency *in vivo* or receptor binding affinity) have been incomplete and confusing.

In this report, we have undertaken a study of the conformations of *meso*- and *dl*-hexestrol and certain hexestrol analogues, using ^{1}H-NMR and force field calculations to arrive at a description of the preferred conformers of such compounds. These studies have shown that *meso*- and *dl*-hexestrol have profoundly different preferred conformations. A comparison between these conformational preferences and the binding affinity of these two diastereomers for the estrogen receptor has led to a model for binding in which only the antiperiplanar conformation is bound, the configuration or state of hybridization at the benzylic centers being of little consequence.

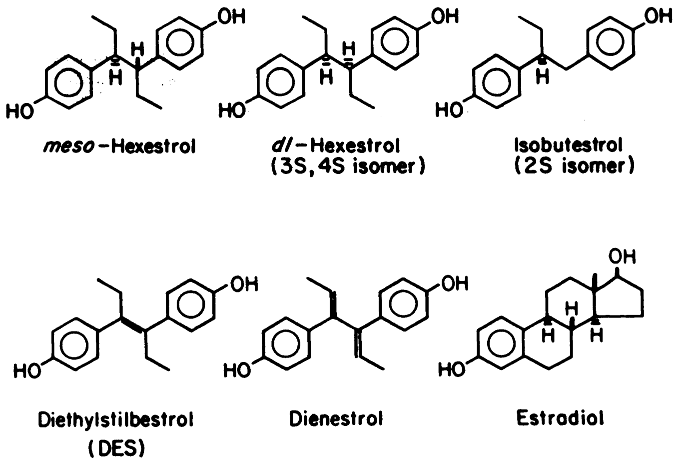


Fig. 1. ***Structures of nonsteroidal estrogens and estradiol***
Systematic names are given in footnote 1.

MATERIALS AND METHODS

^{1}H-NMR spectra were recorded on Varian Associates spectrometers, Models T-60, EM-390, and HR-220. Chemical shifts are reported in parts per million downfield from a tetramethylsilane internal standard (δ scale). Data are reported as δ values of protons (multiplicity of signal, number of protons, identity of protons). IR spectra were recorded on either a Beckmann Model IR-12 or a Perkin-Elmer Model 137 spectrometer. Spectra were obtained from samples prepared as KBr pellets or, for liquids and oils, as neat samples held between salt plates. Data are presented in cm^{-1} and only the important diagnostic bands are reported. Mass spectra were obtained from a Varian MAT CH-5 spectrometer and were at 70 eV unless otherwise indicated. Data are presented in the form *m/e* (intensity relative to base peak). Exact mass determinations by high-resolution mass spectrometry were obtained on a Varian MAT 731 spectrometer. Melting points were determined on a Fisher-Johns hot-stage apparatus, and temperatures reported are uncorrected. Column chromatography was carried out using Brinkman silica gel, 0.05- to 0.2-mm mesh. Elemental analyses were provided by the microanalytical service laboratory of the University of Illinois.

The chemicals and reagents used in this study were obtained from the following sources: trimethylsilyliodide, Aldrich Chemical Company, Milwaukee, Wisc.; triethylsilane, Pfaltz and Bauer, Stamford, Conn.; 5% palladium on charcoal, Englehard, Iselin, N. J.; hexestrol, Sigma Chemical Company, St. Louis, Mo. The following compounds or synthetic precursors were prepared by literature methods: *dl*-hexestrol by hydrogenation of DES (11), (*E*)-1,2-bis(4-methoxyphenyl)-1-butene (12, 13), α-ethyldesoxyanisoin (12, 13), *erythro* and *threo* methyl 3,4-bis(4-methoxyphenyl)hexanoates (14).

MMI calculations were performed on a VAX 11/780 computer using an adapted version of the program of Allinger *et al.* (15, 16). Binding affinity to the uterine estrogen receptor were performed by methods we have described previously (17). Data are taken from our own laboratory or from reports of Ellis.[2]

1,2-Bis(4-methoxyphenyl)butane

Method A. To a solution of (*E*)-1,2-bis(4-methoxyphenyl)-1-butene (12, 13) (400 mg, 1.4 mmoles) in 20 ml of glacial acetic acid were added 20 mg of 5% palladium on charcoal, and the mixture was hydrogenated at ambient pressure for 5 hr. Water was added, the catalyst was filtered off, and the product was extracted into

---

[1] The abbreviations and common names used are: hexestrol, 3,4-bis(4-hydroxyphenyl)hexane; DES, diethylstilbestrol [(E)-3,4-bis(4-hydroxyphenyl)-3-hexene]; isobutestrol, 1,2-bis(4-hydroxyphenyl)butane; dienstrol, (*Z,Z*)-bis(4-hydroxyphenyl)-2,4-hexadiene; ^{1}H-NMR, proton nuclear magnetic resonance spectroscopy; Eu(fod)$_3$, tris-(1,1,1,2,2,3,3,-heptafluoro-7,7-dimethyl-4,6-octanedione)europium (III).

[2] Ellis has determined the binding affinity of 140 steroid derivatives for a partially purified estrogen-binding protein from mature rat uterus, using a competitive binding assay with [^{3}H]estradiol and charcoal-dextran adsorption, similar to the method used in this paper. These data are available from Dr. David J. Ellis, Institute of Biological Sciences, Syntex Research Center, Stanford Industrial Park, Palo Alto, Calif. 94304.

diethyl ether. The ether layer was washed with water and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to yield a yellow oil. Trituration with methanol gave a white solid, which was recrystallized from methanol to yield 350 mg (88%) of 1,2-bis(4-methoxyphenyl)-butane: m.p. 41–42°; ^{1}H-NMR ($CDCl_3$) δ 0.74 (t, 3H, —CH_2CH_3), 1.63 (m, 2H, —CH_2CH_3), 2.55–2.8 (m, 3H, benzylic H atoms), 3.75 (s, 3H, $ArOCH_3$), 3.77 (s, 3H, $ArOCH_3$), and 6.75–7.05 (m, 8H, ArH); mass spectrometry 270 (7.7, M^+), 149 (100), 121 (42). Analysis:

$C_{18}H_{22}O_2$

Calculated: C 79.96, H 8.20
Found: C 79.82, H 8.10

Method B. To a solution of α-ethyldesoxyanisoin (14) (250 mg, 0.88 mmole) in 2 ml of trifluoroacetic acid was added triethylsilane (220 mg, 1.88 mmole). After 2 hr, the reaction was quenched by pouring the mixture into water, and the aqueous solution was extracted with ethyl acetate. The ethyl acetate was washed (saturated aqueous sodium bicarbonate, water) and dried ($MgSO_4$), and the solvent was evaporated to yield a yellow oil. Trituration with methanol gave a yellow solid, which was recrystallized from methanol to give 225 mg (83%) of the same alkane as above.

