J|A|C|S ARTICLES

Published on Web 11/06/2009

17α-Estradiol·1/2 H_2O: Super-Structural Ordering, Electronic Properties, Chemical Bonding, and Biological Activity in Comparison with Other Estrogens

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Abstract: The biological function of steroidal estrogens is related to their electronic properties. An experimental charge density study has been carried out on 17α-estradiol and compared to similar studies on more potent estrogens. High accuracy X-ray data were measured with a Rigaku rotating anode diffractometer equipped with an R-Axis Rapid curved image plate detector at 20 K. The total electron density in the 17α-estradiol·1/2 H_2O crystal was modeled using the Hansen−Coppens multipole model. Topological analysis of the electron density based on Bader's QTAIM theory was performed. The crystal structure, chemical bonding, and molecular properties, including the electrostatic potential (ESP), are reported and discussed. Observed disordering of hydroxyl and water hydrogen atom positions are interpreted as a superstructural ordering in a lower symmetry space group. The ESP's for the resulting four conformers are compared with each other and with that of 17β-estradiol. The relative binding affinities are discussed in terms of the observed potentials.

Introduction

17α-Estradiol (epiestradiol, 3-hydroxy-1,3,5(10)-estratrien-17α-ol) is a natural stereoisomer of the more active estrogen 17β-estradiol (E_2) (Figure 1).[1] Since 17α-estradiol (AE_2) exhibits reduced binding affinity[2,3] for both estrogen receptors (ER_α and ER_β) in comparison to E_2, resulting in decreased estrogenic activity,[4] it is considered a less feminizing estrogen. It is believed that nonfeminizing estrogens, such as AE_2, work primarily through nongenomic pathways in contrast to the feminizing estrogens such as E_2. Because of this trait, it has been recently proposed that AE_2 can also be used in drug cocktails which may find application in the treatment of neurodegenerative disorders (like Alzheimer's disease)[5] as it is known that AE_2, like other estrogens, exhibits neuroprotective properties.[6−9] Since hormonal therapy using E_2 can lead to complications, it has been suggested that AE_2 could be implemented in neuroprotective drugs instead of E_2.[5] In view of the prevailing importance of AE_2 and its immense biological significance, we have undertaken a detailed electron density study of this compound in an attempt to understand the reason for the reduced binding affinity of AE_2 toward the estrogen receptor, based on the topological properties of the electron density. It is our belief that an examination of this sort should provide further insight into the mechanism of action of AE_2.

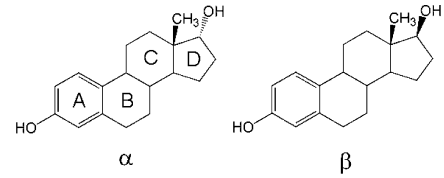

Figure 1. Representation of the 17α-estradiol and 17β-estradiol molecules.

Examination of the structures of compounds[10] having high affinity for estrogen and other steroidal receptors led to the suggestion that steroid-receptor binding is primarily the result of interactions between the receptor and the steroidal A ring,[11−13] whereas the concomitant biological activity of

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10.1021/ja906057z CCC: $40.75

estrogens and related steroids might be controlled by the D ring. The interesting properties and rich applications of steroidal molecules in molecular recognition events has been summarized in a detailed review.[14] It has been suggested[15,16] that the variations in biological activity (for example, promoting or hindering breast cancer) between such compounds are the result of slight alterations in the molecular structure, thereby resulting in differences in the molecular electrostatic potential. A program of study has been initiated in our group to develop correlations between the electronic properties of estrogenic compounds and their biological functions. The current study adds another dimension to the electron density studies on estrogens and pseudoestrogens already performed, namely estrone,[17] 17β-estradiol·urea,[18] genistein,[19] dienestrol[20] (DNS), and diethylstilbestrol[20] (DES).

Experimental Details

Crystals of 17α-estradiol·1/2 H_2O were grown by evaporation of a solution in ethanol (96%) and hexane (1:1 approximately). A clear, colorless crystal ($0.25 \times 0.22 \times 0.10$ mm^3) was oil-mounted (paratone and mineral oil mix) in a 0.3 mm loop made from 0.04 mm nylon fiber. A Rigaku diffractometer equipped with an 18 kW ULTRAX-18 generator, Mo rotating anode, R-Axis Rapid curved image plate detector, flat graphite monochromator and 0.5 mm collimator was used for data collection. The temperature of the crystal was maintained at 20.0(1) K using an open flow helium cryostat.[21−23]

To obtain quality data having sufficiently high resolution and redundancy, 10 different runs[24] (divided into 5 pairs) ranging from 0 to 180° in ω were collected at different χ and φ settings. A 4° ω-scan range was taken in order to avoid significant overlap of reflections in any given image. For each pair of runs, a 2° shift in start angle provided a half oscillation range overlap for precise scaling and avoided the use of partial reflections. A frame time of 220 s was chosen to maximize the intensity of the Bragg reflections and also to avoid saturation of the strongest reflections. The entire experiment was completed in less than two days.

The reflections were indexed using HKL2000[25] and the collected data integrated using the VIIPP data integration program[26,27] based on the reflection positions predicted from HKL2000. The program SORTAV[28] was used for scaling and averaging of reflections in point group 2. HKL-conditions for space group *C*2 were applied following a preliminary structure solution using SHELXTL[29] based on the complete unaveraged data set. Absorption was considered negligible ($\mu = 0.08$ mm^{-1}). Data reduction statistics can be found in Table 1.

Table 1. Crystal Structure Information and Experimental Details for 17α-Estradiol·1/2 H_2O

Formula	$C_{18}H_{24}O_2 \cdot 1/2\ H_2O$
Formula Weight	281.38
Crystal System	Monoclinic
Space Group[a]	*C*2 (*P*2$_1$)
a (Å)	18.9354(4)
b (Å)[a]	7.0470(2) (14.094)
c (Å)	13.3041(3)
β (°)	123.922(1)
Volume (Å^3), *Z*[a]	1473.1, 4 (2946.2, 8)
T (K)	20.0(1)
λ (Å)	0.71073
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	1.323
Reflections integrated	77491
R_{int}/average data multiplicity	0.0199/4.3
Completeness	
$\sin\theta/\lambda < 0.67$ Å^{-1}, %	96.8
all data, %	62.3
Independent reflections	17823
Reflections used ($I > 3\sigma$)	15076
Spherical atom refinement:	
$wR(F^2)$	0.0870
$R(F)$	0.0363
Multipole refinement:	
Refinement based on	*F*
Total number of parameters	881
Final $R(F)$	0.0174
$R_w(F)$	0.0100
$R(F^2)$	0.0180
Goodness_of_fit	1.0302
$\Delta\rho_{min/max}$, eÅ^{-3}	
all data	−0.23/0.20
$\sin\theta/\lambda < 1.0$ Å^{-1}	−0.10/0.10

[a] See text for details.

