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# X-RAY CONFORMATION OF SOME ESTROGENS AND THEIR BINDING TO UTERINE RECEPTORS

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## SUMMARY

Estrogens like estradiol, diethylstilbestrol and 6–7′ methyl 7 ethyl stilbestrol exhibit exactly the same coefficient of binding to uterine receptor sites. Some others like dienestrol, dimethylstilbestrol and cyclofenyl have a slightly weaker affinity. X-Ray analysis of these molecules gives their precise conformations. Only one conformation is found for estradiol whatever its surrounding is. Diethylstilbestrol has two conformations: one is symmetrical ("DES 1") and the other asymmetrical ("DES 2"). The latter one shows a more estradiol-like shape than the former and its hydrogen bonding has the same geometry as estradiol.

Dienestrol and dimethylstilbestrol always resemble symmetrical diethylstilbestrol structurally. Their affinity and other physical and biochemical results allow us to assume that this form, "DES 2", is the active form of diethylstilbestrol on receptor binding sites.

An explanation of the relatively high affinity of cyclofenyl which has a very different shape can be proposed by geometrically superposing on estradiol and diethylstilbestrol ("DES 2").

The weak activity, *in vivo* of cyclofenyl raises the possibility of the role played by each extremity of estradiol. Synthesis and biochemical and biological experiments of new molecules are necessary to confirm this and to postulate some new anti-estrogenic molecules.

## INTRODUCTION

X-Rays analysis allows one to see the tridimensional conformation of a molecule as it is in a crystal. The cohesion of a crystal is due to molecular interactions such as hydrogen bonds and/or dispersion forces. These are the same kinds of interactions which are implicated when a biologically active molecule interacts with a *receptor*. Changing the conditions of crystallization (solvents, composition of solutions, etc.) and consequently the surroundings of the molecules may be a way to describe the conformation of the "active" molecule. In the case of a rigid or quasi rigid molecule, there is no problem. When several conformations are present, the comparison between molecules with different geometry but approximately the same affinity is an important element in establishing a valid hypothesis and in deciding which is the "active" conformation.

Of course, the best information would be obtained when the structure of the receptors and their binding sites, alone and in the presence of ligands, are known. Such studies can be carried out with estrogenic compounds which are molecules as different as steroids, diphenylethylene, and triphenylethylene. In order to avoid the ambiguity necessarily involved with metabolism and transport of the molecules to the sites of action, we have only used the *in vitro* results of binding [1]. These results, given in Table 1, concern studies of the binding at equilibrium of different estrogens with the uterine cytosol *receptor*.

## METHOD

Molecules were crystallized from solutions of pure water, methanol–water or ethanol–water. The crystal structures were analyzed by X-ray diffraction using an automatic diffractometer and Cu Kα radiation. The structures were solved either by direct phase determination (programme Multan [2]) or by study of the Patterson function. Refinements were introduced with a block diagonal least squares method using a programme adapted either for an IBM-360-44 or for a CII-IRIS-80 System.

## EXPERIMENTAL RESULTS

Data about conformations of the different compounds: estradiol [3,4] diethylstilbestrol [5,6], dienestrol [7], dimethylstilbestrol [8], and cyclofenyl [9] have already been published in crystallographic reviews. Some typical features of these structures will now be mentioned briefly to aid in further discussion:

*Estradiol* (EST)

Two different crystals (one with water [3] and the other with propanol [4]) gave the same conformation for the steroid. Dissymmetry of the molecule is extended to hydrogen bonds given by the two extremities. An hydroxyl group of aromatic ring A is involved in the shortest hydrogen bond.

*Diethylstilbestrol* (DES)

Two different conformations were found in the different crystals. One obtained in the crystals grown in a nonpolar solvent and called "DES 1" and the second called "DES 2" was grown in polar solvents such as DMSO, ethanol, or methanol–water (1:1). "DES 1" has a centrosymmetrical conformation with planes of the two aromatic rings parallel and two ethyl groups in *trans* position. The hydrogen bonds