1,2-Bis(4-hydroxyphenyl)butane (Isobutestrol)

Method A. A solution of 1,2-bis(4-methoxyphenyl)butane (100 mg, 0.37 mmole) in 2 ml of acetic acid was added dropwise to a solution of 0.5 ml of 57% hydroiodic acid in 2 ml of acetic acid. The dark red solution was refluxed for 15 hr, cooled to 70°, and diluted with an equal volume of water. The aqueous mixture was extracted with ethyl acetate, and the organic layer was washed (10% sodium bisulfite, 10% sodium hydroxide, water) and dried ($MgSO_4$). Removal of solvent under reduced pressure yielded 87 mg (86%) of an off-white solid. Attempts to recrystallize this substance were unsuccessful. Column chromatography of a portion of the material (methylene chloride as eluant) gave a clear oil which crystallized after 4 months at −4°, yielding 7.5 mg of clean white crystals of isobutestrol (upon attempts to recrystallize this material, the product reverted to an oil): m.p. 102–103°; ^{1}H-NMR ($CDCl_3$) δ 0.75 (t, 3H, $J = 7$ Hz, CH_2CH_3), 1.4–1.9 (m, 2H, —CH_2CH_3), 2.55–2.8 (m, 3H, benzylic H atoms), 5.5 (s, broad, 2H, 2 × $ArOH$), and 6.65–6.94 (m, 8H, ArH); mass spectrometry 242 (4.9, M^+), 135 (58), 78 (100), and 77 (24). Analysis:

$C_{16}H_{18}O_2$

Calculated: mol wt 242.1302
Found: mol wt 242.1305

Method B. Trimethylsilyliodide (440 mg, 2.2 mmoles) was added via syringe to a solution of the bis-ether (200 mg, 0.74 mmole) in 0.5 ml of chloroform. After 12 hr at 55°, the reaction was quenched by addition of 2 ml of methanol. After stirring an additional 2 hr, the solvents were evaporated under reduced pressure, and the oily residue was purified by preparative thin-layer chromatography (3:2 hexane-ethyl acetate) to yield 107 mg (60%) of isobutestrol.

RESULTS

Conformations of Hexestrols: ^{1}H-NMR Studies

^{1}H-NMR has been used extensively in conformational analysis. For substituted ethanes, the conformation can be approximated by measuring the coupling constant between vicinal protons; the Karplus equation (18, 19)

$$J = J^\circ \cos^2 \phi - C \qquad (1)$$

describes a relationship between the dihedral angle ϕ and the magnitude of the coupling constant J. This relationship has been used to predict the preferred solution conformations of a number of physiologically important compounds (20–23), and, in general, results of ^{1}H-NMR studies have agreed with conformation obtained from X-ray crystallography or calculated by molecular orbital theory.

Bothner-By and Naar-Colin (24) used ^{1}H-NMR to study conformations of *meso-* and *dl*-2,3-diphenylbutane. From the Karplus equation, these authors estimated that the *meso*-isomer exists predominantly in the antiperiplanar conformation[3] (≅70%; see Fig. 2, R = CH_3), whereas the *dl*-isomer exists in a 40:60 ratio of (+)-synclinal[4] to antiperiplanar plus (−)-synclinal conformers. Similarly, Reuvers *et al.* (26) examined the ^{1}H-NMR spectra of *meso-* and *dl*-2,3-bis(2,6-dimethylphenyl)-butane, and described the same relationship of preferred antiperiplanar conformation for the *meso*-isomer, and preferred (−)-synclinal for the *dl*-isomer; in these sterically demanding cases, the preferences were even greater.

With these literature precedents, it appeared that the conformations of *meso-* and *dl*-hexestrol and of isobutestrol could be determined by measurement of the vicinal coupling constants between the benzylic hydrogens and application of the Karplus equation. In practice, extraction of the benzylic coupling constants in these compounds is difficult because of the identical (or nearly identical) resonance position of these protons. Furthermore, these resonances are too weak and lie too close to other resonances to permit observation of the vicinal coupling in the C-13 satellites.

The desired coupling information can be extracted from the ^{1}H-NMR spectra of certain derivatives of hexestrol in which the benzylic hydrogens are no longer

---

[3] As is standard practice (25), the terms antiperiplanar and synclinal refer to the phenyl or hydroxyphenyl groups. These groups appear to be of greatest significance in the interaction of the nonsteroidal estrogens with the estrogen receptor.

[4] According to standard practice in conformational analysis, (+) and (−) are used to designate the synclinal conformers in which the back carbon has been rotated clockwise or counterclockwise, respectively, from the syn conformation (both aryl groups eclipsed) (25). In order for the disposition of the other substituents to be considered in an unambiguous manner, it is essential that discussion focus on a single enantiomer of *dl*-3,4-diphenylhexane and of 1,2-diphenylbutane. In Figs. 2, 3, and 6–8 and in the text of this paper, we have chosen the 3S,4S-enantiomer and 2S-enantiomer, respectively. An equivalent discussion can be made using the R enantiomers, but the (+) and (−) designations of the synclinal conformations must then be exchanged.

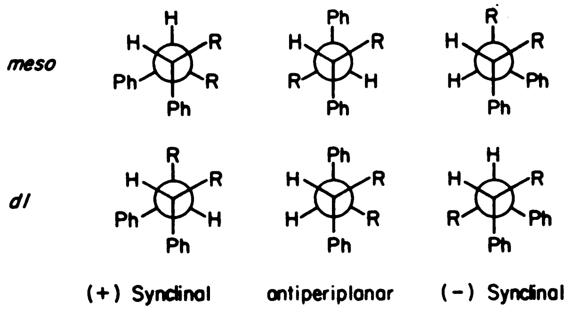
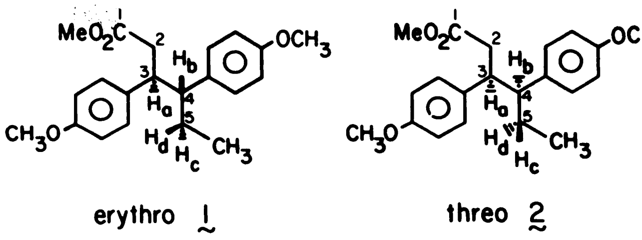


FIG. 2. *Conformational minima for meso- and dl-diaryl alkanes*
For an explanation of names and terms, see footnotes 3 and 4.



	J_{ab}	J_{bc}	J_{bd} (Hz)
erythro	10.5	10.5	5.0
threo	5.0	10.0	5.0

FIG. 3. *Structures and coupling constants for diastereomeric methyl 3,4-bis(4-methoxyphenyl)hexanoates*

equivalent. The benzylic protons in *erythro*- and *threo*-methyl 3,4-bis(4-methoxyphenyl)hexanoate (**1** and **2**, Fig. 3) resonate as complex patterns that overlap with resonances from other protons and thus cannot be readily analyzed (Fig. 4A and C); however, addition of a lanthanide-shift reagent, Eu(fod)$_3$, caused a pronounced downfield shift of the C-2 protons, which leaves the pattern due to the C-4 proton in an unobscured region of the spectrum (Fig. 4B and D). In the undecoupled spectra this signal appears as a triplet of doublets (J = 10.5, 5.0 Hz) in the *erythro*-diastereomer and as a quintet (actually a doublet of triplets; J = 10.0, 5.0 Hz) in the *threo*-diastereomer. Decoupling of the C-5 methylenes left the C-4 methine as a doublet with a coupling of 10.5 Hz in the *erythro*-diastereomer and 5.0 Hz in the *threo*-isomer (Fig. 4B and D *insets*). The coupling constants are summarized in Fig. 3.

