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# Molecular Structures of Metabolites and Analogues of Diethylstilbestrol and Their Relationship to Receptor Binding and Biological Activity

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SUMMARY

A series of indanyl derivatives of diethylstilbestrol (DES) have recently been identified as *inv vivo* metabolites of DES. These compounds are of interest because they possess effective uterine estorgen receptor-binding affinity but poor biological activity. The X-ray crystal structures of three of these derivatives were determined and their conformations were compared with those of estradiol and DES. The more active derivatives, indenestrol A (I) and indenestrol B (II) have nearly identical conformations, in which the overall molecule is highly planar, the phenyl ring is twisted out of the plane of the indene rings by approximately 30°, and the distance between the hydroxyl groups is 11.6 Å. In the least active derivative, idanestrol (III), the methyl, ethyl, and phenyl substituents were found to be in the same side of the indane ring so that the molecule is constrained to an L-shape. The crystallographically observed conformations of I, II, III, DES, and estradiol, their competative binding affinities, and their *in vivo* biological activities are consistent with the proposal that the steroid A-ring plays a dominant role in initiating receptor binding while the D-ring orientation relative to the A-ring has a more decisive influence upon activity. The reduction in estrogen receptor-binding affinity and associated reduced activity of III is almost certainly due to its L-shape conformation. The extended conformation of I and II in which both phenolic rings are exposed permitting ready access to both surfaces of either ring probably accounts for the ability of these derivatives to compete so successfully with estradiol for estrogen receptor binding. There are eight different ways in which the molecules of the racemic mixtures of I and II could initiate receptor binding. The reduced biological activity of I and II is probably due to the fact that not all eight binding orientations are compatible with eliciting estrogenic response. Comparison of the observed conformations of I, II, DES, and estradiol suggests that it is the $\alpha$-ring of I and II that minics the steroid A-ring in receptor binding, and that two of the four possible $\alpha$-ring/A-ring matches are most conducive to eliciting hormone activity.

## INTRODUCTION

Studies in humans and mice (1, 2) have demonstrated an association between *in utero* exposure to DES[4] and cancerous lesions of the reproductive tract. The mechanisms underlying these effects by DES are yet unknown but may be related to the action of the products of oxidative metabolism of DES in its target tissue (3). Previous studies have shown that these oxidative products interact with the uterine estrogen receptor with a variety of binding affinities (4). One series of compounds, termed indanyl-DES derivatives, originally described as DES analogues, have recently been identified as *in vivo* metabolites (4). These compounds are of particular interest since they possess effective receptor-binding affinity but poor biological activity. Structures of the compounds indenestrol A (I) [1-ethyl-2-(4′-hydroxyphenyl)-3-methyl-5-hydroxyindene], indenestrol B (II) [1-methyl-2-(4′-hydroxyphenyl)-3-ethyl-6-hydroxyindene], and indanestrol (III) [1-ethyl-2-(4′-hydroxyphenyl)-3-methyl-5-hydroxyindane], are illustrated in Fig. 1. The binding affinities of these compounds relative to those of $E_2$ and DES as previously determined by competitive equilibrium receptor binding analyses and *in vivo* bioassay (5, 6) are presented in Table 1.

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[4] The abbreviations used are: DES, diethylstilbestrol, (*E*)-3,4-bis(4-hydroxyphenyl)-3-hexene; $E_2$, estradiol, 1,3,5(10)-estratriene-3,17$\beta$-diol.

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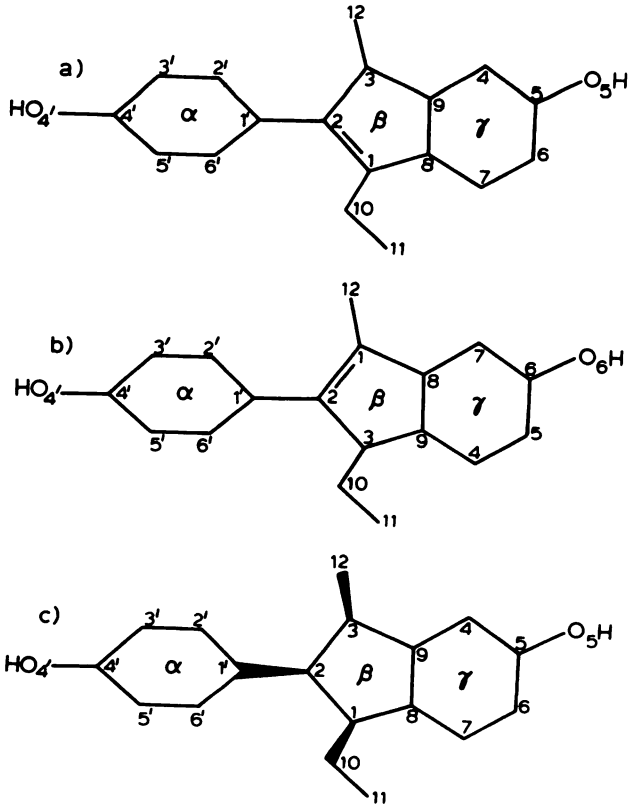


