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Molecular Modeling of Steroidal Estrogens: Novel Conformations and Their Role in Biological Activity

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Since the structure and conformation of many estrogenic ligands cannot be described with X-ray crystallographic studies, molecular modeling techniques must be used to generate their 3-dimensional structures. The potential of three molecular modeling methods to simulate the X-ray crystallographic geometry of estradiol-17β and various analogs (estratrien-1,17β-diol, estratrien-2,17β-diol, estratrien-3,11α,17β-triol, estratrien-3,11β,17β-triol, 9β-estratrien-3,17β-diol-11-one) have been compared. MMP2 molecular mechanics as well as the MOPAC semi-empirical molecular orbital methods, AM1 and PM3, were examined in these studies of estrogens with unique ring distortions. Whereas all three methods were able to simulate reasonable estrogen structures, the MMP2 method was found to reproduce the X-ray geometry of estrogens better than the MOPAC methods. The contribution of crystal packing distortions on the X-ray structures in these comparisons is discussed. Additionally, a molecular modeling dynamics method for the systematic conformational searching of steroidal estrogens is presented. For each estrogen examined, conformational searching produced at least one unique steroid conformation in addition to the X-ray crystallographic geometry. The MMP2 potential energy of predicted conformations and transition barriers of these estrogens has been shown to be less than the free energy of receptor binding. Thus, it is conceivable that estrogen ligands which can exist in a number of conformations may be converted to a preferred geometry by binding within the specific site of receptor. Furthermore, it is suggested that conformational flexibility of estrogens may be an important property of specific ligands for the estrogen receptor.

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INTRODUCTION

Recent characterizations of estrogen receptor mediated gene regulation emphasize the role of the estrogen ligand in the transcription activation process (for review see Ref. [1]). The localization of transactivation function-2 (TAF-2) in the estrogen receptor's ligand-binding domain [1–3] suggests that the estrogen binding event may initiate a process more complex than just receptor association with hormone responsive elements on DNA. Ligand induced alterations in the tertiary structure of the receptor complex [4] may additionally contribute to specific transactivation events [5–7]. In the absence of 3-dimensional structure data for the estrogen receptor, characterization of ligand requirements presents a classic problem of indirect drug design. Modern quantitative structure–activity relationship (QSAR) methods offer potentially useful approaches for characterizing the properties of a ligand responsible for particular receptor activation [8]. However, before implementing such methods on a set of steroidal estrogens, 3-dimensional structure data is required for each compound to be examined.

X-Ray crystallographic coordinates have been determined from a large number of steroidal estrogens [9, 10], but are lacking for many novel compounds that must be included in complete and systematic structure–activity relationship studies (See Refs [11, 12, 13] for example). Furthermore, the A- to B-ring juncture of the 1,3,5(10)-estratriene ring system may be troublesome for molecular modeling optimization. It has been shown that computer generated simulations of steroids

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with Csp3–Csp2 bonds can contain significant structure distortions when compared to X-ray crystallography [14]. Thus, computer modeling simulations of steroidal estrogens must be carefully evaluated by comparison to crystal structure determinations.

In an effort to ascertain molecular modeling methods that accurately simulate steroidal estrogens, the present study compares and evaluates several geometry optimization methods that closely reproduce X-ray crystallographic structures. Estrogens with varying degrees of ring strain induced by strategically located functional groups were employed in this assessment. Also presented is a molecular dynamics method for objectively exploring the conformational space of steroid estrogens. Finally, it is suggested that the conformational flexibility of estrogens might be important in understanding the binding of estrogens to receptor, the initial reaction in their biological activity.

EXPERIMENTAL

Steroids

Estrogens utilized in this study (Fig. 1) were: estratrien-3,17β-diol (E_2), estratrien-1,17β-diol (1OH), estratrien-2,17β-diol (2OH), estratrien-3,11α,17β-triol (11αOH), estratrien-3,11β,17β-triol (11βOH) and 9β-estratrien-3,17β-diol-11-one (11K9β). X-ray derived structures of E_2 that have been cocrystallized with H_2O, propanol or urea (E_2–H_2O, E_2–propanol and E_2–urea) were examined [9]. This laboratory has reported the receptor affinity constants (K_a) and the slightly bent and twisted conformations of the 1-OH and 2-OH X-ray crystal structures [15]. The affinity constants and crystallographic structures of 11αOH, 11βOH and 11K9β have also been determined recently [16].

Structure optimization

All models and X-ray structures were displayed on a Silicon Graphics Iris 4D/20 workstation with the SYBYL 5.5 molecular modeling software (Tripos Assoc., 1699 S. Hanley Rd, St Louis, MO, 63144). In order to evaluate computer modeling of estrogens, X-ray crystal coordinates of each compound in the study were optimized independently by three molecular modeling methods. MMP2 molecular mechanics [MM2(87)-SGRW, The Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN, 47405; Ref. [17]] as well as the AM1 and PM3 semi-empirical molecular orbital Hamiltonians (MOPAC 5.0, The Quantum Chemistry Program Exchange; Ref. [18]) were carried out through SYBYL interface on the Iris 4D/20. All molecular modeling calculations described use default values of the specified software unless indicated. Hydrogens were added to each estrogen structure when not defined by X-ray data. Hydroxyl hydrogens were added such that each rotational position was optimized and compared (three staggered geometries differentiated by 120° for sp3 carbons and two eclipsed orientations 180° apart for aromatic carbons). Structures with hydroxyls in the lowest energy orientation after optimization were used for comparisons. Lone pairs were added to hydroxyl groups while aromatic and sp2 carbons as well as sp2 oxygens were defined as pi atoms for MMP2 calculations. Optimizations utilizing the AM1 and PM3 methods included specification for "precise × 100" and a time limit such that convergence was achieved.