The three staggered conformations of diastereomeric-substituted diphenyl ethanes are depicted in Fig. 2. If the *erythro*-ester **1** is considered a model for the *meso*-diastereomer, then the large vicinal coupling (10.5 Hz) indicates that the predominant conformer is antiplanar, in which the hydrogen atoms have a dihedral angle of 180°. By using the *threo*-ester **2** as a model for the *dl*-diastereomer, the smaller benzylic vicinal coupling (5.0 Hz) indicates that the antiperiplanar or (−)-synclinal conformers (60° dihedral angles) are favored over the (+)-synclinal (180°), although no prediction is made as to which of the former two would be favored.[5]

Although coupling constants have been more widely used in conformational analysis, there is also precedent for the use of chemical shift differences. In their work on 2,3-diphenylbutanes, Bother-By and Naar-Colin (24) reported greater δ values (downfield shifts) for the methyl groups of the *dl*-isomer. The difference in δ values for the ester methyl groups in 3,4-bis(4-methoxyphenyl) adipic acid methyl esters was used (28) to assign *meso*- and *dl*-structures, with the greater chemical shift for the *dl*-isomer attributed to a synclinal conformation. Finally, Barbiuex and Martin (29) assigned *threo*- and *erythro*-configurations to diastereomers of 2,3-dimethyl-4′-methoxydihydrocinnamic acid esters on the basis of the greater downfield shift on the 3-methyl group in the *threo*-isomer. In these examples, the changes in chemical shifts were attributed to changes in the position of the alkyl groups relative to the shielding regions of the aromatic rings.

Side-by-side comparisons of the spectra of *dl* and *meso*-hexestrol showed definite differences in chemical shifts for the methyl triplets and methylene multiplets. These differences in chemical shifts are consistently seen between the diastereomeric *meso*- and *dl*-hexestrol derivatives, and between the related diastereomeric *erythro*- and *threo*-hexestrol derivatives. The average resonance positions of the methyl groups in these compounds are shown in Fig. 5. This signal in the *meso*- and *erythro*-isomers appears consistently at 0.2-ppm higher field than in the *dl*- or *threo*-isomers. The chemical shift values for isobutestrols are remarkably similar to those of *dl*-hexestrol and the *threo*-diastereomers.

Examinations of space-filling (CPK) models of dialkyl diphenyl ethanes show that the alkyl groups are within the shielding cone in an antiperiplanar conformation (for either *meso*- or *dl*-diastereomers), and disposed out and away from the shielding region in synclinal conformers. Based on this analysis and the precedents cited above, the chemical shift differences between *meso*- and *dl*-hexestrol (and *erythro* and *threo* derivatives) are an indication of different solution conformations: a predominance of antiperiplanar for *meso*-hexestrol, a predominance of synclinal for *dl*-hexestrol. For the isobutestrols, the chemical shift values indicate that these molecules also exist in solution as predominantly the synclinal conformer(s).

In summary, the chemical shift data and the benzylic coupling constants for *meso*-hexestrol (and related *meso* and *erythro* derivatives) make identical predictions—the antiperiplanar conformation predominates.[6] With *dl*-hexestrol (and related *dl* and *threo* derivatives), the chemical

---

[5] The magnitude that is observed for the benzylic coupling constant is a weighted average of the coupling in the three staggered conformers; thus, it can be used to predict the population of these conformers, provided accurate values for the coupling of *trans* (J_t) and *gauche* (J_g) vicinal hydrogens in this system are availabale. These couplings are known to depend on electronegativity, strain, and hybridization, and the literature provides a range of values (ref. 27). Nevertheless, if one makes the reasonable assumption that J_t = 12 Hz and J_g = 4.0 Hz, then the conformer populations for the *erythro* ester **1** (J = 10.5 Hz) is 81% antiperiplanar and 29% (+)- and (−)-synclinal and for the *threo* ester **2** (J = 5.0 Hz) is 88% (−)-synclinal and antiperiplanar and 12% (+)-synclinal. These conformer populations are constant with those obtained by calculation for *meso*- and *dl*-hexestrol (cf. Table 4).

[6] Dipole moment measurements have suggested the antiperiplanar conformation for *meso*-hexestrol (30).

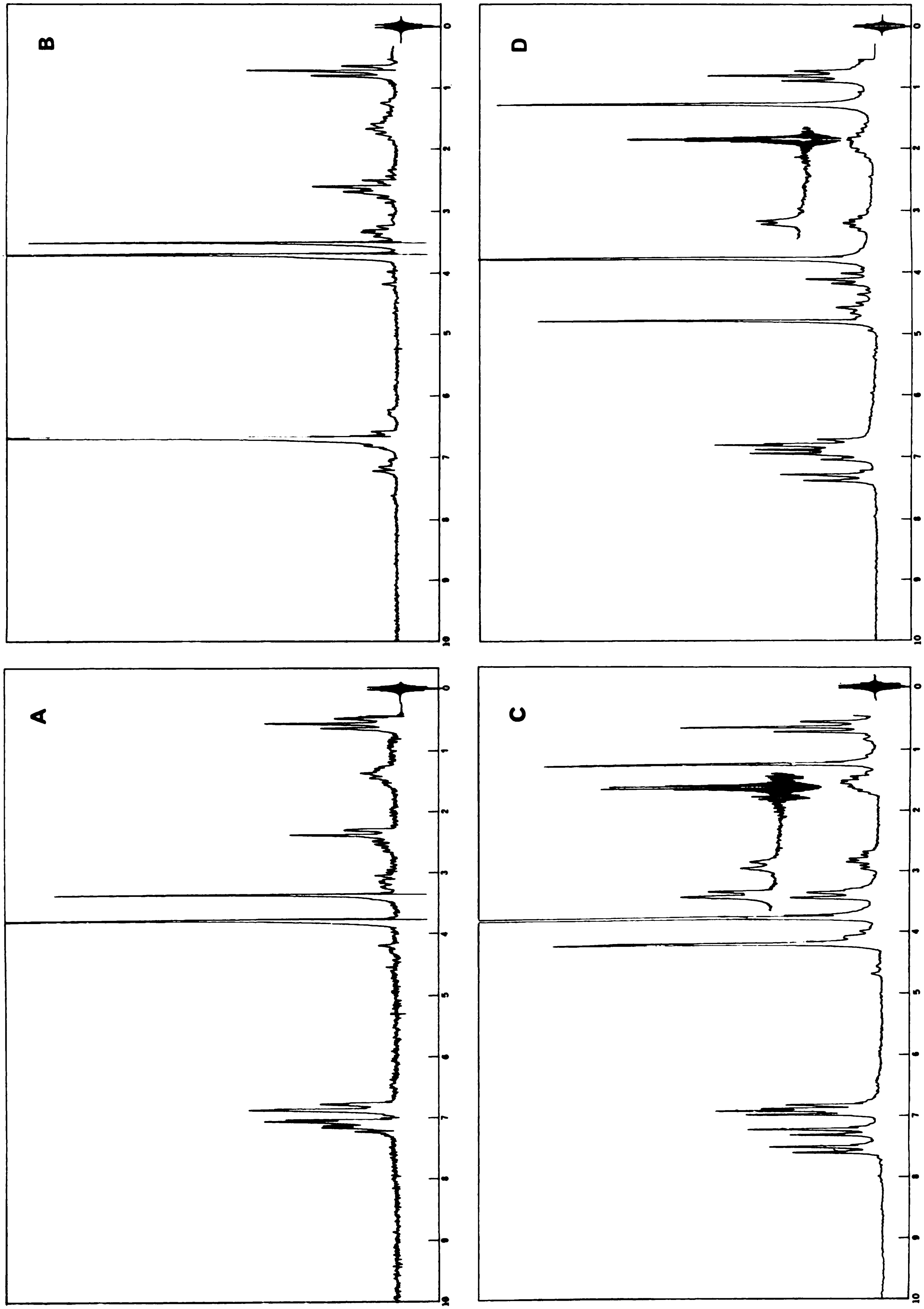


FIG. 4. ^{1}*H-NMR spectra (90 MHz) of erythro (A and C) and threo (B and D) methyl 3,4-bis(4-methoxyphenyl)hexanoates*

A and B are spectra in $CDCl_3$; shift reagent $Eu(fod)_3$ has been added in C and D. *Insets* are from spectra in which the C-5 methylene is decoupled. The signal for the C-4 benzylic hydrogen appears at approximately δ 2.5–2.7 in A and B, at approximately δ 2.8 in C and at approximately δ 3.3 in D.