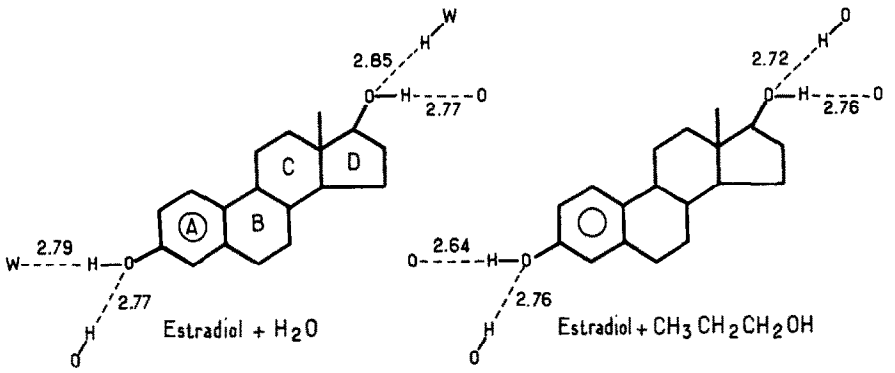



Fig. 1. Hydrogen bonds of estradiol.

of the two extremities of the molecules are symmetrical and therefore exactly identical. "DES 2" has lost its symmetry. The angle between the two phenol rings is 60° and the two ethyl groups are on the same side of the double bond as shown on the Fig. 2. In the same crystals two enantiomers of "DES 2" are present related just by a center of symmetry. Hydrogen bonds of the two extremities of the molecule are asymmetrical: one is strong (2·61 and 2·72 Å) and the other weaker (3·00 and 2·99 Å). If we compare the hydrogen bonding of "DES 2" and estradiol, we could say that the electronic state of ring A of estradiol and ring $\phi$ of "DES 2" are equivalent, just the same as ring D of estradiol and ring $\phi'$ of "DES 2" are equivalent.

*Dimethylstilbestrol* (DMS) *and Dienestrol* (DEE)

DMS and DEE have a symmetrical conformation like "DES 1". This conformation remains the same, regardless of the conditions of crystallization.

6,7′-*Dimethyl-7-Ethyl-Stilbestrol* (DMES) [10]

This molecule is asymmetrical by synthesis; the two aromatic rings have an angle of approximately 50°, and the hydrogen binding is nearer that of "DES 2". Ring $\phi$ with a methyl group plays the part of ring A of estradiol.

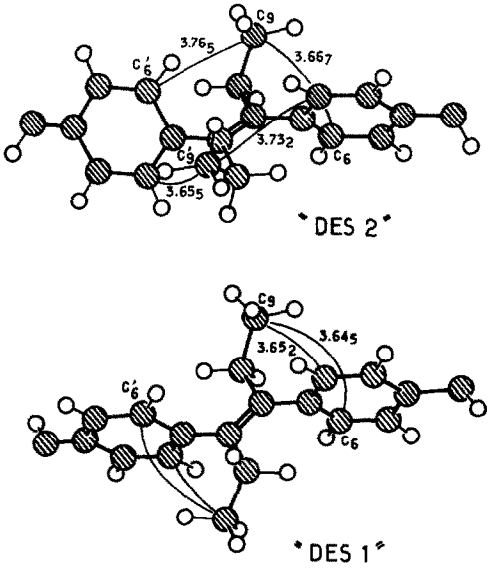



Fig. 2. General sight of "DES 1" and "DES 2".

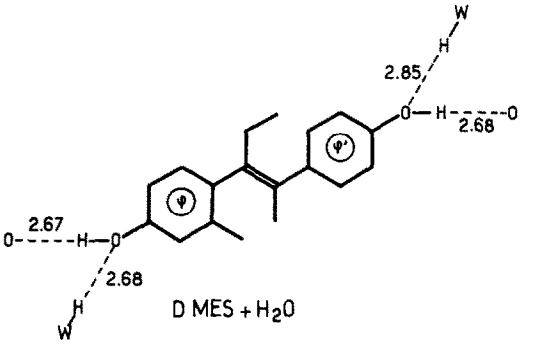



Fig. 4. Hydrogen bonds of DMES.