Structure comparison

Structures in experiments were compared according to conventions established from crystallographic studies of steroidal estrogens [9]. Steroid twist of an estrogen is the measure of the C1-C10-C13-C18 torsion angle. Estrogen ring bowing is the plane angle difference of the A-ring plane (C1, C2, C4, C5) in relation to the B–C–D-ring plane (C6–C12, C14–C17). For this study, estrogen length comparisons are based on a

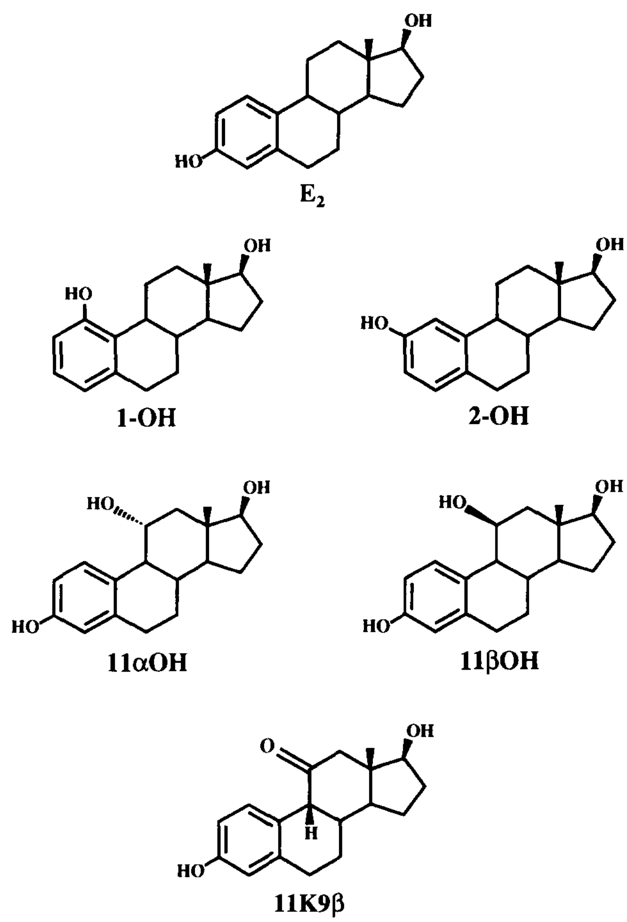

Fig. 1. 2-Dimensional diagrams depicting the specific steroidal estrogens examined in this study. Compounds are abbreviated in the text as follows: estratrien-3,17β-diol (estradiol-17β), E_2; estratrien-1,17β-diol, 1OH; estratrien-2,17β-diol, 2OH; estratrien-3,11α,17β-triol, 11αOH; estratrien-3,11β,17β-triol, 11βOH and 9β-estratrien-3,17β-diol-11-one, 11K9β.

measure of the distance in Å from the A-ring oxygen to the oxygen at 17β. Additionally, steroid models were compared by root mean square (RMS) fitting of all C and O atoms with SYBYL's MATCH command.

Conformational searching

Traditional small molecular conformational analysis usually involves searching for conformers by rotating bonds [19]. Since it was determined that the rotation method could not be efficiently and objectively applied to steroid ring systems [20], a variation of simulated annealing was implemented for searching the estrogens [20–22]. In an effort to explore the conformational space of these compounds, starting geometries were heated and equilibrated to the unrealistically high temperature of 1500 K. It is assumed that molecular models of structures at this temperature contain sufficient energy to overcome all conformational barriers. Many samples of these high energy structures were then "quenched" by energy minimization to the closest stable conformation [20, 22]. If enough high energy models are sampled and optimized, all possible minimum energy conformations will be observed. This procedure does not attempt to simulate a natural event but rather provide a means to objectively produce all geometrically possible conformations of the steroid ring system. It should also be noted that while this reannealing procedure may drastically stretch and bend chemical bonds in a molecular model, initial stereochemistry is preserved.

Each estrogen X-ray derived structure (with hydrogens) was subjected to SYBYL molecular dynamics (no electrostatics) for the production of high energy starting conformations. Initially, boltzman trajectories of the structures were heated to 1500 K for 100 fs (time step = 1.00 fs). This interval was immediately followed by a 4000 fs equilibration interval at 1500 K. Structures corresponding to high energy geometries observed at 200 fs intervals throughout the equilibration step were saved for use as starting conformation for optimization. Equilibration times longer than 4000 fs as well as sampling intervals more frequent than 200 fs were not found to produce additional conformations of these compounds (data not shown). Lone pairs were added to the hydroxyl oxygens of sample structures before each was minimized by MMP2 (see above). After the initial optimization, hydroxyl groups of each structure were manually rotated to each possible conformation followed by re-minimization. Estrogens with their hydroxyl groups in the lowest energy orientations were used for structure comparisons. All "quenched" (optimized) conformations of each analogue were compared to the corresponding X-ray crystal structures by RMS fitting of carbons and oxygens (see above). In addition, ring twist, bowing and length parameters were used to differentiate predicted conformations.

Conformational energy surface data of the estrogen analogs was generated from geometry optimization calculations utilizing the "dihedral driver" option of MMP2. This simulation incrementally rotated torsion angles of the ring system, transforming the steroid from one predicted conformation to another. Estrogen geometry was optimized at fixed, 5° intervals throughout this process resulting in the potential energy profile separating the two conformations. The C5-C6-C7-C8 torsion angle was used to measure the interconversion of the half-chair and boat B-ring conformations of E_2, 1OH, 2OH, 11αOH and 11βOH. A sequential combination of rotations about the C5-C6-C7-C8 and C9-C11-C12-C13 torsion angles was used to measure the structural barriers in the B- and C-rings of the four conformations of 11K9β.

RESULTS AND DISCUSSION

X-Ray derived structures of E_2

Since the prototype for computer simulation of estrogens must be the X-ray crystal structure assigned to these molecules [14, 23], the disparity found between structures of E_2 derived from crystals of varying origin and composition should be considered before the performance of molecular modeling optimization methods are compared. Although it has been reported that testosterone may display different A-ring conformations in independent crystals [24], the three X-ray derived structures of E_2 were considered to have "almost no variation" despite minor inconsistencies in the A/B ring juncture [9]. Nevertheless, when these structures of E_2 are superimposed via RMS fit of their A-rings, dissimilarities are revealed in the relative orientation of the O17 and C18 atoms (Fig. 2). These discrepancies result from subtle alterations among the B-ring geometries of the three structures and produce significant variations in steroid ring twist and bowing (Table 1). The consequence of such structure deformities on the steroidal estrogen's overall shape has been quantified by means of RMS fit (Table 2). Structures of E_2 which were cocrystallized with H_2O or propanol were found to be similar (RMS MATCH = 0.0627), whereas the crystal which included urea displayed significant differences in the X-ray derived geometry of E_2 (RMS MATCH = 0.1569).