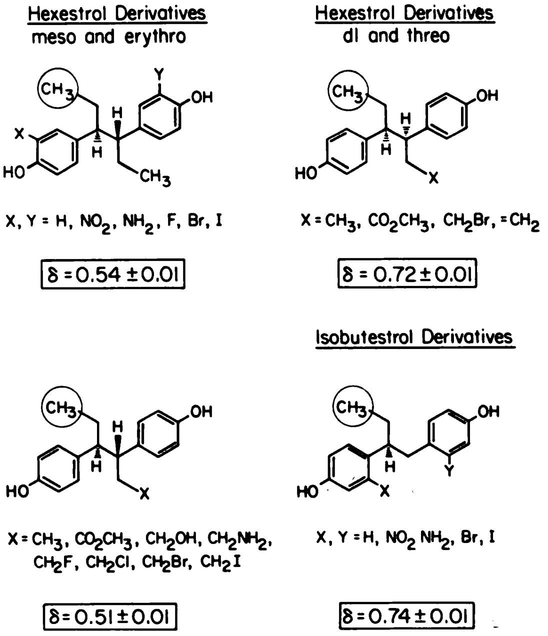


FIG. 5. ***Chemical shifts of methyl groups in diastereomeric hexestrol or isobutestrol derivatives***

^{1}H-NMR spectra were obtained at 90 mHz; data are average chemical shifts ± standard error of the mean of the chain terminal methyl group resonances (*circled*). In certain cases, NMR data were taken from the corresponding phenolic methyl ethers.

shift data indicate a preference for synclinal conformations; since the coupling data suggest (−)-synclinal or antiperiplanar, but not (+)-synclinal, it appears likely that the (−)-synclinal predominates. It is of note that the X-ray structure of *dl*-hexestrol by Weeks *et al.* (10) showed the (−)-synclinal conformer.[7]

Conformation Analysis via Force Field Calculations

Although the analysis of ^{1}H-NMR spectra of hexestrols and isobutestrols was highly suggestive of the predominant conformations of these molecules in solution, such studies do not give detailed information about the relative energy differences between conformers or quantitative data on conformer populations. Such information can be obtained from a variety of theoretical calculations.

Forms of molecular orbital (MO) theory [the PCILO method of Pullman (6), in particular] have been applied to numerous biologically important compounds (3–7). Quantitative data can also be obtained from a second type of theoretical calculation, known as force field calculations, the molecular mechanics method, or the Westheimer method (31), that is based on classical mechanical potential energy functions; the conformational energy is then a function of bond stretching, bond-angle bending, and nonbonded interactions (31). Although it is an empirical method of energy calculation, the force field method has given results in excellent agreement with experimental physical values for a variety of compounds (32, 33). Typically, the conformation energy is calculated at increments of rotation about a single bond, and the results are expressed as a plot of energy versus dihedral angle. Additionally, at each calculated energy value, the conformation of the entire molecule is described in terms of dihedral angles.

Force field calculations were applied here to three compounds, using the MMI program of Allinger *et al.* (15) and Allinger and Chung (16). As models for the hexestrols, the calculations were done on *meso*- and *dl*-3,4-diphenylhexane; as a model for isobutestrol, 1,2-diphenylbutane was used. These model compounds differ from the synthetic estrogens only by the lack of the 4-hydroxyl functions; it was felt that this difference would not seriously affect the results.[8] The energy profiles for these compounds (calculated steric energy vs dihedral angle) are shown in Fig. 6 (*meso*-3,4-diphenylhexane), Fig. 7 (*dl*-3,4-diphenylhexane), and Fig. 8 (1,2-diphenylbutane); each compound is discussed separately below. The structure of each molecule was fully optimized to give the minimal steric compression energy for each dihedral angle value about the double benzylic bond. The numbering systems for the diphenylhexanes and 1,2-diphenylbutane are shown in Fig. 9, and the dihedral angles for the minimal and maximal energy conformers for the three compounds are given in Table 1–3.

meso-3,4-Diphenylhexane. The plot of energy versus dihedral angle between ethyl groups (angle 2-3-4-5) shows the symmetrical shape expected for the *meso*-configuration, a symmetrical molecule for which the synclinal conformers are mirror images, and thus of identical energy (Fig. 6). The lowest energy conformation is the expected antiperiplanar form, with like substituents very nearly 180° apart (the dihedral angles describing the structures at the minima and maxima of the energy curve of the *meso*-isomer are given in Table 1). The two synclinal conformers are each at an energy level 1.29 Kcal/mole higher than the antiperiplanar, with dihedral angles between *gauche* groups quite close to the 60° of an exactly staggered arrangement.

The barrier to rotation from antiperiplanar to synclinal conformer (3.1 Kcal/mole) is significantly less than that between the two synclinal conformers (5.04 Kcal/mole); the latter rotation involves an eclipsing of both the sterically demanding ethyl substituents and the phenyl groups, while the former involves only a single ethyl-phenyl eclipsing.

dl-3,4-Diphenylhexane. As expected, the plot of energy versus dihedral angle (Fig. 7) for the *dl*-isomer is more complex, as the molecule is asymmetrical and the synclinal conformers are different. For this isomer, the (−)-synclinal conformer is that of the lowest calculated energy. The minimum actually occurs at phenyl-phenyl dihedral angle of 68°, slightly greater than the 60° expected of an exactly staggered conformation (data on dihedral angles for each conformer of the *dl*-isomer are given in Table 2).

---

[7] Weeks *et al.* (10) actually give the crystallographic data for the (+)-synclinal conformation of the 3R,4R-enantiomer, which would be equivalent to the (−)-synclinal conformer for the 3S,4S-enantiomer discussed here (see footnote 4).

[8] To simplify the MMI calculations, the hydroxyl groups were omitted from hexestrols and from isobutestrol. Recalculation of the steric energies of the antiperiplanar and synclinal conformations of the diphenylhexanes and butane both with and without the hydroxyl groups show negligible differences (less than 0.2 Kcal/mole).

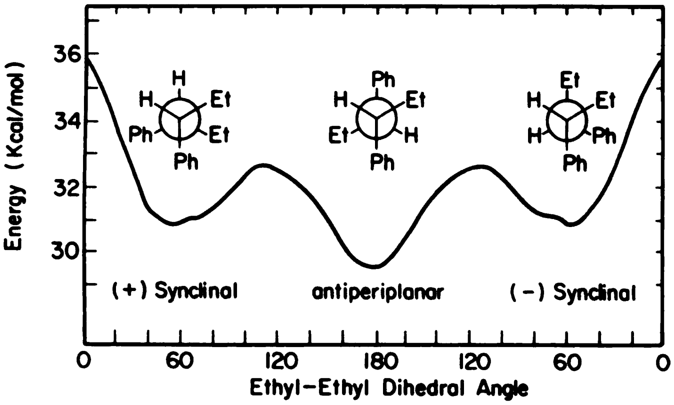


FIG. 6. *Steric energy profile for rotation of meso-3,4-diphenylhexane*

The next lowest energy minimum corresponds to the (+)-synclinal conformer and lies 0.79 Kcal/mole above the (−)-synclinal conformer. The phenyl-phenyl dihedral angle here (43°) is significantly less than 60°; the two phenyl rings move toward partially overlapping positions to relieve the torsional interaction between the *gauche*-oriented ethyl groups.