Crystal Structure Determination and Refinements. The crystal structure of 17α-estradiol·1/2 H_2O (at 100 K) and a preliminary determination of the electron density distribution was previously reported by Parrish;[30] the crystal structure of anhydrous 17α-estradiol in space group $P2_1$ with two independent estradiol molecules has been reported by Busetta et al.[31] In the current work, we first redetermined the crystal structure of the hemihydrate with the program SHELXTL.[29] The asymmetric unit contains one full molecule of the estradiol and a half molecule of water, the second half being generated by the crystallographic 2-fold axis in the monoclinic noncentrosymmetric space group *C*2. In the previous work by Parrish,[30] the disorder of the water molecules was recognized, but not modeled. Initially, our crystal structure was also solved and refined as a completely ordered crystal structure with one symmetry independent hydrogen atom on the water molecule. But preliminary multipole refinements resulted in large negative areas of the total electron density around the hydroxyl groups and the water molecule, and a somewhat enlarged displacement parameter for the hydrogen atom bonded to O(2). In addition, an unreasonable water H−O−H angle of 82.1° was obtained.

In the effort to eliminate these physically unreasonable features, we have found that not only the water hydrogen atoms, but the hydrogen atoms of both hydroxyl groups of the 17α-estradiol

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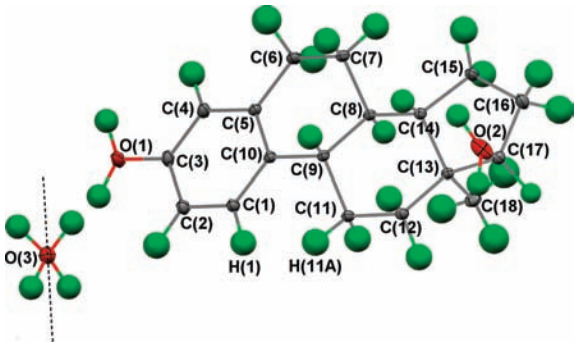

Figure 2. ORTEP drawing of 17α-estradiol·1/2 H_2O at 20 K (including all disordered hydrogen atoms) showing atomic thermal ellipsoids at the 75% probability level after the multipole refinement in space group *C*2. The dashed line depicts the 2-fold axis.

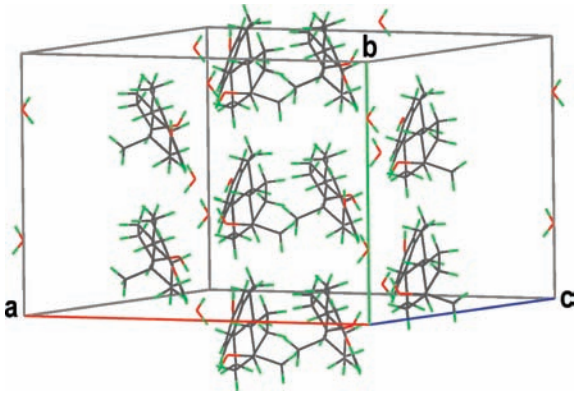

Figure 3. Packing diagram of the ordered 17α-estradiol·1/2 H_2O crystal structure in space group $P2_1$ with the unit cell doubled in the **b** direction (14.094 Å). Oxygen atoms are red, carbon atoms are gray and hydrogens are green. Drawn with the program Mercury.[45]

molecule are disordered over two sites. The high accuracy of our data allowed us to refine the occupancies of the disordered hydrogen positions with SHELXTL. As the refined values were not significantly different from 0.5, these occupancies were fixed to exactly 0.5 in the further multipole refinements. Any related disorder of the oxygen atoms was considered to be small, and each oxygen atom was treated as occupying a single position.

For the charge density analysis, the Hansen-Coppens multipole model[32] was implemented using the XD program.[33] The VM (Volkov and Macchi) data bank using STO atomic relativistic wave functions obtained at PBE/QZ4P level of theory for neutral atoms in their ground state configuration was used.[33] The C−H and O−H distances were extended to the average neutron bond lengths ($C_{aromatic\_sp2}H$ = 1.083 Å, $C_{vinylic\_sp3}H$ = 1.099 Å, $C_{methylene\_sp3}H$ = 1.092 Å, $C_{methyl\_sp3}H$ = 1.059 Å, OH (O1 and O2) = 0.967 Å, OH (O3) = 0.96 Å).[34] Disordered hydroxyl and water hydrogen atoms were chemically constrained to be identical (in terms of both thermal motion and the multipole model). For the 17α-estradiol core structure, the electron density for every atom was modeled using the standard estrogen local coordinate system as previously described.[35] Initially, the number of refined parameters was reduced by using several chemical constraints and refining only the most important multipoles.[35] Then, all multipoles were refined, with the oxygen and carbon atoms treated up to the hexadecapole level. For the hydrogen atoms only D1+, D1−, D0, and Q0 multipole parameters were refined. For the water oxygen atom, only symmetry allowed multipole parameters (12 in total) were refined. Chemical constraints, except for the disordered hydrogen atoms, were released progressively during the final stages of the refinement. However, the monopole populations on hydrogens, H(1, 2, 4), H(8, 9, 14), all H(A, B), and H(18(A, B, C)) were constrained to group values in the final refinement cycles. In the last stage, all parameters were refined at the same time until full convergence was achieved. The electroneutrality condition was imposed on the estradiol and water molecules separately for the entire refinement. Different contraction and expansion parameters (κ' and κ'') were assigned to groups of atoms based on their atom type, hybridization, and chemical environment. A total of eight different kappas were implemented into the refinement. The κ' and κ'' parameters for the hydrogen atoms were fixed (κ_H = 1.2) during the refinement while the displacement parameters were allowed to refine.

A number of parameters from different tests were used to evaluate the quality of the refinement (Table 1). The final $R(F)$ value was 1.74% for all data above $3\sigma(I)$. Reasonable residual density has been obtained in the large volume (16 × 10 × 9 $Å^3$) around both estradiol and water molecules. Averaged (in 0.05 $Å^{-1}$ intervals) ratios of the observed and calculated structure factors were very close to unity (within 0.5% deviation up to sin θ/λ of 1.323 $Å^{-1}$) based on F. The rigid-bond test[36] demonstrated that the differences of mean-square displacement amplitudes along the interatomic vectors were less than 5×10^{-4} $Å^2$. The total electron density was now found to be non-negative in the entire region of space. For the topological characterization of one-electron properties the program packages XDPROP[33] and WinXPRO[37,38] have been used.