FIG. 1. *Chemical diagrams, atomic numbering, and ring identification for (I) indenestrol A, (II) indenestrol B, and (III) indanestrol*

TABLE 1

*Summary of reported binding affinities and uterotropic activities of estradiol, DES, indenestrol A (I), indenestrol B (II), and indanestrol (III)*

| Compound | $IC_{50}$[a] | Doubling dose[b] |
|---|---|---|
| | | *μg/kg* |
| $E_2$ | 1.0 ± 0.1 | 10 |
| DES | 0.5 ± 0.1 | 7 |
| I | 0.7 ± 0.1 | 107 |
| II | 0.7 ± 0.2 | 111 |
| III | 50 ± 5 | 1120 |

[a] Molar excess concentration of competitor required for 50% inhibition of specific binding of [$^3$H]estradiol. Results are expressed as the mean ± standard error for a minimum of four determinations. Competitive binding values were obtained from equilibrium binding experiments. Mouse uterine cytosol (100 μl) was incubated for 18 hr at 4° with 10 nM [$^3$H]estradiol in the presence of various unlabeled competitors (5). The concentration of competitor ranged from 0.1–1000-fold molar excess. Receptor binding was assessed by precipitation of the receptor with a 0.5-ml aliquot of 4 mg/ml solution of protamine sulfate. The $IC_{50}$ value is calculated as the molar excess of unlabeled competitor required to inhibit 50% of the specific receptor binding.

[b] Values are expressed as dose of compound required to produce a 2-fold increase above the control of uterine weight/body ratio in 21-day-old CD1 mice treated for 3 days. Data are taken from refs. 5 and 6.

On the basis of a previous analysis of the structures of a variety of compounds that compete for binding to the estrogen receptor, we have proposed that the phenolic A-ring of steroidal estrogens is primarily responsible for initiating receptor binding and that the D-ring end of the molecule is primarily responsible for controlling activity (7).

The following observations support such a model. The only common feature of compounds that compete with estradiol for binding to the estrogen receptor with relatively high affinity is a phenolic ring. Simple compounds such as tetrahydronaphthol and *p-sec*-amyl phenol prevent or compete for the binding of estradiol to its receptor (8). Chernyaev *et al.* (9) have demonstrated that the removal of the 3-hydroxyl substituent significantly decreases receptor binding while retaining some portion of biological activity. Removal of the 17-hydroxyl was shown to decrease binding to a lesser extent than 3-hydroxy removal but to almost totally abolish biological activity (9). The indanyl compounds are an interesting series with which to further test this hypothesis since a minor chemical variation in III, the saturation of the indene ring of I and II, produces a significant decrease in binding activity. A comparison of the crystallographically observed structures of I, II, and III with estradiol and DES may provide further information on the relative orientation of these molecules when bound to the receptor and the basis for the observed variation in binding and activity. Examination of the X-ray crystal structures of I, II, and III was undertaken in order to determine unambiguously the relative configuration of the ethyl and methyl substituents on III, the overall conformations of all three of the molecules, and the structural basis for the observed variation in receptor binding and activity.

## MATERIALS AND METHODS

Single crystals were obtained by slow evaporation from ethanol for I and III and chloroform of II. Lattice parameters were calculated by least squares fitting the $\theta$ values of 31, 25, and 24 diffractometer-centered reflections for I, II, and III, respectively. A Nicolet P3 automated diffractometer was used to collect the intensity data. Because of a pseudo-R-centering in the structure of I, the data were collected at 90°K to improve the observed to unobserved ratio. Crystal data for the three compounds are listed in Table 1. The $\theta$–$2\theta$ scan data were corrected for Lorentz and polarization factors but not for absorption.

The structure of each compound was determined using the direct methods computer program MULTAN (10) and the atomic parameters were refined by anisotropic full matrix least squares methods. The hydrogen atom coordinates were taken from difference electron density maps and refined isotropically. The crystal structure of II includes a hydrophobic channel with disordered partially occupied solvent positions which were refined isotropically.

The crystal and refinement data and reliability indices are given in Table 2 and the atomic coordinates and equivalent $B_{iso}$ for nonhydrogen atoms are given in Table 3. Atomic coordinates for hydrogen atoms, disordered solvent in II, anisotropic thermal parameters, lists of the bond distances, bond angles and torsion angles for the three compounds, and structure factor tables are available from the corresponding author.