Finally, the highest energy conformation is that with the phenyl rings in the antiperiplanar positions. This conformer is 2.22 Kcal/mole higher than the (+)-synclinal conformer, and 3.01 Kcal/mole higher than the (−)-synclinal conformer. Although termed here antiperiplanar, the structure deviates significantly from an exactly staggered conformation: the sterically demanding ethyl groups, which are *gauche* to one another, are twisted 78.4° apart; this forces the phenyl rings toward the hydrogen atoms, reducing the phenyl-phenyl dihedral angle to 173°.

Just as there are significantly different energy minima, there are also different barriers to rotation between conformers. Rotation from the (−)-synclinal to antiperiplanar conformer incurs the highest energy barrier (6.22 Kcal/mole) because of two unfavorable ethyl-phenyl interactions in the eclipsed form. The barrier from (+)-synclinal to antiperiplanar is lower (4.20 Kcal/mole), involving only a single significant eclipsing of substituents (ethyl with ethyl). The barrier to rotation between synclinal conformers involves only a phenyl-phenyl eclipsing and is quite low.

1,2-Diphenylbutane. This is again an unsymmetrical molecule, and an unsymmetrical steric energy curve is seen (Fig. 8). Unlike the diphenylhexanes, the antiperiplanar conformer is of intermediate energy, and all conformers are of lower energy than those of the more sterically crowded diphenylhexanes.

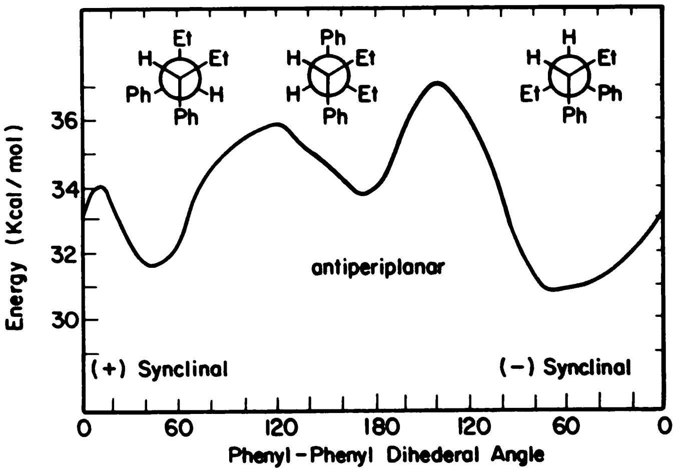


FIG. 7. *Steric energy profile for rotation of (3S,4S)-3,4-diphenylhexane*

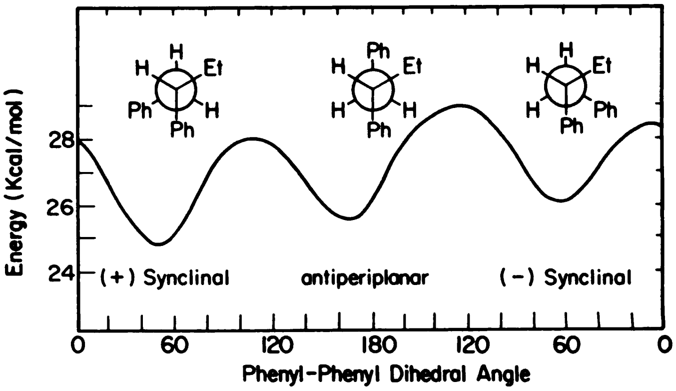


FIG. 8. *Steric energy profile for rotation of (2S)-1,2-diphenylbutane*

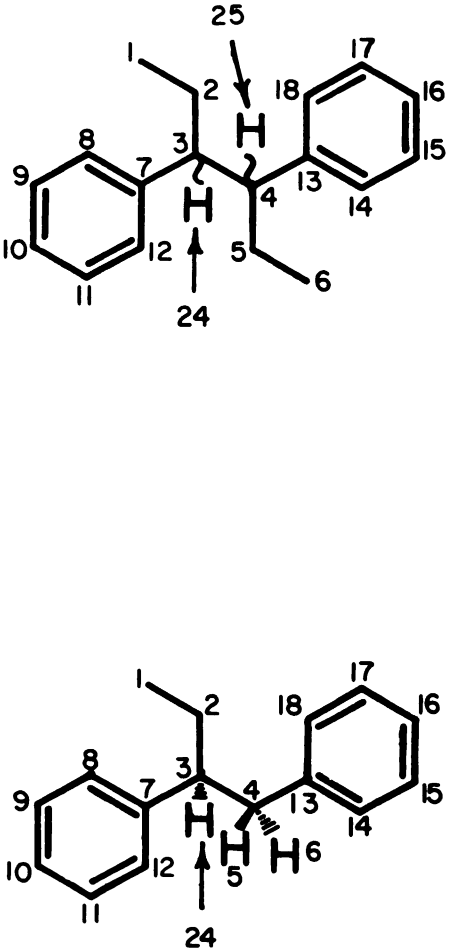


FIG. 9. *Numbering for carbons in the diphenylhexanes and diphenylbutane*

TABLE 1

Calculated dihedral angles for minimal and maximal energy conformers of meso-3,4-diphenylhexane

Dihedral angle[a]	Conformer energy (Kcal/mole)			
	30.83	35.87	29.54	32.66
1-2-3-4	178.2°	175.8°	179.6°	178.5°
1-2-3-7	50.8	50.2	49.7	49.3
2-3-4-5	58.2	0.8	177.8	112.4
2-3-7-8	130.7	127.4	119.4	116.4
2-3-4-13	75.0	134.9	51.0	19.4
2-3-7-12	49.9	53.0	60.5	63.5
3-4-5-6	62.9	83.9	84.2	70.3
3-4-13-14	93.5	87.7	107.2	96.5
4-3-7-8	95.4	95.2	110.2	109.5
4-3-7-12	83.8	84.0	69.8	70.5
5-4-13-14	131.3	135.2	123.3	130.1
5-4-3-7	169.3	133.5	53.0	115.9
6-5-4-13	164.5	143.4	149.0	156.9
7-3-4-13	57.5	0.6	179.7	112.3
25-3-4-24	54.8	1.8	179.8	111.1

[a] See Fig. 9 for numbering scheme.

The lowest energy conformer is the (+)-synclinal, and occurs at a phenyl-phenyl dihedral angle of 49.4° (dihedral angles for the minimal and maximal energy conformers are given in Table 3), with the ethyl-phenyl dihedral angle nearly 180°. In this conformer the ethyl group is *gauche* only to hydrogen atoms. The next lowest energy conformer is the antiperiplanar, at 0.73 Kcal/mole higher than the minimum for the (+)-synclinal. In this conformer the phenyl-phenyl dihedral angle is 166.7°, not the 180° which might be expected, and is not unlike that observed for the antiperiplanar conformer of *dl*-3,4-diphenylhexane. The highest energy conformer is thus the (−)-synclinal, at a point 1.27 Kcal/mole higher than the (+)-synclinal form; here, all three of the large substituents are *gauche*, and the angles between them are both more than the 60° of an exactly staggered conformation. In general terms, the diphenylbutane is more like the *dl*-isomer of the diphenylhexane in that the lowest energy conformer is synclinal rather than antiperiplanar.