Results and Discussion

Structure. An ORTEP drawing of the 17α-estradiol·1/2 H_2O molecule is shown in Figure 2. The bond lengths and angles are unremarkable, and are similar to those reported by Busetta et al. for the anhydrous compound,[31] and have been deposited. An important intramolecular distance in estrogens is the O(1)···O(2) distance, which should typically lie between 9.7−12.3 Å for a given molecule to exhibit optimum biological activity.[39,40] In the present case the O(1)···O(2) distance has a value of 10.3779(3), which can be compared with the O(1)···O(2) distance in β-estradiol (11.036 Å),[18] estrone (10.833 Å),[17] genistein[19] (11.982 Å), dienestrol[20] (11.907 Å), and diethylstilbestrol (12.152 Å).[20]

Since the refinement of occupancies of positions of the disordered hydrogen atoms gave almost a 50:50% ratio (within 3σ), this may be an indication that the atomic disorder is not random, but that an ordered superstructure exists with hydroxyl and water hydrogen atoms alternately 100% occupying one of the two refined positions. This ordered superstructure would

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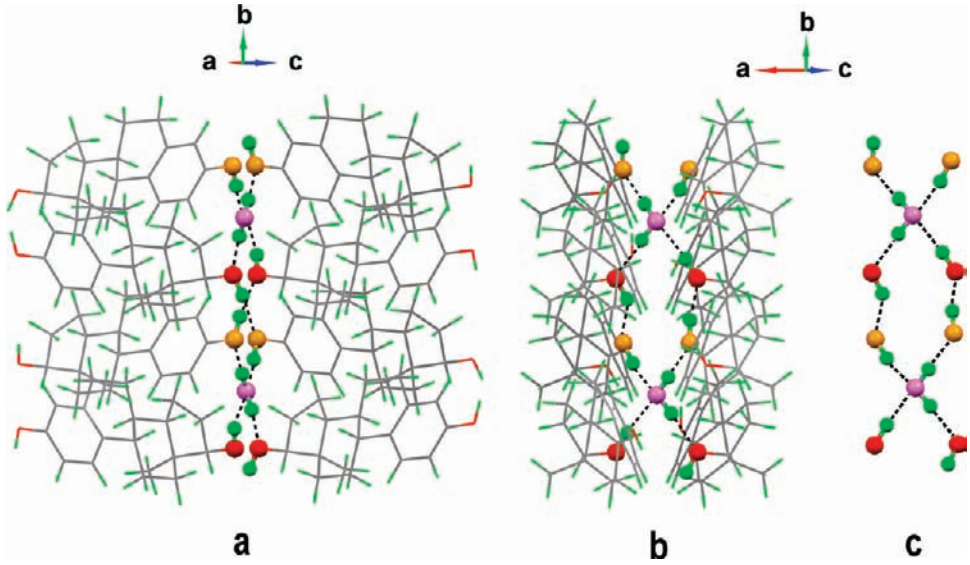

Figure 4. Fragments of 17α-estradiol·1/2 H_2O ordered molecular packing in space group $P2_1$ with the unit cell doubled in the **b** direction. Selected atoms of the hydroxyl groups and of water molecules are shown as colored spheres. O(1) (bonded to the A ring) atoms are orange, O(2) (bonded to the D ring) are red, water oxygen atoms are purple and hydrogen atoms are green. (a) Projected onto the "plane" of the steroid molecules, (b) projected to show the hydrogen bonded water column, (c) as for (b) with the steroid core stripped away. Strongest hydrogen bonds are shown as dashed lines. Drawn with the program Mercury.[45]

have a logical hydrogen bonding scheme, and may be constructed in the lower symmetry space group $P2_1$ with the crystal unit cell doubled in the **b** direction (Figure 3).

We do not observe experimental superstructure reflections, however this could be explained by the fact that in space group *C2* only three hydrogen atoms (out of 46 total atoms) are disordered. An alternative explanation would consider only short-range ordering. Accomplishing a neutron diffraction study would resolve this ambiguity.

To clarify the discussion below, the results of the multipole refinement have been transformed into the proposed ordered structure in the new unit cell (a = 18.9354 Å, b = 14.094 Å, c = 13.3041 Å, β = 123.922°) containing 8 estradiol and 4 water molecules. The refined water H−O−H angle[41] is now satisfactory at 104.506(2)°. The estradiol molecules are linked with strong hydrogen bonds through water molecules, and also directly to each other (Figure 4), creating chains of hydrogen-bonded molecules along the **b** direction.

Electron Density and Topological Analysis. The following discussion has been divided into four parts. First all of the hydrogen bonds and other interactions involving hydrogen atoms are analyzed. Then the atomic charge distribution and the derived dipole moment is discussed and compared with other estrogens. All covalent bonds are characterized by their topological properties, including an estimation of the topological bond order. Finally, the molecular electrostatic potential and its relation to binding and biological activity are discussed.

Critical point properties for all hydrogen bonds are listed in Table 2. In each of these bonds, the bond path and the virial path[42,43] have been found verifying the bonding nature of these interactions. The O···H bond lengths vary between 1.827 and 1.930 Å; these relatively short distances and high dissociation energies demonstrate strong hydrogen bonding in the superstructure of 17α-estradiol·1/2 H_2O crystal. Although all listed interactions are characterized by positive Laplacian values, O(3)···H(1O1) (water···estradiol) and O(2)···H(1O2) (estradiol···estradiol) bonds have highest electron density values and negative electronic energy density values. The $h_e < 0$ and $|v|/g > 1$ suggest incipient (incomplete) covalent bonding or partially covalent character of these two hydrogen bonds.[44] All other hydrogen bonds listed in Table 2 belong to the closed-shell interaction type.

Other (weaker) hydrogen bonds, H···H and C···H interactions are also listed in Table 2. One of the interesting features of the 17α-estradiol molecule is the formation of an intramolecular H(1)-H(11A) chemical bond. Similar bonding interactions have been found in 17β-estradiol[18] and estrone.[17] This interaction is characterized by relatively high electron density at the critical point, positive Laplacian, and positive electronic energy density, which are indicators of the closed-shell character of this interaction (Table 2). The H(1)···H(11A) distance is found to be 2.004 Å, a distance that is significantly shorter than the double of the van der Waals radius of the hydrogen atom[47] (2.4 Å). A significant *local* stabilization has been ascribed to this type of bonding wherein each hydrogen atom can be stabilized by up to ca. 2−7 kcal/mol.[48,49] Although a (3,−1) critical point and bond path have been found for this interaction (Figure 6), no virial path could be located using the Kirzhnits approximation[50,51] for calculations of the kinetic and potential energy densities.

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Table 2. Hydrogen Bonds and Other Noncovalent Bonding Interactions in the 17α-Estradiol·1/2 H_2O Crystal[a]