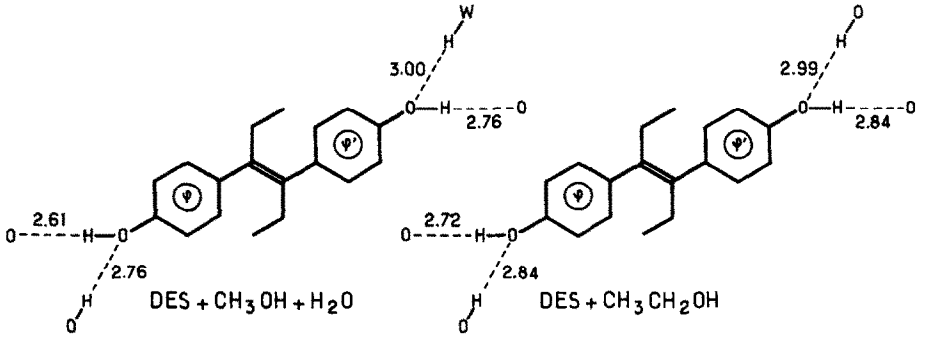



Fig. 3. Hydrogen bonds of "DES 2".

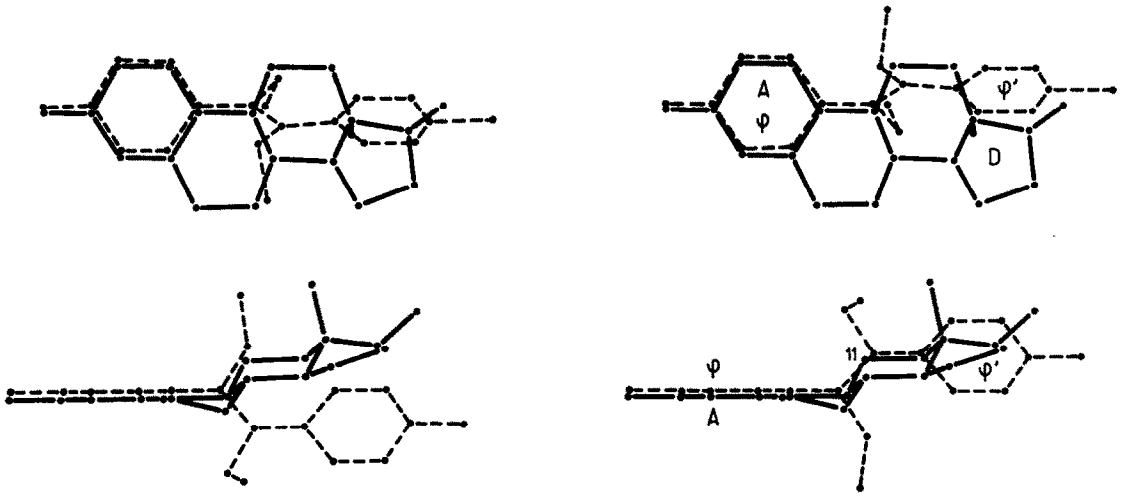



Fig. 5. Superposition: "DES 2"—estradiol: ring $\phi$ of "DES 2" is superposed on ring A of estradiol.

*Cyclofenyl*

The two aromatic rings have an angle of 80° as it is generally found for the contiguous ring of triphenylethylene molecules like broparestrol [11] or ICI 47499 [12].

## DISCUSSION

*Comparison of estradiol and diethylstilbestrol*

Estradiol and diethylstilbestrol have the same affinity coefficient for the uterine *receptor*. This structure-affinity relationship has been discussed previously [13]. It was assumed that "DES 2" was the more active form of DES. The main reasons for such an hypothesis were the following:

In biology, a symmetrical "active" molecule is very rare.

"DES 2" conformation is found in a polar solution which is closer to a biological medium.

"DES 2" has a more estradiol-like geometry, particularly in the thickness in the center of the molecule at the level of the ethyl groups.

The two hydroxyl groups of "DES 2" and estradiol (Fig. 3 and 1) have the same electronic state. This identity allows one to superimpose both molecules. "DES 2" has two enantiomers and their superposition with estradiol is shown on Fig. 5.

The enantiomers situated on the right give the best overlapping and by adding to estradiol a methoxy in 11$\beta$ position (corresponding to the ethyl group of "DES 2"), a new molecule (11$\beta$ methoxy estradiol) is obtained which resembles "DES 2" more closely than estradiol itself. Its coefficient of affinity is of the same order of magnitude.

A previous theory has been proposed based on the "non planar structure" of the estrogenic molecule after U.V. study of solutions of unsymmetrical diphenylethylenes and triphenylethylenes [14] in relation to their *in vivo* activity.