TABLE 2

Calculated dihedral angles for minimal and maximal energy conformers of (3S,4S)-3,4-diphenylhexane (dl-3,4-diphenylhexane)

Dihedral angle[a]	Conformer energy[b] (Kcal/mole)				
	31.65	35.85	33.82	37.08	30.86
1-2-3-4	93.0°	70.1°	87.3°	78.4°	70.9°
1-2-3-7	140.8	155.6	141.1	147.9	158.3
2-3-4-5	63.0	32.2	78.4	121.0	165.1
2-3-7-8	60.2	40.9	41.7	54.8	47.3
2-3-4-13	169.9	102.5	54.4	11.0	63.4
2-3-7-12	120.2	139.5	139.0	124.4	132.9
3-4-5-6	93.1	70.1	87.2	78.6	70.9
3-4-13-14	108.5	82.2	86.1	99.3	93.8
4-3-7-8	70.7	96.7	92.9	81.6	85.5
4-3-7-12	108.9	82.9	86.4	99.1	94.3
5-4-13-14	120.6	140.2	139.3	124.3	133.3
5-4-3-7	169.9	102.5	54.4	11.1	63.4
6-5-4-13	140.9	155.8	141.3	147.9	158.2
7-3-4-13	42.9	122.8	172.8	143.1	68.1
25-3-4-24	171.4	101.7	55.4	13.4	61.7

[a] See Fig. 9 for numbering scheme.

[b] Dihedral angle data for the maximal energy conformer at the phenyl-phenyl dihedral angle of zero are not available because of the inaccurate optimization of steric energies for the two phenyl groups whose position was set in these conditions.

TABLE 3

Calculated dihedral angles for minimal and maximal energy conformers of (2S)-1,2-diphenylbutane

Dihedral angle[a]	Conformer energy (Kcal/mole)					
	24.73	27.98	25.46	28.92	26.00	28.23
1-2-3-4	179.5°	177.9°	179.8°	178.9°	176.6°	179.3°
1-2-3-7	54.0	52.4	52.5	50.9	52.9	50.4
2-3-7-8	122.3	117.9	115.8	114.8	132.7	125.2
2-3-4-13	177.8	122.2	65.1	5.8	70.5	120.5
2-3-7-12	58.5	62.4	64.2	65.1	47.9	55.2
3-4-13-14	78.1	100.9	99.0	94.4	88.5	79.4
4-3-7-8	109.3	110.0	115.2	113.3	95.6	103.8
4-3-7-12	69.9	68.6	64.8	66.8	83.7	75.7
5-4-13-14	159.6	137.7	139.4	140.1	146.5	155.5
5-4-3-7	73.4	10.7	46.5	113.2	174.0	134.6
7-3-4-13	49.4	107.3	166.7	124.2	61.7	10.1
25-4-3-24	169.8	127.5	70.3	3.4	58.4	108.7

[a] See Fig. 9 for numbering scheme.

The barriers to rotation between conformers of this molecule are all very similar. The highest barrier is for rotation from (+)- to (−)-synclinal conformer (3.50 Kcal/mole), but this is only slightly higher than that between antiperiplanar and (−)-synclinal (3.46 Kcal/mole); the former rotation involves eclipsing of the phenyl groups, the latter an eclipsing of a phenyl with the ethyl group. The lowest barrier (3.25 Kcal/mole) is for rotation from (+)-synclinal to antiperiplanar, which involves only eclipsing of large groups with hydrogens. It should be noted that all of these barriers are significantly lower than those found for the more sterically crowded diphenylhexanes.

Conformer energies and conformer populations. The relative populations of different conformational states can be calculated using the steric energeis of each conformer; assuming a Boltzmann distribution, the conformer populations then can be calculated by Eq. 2

$$\frac{N_i}{N} = \frac{g_i e^{-(\epsilon_i-\epsilon_0)/kT}}{\sum_i g_i e^{-(\epsilon_i-\epsilon_0)/kT}} \qquad (2)$$

where ϵ_0 is the energy of the lowest energy conformer, ϵ_i the energy of the i conformer, and g_i a weighting factor for degenerate energy levels (T is assumed to be 310° K or 37°). Application of Eq. 2 to the steric energies for the diphenylhexanes and diphenylbutane gives the conformation populations that are summarized in Table 4 (Method A).

Recently, Farnell and co-workers (34) have questioned the use of calculated steric energies for the determination of conformer populations, since such calculations do not reflect the shapes of the energy wells and entropy consid-

TABLE 4

Conformer populations for 3,4-diphenylhexanes and 1,2-diphenylbutane

Compound	Method[a]	Conformer population		
		(+)-Synclinal	Antiperiplanar	(−)-Synclinal
			%	
meso-3,4-	A	9	82	9
Diphenylhexane	B	9	82	9
dl-3,4-	A	21.6	0.6	77.8
Diphenylhexane	B	13.1	0.5	86.4
1,2-Diphenylbutane	A	73.3	19.4	7.2
	B	70	21.2	8.8

[a] Method A is based on steric energy differences, Method B on the differences in free energy.

erations would indicate that the group of conformers describing a wide energy well would be more populated than those describing a narrow well of equal energy. These workers have suggested using instead the free energy differences, which can be calculated by Eq. 3

$$\Delta G = -kT \ln (Z_x/Z_y) \tag{3}$$

where Z_x and Z_y are the classical Boltzmann partition functions for each conformation, calculated by Eq. 4:

$$Z = \sum_{\phi} e^{-\epsilon_\phi kT} \tag{4}$$

In Eq. 4, ϵ_ϕ is the calculated steric energy for dihedral angle ϕ; the summation is then made over an arbitrary range of ϕ values, taken to describe adequately the shape of the energy well.

This method of calculation of conformer populations has also been applied to the data for the diarylalkanes (see Table 4, Method B). To calculate the partition functions, values of ϕ were chosen to encompass the range 1.5 Kcal/mole above each of the three minima in the curves for the diphenylhexanes and 2 Kcal/mole above the minima of diphenylbutane, and integration was made by summation over 5° increments (rectangular rule). According to Method B, the populations of the antiperiplanar conformer for *meso*-3,4-diphenylhexane and 1,2-diphenylbutane are very similar to those obtained by Method A, while the (+)-synclinal conformer population for *dl*-3,4-diphenylhexane is significantly lower than the previous estimate because its energy well is significantly narrower than those of the other two conformers.

Extension of force field calculations results to nonsteroidal estrogens. Although the force field calculations of conformer energies, structures, and populations were made on model hydrocarbons (lacking the phenolic functions), the extension of the results to the nonsteroidal estrogens (hexestrols and isobutestrol) appears quite reasonable.[8] The calculated preferred conformers are in agreement with the results of the ^{1}H-NMR study [and a published X-ray structure (10)]: (−)-synclinal as the predominant conformer for *dl*-hexestrol and antiperiplanar as the predominant conformer in solution for *meso*-hexestrol. For isobutestrol, the predominant conformer is (+)-synclinal, which is consistent with the shielding of the methyl group observed in the ^{1}H-NMR. The structures calculated by the force field method (as defined by the dihedral angles) may indeed be very good descriptions of the conformers of the nonsteroidal estrogens; comparison of the computed dihedral angles for the (−)-synclinal conformer of *dl*-3,4-didphenylhexane with those measured by X-ray chrystallography for *dl*-hexestrol (10) shows essentially the same structure, differing only in the orientation of the chain-terminal methyl groups. Thus, the conformer populations shown in Table 4 may be considered as good estimates of the solution populations for the nonsteroidal estrogens.

DISCUSSION

Conformer population and the binding affinity of conformationally flexible ligands for the estrogen receptor. Although many compounds of natural and synthetic origin have been found to have an affinity for the estrogen receptor, very little is known about the detailed characteristics of the binding site. The high binding affinity of estradiol (K_D = 0.2–0.4 nM) is attributed to a combination of hydrophobic bonding (aromatic ring and B—C—D rings) with hydrogen bonding of the 3- and 17-hydroxyl groups; modifications which change either feature alter the affinity of the ligand for the binding site. For the nonsteroidal estrogens, a similar dependence on hydrogen bonding capability and lipophilicity is observed.