bond	ρ, eÅ^{-3}	$\nabla^2\rho$, eÅ^{-5}	R_{ij}, Å	d_1, Å	d_2, Å	g, a.u.	v, a.u.	h_e, a.u.	D_e, kJ/mol
Strongest hydrogen bonds:									
O(3)···H(1O1)	0.228	2.33	1.846	1.201	0.646	0.0263	−0.0283	−0.0021	37.2
O(3)···H(2O2)	0.090	2.84	1.925	1.321	0.620	0.0218	−0.0141	0.0077	18.5
O(1)···H(3O2)	0.181	2.57	1.836	1.218	0.648	0.0247	−0.0226	0.0020	29.7
O(2)···H(3O1)	0.141	2.14	1.912	1.263	0.683	0.0194	−0.0165	0.0028	21.7
O(1)···H(2O1)	0.109	2.64	1.930	1.288	0.694	0.0213	−0.0151	0.0062	19.8
O(2)···H(1O2)	0.229	2.39	1.827	1.201	0.632	0.0268	−0.0287	−0.0020	37.7
Other bonding interactions:									
H(1)···H(11A)*	0.098	1.27	2.004	1.056	1.090	0.0112	−0.0093	0.0019	−
O(3)···H(14)	0.064	0.66	2.695	1.665	1.051	0.0058	−0.0048	0.0011	6.3
O(3)···H(2)*	0.040	0.54	2.764	1.611	1.203	0.0043	−0.0030	0.0013	3.9
O(2)···H(4)	0.041	0.57	2.729	1.609	1.185	0.0045	−0.0031	0.0014	4.1
O(1)···H(12B)	0.033	0.47	2.843	1.698	1.162	0.0036	−0.0024	0.0012	3.2
O(2)···H(12B)*	0.020	0.25	3.063	1.786	1.280	0.0019	−0.0012	0.0007	1.6
C(2)···H(7A)	0.052	0.52	2.694	1.637	1.061	0.0044	−0.0035	0.0009	−
H(7B)···H(11B)	0.050	0.71	2.289	1.266	1.096	0.0057	−0.0041	0.0016	−
C(4)···H(15A)	0.050	0.48	2.702	1.661	1.052	0.0041	−0.0032	0.0008	−
H(7A)···H(11A)*	0.042	0.58	2.467	1.232	1.313	0.0046	−0.0032	0.0014	−
H(16B)···H(17)	0.042	0.56	2.162	1.162	1.085	0.0045	−0.0031	0.0013	−
H(6A)···H(15A)	0.042	0.54	2.231	1.141	1.094	0.0043	−0.0031	0.0013	−
H(8)···H(8)	0.041	0.35	2.283	1.140	1.144	0.0030	−0.0024	0.0006	−
H(6A)···H(7B)	0.038	0.39	2.287	1.139	1.174	0.0032	−0.0024	0.0008	−
H(12A)···H(7B)	0.037	0.48	2.592	1.272	1.359	0.0038	−0.0027	0.0012	−
H(1)···H(9)	0.035	0.45	2.373	1.272	1.140	0.0036	−0.0024	0.0011	−
H(1)···H(6B)	0.032	0.38	2.348	1.265	1.133	0.0030	−0.0021	0.0009	−
H(2)···H(9)	0.031	0.36	2.401	1.302	1.170	0.0028	−0.0020	0.0008	−
H(12A)···H(15B)	0.020	0.29	2.403	1.253	1.207	0.0022	−0.0014	0.0008	−
H(15B)···H(18A)	0.015	0.23	2.558	1.339	1.294	0.0017	−0.0010	0.0007	−

[a] Atomic labeling for oxygen and hydrogen atoms is shown in Figure 5. ρ is the electron density; $\nabla^2\rho$ is the Laplacian; d_1, d_2 are the distances from the critical point to atoms 1 and 2; R is the interatomic distance; g, v, and h_e are the kinetic, potential, and total electronic energy densities; D_e is the dissociation energy calculated as $D_e = -v/2$.[46] Interactions with no virial path found are labeled with *.

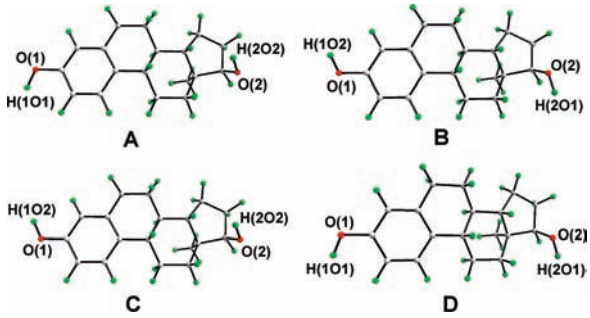

Figure 5. Molecular and atomic labels in 17α-estradiol·1/2 H_2O crystal structure showing the four different conformations used to build the superstructure.

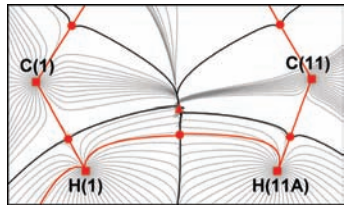

Figure 6. Electron density gradient line map showing the H−H interaction in the 17α-estradiol molecule (molecule A is shown). (3,−3) Critical points are red squares, (3,−1) (bonding) critical points are red circles, (3,+1) critical point is a red triangle. Bond paths are red and atomic zero-flux surfaces are black.

Table 3 lists atomic charges and volumes integrated over the atomic basins delimited by the zero-flux surfaces[42] The atomic properties of H(1) and H(11A) are typical of hydrogen atoms involved in a H−H bonding interaction. Although these atoms carry slightly positive (H(1)) and slightly negative (H(11A)) charges, these charges are very small, and H(1) and H(11A) atoms can be considered as essentially neutral. As in the other cases of H−H bonding interactions, the atomic volumes of these atoms are significantly smaller compared to other hydrogen atoms bonded to carbon:[17,18,48,49] H(1) is much smaller in volume than H(2) and H(4), and H(11A) is smaller than any other methylene hydrogen.

There is a significant difference in atomic charges and volumes between four different conformers of estradiol, especially for the oxygen atoms (Table 3). When the hydroxyl hydrogen attached to the O(2) atom (H(2O2)) points above the 5-membered ring (molecules A and C) and toward the water O(3) oxygen atom (Figure 5, Table 2)), the O(2) atomic basin becomes bigger which results in a higher charge and a larger volume of the O(2) atom. When the hydrogen atom (H(2O1)) points in the other direction (toward the O(1) atom of another estradiol molecule), the O(2) atomic basin and the integrated charge become smaller (molecules B and D). Charges and volumes of H(2O1) and H(2O2) follow the opposite trend: the bigger is the charge and volume of O(2), the smaller is the charge and volume of the hydrogen atom and vice versa.

For the O(1) atom, the picture is different. When hydrogen atom (H(1O1) in molecules A and D) points toward the water O(3) atom, the O(1) atomic basin becomes bigger, but the O(1) integrated charge is smaller. When the hydrogen atom (H(1O2) in molecules B and C) points toward the O(2) atom of another estradiol molecule, the O(1) atomic basin becomes smaller, but its integrated charge becomes bigger due to some charge redistribution in the crystal. The difference in the values of the charges and volumes is smaller for the O(1)

(52) DES and DNS molecules have zero dipole moment due to the presence of the center of symmetry.
(53) Flensburg, C.; Madsen, D. *Acta Crystallogr.* **2000**, *A56*, 24–28.

