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The Crystal and Molecular Structure of Diethylstilbestrol

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The potent artificial estrogen, diethylstilbestrol ($C_{18}H_{20}O_2$), crystallizes in the orthorhombic space group *Pbca* with cell dimensions $a=18{\cdot}954$, $b=14{\cdot}929$, and $c=5{\cdot}291$. The four molecules in the unit cell each lie on a crystallographic center of symmetry. The separation of the phenolic oxygen atoms, a parameter which is generally accepted as being important in determining estrogenic activity, is 12·13 Å, and the plane of each benzene ring forms a dihedral angle of 62·8° with the plane passing through the atoms comprising the central ethylenic linkage. Weak hydrogen bonding is observed in the crystals, with H···O=2·11 Å and an angle O–H···O of 171°.

Introduction

Diethylstilbestrol* was one of the first synthetic substances found to possess estrogenic activity (Dodds, Goldberg, Lawson & Robinson, 1938), and it still is one of the most potent estrogens known. The mechanism by which the estrogens control the development of the secondary sex characteristics in the female is unknown, and only recently has any progress been made towards the identification of the component of sensitive tissue responsible for initial interaction with hormone molecules (Jensen & Jacobson, 1962). It has been found that the natural hormone, 17β-estradiol, is bound to a stereospecific, and probably proteinaceous receptor in uterine cells (Noteboom & Gorski, 1965). Diethylstilbestrol inhibits the binding of 6,7-H^3-17β-estradiol to this specific estrogen receptor (Noteboom & Gorski, 1965; Brecher, Vigersky, Wotiz & Wotiz, 1967). Although it is possible to draw the diethylstilbestrol molecule in such a way (Fig. 1) as to show a superficial resemblance to a steroid, steric hindrance between the *ortho* hydrogen atoms and those of the alkyl group precludes the existence of this conformation (Koch, 1948). The X-ray analysis of diethylstilbestrol was undertaken in order to elucidate its three-dimensional configuration, and to help pinpoint the structural features necessary for estrogenic activity.†

* Throughout this communication 'diethylstilbestrol' will be used as an abbreviation for *trans*-diethylstilbestrol, and refers to the physiologically active isomer which melts at 171°C.

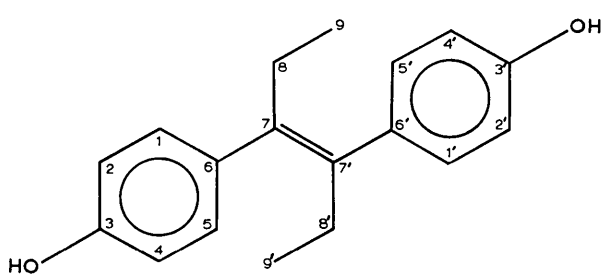


Fig. 1. The diethylstilbestrol molecule drawn to resemble a steroid. The numbering system to be used in this communication is also illustrated.

Experimental

Single crystals were grown by slowly cooling a solution of diethylstilbestrol in a 0·01 *M* solution of *p*-chlorophenol in iso-octane. The molar ratio of *p*-chlorophenol to diethylstilbestrol in this solution was 2:1. The systematic absences (0*kl* for *k* odd, *h*0*l* for *l* odd, and *hk*0 for *h* odd) in the diffraction pattern were consistent with the orthorhombic space group *Pbca*, and the crystal data are:

Diethylstilbestrol ($C_{18}H_{20}O_2$), $M=268{\cdot}34$,
$a=18{\cdot}954\pm0{\cdot}004$, $b=14{\cdot}929\pm0{\cdot}001$,
$c=5{\cdot}291\pm0{\cdot}001$ Å (at 20°C; λ Cu $K\alpha_1=1{\cdot}50451$ Å)
$V=1497{\cdot}29$ Å^3, $D_m=1{\cdot}14$ g.cm^{-3}
(by flotation), $Z=4$, $D_c=1{\cdot}19$ g.cm^{-3}, $\mu=6{\cdot}1$ cm^{-1}.
Space group *Pbca*, D_{2h}^{15} no. 61.