Surprisingly, these deviations result in only small variation in the O3 to O17 distance among the three E_2 structures (Table 1). Although each crystal of E_2 did maintain the characteristic "head to tail" estrogen hydrogen bonding pattern, each hydroxyl group is involved in two hydrogen bonds in the similar H_2O and propanol complexes (one with steroid, one with solvent component [25, 26], compared to three hydrogen bonds observed in the unique urea complex (one with steroid, two with solvent component) [27].

Comparison of these X-ray derived structures of E_2 provided an example of the type and magnitude of crystal packing distortions that may be encountered in X-ray derived geometries of 1,3,5,(10)-estratriene

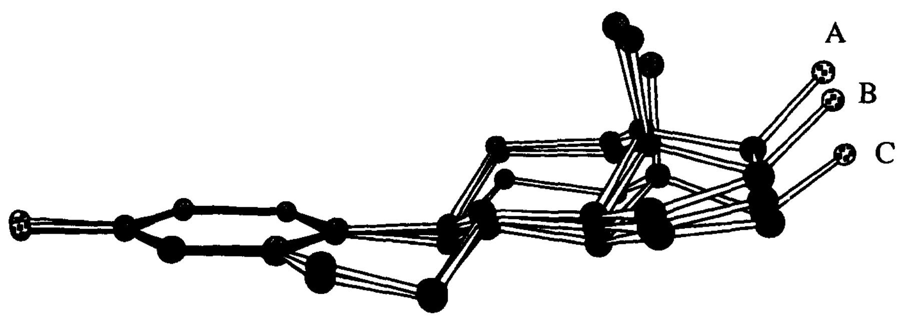

Fig. 2. View of X-ray derived structures of E_2 cocrystallized with H_2O (A), propanol (B) or urea (C). Carbons and oxygens are shown. Compounds were RMS fit relative to each other by A-ring carbons only. View is from slightly above with carbons 6 and 7 in foreground.

derivatives. Thus is appears that in the solid state, the B-ring of E_2 can be significantly distorted by differential crystal packing forces originating from the cocrystallized solvent components (H_2O, propanol or urea).

Optimization of E_2 X-ray structures

Each of the E_2 structures resulting from X-ray crystallography were optimized by the three molecular modeling methods (MMP2, AM1, PM3). All methods generated similar structures, regardless of the X-ray crystal geometry used as starting point for optimization. Furthermore, the modeling techniques produced an E_2 geometry which corresponded closely with the E_2–H_2O or E_2–propanol X-ray crystal derived structures (Table 2). Of the three computer methods used, MMP2 optimization of E_2 was found to have the best RMS MATCH to the crystal structures of E_2–H_2O and E_2–propanol (0.0587 and 0.0458, Table 2). Although the PM3 modeling method duplicated the E_2–propanol X-ray derived structure better than AM1 (RMS MATCH = 0.0762 vs 0.1240), this simulation was slightly less accurate than that obtained from MMP2 (RMS MATCH = 0.0458, Table 2). The MMP2, AM1 and PM3 minimizations of E_2 each produced ring bowing close to the 12.9° of the E_2-propanol X-ray structure (Table 1). The MMP2 and PM3 methods also generated a twist in the steroid molecule (86.3 and 89.4°) which was most similar to the E_2–H_2O and E_2–propanol X-ray structures (88.1 and 89.3°). As observed with the E_2–X-ray structures, all three modeling techniques produced a similar O3 to O17 length (10.9 Å, Table 1).

Whereas the MMP2 molecular modeling optimization technique generated E_2 structures most similar to the closely related E_2–H_2O and E_2–propanol X-ray geometries (RMS MATCH $\leq$ 0.0587, Table 2), it is significant that none of the molecular modeling methods closely simulated the relatively flat E_2–urea X-ray structure (ring bowing = 5.6°, Table 1 and RMS MATCH $\geq$ 0.1231, Table 2). This could mean that the crystal packing distortions in this particular X-ray structure are so extreme that modeling methods would not be expected to reproduce their effects. The E_2–urea X-ray structure may be the most strained (least relaxed) of the crystal derived E_2 structures.

Optimization of estradiol analog X-ray structures

Structure simulation of the five estrogen analogs proved more challenging than the optimization of E_2. X-ray crystallographic data was used for the initial structures of the 1OH, 2OH, 11αOH, 11βOH and 11K9β analogs of E_2. Unlike E_2, all but one of these analogs formed solvent-free crystals. The exception, 1OH, contained acetone in the crystal complex.

The relationship of the modeled structures to the corresponding X-ray derived geometries are listed in Tables 1 and 2. Structures which most closely resembled the X-ray data in terms of RMS deviation of C and O atoms were produced by the MMP2 optimization method for the 1OH, 11αOH, 11βOH and 11K9β estrogen analogs. When models of these compounds were compared in terms of steroid twist and bowing, no one modeling method simulated the X-ray crystallographic data consistently better than another (Table 1). In addition, even though all the computer generated geometries produced in this study varied from X-ray data over a considerable range of RMS MATCH values of C and O atoms, only rarely did the optimization methods generate estrogen analog structures with an A-ring O to O17 dimension significantly different from the corresponding X-ray data (Table 1). This clearly illustrates that the distance between the A- and D-ring hydroxyl groups is not seriously altered by subtle geometric distortions in the steroid nucleus of these estrogens. Thus, compared to RMS fitting of C and O atoms, the A-ring O to O17 dimension as well as the steroid twist and ring bowing parameters failed to highlight discrepancies in the overall molecular shape of these models (Table 1).

Only two analogs (1OH and 11K9β) yielded MMP2 modeled structures that fit their X-ray derived geometries as well as E_2 simulations (RMS MATCH = 0.0652 and 0.0619, Table 2). In fact, when some of the optimized models were compared to their

X-ray structures, the RMS deviation reached values as high as 0.2424 (Table 2). Nevertheless, all modeling simulations of these compounds resulted in structures with ring conformations similar to the X-ray data. With the presently available information, it is impossible to determine if discrepancies observed between computer optimization and X-ray data of these estrogen analogs are the result of crystal packing distortions.