## RESULTS

There are three crystallographically independent molecules of I present in the crystal lattice. Although the molecules have slightly different crystalline environments and distinctly different hydrogen bonding geometries (Table 4), their conformations are nearly identical. The conformation of one molecule of I is illustrated in Fig. 2a. The atoms of the indene molecule (rings β and

TABLE 2

*Crystal and refinement data*

| | I | II | III |
|---|---|---|---|
| Molecular formulas | $C_{18}H_{18}O_2$ | $C_{18}H_{18}O_2 \cdot \frac{1}{2}CHCl_3$ | $C_{18}H_{20}O_2$ |
| Molecular weight | 266.36 | 319.54 | 268.38 |
| Crystal system | Trigonal | Monoclinic | Monoclinic |
| Space group | $P\bar{3}$ | $P2_{1/n}$ | $P2_{1/c}$ |
| Cell dimensions | | | |
| $a$ (Å) | 17.355 (3) | 10.322 (1) | 8.140 (1) |
| $b$ | 17.355 | 13.288 (1) | 21.813 (2) |
| $c$ | 24.816 (4) | 25.048 (2) | 8.522 (1) |
| $\beta$ (°) | [$\gamma$ = 120°] | 100.57 (1) | 92.34 (1) |
| $V$ (Å$^3$) | 6,473.0 | 3,377.2 | 1,512.0 |
| $Z$ | 18 | 8 | 4 |
| Crystal size (mm) | 0.20 × 0.20 × 0.20 | 0.12 × 0.24 × 0.38 | 0.08 × 0.48 × 0.64 |
| Density calculated (g/cm$^3$) | 1.23 | 1.26 | 1.18 |
| $\lambda K\bar{a}$ | MO 0.7107 | Cu 1.54 | Cu 1.54 |
| $\theta_{max}$ (°) | 27 | 79 | 58.5 |
| Total no. of data collected/averaged | 16,861/8,864 | 9,117/6,596 | 2,516/1,990 |
| Data used in refinement | 4,567 | 5,111 | 1,824 |
| $R$ | 0.119 | 0.098 | 0.058 |
| $R\omega$ | 0.077 | 0.114 | 0.091 |

$\gamma$) are coplanar and the $\alpha$-ring is twisted 31°, 31°, and 35° out of that plane in the three crystallographically independent molecules. The C-3-methyl substituent and the terminal methyl of the ethyl substituent are on the same side of the plane of the $\beta$-ring. The compound is a *dl* mixture and the crystals contain the molecule illustrated in Fig. 2a and its mirror image. Unless designated otherwise, the enantiomorph used in all illustrations of I and II is the one that proved to show greatest structural similarity with estradiol and DES in the comparative analysis described below. The bond lengths and angles are unexceptional. It should be noted that for all three molecules the hydrogen bonds formed by the O-5 hydrogens are shorter and consequently stronger than those formed by the O-4′ hydrogens. In all of these shorter hydrogen bonds, the acceptor as well as the donor is an O-5-hydroxyl and the refined position of the hydrogen atom is shifted toward the center of the hydrogen bond. This may reflect an average of two partial occupancy hydrogen positions in these shorter hydrogen bonds. The hydrogen atom geometry of the O-4 by hydroxyls is unexceptional.

There are two crystallographically independent molecules of II present in the crystal lattice. Once again, despite different crystalline environments and hydrogen bonding (Table 4), the molecules have nearly identical conformations. The conformation of one of the molecules is illustrated in Fig. 2b. The $\beta$- and $\gamma$-rings are coplanar and the $\alpha$-ring is rotated 33° out of that plane in both conformers. The double bond forces the methyl substituent to be coplanar with the indene molecule. The two conformers of II differ only in the orientation of the C-methyl atom of the ethyl substituent which is *trans* to the C-2—C-3 bond in one molecule and oriented over the $\beta$-ring, *gauche* to the C-2—C-3 and C-3—C-9 bonds in the other. Once again the compound is a *dl* mixture and the crystals contain the molecules illustrated in Fig. 2b and their mirror images. The bond lengths and angles are unexceptional, although the crystallographically determined hydrogen atom location in the hydrogen bond between the indene-hydroxyls in structure II is consistent with those observed in the hydrogen bonding between the indene-hydroxyls in I, and O ... O distance is not as short as in the case of structure I. In this case the shortest, strongest hydrogen bond is between the O-4-hydroxyls of the two molecules of II.

The observed conformation of III is illustrated in Fig. 2c. The methyl, ethyl, and phenyl substituents are all on the same side of the five-membered ring with the phenyl in an axial position and the ethyl and methyl substituents in equatorial positions. Because of the axial orientation of the phenyl substituent, the molecule has an L-shaped conformation, uncommon for DES analogues. The bond lengths and angles are unexceptional. Both of the hydroxyls form strong hydrogen bonds whose geometry is described in Table 4.