The unit-cell dimensions were determined by a least-squares analysis of 31 independent measurements of 2θ for high-angle data. Since space group *Pbca* requires the presence of eight symmetry-related units within the unit cell, the preliminary X-ray data and the density measurement were sufficient to reveal that the individual molecule must possess a center of symmetry.

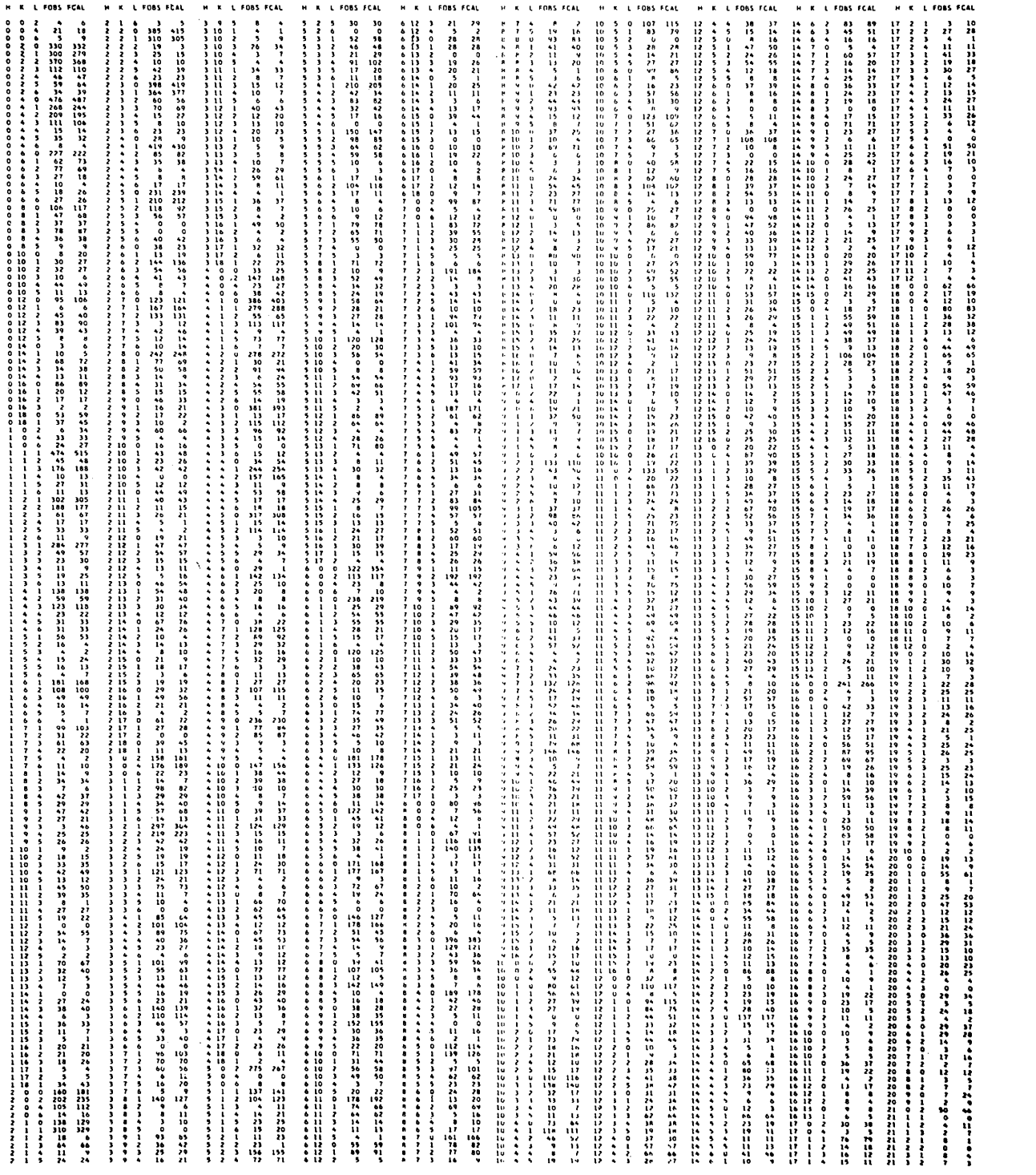
The intensities of 1500 independent spectra with 2θ less than 145° were measured on a General Electric single-crystal orienter with Cu $K\alpha$ radiation monochromatized by balanced nickel and cobalt filters. The crys-