All three optimization techniques produced similar geometries for the 2OH compound which were significantly different from the bowed X-ray derived conformation (Tables 1 and 2 and Ref. [15]). In fact, each optimization method failed to reproduce the X-ray geometry of the 2OH estrogen within RMS MATCH of 0.1694 (Table 2). Rather, molecular modeling always generated 2OH structures with twist and bowing characteristics similar to that obtained from the optimization of E_2 (Table 1). Thus, it is suggested that the bent and twisted structure of 2OH observed with X-ray crystallography might be the product of intermolecular hydrogen bonding and/or other crystal packing distortions. The proposition that the 2-hydroxy estrogen contains unique structural features resulting from particular A-ring electronic effects acting on the B-ring is also possible [15, 28].

These data suggest that X-ray derived structures of some steroid estrogens could be influenced by crystal packing forces such that typical molecular modeling methods are not able to exactly reproduce their geometry. Nevertheless, since the E_2 steroid ring system is inherently rigid, X-ray determinations of such compounds appear to have relatively small crystal packing distortions [9, 10, 24]. Therefore, the molecular modeling simulations which generate estrogen structures with RMS fit close to X-ray derived geometries (e.g. within 0.1) would be expected to be useful. Still, while

Table 1. Comparison of modeled structures to X-ray structures: geometric properties

Estrogen X-ray structure	Optimization method	Twist[a]	Ring bowing[b]	Length[c]
Estratrien-3,17β-diol (E_2)				
E_2-H_2O	X-ray	88.1	15.6	10.9
E_2-propanol	X-ray	89.3	12.9	11.0
E_2-urea	X-ray	82.8	5.6	11.0
	MMP2	86.3	12.6	10.9
	AM1	82.2	11.8	10.9
	PM3	89.4	9.9	10.9
Estratriene-1,17β-diol				
(1OH)	X-ray	99.8	16.7	7.3
	MMP2	98.1	15.7	7.5
	AM1	96.6	16.3	7.5
	PM3	94.8	12.8	7.4
Estratriene-2,17β-diol				
(2OH)	X-ray	74.9	20.3	9.6
	MMP2	84.3	12.3	9.9
	AM1	82.1	11.8	9.7
	PM3	89.4	9.7	9.8
Estratrien-3,11α,17β-triol				
(11αOH)	X-ray	54.6	28.7	10.8
	MMP2	48.7	31.3	10.8
	AM1	58.5	26.3	10.7
	PM3	52.8	29.8	10.8
Estratrien-3,11β,17β-triol				
(11βOH)	X-ray	95.9	8.7	11.1
	MMP2	89.4	12.8	10.9
	AM1	84.4	8.7	10.9
	PM3	96.4	10.4	11.0
9β-Estratrien-3,17β-diol-11-one				
(11K9β)	X-ray	104.6	65.5	8.9
	MMP2	103.4	64.2	9.2
	AM1	100.9	57.4	9.6
	PM3	107.3	61.7	9.4

[a]Twist is a measure of the C1-C10-C13-C18 torsion angle.
[b]Ring bowing measured as the plane angle difference of the A-ring plane (C1, C2, C4, C5) in relation to the B–C–D-ring plane (C6–C12, C14–C17).
[c]Length measured as distance in Å from A-ring O to O17.

Table 2. Comparison of modeled structures to X-ray structures: overall molecular shape

Estrogen X-ray structure	Optimization method	RMS MATCH[a]
Estratrien-3,17β-diol		
E_2–H_2O	MMP2	0.0587
	AM1	0.1063
	PM3	0.0981
E_2–propanol	X-ray (H_2O)	0.0627
	MMP2	0.0458
	AM1	0.1240
	PM3	0.0762
E_2–urea	X-ray (H_2O)	0.1569
	MMP2	0.1231
	AM1	0.1784
	PM3	0.1295
Estratriene-1,17β-diol		
(1OH)	MMP2	0.0652
	AM1	0.1028
	PM3	0.0957
Estratriene-2,17β-diol		
(2OH)	MMP2	0.1900
	AM1	0.1694
	PM3	0.2424
Estratrien-3,11α,17β-triol		
(11αOH)	MMP2	0.1048
	AM1	0.1260
	PM3	0.1253
Estratrien-3,11β,17β-triol		
(11βOH)	MMP2	0.1320
	AM1	0.2381
	PM3	0.1355
9β-Estratrien-3,17β-diol-11-one		
(11K9β)	MMP2	0.0619
	AM1	0.2217
	PM3	0.1467

[a]RMS MATCH is the root mean square fit of the C and O atoms in modeled structures to the corresponding atoms of that estrogen's X-ray structure.
For supplementary material to Table 2 see Appendices A and B.

RMS MATCH determination may be able to quantify the relationship between the overall shapes of two molecular models, it does not indicate which structure is deviant. Considering the disparity found in the three X-ray derived structures of E_2, exact reproduction of particular crystallographic data by computer modeling could sometimes be misleading. Therefore, since the most biologically relevant structure of E_2 may only be found in physiologic solutions or in the receptor binding site, all possible conformations of an estrogen ligand must be examined.

Alternate conformations of estrogens

The various possible ring conformations of E_2 and its analogs were thoroughly explored via the quench reannealing method. Conformational searching of E_2, 1OH, 2OH, 11αOH, 11βOH and 11K9β generated models with at least two different steroid ring conformations for each compound. The X-ray derived structure of each estrogen was reproduced as one of the computer predicted conformations (Figs 3–6, Table 3). These conformers have been designated I–IV in accordance with their increasing relative MMP2 potential energy after optimization (Figures 3–6).