In the bis(4-hydroxyphenyl)alkane series of estrogens, it has long been known that the *meso*- and *erythro*-diastereomers have high activity, while the *dl*- and *threo*-diastereomers are only weakly active (35). More recently, measurements of binding to the estrogen receptor have confirmed that the difference in biological potency of these diastereomeric pairs is a reflection of very large differences in their affinity for the receptor (Table 5, entries 4 and 5). While discussion has been made of the possible importance of achieving in the nonsteroidal estrogen a stereochemical "congruence" of the benzylic centers with the corresponding atoms of estradiol (C-8 and C-9) (37), a satisfactory explanation for these dramatic differences in affinity has not been advanced.

On the basis of our conformational analysis of nonsteroidal estrogens, the following model for receptor binding can be proposed: *The estrogen receptor binds conformationally flexible ligands of the bis(4-hydroxyphenyl)alkane class only in the antiperiplanar conformation.* From this, one would expect that the relative binding affinities of the diastereomeric pairs should reflect to some degree the population of the antiperiplanar conformation; this appears to be the case (see below).

The influence of conformation populations on the relative energies of transition states for organic reactions has been the subject of much discussion (38). Although the Curtin-Hammett principle (38) cautions against utilizing conformational preferences of a single molecule to predict the relative energies of transition states that are different in structure (that is, lead to different products), it appears to be generally true that when different diastereomers are constrained by stereoelectronic factors to adopt equivalent transitions states (e.g., the antiorientation required for an E2 elimination), then the rates of the

Table 5

Relative binding affinities of various ligands for the uterine estrogen receptor

Compound	Relative binding affinity[a]
	%
1. 17β-Estradiol	100
2. 8α,17β-Estradiol	57[b]
3. DES	300
4. *meso*-Hexestrol	300[c]
5. *dl*-Hexestrol	3.2[c]
6. *erythro*-2,3-bis (4-Hydroxyphenyl)pentane	229[c]
7. *threo*-2,3-bis(4-Hydroxyphenyl)pentane	2.24[c]
8. Isobutestrol	25
9. Methyl *dl-erythro*-3,4-bis(4-Hydroxyphenyl)hexanoate	8.8[d]
10. Methyl *d-erythro*-3,4-bis(4-Hydroxyphenyl)hexanoate	7.0[d]
11. Methyl *l-erythro*-3,4-bis(4-Hydroxyphenyl)hexanoate	8.8[d]
12. Pentyl *dl-erythro*-3,4-bis(4-Hydroxyphenyl)hexanoate	3.8[d]
13. Pentyl *d-erythro*-3,4-bis(4-Hydroxyphenyl)hexanoate	4.0[d]
14. Pentyl *l-erythro*-3,4-bis(4-Hydroxyphenyl)hexanoate	4.2[d]

[a] Binding affinities were determined in a competitive binding assay using $[^3H]$estradiol and charcoal-dextran adsorption with either rat or lamb uterine cytosol, by the method described in ref. 17. The affinity of estradiol is 100% by definition and this corresponds to a K_d of 0.2–0.4 nM when measured by Scatchard analysis under the same conditions. Unless noted otherwise, values are from our laboratory.

[b] From ref. 36.

[c] Values from Ellis; see text footnote 2.

[d] From ref. 37.

reactions do reflect the population of the corresponding ground state conformers (38).

The situation with the binding of the nonsteroidal estrogens to the estrogen receptor is strictly analogous. If as a corollary to the model for binding advanced above it is assumed that *the estrogen receptor forms an equally satisfactory interaction with the antiperiplanar conformation of both meso- and dl-hexestrol* (see below), then the relative affinities of these diastereomers should simply reflect the ratio of population of their respective antiperiplanar conformations. Depending on the method of calculation, this ratio ranges from 137:1 to 164:1 for *meso*- and *dl*-3,4-diphenylhexane, respectively (Table 4); this is to be compared with the ratio of 94:1 for the ratio of binding affinities of the *meso*- and *dl*-hexestrol diastereomers for the estrogen receptor (Table 5, entries 4 and 5). The diastereomers of the next lower hexestrol homologue, *erythro*- and *threo*-2,3-bis(4-hydroxyphenyl)pentane, which would most likely have a similar ratio of populations of the antiperiplanar conformations, also have a similar ratio of binding affinities (Table 5, entries 6 and 7).

Despite the very good agreement between the ratio of binding affinities and the ratios of antiperiplanar conformation populations of the different diastereomers, it may still seem unwarranted to propose that both diastereomers in the antiperiplanar conformation form equally satisfactory complexes with the estrogen receptor, as the complexes are still diastereomeric. There are, however, several independent pieces of evidence that support this proposal and indicate that, with these nonsteroidal estrogens, the receptor appears to be relatively insensitive to the absolute and relative configuration of the benzylic centers and to changes in their hybridization.

First, although they too form diastereomeric complexes with receptor, the pure, resolved enantiomers *d*- and *l*-hexestrol (39) have nearly equivalent affinities (ratio of affinities: d/l = 1.6:1).[2] Second, DES, in which both of the benzylic carbons are sp^2 hybridized, has an affinity for the receptor very comparable to that of *meso*-hexestrol (Table 5, entry 3). Third, in recent studies (37) we have found that both enantiomers of *erythro*-methyl and pentyl-3,4-bis(4-hydroxyphenyl)hexanoate have identical affinities for the receptor (Table 5, entries 4–14); the most likely explanation for this is that the receptor cannot distinguish between a hexestrol analogue of this type that is bound in the "normal" mode [antiperiplanar, with the configurations of the benzylic centers congruent with those of the 8β and 9α centers of (+)-estradiol], and in the "reversed" mode (with the benzylic centers having configurations equivalent to an 8α,9β-estrogen) (37). Finally, even 8α-estradiol, which has a *cis*-B—C— ring fusion, has a binding affinity 60% that of estradiol (36) (Table 5, entry 2).

This model also satisfactorily explains the relative binding affinity for isobutestrol. The antiperiplanar population for 1,2-diphenylbutane has been calculated here to be 20% (Table 4). Since this is 25% of the antiperiplanar conformer population of *meso*-3,4-diphenylhexane (Table 4), it would suggest that isobutestrol should have a relative binding affinity around 25% that of *meso*-hexestrol (or relative binding affinity = 75), except that isobutestrol lacks one ethyl group and thus the corresponding free energy of hydrophobic bonding with the receptor site. The lower relative binding affinity of isobutestrol (25%; Table 5, entry 8), then, reflects the need for additional hydrophobic bonding.

Conclusion. The close correspondence between the relative population of the antiperiplanar conformation of *meso*- and *dl*-hexestrol and their relative affinities for the estrogen receptor has led to a model for the binding of nonsteroidal estrogens to the receptor. This model has two essential features: (1) only the conformation in which the two 4-hydroxyphenyl groups are antiperiplanar is bound, and (2) the configuration or state of hybridization (sp^3 versus sp^2) of the benzylic centers in these nonsteroidal estrogens have little influence on the nature of the complex.