Table 3. Atomic Charges and Volumes in the 17α-Estradiol·1/2 H_2O Crystal[a]

	molecule A		molecule B		molecule C		molecule D	
atom	$q(\Omega)$, e	$V(\Omega)$, Å^3	$q(\Omega)$, e	$V(\Omega)$, Å^3	$q(\Omega)$, e	$V(\Omega)$, Å^3	$q(\Omega)$, e	$V(\Omega)$, Å^3
				17α-Estradiol molecules:				
O(1)	−1.108	18.68	−1.124	18.51	−1.124	18.50	−1.108	18.69
O(2)	−1.197	17.51	−1.172	17.25	−1.197	17.51	−1.172	17.25
C(1)	−0.038	11.97	−0.038	11.98	−0.038	11.98	−0.038	11.98
C(2)	−0.095	13.08	−0.100	13.10	−0.100	13.09	−0.095	13.09
C(3)	0.362	8.97	0.361	8.98	0.361	8.98	0.362	8.97
C(4)	−0.079	11.73	−0.077	11.68	−0.076	11.65	−0.080	11.74
C(5)	−0.067	10.05	−0.067	10.06	−0.067	10.06	−0.067	10.06
C(6)	0.134	8.46	0.136	8.44	0.134	8.46	0.136	8.44
C(7)	0.073	7.74	0.070	7.77	0.073	7.74	0.071	7.78
C(8)	0.081	6.58	0.081	6.57	0.080	6.59	0.081	6.57
C(9)	0.028	6.92	0.028	6.91	0.028	6.92	0.028	6.91
C(10)	−0.175	10.60	−0.175	10.60	−0.175	10.60	−0.175	10.60
C(11)	0.078	7.39	0.076	7.50	0.078	7.39	0.076	7.50
C(12)	0.043	7.84	0.043	7.85	0.043	7.84	0.043	7.85
C(13)	0.071	6.06	0.070	6.07	0.071	6.06	0.070	6.07
C(14)	0.024	6.60	0.025	6.55	0.024	6.60	0.025	6.56
C(15)	0.012	9.27	0.013	9.15	0.012	9.26	0.013	9.10
C(16)	0.052	9.18	0.051	9.17	0.052	9.18	0.051	9.16
C(17)	0.411	6.25	0.410	6.27	0.411	6.24	0.410	6.27
C(18)	0.165	9.12	0.164	9.10	0.165	9.12	0.165	9.08
H(1O1)	0.610	1.83	–	–	–	–	0.610	1.83
H(1O2)	–	–	0.611	1.85	0.611	1.85	–	–
H(2O1)	–	–	0.784	1.02	–	–	0.784	1.02
H(2O2)	0.775	0.93	–	–	0.775	0.93	–	–
H(1)	0.037	5.95	0.037	5.95	0.037	5.96	0.037	5.95
H(2)	0.058	7.45	0.055	7.39	0.055	7.40	0.058	7.45
H(4)	0.067	8.42	0.069	8.39	0.069	8.36	0.067	8.42
H(6A)	0.006	7.00	0.006	7.00	0.006	7.02	0.006	7.00
H(6B)	−0.016	7.77	−0.016	7.77	−0.016	7.77	−0.016	7.77
H(7A)	−0.035	6.16	−0.035	6.18	−0.035	6.16	−0.035	6.18
H(7B)	−0.033	6.96	−0.033	6.96	−0.033	6.96	−0.033	6.96
H(8)	−0.045	6.64	−0.045	6.65	−0.045	6.64	−0.045	6.65
H(9)	−0.032	7.32	−0.032	7.31	−0.032	7.32	−0.032	7.31
H(11A)	−0.056	6.01	−0.056	6.00	−0.056	6.01	−0.056	6.00
H(11B)	−0.059	7.27	−0.059	7.26	−0.059	7.27	−0.059	7.27
H(12A)	−0.041	8.00	−0.041	7.87	−0.041	8.00	−0.041	7.87
H(12B)	−0.012	6.80	−0.012	6.65	−0.012	6.80	−0.012	6.65
H(14)	−0.020	6.49	−0.025	6.30	−0.020	6.49	−0.025	6.30
H(15A)	0.026	6.06	0.026	6.06	0.026	6.06	0.026	6.09
H(15B)	0.017	7.94	0.017	7.99	0.017	7.94	0.017	7.98
H(16A)	0.055	7.91	0.054	7.90	0.055	7.91	0.054	7.90
H(16B)	0.051	7.51	0.051	7.50	0.051	7.50	0.051	7.50
H(17)	0.076	6.91	0.074	6.95	0.076	6.93	0.074	6.95
H(18A)	−0.065	9.63	−0.065	9.62	−0.065	9.62	−0.065	9.62
H(18B)	−0.062	6.45	−0.062	6.45	−0.062	6.45	−0.062	6.45
H(18C)	−0.061	8.08	−0.060	8.09	−0.061	8.08	−0.061	8.08
Total	0.019	355.49	0.021	354.62	−0.001	355.19	0.042	354.91
				Water molecules:				
O(3)	−1.378	21.30	−1.378	21.30				
H(3O1)	0.662	1.58	0.662	1.58				
H(3O2)	0.670	1.41	0.670	1.42				
Total	−0.047	24.30	−0.047	24.29				

[a] $L_{err} = (\Sigma L_\Omega^2/N_{atoms})^{1/2} = 0.00013$ a.u. for all 182 atoms, $L_\Omega = -1/4(\nabla^2\rho)_\Omega$[53]; $L_{max} = 0.00046$ a.u. Total molecular volume = 1468.80 Å^3; unit cell volume/2 = 1473.1 Å^3. Molecular labels are as shown in Figures 2 and 5.

atom compared to the O(2) atom. There is practically no difference between the charges and volumes of the H(1O1) and H(1O2) atoms.

This difference in the hydroxyl groups has only a small effect on the core of the 17α-estradiol molecule, however there is a small difference in the atomic volumes for some of the carbon (C(11), C(14), and C(15)) and hydrogen (H(2), H(12A), H(12B), H(14), and H(15B)) atoms (up to 2.9% for H(14)).

Molecular dipole moments calculated using both the multipole model parameters as well as the atomic integrated charges and dipole moments are listed in Table 4, along with those for all estrogen molecules studied previously.[52] All four 17α-estradiol conformers exhibit similar values of the molecular dipole moment, which are somewhat larger than those of 17β-estradiol, estrone, and genistein. This feature is consistent with the distribution of the molecular electrostatic potential (see below).

Critical point properties for the covalent intramolecular bonds are shown in Table 5. When the hydroxyl hydrogen atoms are hydrogen-bonded to the water O(3) oxygen atom, the O−H bonds within the hydroxyl groups (O(1)−H(1O1) and O(2)−H(2O2)) become weaker which results in lower values of the electron density and the Laplacian. In contrast, when hydrogen bonds

(54) Calculated in this work using data from refs 17−20.

Table 4. Molecular Dipole Moments of Some Estrogens[a]

compound	D_{mult}, D	D_{Ω}, D
17α-estradiol: molecule A	13.49	10.44
17α-estradiol: molecule B	15.18	13.15
17α-estradiol: molecule C	14.18	12.28
17α-estradiol: molecule D	15.50	12.97
17β-estradiol[54]	9.75	8.36
estrone[54]	9.27	11.17
genistein[54]	7.69	7.87

[a] D_{mult} is a molecular dipole moment calculated from the multipole model parameters; D_{Ω} is a molecular dipole moment calculated from atomic charges and dipole moments integrated over atomic basins. Dipole moments of both water molecules in the 17α-estradiol·1/2 H_2O crystal are $D_{mult} = 2.41$ D and $D_{\Omega} = 2.65$ D.

are created between two estradiol molecules, the O−H hydroxyl bonds (O(1)−H(1O2) and O(2)−H(2O1)) become stronger and possess higher electron density and Laplacian values. Except for the O−H bonds, there is no significant difference in the critical point properties between the four different 17α-estradiol molecules, or between the two water molecules.