† Since submission of this manuscript it has come to our attention that recent communications by Smiley & Rossmann (1969) and Busetta & Hospital (1969*a*) have outlined the geometry of the orthorhombic modification of diethylstilbestrol. The present communication confirms and extends their results In addition, the structure of a triclinic diethylstilbestrol–methanol–water complex has been solved (Busetta & Hospital, 1969*b*).

tals used for intensity measurements had dimensions of approximately $0{\cdot}2\times0{\cdot}15\times0{\cdot}15$ mm with $\mu=6{\cdot}1$ cm^{-1}. A three-dimensional Patterson synthesis revealed the orientation of the aromatic ring and the locations of the attached phenolic oxygen and ethylenic carbon atoms. A Fourier synthesis calculated using these eight

Table 1. *Comparison of $|F_o|$ and $|F_c|$ for the refined atomic parameters*

The structure amplitudes are given on five times absolute scale.



atoms as a trial structure produced the carbon atoms comprising the ethyl group. The positions and isotropic thermal parameters of the nonhydrogen atoms were refined, using a block-diagonal approximation to the normal equations, until the reliability index, R, was 15%.* A Fourier difference synthesis was then computed, and the hydrogen atoms located. The positional and anisotropic thermal parameters of the nonhydrogen atoms were further refined keeping the hydrogen atoms fixed ($B=2{\cdot}0$) until the calculated shifts in all parameters were less than $\frac{1}{3}$ their respective standard deviations. At the end of the refinement, the R value was 9·1% for all 1500 reflections. Table 1 shows the agreement between the observed and calculated structure factor amplitudes, and the refined atomic coordinates and thermal parameters are listed in Table 2.

Discussion

Schueler (1946) suggested that an estrogen is a substance whose individual molecules are large, rigid, lipophilic surfaces with hydrogen bond-forming groups on opposite ends of the molecule. Giacomello & Bianchi (1941) examined a triclinic modification of diethylstilbestrol which included a molecule of ethanol as solvent of crystallization. Their Patterson projection on (010) revealed that the oxygen–oxygen distance in diethylstilbestrol was about 12·2 Å. Unfortunately, the structure was not refined, and further information about the molecular geometry was not obtained. The present investigation shows that the oxygen–oxygen distance is 12·13 Å, which is somewhat longer than the corresponding distances of 10·7–11·1 Å observed in the steroidal estrogens (Cooper, Norton & Hauptman, 1969). Examination of space-filling models shows that it is very unlikely that the molecules are able to undergo distortion, under any circumstances, in such a way as to bring the terminal oxygen atoms 1 Å closer together so that the oxygen–oxygen distance more closely resembles that in the natural estrogens. Consequently, it seems that the estrogen receptor protein must have sufficient flexibility to allow for these differences in the separation of the functional groups in substrate molecules.

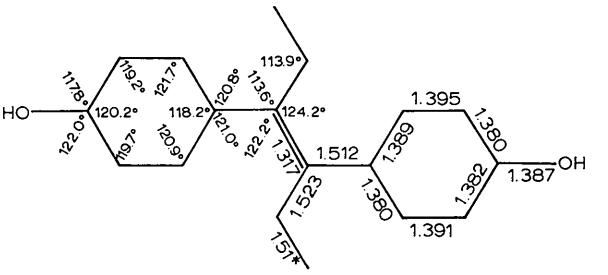


Fig. 2. Interatomic distances and valency angles. Corrected for thermal motion.