When the conformations of I and II are compared, the orientation of the $\alpha$-ring relative to the $\beta$- and $\gamma$-rings is found to be nearly identical in the two structures despite the shift in location of the double bond. The consistency of the orientation of the plane of the $\alpha$-ring relative to the plane of the $\beta$- and $\gamma$-rings in the five crystallographically independent observations suggest that this conformation represents a very stable minimum energy position. An approximate 35° rotation of the plane of the $\alpha$-ring relative to the plane of the $\beta$- and $\gamma$-rings could be achieved in a positive or negative sense. The direction of this rotation appears to be controlled by intermolecular interaction between the phenyl ring and the substituent on the chiral carbon (C-3) of the $\beta$-ring. The observed rotations allow maximum separation between the atoms indicated by the two-headed arrows of Fig. 2a and b.

In order to identify the structural basis for their competition for binding to the estrogen receptor site, it is of interest to compare the overall conformations of I, II, and III with $E_2$ and DES.

There are at least eight different ways to compare each of the molecules I, II, and III to estradiol. There are two

TABLE 3

*Atomic coordinates (×10⁴) and isotropic thermal parameters (×10) for the nonhydrogen atoms*

Compound I

| ATOM | X/A(σ) | Y/B(σ) | Z/C(σ) | BISO(σ) | ATOM | X/A(σ) | Y/B(σ) | Z/C(σ) | BISO(σ) |
|---|---|---|---|---|---|---|---|---|---|
| C(1A) | 8783(3) | 3598(3) | 5667(2) | 17(2) | C(11B) | 91(4) | 3120(4) | -582(3) | 26(2) |
| C(2A) | 8538(3) | 4151(3) | 5873(2) | 17(2) | C(12B) | 3618(4) | 4753(4) | -1287(2) | 17(2) |
| C(3A) | 8209(4) | 4511(3) | 5423(2) | 15(2) | C(1'B) | 2555(3) | 4844(3) | -58(2) | 16(2) |
| C(4A) | 8282(4) | 4227(3) | 4387(2) | 17(2) | C(2'B) | 1948(4) | 4239(4) | 325(2) | 19(2) |
| C(5A) | 8408(4) | 3765(4) | 4085(2) | 21(2) | C(3'B) | 2182(4) | 4328(4) | 867(2) | 19(2) |
| C(6A) | 8714(4) | 3207(4) | 4147(2) | 21(2) | C(4'B) | 3007(4) | 4996(4) | 1031(2) | 17(2) |
| C(7A) | 8856(4) | 3098(4) | 4684(2) | 22(2) | C(5'B) | 3689(4) | 5565(4) | 655(2) | 22(2) |
| C(8A) | 8684(4) | 3553(4) | 5077(2) | 21(2) | C(6'B) | 3382(3) | 5489(3) | 121(2) | 14(2) |
| C(9A) | 8348(3) | 4111(3) | 4923(2) | 15(2) | O(5B) | 2261(3) | 5869(3) | -3358(1) | 22(2) |
| C(10A) | 9101(4) | 3035(4) | 5963(2) | 22(2) | O(4'B) | 3185(3) | 5076(3) | 1582(1) | 26(2) |
| C(11A) | 10110(4) | 3447(5) | 5963(3) | 25(2) | C(1C) | 7975(3) | -181(3) | 7618(2) | 12(2) |
| C(12A) | 8685(4) | 5521(4) | 5421(2) | 24(2) | C(2C) | 8733(3) | 558(3) | 7387(2) | 14(2) |
| C(1'A) | 8503(4) | 4382(3) | 6440(2) | 18(2) | C(3C) | 9428(3) | 1057(3) | 7829(2) | 15(2) |
| C(2'A) | 9115(4) | 4425(3) | 6819(2) | 18(2) | C(4C) | 9213(4) | 703(4) | 8868(2) | 17(2) |
| C(3'A) | 9837(4) | 4571(4) | 7354(2) | 21(2) | C(5C) | 8608(4) | 259(4) | 9262(2) | 19(2) |
| C(4'A) | 8335(4) | 4677(3) | 7526(2) | 19(2) | C(6C) | 7766(4) | -435(4) | 9142(2) | 18(2) |
| C(5'A) | 7736(4) | 4687(4) | 7161(2) | 19(2) | C(7C) | 7502(3) | -618(4) | 8594(2) | 18(2) |
| C(6'A) | 7824(4) | 4532(4) | 6621(2) | 18(2) | C(8C) | 8081(3) | -87(3) | 8202(2) | 16(2) |
| O(5A) | 8304(3) | 3876(3) | 3451(1) | 22(2) | C(9C) | 8932(3) | 595(3) | 8332(2) | 17(2) |
| O(4'A) | 8246(3) | 4769(3) | 8074(1) | 24(2) | C(10C) | 7121(4) | -737(4) | 7353(2) | 19(2) |
| C(1B) | 1484(3) | 4496(3) | -853(2) | 13(2) | C(11C) | 6446(4) | -391(4) | 7364(2) | 22(2) |
| C(2B) | 2315(3) | 4815(3) | -637(2) | 15(2) | C(12C) | 9742(4) | 2861(4) | 7828(2) | 21(2) |
| C(3B) | 3016(4) | 5166(4) | -1077(2) | 15(2) | C(1'C) | 8948(3) | 768(3) | 6821(2) | 14(2) |
| C(4B) | 2727(4) | 5173(4) | -2115(2) | 18(2) | C(2'C) | 8385(4) | 631(3) | 6431(2) | 18(2) |
| C(5B) | 2067(4) | 4927(4) | -2585(2) | 19(2) | C(3'C) | 8586(3) | 774(3) | 5886(2) | 15(2) |
| C(6B) | 1181(4) | 4533(3) | -2368(2) | 19(2) | C(4'C) | 9365(3) | 1088(3) | 5723(2) | 15(2) |
| C(7B) | 921(4) | 4367(4) | -1828(2) | 22(2) | C(5'C) | 10222(4) | 1253(4) | 6093(2) | 16(2) |
| C(8B) | 1578(3) | 4602(3) | -1435(2) | 14(2) | C(6'C) | 9887(3) | 1100(3) | 6632(2) | 16(2) |
| C(9B) | 2467(4) | 4996(3) | -1578(2) | 17(2) | O(5C) | 8819(3) | 411(3) | 9089(1) | 22(1) |
| C(10B) | 614(4) | 4136(4) | -573(2) | 19(2) | O(4'C) | 9542(3) | 1213(3) | 5173(1) | 20(1) |