For the O−C and C−C bond types, topological bond orders[55] have been calculated. The conjugated bonds in the aromatic ring are represented by the higher values of the topological bond orders (1.43 (ave.) compared with 0.90 (ave.) for the single C−C bonds). The O(1)−C(3) bond order (1.01) is slightly higher than the O(2)−C(17) bond order (0.81), which may be an indication of a small interaction between the orbitals of the O(1) oxygen atom and the π-orbitals of the aromatic ring. The electron density at the critical point and the bond ellipticity are also higher for the O(1)−C(3) bond compared to the O(2)−C(17) bond. The same effect was observed for other estrogen molecules: the topological bond order for the O(1)−C(3) bond is 1.05 in 17β-estradiol and 1.07 in estrone; for the O(2)−C(17) bond $n_{topo} =$ 0.75 in 17β-estradiol.[54] Pseudoestrogens having similar O−C_{ar} bonds exhibit bond orders of 1.04 (ave.) in genistein, 0.98 in diethylstilbestrol and 1.00 in dienestrol. At the same time, the observation of two oxygen lone pairs in every estrogen previously studied[17−20] reflects that the possible interaction between hydroxyl groups and the π-orbitals of the aromatic ring is fairly small.

As stated above, there is a body of evidence that the binding and subsequent biological activity of estrogens is intimately tied to the details of the molecular electrostatic potential, and its relation to that of the receptor. Electrostatically the negative areas in the molecule will be attracted toward the positive areas in the estrogen receptor cavity (and vice versa). Thus it is instructive to first compare the electrostatic potential (ESP) maps of the four conformers of 17α-estradiol taken from the crystal as shown in Figures 7 and 8 (either projected onto the molecular surface, or as an isosurface), with the same properties of 17β-estradiol (Figure 9).[18] Large negative ESP areas around the aromatic hydroxyl group and above and below the aromatic ring are observed in all cases. This is a very common feature of all estrogen molecules previously studied.[20] In contrast to the ESP of 17β-estradiol (Figure 9), the negative ESP in areas around the aliphatic hydroxyl group attached to the D-ring is significantly reduced, which may be responsible for the reduced relative binding affinity (RBA) of 17α-estradiol compared to 17β-estradiol.[2,3] Indeed, there is evidence from gel mobility shift assays on estrogens bound to ER-estrogen response elements, that the 17α-hydroxy group does not contribute to the binding.[56] The ESP pattern is very similar to that previously calculated theoretically for 17α-estradiol,[57] but in the present case the experimental ESP area around the aliphatic hydroxyl group is significantly less negative than obtained computationally.

Table 5. Properties of Intramolecular Shared-type Interactions in the 17α-Estradiol·1/2 H_2O Crystal

bond	ρ, eÅ^{-3}	$\nabla^2\rho$, eÅ^{-5}	R_{ij}, Å	ε	n_{topo}	bond	ρ, eÅ^{-3}	$\nabla^2\rho$, eÅ^{-5}	R_{ij}, Å
O(1)−C(3)[a,d]	2.050	−21.39	1.370	0.096	1.007	O(1)−H(1O1)[a,d]	2.177	−33.77	0.967
O(1)−C(3)[b,c]	2.051	−21.42	1.370	0.097	1.008	O(1)−H(1O2)[b,c]	2.196	−35.04	0.967
O(2)−C(17)[a,c]	1.717	−13.57	1.435	0.089	0.807	O(2)−H(2O2)[a,c]	2.157	−47.33	0.967
O(2)−C(17)[b,d]	1.717	−13.56	1.435	0.090	0.807	O(2)−H(2O1)[b,d]	2.234	−55.62	0.967
C(1)−C(2)	2.141	−19.13	1.392	0.196	1.428	C(1)−H(1)	1.834	−17.73	1.083
C(1)−C(10)	2.124	−19.06	1.402	0.174	1.404	C(2)−H(2)[a,d]	1.831	−18.03	1.083
C(2)−C(3)	2.130	−19.28	1.394	0.190	1.434	C(2)−H(2)[b,c]	1.832	−18.03	1.083
C(3)−C(4)[a,d]	2.162	−20.84	1.393	0.198	1.499	C(4)−H(4)[a,d]	1.836	−17.45	1.083
C(3)−C(4)[b,c]	2.163	−20.83	1.393	0.197	1.500	C(4)−H(4)[b,c]	1.836	−17.46	1.083
C(4)−C(5)	2.126	−19.19	1.403	0.194	1.398	C(6)−H(6A)	1.830	−19.48	1.092
C(5)−C(6)	1.710	−12.70	1.515	0.023	0.903	C(6)−H(6B)	1.821	−18.70	1.092
C(5)−C(10)	2.084	−18.06	1.409	0.166	1.329	C(7)−H(7A)	1.863	−19.01	1.092
C(6)−C(7)	1.664	−12.60	1.528	0.774	0.901	C(7)−H(7B)	1.852	−18.97	1.092
C(7)−C(8)	1.679	−13.67	1.526	0.033	0.946	C(8)−H(8)	1.820	−18.34	1.099
C(8)−C(9)	1.633	−11.89	1.545	0.007	0.860	C(9)−H(9)	1.797	−17.43	1.099
C(8)−C(14)	1.713	−13.71	1.525	0.019	0.968	C(11)−H(11A)	1.854	−18.97	1.092
C(9)−C(10)	1.715	−12.94	1.522	0.083	0.904	C(11)−H(11B)	1.840	−18.63	1.092
C(9)−C(11)	1.634	−11.64	1.538	0.027	0.856	C(12)−H(12A)[a,b]	1.807	−17.48	1.092
C(11)−C(12)	1.638	−12.02	1.536	0.013	0.866	C(12)−H(12A)[c,d]	1.805	−17.44	1.092
C(12)−C(13)	1.674	−13.06	1.529	0.006	0.923	C(12)−H(12B)	1.844	−18.91	1.092
C(13)−C(14)	1.628	−11.74	1.544	0.022	0.858	C(14)−H(14)	1.806	−18.01	1.099
C(13)−C(17)	1.720	−14.05	1.543	0.030	1.010	C(15)−H(15A)	1.844	−18.64	1.092
C(13)−C(18)	1.637	−12.06	1.540	0.024	0.868	C(15)−H(15B)	1.823	−17.39	1.092
C(14)−C(15)	1.650	−12.21	1.537	0.014	0.880	C(16)−H(16A)	1.791	−17.26	1.092
C(15)−C(16)	1.569	−10.85	1.556	0.026	0.781	C(16)−H(16B)	1.799	−18.50	1.092
C(16)−C(17)	1.666	−13.44	1.545	0.039	0.951	C(17)−H(17)	1.860	−21.43	1.099
						C(18)−H(18A)[a,b]	1.794	−16.85	1.059
						C(18)−H(18A)[c,d]	1.792	−16.80	1.059
O(3)−H(3O1)	2.182	−38.97	0.960			C(18)−H(18B)	1.857	−18.35	1.059
O(3)−H(3O2)	2.201	−40.47	0.960			C(18)−H(18C)	1.849	−18.12	1.059

[a] Molecule A. [b] Molecule B. [c] Molecule C. [d] Molecule D (Figure 5). ρ is the electron density at the bond critical point; $\nabla^2\rho$ is the corresponding Laplacian; R_{ij} is the interatomic distance; ε is bond ellipticity; n_{topo} is topological bond order.