The two predicted conformations of E_2 (Fig. 3) have MMP2 potential energies of 23.777 (I) and 27.093 (II) kcal/mol (Table 3). The low energy conformer corresponded to the X-ray structure (RMS MATCH 0.0458, Table 3). Differences between these two computer models of E_2 reside entirely in altered B-ring conformation. The B-ring of E_2-I (lowest energy) is a distorted 7α,8β-half-chair while E_2-II has a B-ring in the boat conformation. The latter B-ring conformation results in a markedly different orientation of C7 and produces a twisting of the C- and D-rings relative to the A-ring (Fig. 3). Comparing specific geometric properties of the two E_2 structures reveals that the E_2-II conformer was twisted 32° more than E_2-I, whereas the steroid bowing in the E_2-II conformer was increased 15° from E_2-I and the X-ray data (Table 4). The O3 to O17 dimension did not differ between the I and II conformers of E_2 (Table 4).

Conformational searching of the 1OH and 2OH estrogens resulted in the same predicted steroid structure patterns as was found for E_2 (Table 4, models not shown). The steric constrains on the 1-hydroxyl group resulted in a slightly higher potential energy in the 1OH-I and -II conformations relative to that of E_2. It is also of note that the 2OH X-ray derived structure, which has been shown to be inconsistent with computer optimizations (see above), can be characterized as maintaining a geometry somewhat intermediate to both of its predicted conformations (Table 4).

Table 3. Alternate conformations of estrogens

Estrogen	Conformer[a]	Potential energy[b] (kcal/mol)	RMS MATCH[c]
Estratrien-3,17β-diol			
(E_2)	I*	23.777	0.0458
	II	27.093	0.3736
Estratrien-1,17β-diol			
(1OH)	I*	24.952	0.0652
	II	27.806	0.5342
Estratrien-2,17β-diol			
(2OH)	I*	23.807	0.1900
	II	27.097	0.2624
Estratrien-3,11α,17β-triol			
(11αOH)	I	24.812	0.2129
	II	25.192	0.5248
	III*	25.783	0.0986
Estratrien-3,11β,17β-triol			
(11βOH)	I*	23.145	0.1695
	II	28.279	0.3817
9β-Estratrien-3,17β-diol-11-one			
(11K9β)	I*	24.412	0.0619
	II	27.533	0.4382
	III	28.000	1.4231
	IV	29.369	1.1462

[a]Conformers designated by potential energy. "I" corresponding to the lowest energy structure of each set. Asterisk designates conformer with same ring geometry as X-ray structure.
[b]Potential energy from MMP2 minimization.
[c]RMS MATCH is the root mean square fit of the conformers C and O atoms to the corresponding atoms of the X-ray structure.
For supplementary material to Table 3 see Appendix C.

I

II

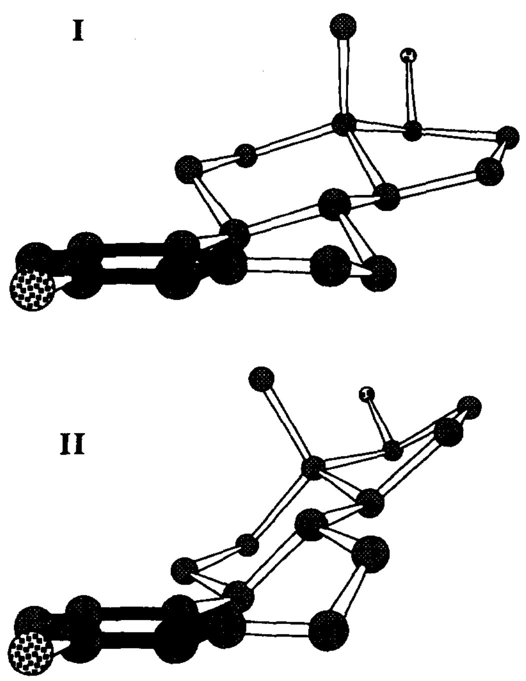

Fig. 3. View of E_2 conformations predicted from the simulated annealing search method. Carbons and oxygens are shown. Conformer "I" (similar to X-ray, 23.777 kcal/mol) and conformer "II" (27.093 kcal/mol) are aligned relative to each other by RMS fit of A-ring carbons. View is from slightly above with carbons 3 and 4 in foreground.

The simulated annealing search method generated three different conformers of the 11αOH compound (Fig. 4). These geometries differed by only 0.971 kcal/mol as determined by MMP2 (Table 3). Interestingly, the conformation shared with X-ray crystallography had the highest potential energy of the three structures (11αOH-III in Fig. 4 and Table 3). As was observed with E_2, the differences in the predicted conformations of 11αOH could be ascribed to variations in the steroid B-ring. Conformer 11αOH-III maintained the B-ring in a boat configuration (Fig. 4). On the other hand, the energetically more favorable conformers I and II of 11αOH had B-rings in the 8β-sofa and 7α,8β-half chair configurations, respectively. The most dramatic difference found between the three predicted structures of 11αOH was displayed by the position of the 11α hydroxyl in relation to the A-ring. Conformations I and III (highest and lowest energy) both maintained the O11 below the A-ring plane while the 11αOH-II structure (intermediate energy) had the O11 located in a position above the A-ring. Thus, in terms of 11α hydroxyl orientation, the I and III conformations were most closely related to each other, differing only in B-ring geometry.

Table 4. Geometric properties of alternate conformers

Estrogen	Conformer[a]	Twist[b]	Ring bowing[c]	Length[d]
Estratrien-3,17β-diol				
(E_2)	X-ray (propanol)	88.1	12.9	11.0
	I*	86.3	12.6	10.9
	II	54.3	28.1	10.9
Estratrien-1,17β-diol				
(1OH)	X-ray	99.8	16.7	7.3
	I*	98.1	15.7	7.5
	II	52.5	28.0	7.3
Estratrien-2,17β-diol				
(2OH)	X-ray	74.9	20.3	9.6
	I*	84.3	12.3	9.9
	II	54.0	27.5	9.7
Estratrien-3,11α.17β-triol				
(11αOH)	X-ray	54.6	28.7	10.8
	I	68.1	16.6	10.9
	II	104.0	19.2	11.1
	III*	48.7	31.3	10.8
Estratrien-3,11β,17β-triol				
(11βOH)	X-ray	95.9	8.7	11.1
	I*	89.4	12.8	10.9
	II	66.0	20.1	11.0
9β-Estratrien-3,17β-diol-11-one				
(11K9β)	X-ray	104.6	−65.5	8.9
	I*	103.4	−64.2	9.2
	II	127.0	−63.2	9.6
	III	82.0	−10.3	11.1
	IV	59.9	−38.0	10.9

[a]Asterisk designates conformer with same ring geometry as X-ray structure.
[b]Twist is measure of the C1-C10-C13-C18 torsion angle.
[c]Ring bowing measured as the plane angle difference of the A-ring plane (C1, C2, C4, C5) in relation to B–C–D-ring plane (C6–C12, C14–C17).
[d]Length measured as distance in Å from A-ring O to O17.