The interatomic distances and valency angles involving nonhydrogen atoms are shown in Fig. 2 (standard deviations are in the ranges 0·006–0·01 Å and 0·2–0·7° respectively). An unusually short apparent distance of 1·498 Å between C(8) and C(9) results from the large thermal motion of C(9); assuming a riding model (Busing & Levy, 1964), a corrected bond distance in the range 1·50 to 1·67 Å is obtained. C–H distances lie in the range 0·91 to 1·19 Å, with an average value of 1·00 Å. The O–H distance is 0·92 Å.

A close intermolecular contact of 3·03 Å occurs between oxygen atoms. The angle O–H···O is 171°, and the H···O distance is 2·11 Å compared with an upper limit of 2·4 Å for hydrogen bonds (Hamilton & Ibers, 1968). Therefore, it seems reasonable to regard this interaction as a hydrogen bond although the O···O

* Quantity minimized: $\sum w(|F_o|-|F_c|)^2$. Weighting scheme: $\sqrt{w}=1$ if $|F_o|<12$, $\sqrt{w}=12/|F_o|$ otherwise. Scattering factors: *International Tables for X-ray Crystallography* (1962), Table 3·3·1 A.

Table 2(*a*). *Atomic coordinates and anisotropic thermal parameters for the nonhydrogen atoms*

Thermal parameters are of the form

$$\exp\left[-2\pi^2(U_{11}h^2a^{*2}+2U_{12}hka^*b^*+\ldots)\right].$$

The standard deviations of the last two figures are given in parentheses.

	X/A	Y/B	Z/C	U11	U22	U33	U12	U13	U23
C(1)	0.15324(27)	0.03507(33)	-0.0921 (9)	0.0680(30)	0.0474(24)	0.0533(26)	-0.0020(22)	-0.0058(22)	-0.0081(20)
C(2)	0.21313(25)	0.08731(33)	-0.0548 (9)	0.0576(26)	0.0505(24)	0.0548(26)	-0.0056(21)	0.0069(21)	-0.0056(20)
C(3)	0.21507(22)	0.14652(28)	0.1462 (8)	0.0445(22)	0.0378(21)	0.0587(26)	-0.0001(17)	-0.0087(19)	0.0033(18)
C(4)	0.15896(25)	0.15144(31)	0.3118 (9)	0.0563(27)	0.0523(27)	0.0556(27)	-0.0082(22)	-0.0093(21)	-0.0106(20)
C(5)	0.09949(23)	0.09820(31)	0.2722 (9)	0.0479(24)	0.0518(25)	0.0581(26)	-0.0071(22)	0.0011(21)	-0.0102(21)
C(6)	0.09571(23)	0.04043(28)	0.0669 (8)	0.0555(24)	0.0357(20)	0.0471(23)	0.0021(18)	-0.0110(19)	0.0021(17)
C(7)	0.03131(24)	-0.01726(25)	0.0233 (8)	0.0601(23)	0.0291(21)	0.0536(24)	-0.0040(17)	-0.0113(21)	-0.0004(17)
C(8)	0.04616(27)	-0.11730(29)	0.0418 (10)	0.0633(28)	0.0302(21)	0.0811(34)	0.0042(20)	-0.0140(25)	-0.0012(21)
C(9)	0.07880(32)	-0.14524(35)	0.2875 (12)	0.0818(36)	0.0482(28)	0.0876(41)	0.0092(26)	-0.0088(30)	0.0213(27)
O*3)	0.27596(16)	0.20085(20)	0.1702 (6)	0.0508(17)	0.0456(16)	0.0790(23)	-0.0126(14)	0.0014(16)	-0.0056(16)

Table 2(*b*). *Atomic coordinates of the hydrogen atoms*

	x/a	*y/b*	*z/c*
H(1)	0·1543	−0·0025	−0·2554
H(2)	0·2524	0·0827	−0·1638
H(4)	0·1707	0·1876	0·4613
H(5)	0·0618	0·1044	0·3899
H(8A)	0·0849	−0·1348	−0·0820
H(8B)	−0·0118	−0·1470	0·0545
H(9A)	0·0630	−0·1087	0·4219
H(9B)	0·1342	−0·1313	0·2757
H(9C)	0·0804	−0·2026	0·3422
HO(3)	0·2729	0·2250	0·3303

distance is larger than the usually accepted range of 2·5–2·9 Å for oxygen–oxygen hydrogen bonds (Donohue, 1968). The system of hydrogen bonds and the packing of the molecules are illustrated in Fig. 3 which is a projection of one unit cell down the *c* axis.