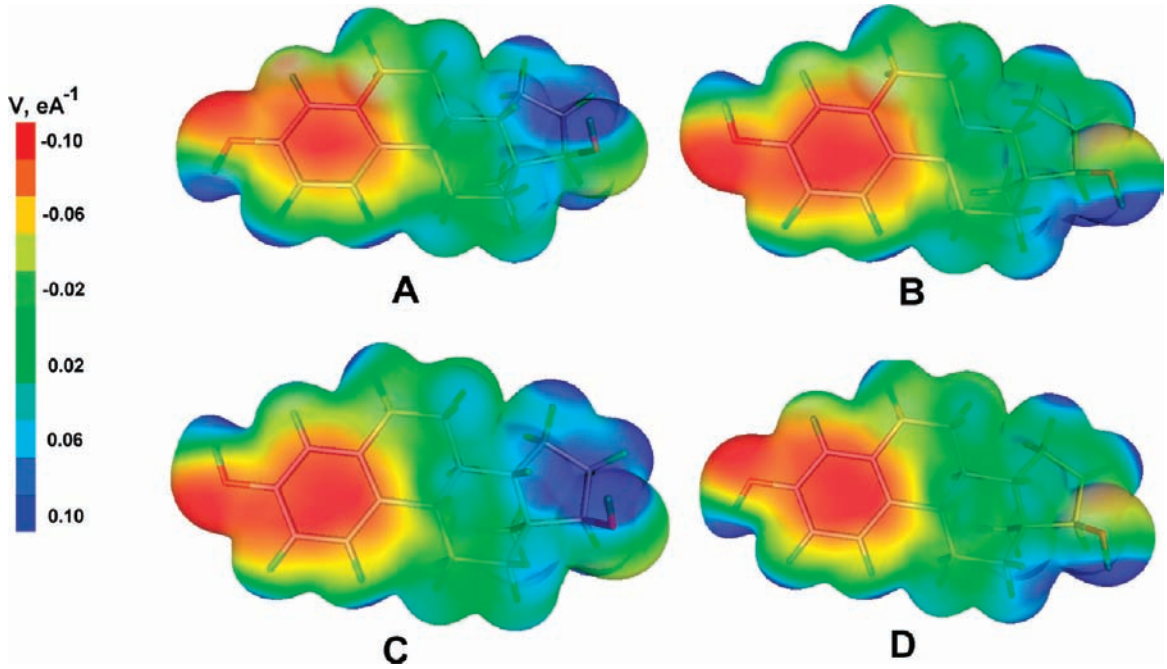

Figure 7. Electrostatic potentials of single 17α-estradiol molecules taken from the solid state plotted on the molecular ($\rho(r) = 0.001$ au) surface. Molecular labeling as in Figure 5. The gOpenMol[61] program was used.

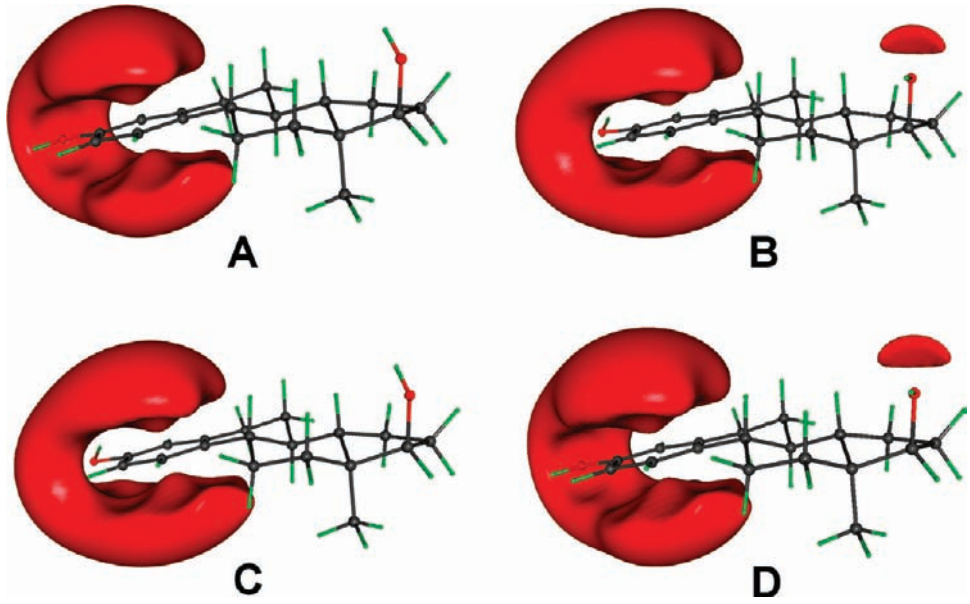

Figure 8. Electrostatic potential isosurfaces (-0.1 eÅ^{-1}) of single 17α-estradiol molecules. The 3DPlot module of WinXPRPO[38] has been used.

On the basis of the reported structure of 17β-estradiol in the ligand binding domain,[58,59] after an estradiol molecule (α or β) approaches the ER, several hydrogen bonds between the O(1)H hydroxyl group and the Glu353, and Arg394 residues, and a water molecule, are created. The high positive charge of the hydrogen atom and the highly negative charge of oxygen (Table 3) will provide strong hydrogen bonding to the estrogen receptor for this end of the molecule. Additional stability for the bound complex is provided by a CH$\cdots\pi$ interaction between Phe778 and the A ring (see Figure 10). In the case of 17β-estradiol, another hydrogen bond between the O(2)H group of the estradiol and the NH group of His524 is formed. However, there is some uncertainty which nitrogen atom of the histidine carries a hydrogen atom, and hence whether the OH group is

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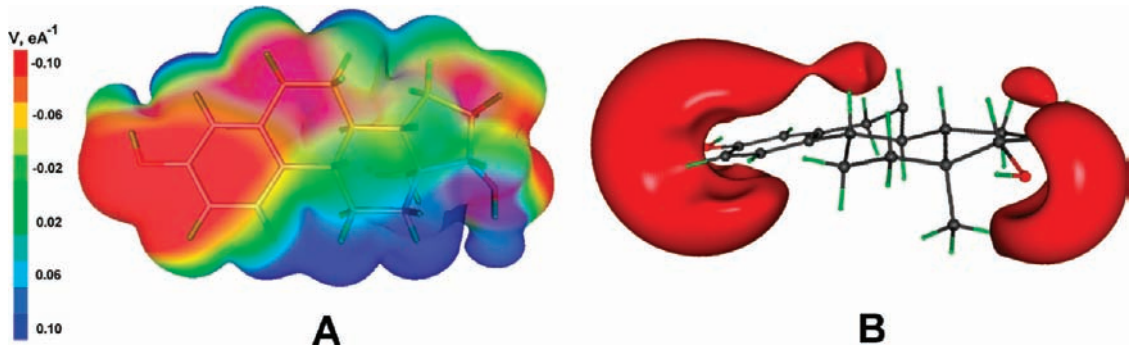