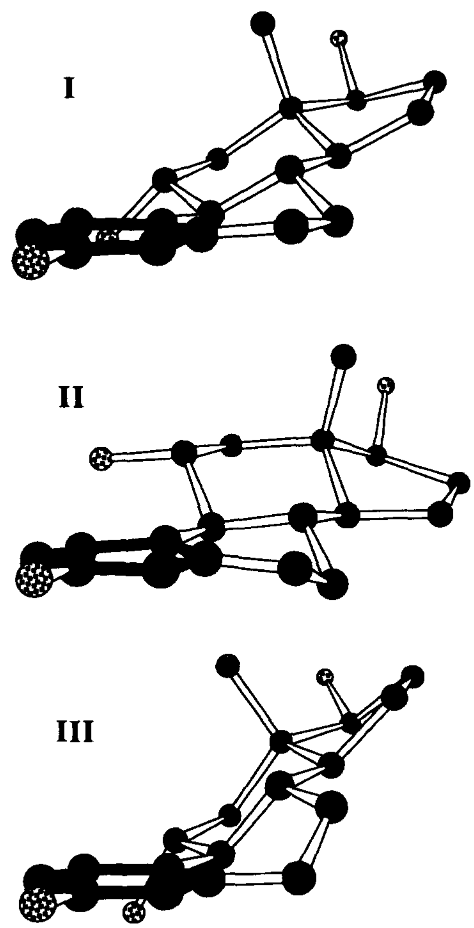

Fig. 4. View of 11αOH conformations predicted from the simulated annealing search method. Carbons and oxygens are shown. Conformers "I" (24.812 kcal/mol), "II" (25.192 kcal/mol) and "III" (similar to X-ray, 25.783 kcal/mol) are aligned relative to each other by RMS fit of A-ring carbons. View is from slightly above with carbons 3 and 4 in foreground.

Utilizing the searching method, two conformations of the 11βOH compound were found which differed by an energy of 5.134 kcal/mol as defined by MMP2 minimization (Fig. 5, Table 3). Again, the essential differences found between the two 11βOH conformations were derived from alterations in the B-ring configuration. As was the case for the highest energy model of E_2, the B-ring in 11βOH-II was in the boat geometry rather than the 7α,8β-half chair observed in the X-ray data and the computer generated conformer I. Nevertheless, in both 11βOH-I and -II, the 11-hydroxyl group was located above the plane of the A-ring. Significant ring twist and bowing differences were also observed between the 11βOH-I and -II structures (Table 4).

Of the estrogens subjected to conformational searching, the 11K9β compound presented the most unique

X-ray derived initial structure. This characteristic "L" shape of 9β estrogens was predicted by molecular mechanics analysis [29] and has been observed by X-ray crystallography of 9β-estratrien-3-ol-11,17-dione [30] as well as 9β-estratrien-3-ol-17-one [31]. Simulated annealing of 11K9β generated four distinct conformers shown in Fig. 6. Even though some of these models were very different from the X-ray structure (RMS MATCH to X-ray = 1.4231), their optimized MMP2 potential energies differed by only 4.957 kcal/mol (Table 3). The lowest energy structure of 11K9β (I) was found to reflect the X-ray conformation. Both the I and II predicted structures of 11K9β maintain the "L" shape and have ring bowing values similar to the $-65.5°$ observed in the crystal data (Table 4). Only the boat conformation in the B-ring of 11K9β-II differentiates it from the energetically more favorable 7β,8α-half-chair B-ring of conformer I. The higher energy models of 11K9β (III and IV) have relatively flat geometry (more like E_2) with ring bowing of $-10.3°$ and $-38.0°$, respectively. Conformations III and IV result from a combination of unique B and C-ring distortions. Whereas the two lowest energy geometries of 11K9β (as well as other estrogens in this study) have their C-ring in the chair conformation, 11K9β-III and -IV combine a twisted C-ring configuration with either a 7α-sofa or 7β,8α-half-chair B-ring. Ring twist, as well as, the O3 to O17 distance did vary between models predicted for 11K9β, but were not found to be good descriptors of observed structural differences (Fig. 6) when compared to RMS MATCH and ring bowing (Tables 3 and 4). As with the predicted conformations of all estrogens in this study, the four structures of 11K9β maintained a planar A-ring as well as a 13β-envelope D-ring structure. Even though it was surprising to find that no D-ring pseudo rotation was observed in the conformational

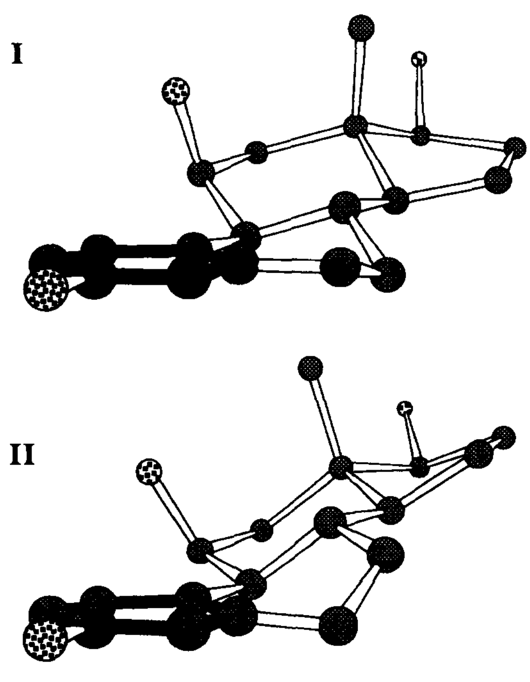

Fig. 5. View of 11βOH conformations predicted from the simulated annealing search method. Carbons and oxygens are shown. Conformers "I" (similar to X-ray, 23.145 kcal/mol) and "II" (28.279 kcal/mol) are aligned relative to each other by RMS fit of A-ring carbons. View is from slightly above with carbons 3 and 4 in foreground.