As predicted from steric consideration, neither the benzene rings nor the alkyl groups lie in the plane of the central ethylenic linkage. Since the atoms comprising the benzene ring lie nearly in a plane as do those attached to the central double bond, the geometry of the molecule is fixed when the torsional angles about the C(6)–C(7) and C(7)–C(8) bonds are defined. These angles are listed in Table 3. The angle formed by the least-squares plane through the ethylenic linkage and the plane through the benzene ring is 62·8°. Since the molecules lie on crystallographic centers of symmetry, the angles of rotation of the two rings are identical. The three-dimensional configuration of the molecule is illustrated in Fig. 4. Rotation of the rings out of the central plane gives the molecule a thickness of about 4·5 Å, which is comparable to the thickness of a steroidal estrogen at C(18). Oki & Urushibara (1952) were the first to suggest that estrogenic activity in the stilbene type of artificial estrogen is correlated with molecular thickness, which, in turn, is determined by the degree of rotation of the rings out of the central plane.

Table 3. *Torsional angles for one asymmetric unit of the structure**

	Angle
C(1)–C(6)–C(7)–C(7′)	−118·6°
C(1)–C(6)–C(7)–C(8)	62·1
C(5)–C(6)–C(7)–C(7′)	63·4
C(5)–C(6)–C(7)–C(8)	−115·9
C(6)–C(7)–C(8)–C(9)	56·0
C(9)–C(8)–C(7)–C(7′)	−123·3

* The sign convention for the torsional angles in that of Klyne & Prelog (1960). The center of symmetry creates angles in the other half of the molecule which have the opposite sign.

The results of this X-ray investigation confirm that the artificial estrogen, diethylstilbestrol, has a nonplanar conformation. As shown by the distance between the phenolic oxygen atoms, it is a molecule slightly