Figure 9. 17β-Estradiol molecule. (A) Electrostatic potential mapped onto the molecular surface, and (B) ESP isosurfaces of -0.1 eÅ^{-1}.[54]

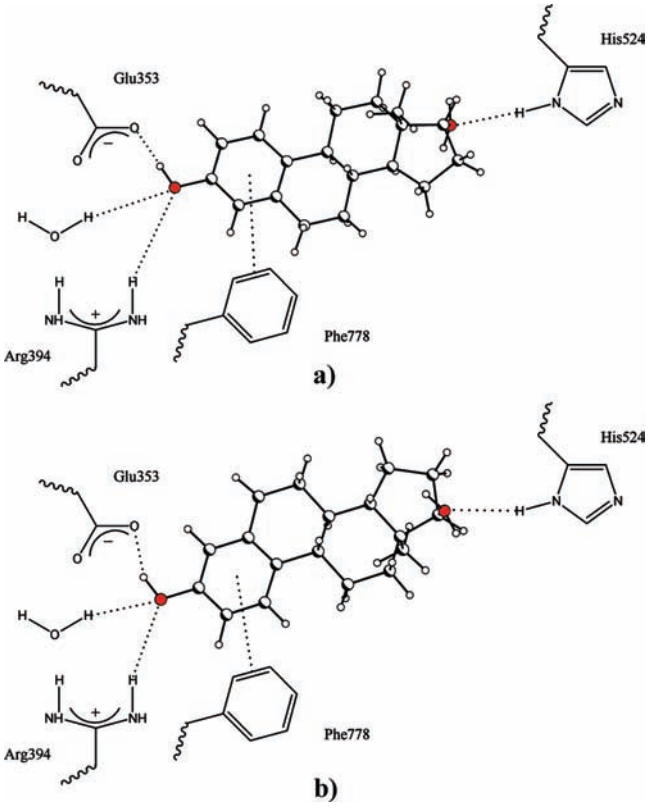

Figure 10. AE_2 binding to the receptor (a) superimposed conformers A/D, (b) superimposed conformers B/C. The molecule in (b) has been rotated by 180° about the long axis of the steroid with respect to (a). Potential hydrogen bonds and CH$\cdots\pi$ interactions are indicated by dashed lines.

acting as a hydrogen bond donor or acceptor. From the ESP, both possibilities clearly exist. Unfortunately, we cannot know from the macromolecular structure determination, which of the three possible orientations the O(2)H group adopts. In the case of 17α-estradiol, without a strong driving force toward the nitrogen atom of the side chain of His525 (provided by the negative ESP around the aliphatic hydroxyl moiety (at C17) in 17β-estradiol) it is less likely that this group acts as a hydrogen bond acceptor. This, coupled with the different orientation of the O(2)H group, suggests that the orientation of the 17α-estradiol ligand inside the ER cavity may be different from that of 17β-estradiol.

When examining the ESP of the four different conformers of 17α-estradiol, we observe that there is little difference in the ESP above and below the A-ring. However, it is clear that the orientation of the hydrogen atom on the O(1)H group significantly effects the ESP, moving the negative region around O(1) closer to H(2) or H(4). Based on the structure proposed from the macromolecular structure,[58,59] conformations A or D appear the most likely for the bound ligand. Due to the uncertainty of the nature of the hydrogen bonding at the D-ring, it is not possible to make a definitive analysis for this end of the molecule. Indeed, without a hydrogen bond between the 17α hydroxyl group and His524, because of the similarity of the two "faces" of the molecule, it is possible that conformations B or C may bind. This would effectively rotate the molecule 180° about its long axis to generate the same hydrogen bonding motifs (Figure 10).

To obtain some further insight into which of the possible conformers might be the most favored, single molecule calculations (DFT, B3LYP/6-311G(d,p), experimental geometry) using the Gaussian98 program[60] showed that the maximum energy difference between the lowest (negative) energy (molecule D) and the highest energy (molecule C) is only 2.8 kJ/mol (0.68 kcal/mol). Thus, we cannot predict which conformer is the most appropriate for binding based on the energies of the isolated molecules. In addition, in solution prior to binding to the ER, the exchange in the hydroxyl hydrogen atom positions may easily be accomplished through proton exchange mediated by formation and dissociation of hydrogen bonds with surrounding water molecules.

It is now possible to make a qualitative analysis of the relationship between binding affinity and surface electrostatic potential. The relative binding affinities for the series of molecules that we have studied to date are DES > DNS > E_2 > estrone $\approx$ AE_2 > genistein.[2] As described above, the two most important contributions to ligand binding are hydrogen bonds to the "A" and "D" rings (here we refer to the "A" and "D" rings as those parts of the molecule that most closely resemble the A and D rings of the parent steroid, E_2). For both DES and DNS,[20] the "A" ring potential is very similar to E_2[18] (DNS being slightly less that DES in the negative areas). However, both DES and DNS have a more extended negative area on the "D" ring, which would favor hydrogen bond formation leading to enhanced binding. Estrone[17] and AE_2 bind more weakly than E_2, but for different reasons. The A ring potential for estrone is much less negative than E_2, but the potential around the keto group of the D ring is very negative. Hence we anticipate reduced binding of the A ring and enhanced binding of the D ring. As described above, the situation is the opposite in the case of AE_2, the A ring behaving much as in E_2, but the D ring having much reduced binding. In the case of genistein,[19] the negative potential close to the OH group of the "A" ring is reduced compared to E_2, and that of the "A" ring is shifted somewhat toward the center of the molecule. This will reduce the OH hydrogen bonding, while maintaining the CH$\cdots\pi$

interactions. The "D" ring potential is also significantly reduced suggesting a lower contribution from hydrogen bonding, in agreement with the lower binding affinity.

Conclusion

The disorder of the hydroxyl hydrogen atoms in the 17α-estradiol crystal has been recognized based mostly on the observation of large areas of negative total electron density around the hydroxyl groups and water molecules in the absence of a disorder model. Thus, in the multipole refinements, the analysis for the non-negativity of the total electron density is an essential criterion for the meaningfulness of the model obtained. The 17α-estradiol•1/2 H_2O crystal structure can be described as a completely ordered structure in a lower symmetry space group ($P2_1$ vs $C2$ for the disordered model) with a unit cell doubled in the **b** direction, containing four different 17α-estradiol conformers and two water molecules. Strong hydrogen bonds (some with partially covalent type of bonding) have been characterized for the superstructure. The increased C(3)−O(1) topological bond order, compared with that for the C(17)−O(2) bond, is an indication of a probable weak interaction between the orbitals of the oxygen atom and the π-density of the aromatic ring. The molecular ESP distributions of the 17α-estradiol conformers have insignificant negative ESP areas around the aliphatic (C17) hydroxyl group, which may explain the relatively low relative binding affinity of this stereoisomer. The differences in the ESP for the four conformers illustrates how subtle changes in ligand conformation may have significant impact on binding affinity. The relative binding affinity for six estrogen or pseudoestrogen molecules has been rationalized on the basis of the observed molecular surface electrostatic potentials.

Supporting Information Available: Figures S1 and S2, complete ref 60, and crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

JA906057Z