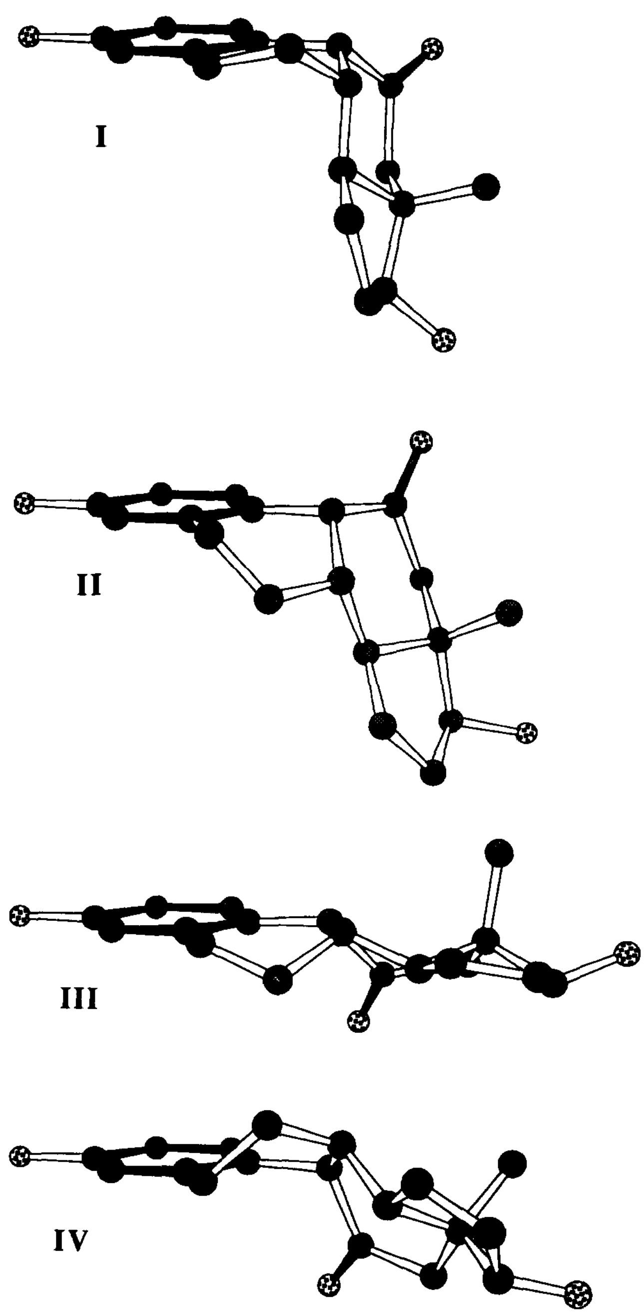

Fig. 6. View of 11K9β conformations predicted from the simulated annealing search method. Carbons and oxygens are shown. Conformers "I" (similar to X-ray, 24.412 kcal/mol), "II" (27.533 kcal/mol), "III" (28.000 kcal/mol) and "IV" (29.369 kcal/mol) are aligned relative to each other by RMS fit of A-ring carbons. View is from slightly above with carbons 6 and 7 in foreground.

searching of these estrogens [9], it is unknown if this result is due to inadequacies in the computational methods.

The observation that the predicted alternate conformations of E_2, 1OH, 2OH, 11αOH, 11βOH and 11K9β may display B-ring conformations different from the X-ray generated structures suggests that the B-ring is a flexible portion of 1,3,5(10)-estratriene derivatives (Figs 3–6). In the case of 11-hydroxylated estrogens, the B-ring boat conformation may be stabilized by steric interactions between the C-11 substituent and the C-1 hydrogen. A similar B-ring stabilizing interaction has been reported for 11β-hydroxy-3-methoxy-11α-methyl-1,3,5(10)-estratrien-17-one, (ES 70 in Ref. [10]). Additionally, X-ray data from our laboratory depicts the 11-OH to be oriented below the plane of the A-ring with the B-ring in the boat conformation [16]. The energy barrier between alternate orientations of the 11-hydroxyl (above or below the A-ring) could be significant enough that a transition from the 11αOH-I or -III conformers to the 11αOH-II geometry (11-OH above the A-ring plane) may be unlikely (Fig. 3, see below). However, it was surprising to find that the three distinct conformers of 11αOH differed by < 1 kcal/mol potential energy following MMP2 optimization (Fig. 4 and Table 3).

Relation to receptor binding

It is of interest to consider the possibility that estrogen receptor may alter ligand geometry during binding, while the complex is undergoing dimerization or during transactivation. Both binding site recognition and alignment of the ligand appear to require hydrogen bonding of the A- and/or D-ring hydroxyl groups which have been shown to maintain their linear distance in the various analogous conformers of 9α estrogens examined (Tables 1 and 4). However, once steroid binding site recognition has occurred, conformational changes in the ligand and as well as the receptor may both contribute to the estrogen regulation process. Conceivably, subtle differences in the estrogenicity of various steroid and non-steroid estrogen receptor complexes may be due to the potential of each ligand to undergo conformational flexing while interacting with receptor.