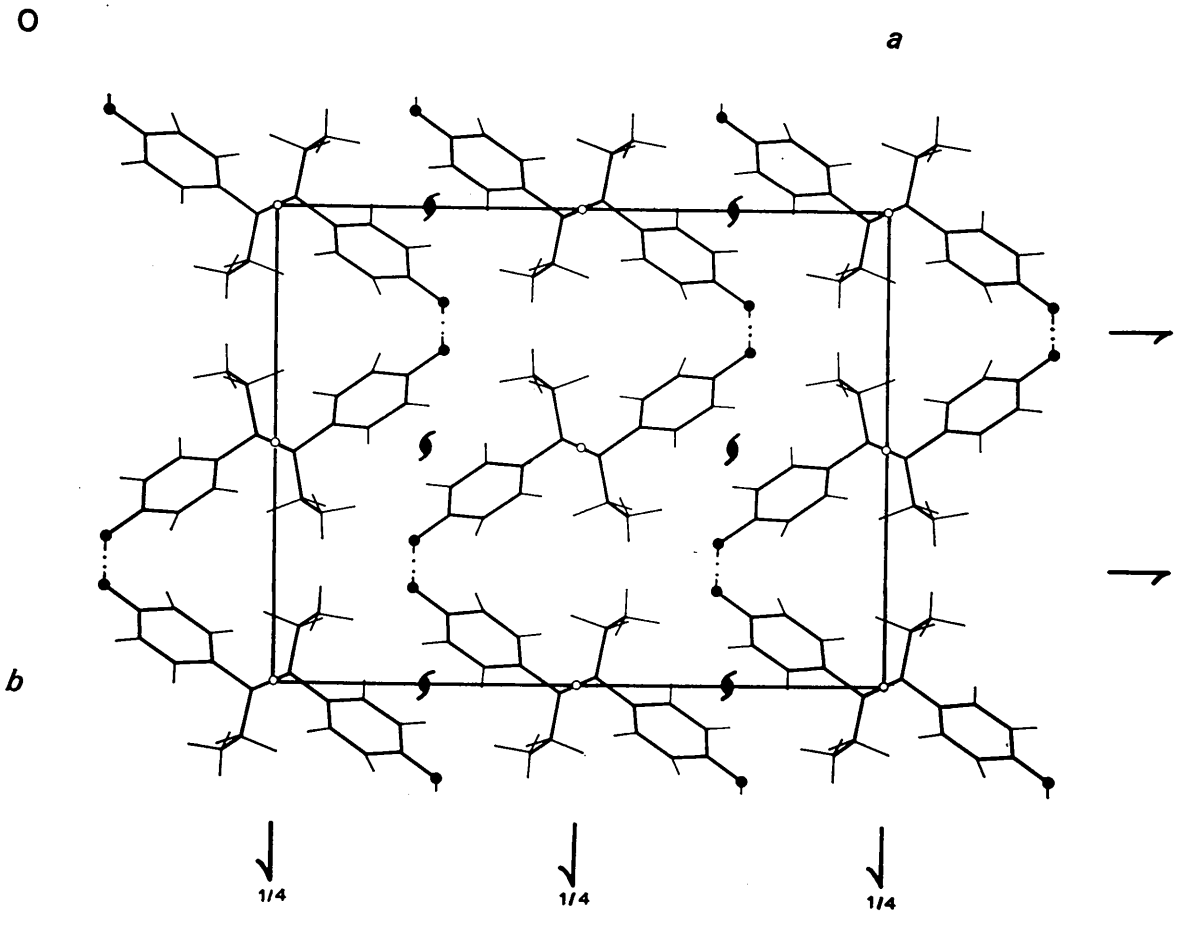


Fig. 3. Molecular packing seen in projection onto (001). Hydrogen bonds are indicated by dotted lines. ● = Oxygen.

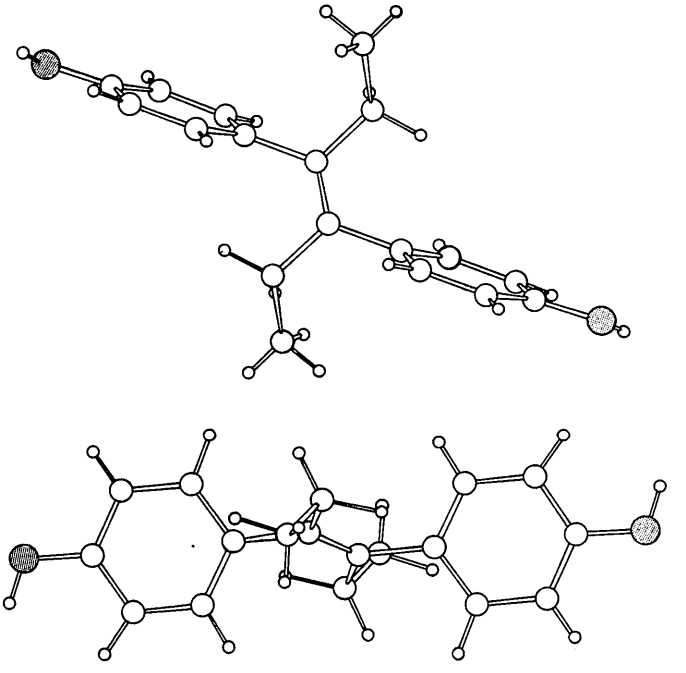


Fig. 4. Three-dimensional conformation of diethylstilbestrol.

longer than the natural estrogens, but the rotation of the benzene rings out of the plane of the central double bond results in a molecular dimension similar to the thickness of a steroidal estrogen.

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