Compound II

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| C(1A) | 8776(4) | 2636(4) | 7618(2) | 44(1) | C(1B) | 6698(4) | 6946(4) | 2021(2) | 45(1) |
| C(2A) | 9369(4) | 2179(4) | 8067(1) | 41(1) | C(2B) | 5960(5) | 7668(4) | 2238(2) | 48(1) |
| C(3A) | 9838(5) | 1128(4) | 7946(2) | 45(1) | C(3B) | 4847(6) | 8084(5) | 1758(2) | 57(2) |
| C(4A) | 9522(5) | 285(5) | 6978(2) | 54(1) | C(4B) | 4426(6) | 7343(5) | 753(2) | 58(2) |
| C(5A) | 9031(5) | 435(5) | 6428(2) | 51(1) | C(5B) | 4881(6) | 6788(4) | 382(2) | 56(1) |
| C(6A) | 8431(4) | 1333(4) | 6248(2) | 50(1) | C(6B) | 5968(5) | 6116(4) | 547(2) | 48(1) |
| C(7A) | 8288(5) | 2136(5) | 6688(2) | 50(1) | C(7B) | 6635(5) | 6135(4) | 1280(2) | 48(1) |
| C(8A) | 8751(4) | 1945(4) | 7154(1) | 43(1) | C(8B) | 6178(4) | 6772(4) | 1445(2) | 44(1) |
| C(9A) | 9378(4) | 1842(4) | 7336(1) | 44(1) | C(9B) | 5268(5) | 7377(4) | 1281(2) | 51(1) |
| C(10A) | 9332(7) | 315(5) | 8288(2) | 61(2) | C(10B) | 4815(9) | 9129(6) | 1654(3) | 81(2) |
| C(11A) | 10893(11) | -691(6) | 8287(3) | 98(5) | C(11B) | 6823(14) | 9504(10) | 1496(4) | 97(3) |
| C(12A) | 8284(8) | 3783(5) | 7538(2) | 66(2) | C(12B) | 7887(6) | 6323(5) | 2328(2) | 56(2) |
| C(1A') | 9636(4) | 2576(4) | 8621(2) | 46(1) | C(1B') | 6145(5) | 8073(4) | 2759(2) | 50(1) |
| C(2A') | 10702(5) | 2322(4) | 8988(2) | 48(1) | C(2B') | 5868(5) | 8369(4) | 2989(2) | 53(1) |
| C(3A') | 11865(5) | 2738(4) | 9503(2) | 53(1) | C(3B') | 5236(5) | 8726(5) | 3518(2) | 57(2) |
| C(4A') | 10107(5) | 3361(4) | 9678(2) | 51(1) | C(4B') | 6472(5) | 8817(4) | 3825(2) | 51(1) |
| C(5A') | 9826(5) | 3623(5) | 9333(2) | 61(2) | C(5B') | 7562(5) | 8561(5) | 3683(2) | 59(2) |
| C(6A') | 8756(5) | 3234(5) | 8812(2) | 58(2) | C(6B') | 7396(5) | 8234(4) | 3272(2) | 52(1) |
| O(6A) | 7960(4) | 1535(3) | 5695(1) | 68(1) | O(6B) | 6388(4) | 5524(3) | 164(1) | 55(1) |
| O(4A') | 10415(4) | 3774(4) | 10190(1) | 74(1) | O(4B') | 6682(4) | 9167(4) | 4358(1) | 66(1) |