REFERENCES

1. Roberts, G. C. K. Orbitals, conformation and biological activity. *Trends Biochem. Sci.* **1**:N80–N82 (1967).
2. Richards, W. G., R. Clarkson, and C. R. Gannelin. Molecular-receptor specificity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **272**:75–85 (1975).
3. Burgen, A. S. V., G. C. K. Roberts, and J. Feeney. Binding of flexible ligands to macromolecules. *Nature (Lond.)* **253**:753–755 (1975).
4. Humblet, C., and G. R. Marshall. Pharmacophore identification and receptor mapping. *Annu. Rep. Med. Chem.* **15**:267–276 (1980).
5. Christofferson, R. E. Molecules of pharmacological interest, in *Quantum Mechanics of Molecular Conformations* (B. Pullman, ed.). Wiley, New York, 193–293 (1976).
6. Pullman, B. (ed.). *Quantum Mechanics of Molecular Conformations.* Wiley, New York, 295–383 (1976).

7. Green, J. P., C. L. Johnson, and S. Kang. Application of quantum chemistry to drugs and their interactions. *Annu. Rev. Pharmacol.* **14**:319–342 (1974).
8. Hospital, M. B., B. Busetta, C. Courseille, and G. Precigoux. X-ray conformation of some estrogens and their binding to uterine receptors. *J. Steroid Biochem.* **6**:221–225 (1975).
9. Doyle, T. D., J. M. Steward, N. Filipescu, and W. R. Benson. Configuration of dienestrol. *J. Pharm. Sci.* **64**:1525–1528 (1975).
10. Weeks, C. M., S. Pokryweiki, and W. L. Daux. (+)-Hexestrol, analog of a synthetic estrogen. *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **29**:1729–1731 (1973).
11. Schwenk, E. D. Papa, B. Whitman, and H. F. Ginsberg. Reductions with nickel-aluminum alloy and aqueous alkali. IV. The carbon-carbon double bond. *J. Org. Chem.* **9**:175–177 (1944).
12. Campbell, N. R., E. C. Dodds, and W. Lawson. Estrogenic activity of dianol, a dimeride of *p*-propenylphenol. *Nature (Lond.)* **141**:78 (1938).
13. Shopee, C. W., J. C. Craig, and R. E. Lack. Acetylenic compounds related to stilbestrol. Part IV. The preparation of *α*-alkyl-*β*-ethynyl-4,4′-dihydroxystilbenes. *J. Chem. Soc.* 2291–2298 (1961).
14. Goswami, R., S. G. Harsy, D. F. Heiman, and J. A. Katzenellenbogen. Estrogen receptor-based imaging agents. 2. Synthesis and receptor binding affinity of side chain-halogenated hexestrol derivatives. *J. Med. Chem.* **23**: 1002–1008 (1980).
15. Allinger, N. J., M. T. Tribble, M. A. Miller, and D. H. Wertz. Conformational analysis. LXIX. An improved force field for the calculation of the structures and energies of hydrocarbons. *J. Am. Chem. Soc.* **93**:1637–1648 (1971).
16. Allinger, N. J., and D. Y. Chang. Conformational analysis. 118. Application of the molecular-mechanics method to alcohols and ethers. *J. Am. Chem. Soc.* **98**:6798–6803 (1976).
17. Katzenellenbogen, J. A., H. J. Johnson, Jr., and H. N. Myers. Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* **12**:4085–4092 (1973).
18. Karplus, M. Contact electron-spin coupling of nuclear magnetic moments. *J. Chem. Phys.* **30**:11–15 (1959).
19. Karplus, M. Vicinal proton coupling in nuclear magnetic resonance. *J. Am. Chem. Soc.* **85**:2870–2871 (1963).
20. Ganellin, C. R. Conformation of histamine derivatives. 3. A relationship between conformation and pharmacological activity. *J. Med. Chem.* **16**:620–623 (1973).
21. Kier, L. B., and E. B. Truitt, Jr. Preferred conformation of dopamine from molecular orbital theory. *J. Pharmacol. Exp. Ther.* **174**:94–98 (1970).
22. Hyne, J. B. Preferred residence conformations of diastereoisomeric-amino alcohols: an N.M.R. study of ephedrines. *Can. J. Chem.* **39**:2536–2542 (1961).
23. Cushley, R. J., and H. G. Mautner. N.M.R. studies on the conformation of acetylcholine isologs. *Tetrahedron* **26**:2151–2159 (1970).
24. Bothner-By, A. A., and C. Naar-Colin. The proton magnetic resonance spectra of 2,3-disubstituted *n*-butanes. *J. Am. Chem. Soc.* **84**:743–747 (1962).
25. Klyne, W., and V. Prelog. Description of steric relationships across single bonds. *Experientia (Basel)* **16**:521–523 (1960).
26. Reuvers, A. J., A. Sinnema, F. van Rantwijk, J. D. Remijnse, and H. van Bekkum. Synthesis and proton magnetic resonance studies of some 1,2-dialkyl-1,2-bis(2,6-dimethylphenyl)ethanes. *Tetrahedron* **25**:4455–4465 (1969).
27. Jackman, L. M., and S. Sternhell. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Ed. 2, Chap. 4.2. Pergamon Press, Oxford (1969).
28. Curtin, D. Y., and S. Dayagi. Effects on the N.M.R. spectra of the stereochemistry of 3,4-diaryladipic ester and 1,2-diarylcyclopentanes. *Can. J. Chem.* **42**:867–877 (1964).
29. Barbieux, M., and R. H. Martin. *α,β*-Dialkyl-*p*-methoxydihydrocinnamates d'ethyle. Attribution des configurations *threo* et *erythro* par spectrographie de resonance magnetique nucleaire. *Tetrahedron Lett.* 2919–2925 (1965).
30. Suetaka, A. On the molecular structures of synthetic estrogens. I. On *meso*-hexestrol dimethyl ether and *trans-α,α′*-bis(methylthio)stilbene. *Bull. Chem. Soc. Jpn.* **25**:402–404 (1952).
31. Westheimer, F. H. Calculation of the magnitude of steric effects, in *Steric Effects in Organic Chemistry* (M. S. Newman, ed.). Wiley, New York, 523–555 (1956).
32. Williams, J. E., P. J. Strang, and P. von R. Schleyer. Physical organic chemistry: quantitative conformational analysis; calculation methods. *Annu. Rev. Phys. Chem.* **19**:531–558 (1968).
33. DuChamp, D. J. Molecular mechanics and crystal structure analysis in drug design, in *Computer Assisted Drug Design* (E. C. Olson and R. E. Christofferson, eds.), ACS Symposium Ser. 112,. Chap. 3. American Chemical Society, Washington, D. C. (1979).
34. Farnell, L., W. G. Richards, and C. R. Ganellin. Calculation of conformational free energy of histamine. *J. Theor. Biol.* **43**:389–392 (1974).
35. Grundy, J. Artificial estrogens. *Chem. Rev.* **57**:281–416 (1957).
36. Payne, D. W., and J. A. Katzenellenbogen. Differential effects of estrogens in tissues: a comparison of estrogen receptor in rabbit uterus and vagina. *Endocrinology* **106**:1345–1352 (1980).
37. Landvatter, S. W., and J. A. Katzenellenbogen. Stereochemical considerations in the binding of nonsteroidal estrogens to the estrogen receptor. *Mol. Pharmacol.*, in press (1981).
38. Eliel, E. L., N. L. Allinger, S. J. Angyal, and G. A. Morrison. *Conformational Analysis*. Interscience, New York, 21–27 (1965).
39. Wessely, F. V., and H. Welleba. Synthetic estrogens. II. Configuration of the synthetic estrogens. *Chem. Ber.* **74**:777–785 (1941).

Send reprint requests to: Dr. John A. Katzenellenbogen, The Roger Adams Laboratory, School of Chemical Sciences, University of Illinois, 1209 West California Street, Urbana, Ill. 61801.