*Comparison of estradiol-diethylstilbestrol and other estrogens*

Some other valuable information can be added by comparing the conformation of other estrogens that have more or less the same biochemical action. Smaller affinity (Table 1) of dienestrol and DMS which have conformations like "DES 1" is a good argument in favour of our hypothesis. By superposing estradiol and asymmetrical DMES, the conformation and hydrogen bonding of which is close to that of "DES 2", the 6 methyl group of the ring $\phi$ begins to mime ring B of estradiol. In these conditions, it is not surprising to find the same coefficient of affinity for the receptor as that found for DES.

A difference of about 1·2 Å remains in the overall length of estradiol and different stilbestrol derivatives; but this is very little considering that a molecule like cyclofenyl has a good affinity ($K_i$ = 7 nM). Direct superposition of one aromatic ring with ring A of estradiol leads to very bad overlapping of the molecules and it is practically impossible to explain its

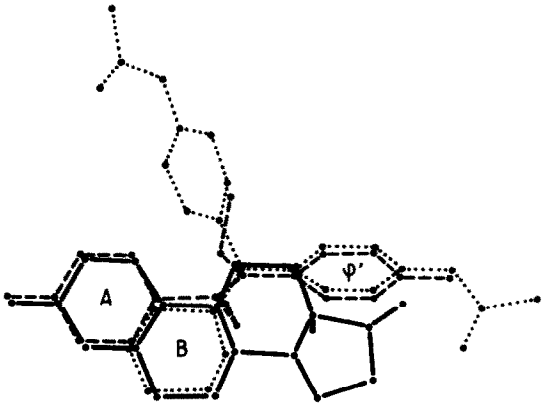
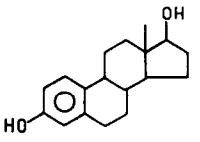
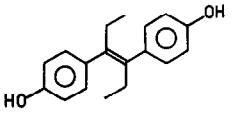
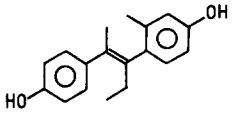
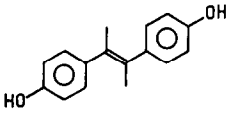
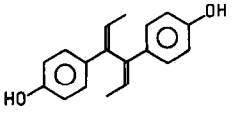
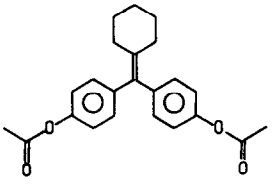



Fig. 6. Superposition: "DES 2"—estradiol–cyclofenyl: ring $\phi$ of "DES 2" is superposed on ring A of estradiol and then, aromatic ring of cyclofenyl on ring $\phi'$ of "DES 2".

Table 1. Binding of different estrogens with cytosol receptor

| Formula | name | $K_D$ or $K_i$ (nM) |
|---|---|---|
|  | Estradiol | 0.2–0.5 |
|  | Diethylstilbestrol (DES) | 0.2 |
|  | 6 7'-dimethyl-7 ethyl stilbestrol | 0.2 |
|  | Dimethylstilbestrol (DMS) | 7 |
|  | Dienestrol | 8 |
|  | Cyclofenyl | 7 |

high affinity for the uterine receptor. On the other hand, if one aromatic ring is superposed to ring $\phi'$ of "DES 2" and then if this is projected on estradiol it is clear that the hexanic ring occupies the position of ring B of estradiol (Fig. 6). The second aromatic ring is in the 11 position of estradiol, the modification of which has less influence on the activity. Cyclofenyl has acetate groups on the aromatic rings, and it is well established that the presence of an hydroxyl group in this position increases considerably the estrogenic activity. It would therefore be of interest to see if the presence of hydroxyl groups would also modify the affinity for the receptor.

The rather weak *in vivo* activity of cyclofenyl [15] and its rather high affinity suggested the following hypothesis about fixation and action of estrogens. The part of the molecule which binds the receptor is represented by the ring D of estradiol. The site is loose enough to allow differences of 1·2 Å. The part of the molecule responsible for the activity is represented by ring A of estradiol. The site must receive a phenolic ring with precise orientation. Such an hypothesis would be partly verified if the molecule composed of a cyclofenyl, on which we have added a phenolic ring at the hexanic ring side to mime the ring A of estradiol, shows high affinity and good activity. The addition of an aromatic ring without an hydroxyl increases slightly the activity of the molecule, giving preliminary proof of this theory (16). If synthesis of new molecules confirms the hypothesis, it will be possible to imagine and build new anti-estrogenic molecules with a higher efficiency.

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