Compound III

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| C(1) | 6180(2) | 5532(1) | 6505(3) | 47(1) | C(11) | 9064(3) | 5197(1) | 6931(4) | 74(1) |
| C(2) | 5670(2) | 5611(1) | 8241(2) | 43(1) | C(12) | 2961(3) | 6030(1) | 9424(3) | 59(1) |
| C(3) | 3767(2) | 5733(1) | 8042(2) | 46(1) | C(1') | 6620(2) | 6101(1) | 9126(2) | 48(1) |
| C(4) | 2417(2) | 6470(1) | 5969(2) | 46(1) | C(2') | 7715(2) | 5949(1) | 10341(2) | 44(1) |
| C(5) | 2561(3) | 6750(1) | 4529(2) | 49(1) | C(3') | 8702(2) | 6370(1) | 11119(2) | 46(1) |
| C(6) | 3906(3) | 6638(1) | 3622(2) | 56(1) | C(4') | 8593(2) | 6940(1) | 10687(2) | 44(1) |
| C(7) | 5133(3) | 6241(1) | 4169(3) | 51(1) | C(5') | 7485(3) | 7155(1) | 9517(3) | 58(1) |
| C(8) | 5015(2) | 5965(1) | 5627(2) | 42(1) | C(6') | 6515(3) | 6724(1) | 8739(3) | 51(1) |
| C(9) | 3654(2) | 6080(1) | 6515(2) | 41(1) | O(5) | 1385(2) | 7153(1) | 3960(2) | 66(1) |
| C(10) | 8080(3) | 5611(1) | 6190(3) | 59(1) | O(4') | 9597(2) | 7427(1) | 11343(2) | 56(1) |

enantiomers of each of the DES analogues, two phenolic rings in each molecule, and two ways to fit the phenolic ring to the A-ring of estradiol. The last two orientations are related by 180° rotation about the C-3—O-3 bond.

The results of a least squares fit of the A-ring of estradiol to the $\alpha$- and $\gamma$-rings of one enantiomer of I are illustrated in Fig. 3. Superposition of the A-ring of estradiol and the $\alpha$-ring of I (Fig. 3a and b) clearly produces the best overall fit and relative positioning of the second hydroxyl. Although the overall fit of hydrophobic bulk and hydroxyl positions of the molecules compared in Fig. 3c and d could be improved, it would occur at the expense of the good fit of the phenolic ring. Not only is the best fit achieved in Fig. 3a, but it is clear that minor reorientation of the molecules will produce a simultaneous improvement in the fit of the hydrophobic bulk and relative location of the second hydroxyl with only minor reduction in the A-ring overlap. This is illustrated in Fig. 4a where the atoms included in least squares fitting process are C-2, C-3, C-4, O-3, and O-17 of estradiol and the corresponding atoms of a molecule of I. Attempting a comparable least squares fit to the molecular overlap shown in fig. 3b significantly reduces hydrophobic bulk overlap. The best least squares fits of the A-ring of the

TABLE 4

*Hydrogen bond donor (D) and acceptor (A) geometry*

| | D | A | D...A | D-H | A...H | D-H...A |
|---|---|---|---|---|---|---|
| I. | O5A | O5B | 2.667 Å | 1.30 Å | 1.39 Å | 165° |
| | O5B | O5A | 2.676 | 1.16 | 1.55 | 160 |
| | O5C | O5C | 2.659 | 1.28 | 1.42 | 162 |
| | O4'A | O4'B | 2.762 | 0.92 | 1.86 | 165 |
| | O4'B | O4'A | 2.774 | 1.03 | 1.89 | 142 |
| | O4'C | 04'C | 2.733 | 1.04 | 1.72 | 164 |
| II. | O6A | O6B | 2.704 | 1.22 | 1.54 | 157 |
| | O6B | O4'B | 2.794 | 0.90 | 1.91 | 163 |
| | O4'A | O6A | 2.724 | .89 | 1.83 | 177 |
| | O4'B | 04'A | 2.683 | 1.00 | 1.75 | 152 |
| III. | O5 | O4 | 2.707 | .94 | 1.79 | 164 |
| | O4' | O5 | 2.680 | .89 | 1.85 | 154 |

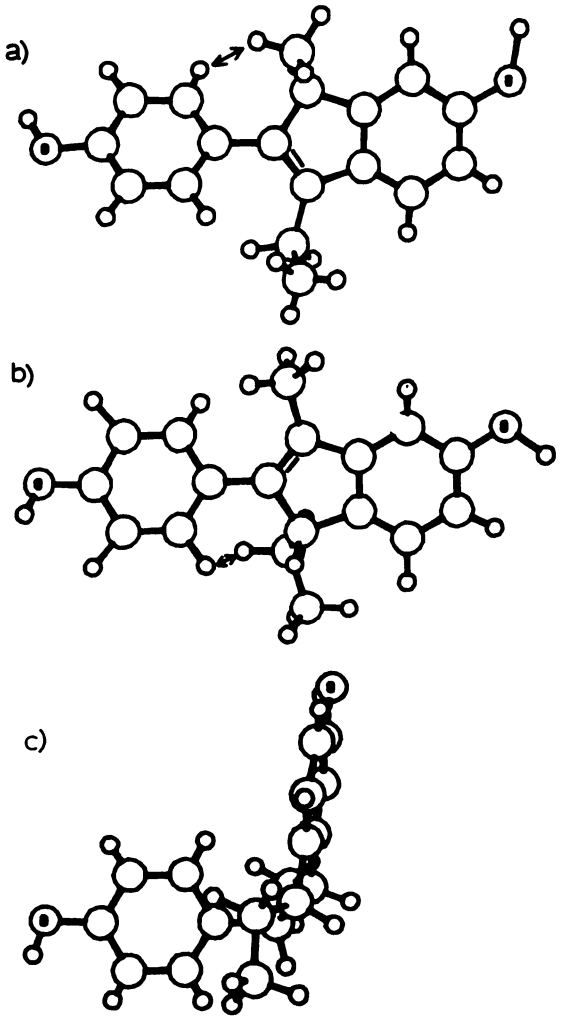


FIG. 2. *ORTEP illustrations of the observed conformations of I, II, and III*

other enantiomer of I with the A-ring of estradiol is illustrated in Fig. 4b.

Of the eight possible orientations of I relative to estradiol, that shown in Fig. 4a would appear to be the most likely to account for their binding to the same site on a receptor and that shown in Fig. 4b is a possible second choice. Similar comparisons between the structure of estradiol and II reveal that the best fit is achieved with

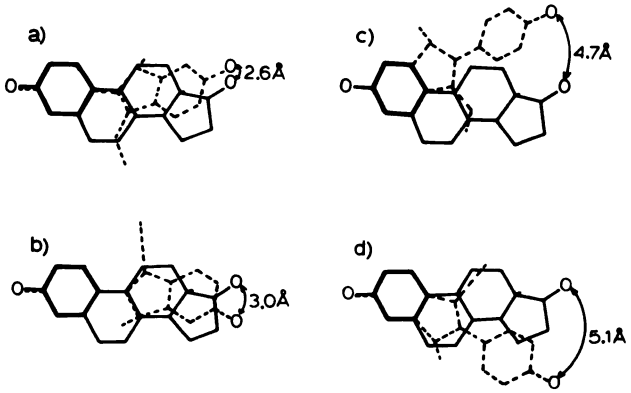


FIG. 3. *Comparison of the conformations of I and estradiol*

In a and b, the $\alpha$-ring is superimposed on the A-ring of estradiol in the two orientations related by 180° rotation about the C-4'—O-4' bond. In c and d, the $\gamma$-ring of I is superimposed on the A-ring of estradiol in the two orientations related by 180° rotation about the C-5—O-5 bond. The molecule of I used for this comparison has the *R* configuration as the chiral C-3 atom. A comparison with the C-3-S isomer is illustrated in Fig. 4b. The comparisons were done by least squares fit to the $\alpha$- or $\gamma$-rings and the attached hydroxyl oxygen. The distances separating the oxygens not included in the least squares fit are indicated.

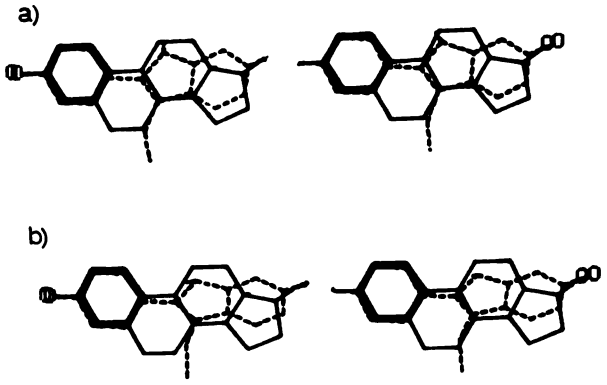


FIG. 4. *Stereo views*

(a) Comparison of the molecular overlap between I and estradiol achieved by relaxing the fit of the $\alpha$-ring shown in Fig. 3a. (b) View of the molecular overlap between estradiol and the C-3-S isomer of I that maximizes overlap of the A-rings of estradiol with the $\alpha$-ring of I, the overlap of the hydroxyl groups and the overlap in the hydrophobic bulk. The least squares fit is between C-2, C-3, C-4, O-3, and O-17 of estradiol and the appropriately related atoms in I.

enantiomers of II that have the orientation comparable to those found for I in Figs. 3 and 4, in which the $\alpha$-ring is the A-ring analogue.

The phenolic rings in DES are chemically constrained to be extended and the oxygen-oxygen distance is fixed at 12.1 Å. This is longer than the 10.9, 11.6, and 11.6 Å distances observed in estradiol, I, and II, respectively. An attempt was made to achieve good agreement in the relative position of both hydroxyl substituents and the hydrophobic bulk of E, DES, and I. The best fit (Fig. 5) requires a relaxation of the constraint of coplanarity of the phenol rings in order to bring the hydroxyl groups into near coincidence.

## DISCUSSION

The crystallographically observed conformations of I, II, III, DES, and estradiol, their competitive receptor-

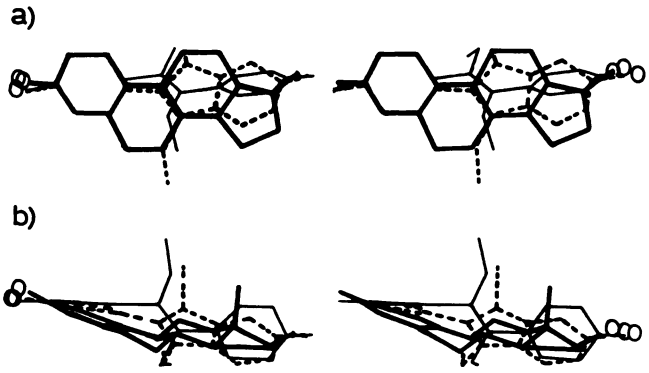


FIG. 5. *Stereo views illustrating the best fit of the hydroxyl groups and the hydrophobic bulk of the structure of E (dark solid lines), DES (light solid lines), and I (dashed lines)*

binding affinities, and their *in vivo* biological activities are consistent with the proposal that the A-ring is most important for receptor binding while the D-ring primarily influences expression of estrogen hormone action. Because I, II, III, and DES all contain two phenolic rings capable of mimicking the A-ring of estradiol in its interaction with the receptor, each could conceivably bind in four different orientations. Because I, II, and III are racemic mixtures and there are four orientations possible for each enantiomer, there are eight ways that each could bind. The presence of the double bond in the five-membered ring of I and II forces them to have relatively flat, fully extended conformations in which both phenolic rings are exposed permitting ready access to either surface of either ring. For this reason, they are found to compete for the estrogen receptors with affinity comparable to that of DES (5, 6). The reduction in binding of III is almost certainly due to its bent ring L-shaped conformation. Although the phenolic rings could be expected to have some affinity for the binding site, the L-shape appears to interfere with optimal receptor interaction. It could be assumed using the analogy with estradiol and estriol that the differences in affinity of the ligands for the receptor are due to a more rapid dissociation rate of the complex. In the case of III, the phenol ring structure would still allow the proper association rate, but the overall structure results in an increased dissociation rate, thereby leading to the overall lower binding constant previously reported (5, 6). Experiments to prove directly this possibility will await radiolabeled III to determine the respective rate constants. The poor *in vivo* biological activity of III would appear to be a natural consequence of its reduced binding affinity and lack of orientation for D-ring activity.

Despite the fact that I and II compete successfully with DES for binding to the estrogen receptor, they are found to have approximately 1/15 the uterotropic biological activity of DES. This significant reduction in activity could be due to the fact that 1) both enantiomers are not equivalent in biological activity and 2) not all of the four possible ligand-binding orientations (Fig. 3) in the receptor are capable of inducing the biological activity of the receptor complex. If the $\gamma$-ring of I or II were to mimic the A-ring of estradiol (Fig. 3c and d) and bind to the receptor, the hydroxyl on the $\alpha$-ring would be so far displaced from the O-17 position that it would fail to fulfill the role of O-17 in promoting hormone action. If only one of the eight binding modes possible for a racemic mixture of I (or II) has the proper orientation of the second hydroxyl group, it would account for the reduction in biological activity that is observed despite evidence that I, II, and DES compete equally well *in vitro* for the estrogen steroid hormone-binding site on the receptor. Difference in the strengths of the hydrogen bonds formed by the hydroxyls in the $\alpha$- and $\gamma$-ring, suggested by the difference in hydrogen bond lengths recorded in Table 4, may also have an influence upon the extent to which a molecule of I, II, or III binds to the receptor in their various possible orientations. Examination of dozens of steroids having common hydrogen bond donors reveals consistent patterns in the orientation of the hydrogen bonds formed by these donors despite extensive variation in the identity of the hydrogen bond acceptors and molecular packing in the crystals, suggesting that the patterns may have relevance to receptor interaction (12). The strongest hydrogen bond formation may or may not correspond to the relative orientation most conducive to induction of hormonal response. Selective differences in stimulating individual hormone responses have been reported (13) from studies using I, II, and III and indicate that these differences may be related to how well a particular ligand receptor complex can interact at specific genomic sites. It should be possible with this approach, in which correlations are developed between estrogen receptor binding, biological activity, and structural modifications in closely related stilbene estrogens, to better understand the structural basis of estrogenicity.

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