The free energy involved in binding of estrogens within the receptor site can be derived from their affinity [32]. ΔG for the E_2–receptor reaction in cytosol from MCF-7 cells is -12.1 kcal/mol at 4°C ($K_a = 3.7 \times 10^9$ M^{-1}). Of this energy, an estimated 3.4 to 5.0 kcal/mol is contributed by hydrogen bonding of the 2 hydroxyl groups with specific functions within the binding site [32, 33]. Therefore, at least 7.1 kcal/mol of the binding energy is involved in hydrophobic attraction of the estrogen skeleton within this binding site [34]. This energy is sufficient for the interconversion of E_2-I and -II (3.6 kcal/mol transition energy for the conformational change resulting from the C5-C6-C7-C8 torsion angle difference of 46.0° to -30.4°, Fig. 7). Free energies of binding required

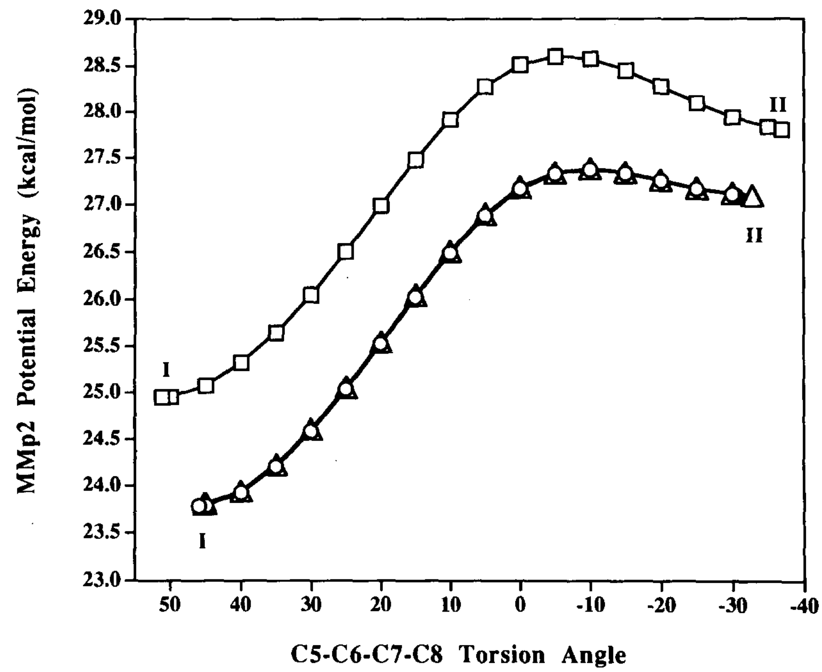

Fig. 7. Conformational energy surface of E_2, 1OH and 2OH. Predicted conformations are designated by I or II (see Fig. 3). The range of torsion angle (C5-C6-C7-C8) increments used in dihedral driving experiments was: E_2 (○), 46.0 to -30.4°; 1OH (□), 51.2 to -36.9° and 2OH (△), 45.4° to -32.8°. Potential energy of conformational minima and barriers was: E_2-I = 23.777 to 27.369 kcal/mol; E_2-II = 27.093 kcal/mol; 1OH-I = 24.952 to 28.601 kcal/mol to 1OH-II = 27.806 kcal/mol and 2OH to I = 23.807 to 27.382 kcal/mol; 2OH-II = 27.097 kcal/mol.

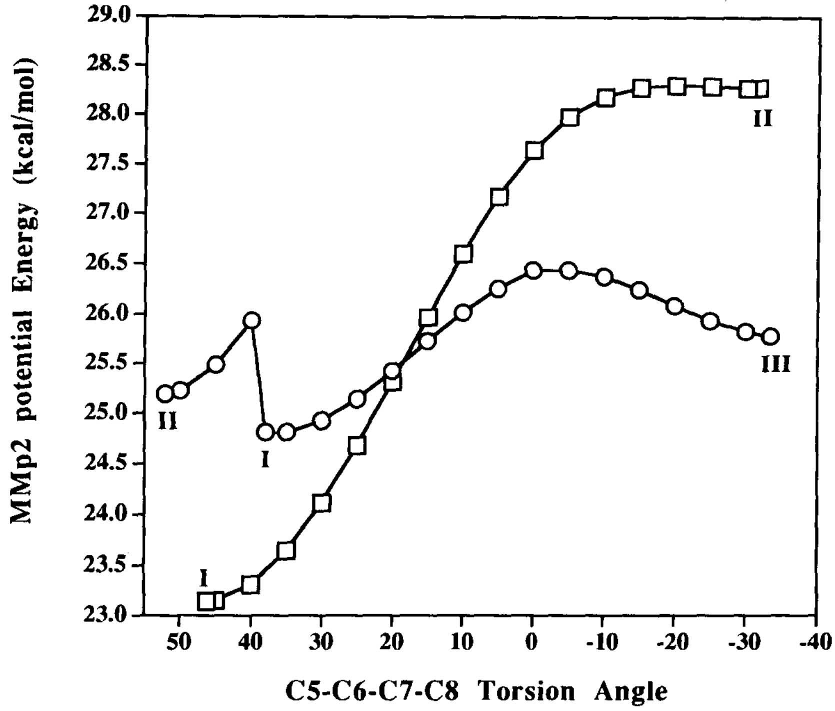

Fig. 8. Conformation energy surface of 11αOH and 11βOH. Predicted conformations are designated by I, II and III (see Figs 4 and 5). The range of torsion angle (C5-C6-C7-C8) increments used in dihedral driving experiments was: 11αOH (○), 52.1 to −33.5° and 11βOH (□), 46.2 to −31.6°. Potential energy of conformational minima and barriers was: 11αOH-II = 25.192 to 25.932 kcal/mol to 11αOH-I = 24.182 to 26.444 kcal/mol to 11αOH-III = 25.783 kcal/mol; 11βOH-I = 23.145 to 28.304 kcal/mol to 11βOH-II = 28.279 kcal/mol.

for the conformational changes of 1OH ($K_a = 1.8 \times 10^7\,M^{-1}$, ΔG of binding = −9.2 kcal/mol) and 2OH ($K_a = 2.6 \times 10^9\,M^{-1}$, ΔG of binding = −11.9 kcal/mol) are similar (transition energy = 3.7 and 3.6 kcal/mol, respectively). The interconversion of 11αOH ($K_a = 1.2 \times 10^7\,M^{-1}$, ΔG of binding = −8.9 kcal/mol, transition energies = 1.1 and 1.6 kcal/mol), and 11βOH ($K_a = 6.22 \times 10^7\,M^{-1}$, ΔG of binding = −9.8 kcal/mol, transition energy = 5.2 kcal/mol) conformers are equally facile (Fig. 8).

One example of the effect of receptor binding on ligand conformation appears to be some of the 9β estrogens which are known to have very low estrogen receptor affinity in chilled *in vitro* assays, but possess significant uterotrophic activity *in vivo* [35, 36]. We have shown that these "L" shaped estrogens may exist in various conformational states including more planar structures which are similar to E_2 (Fig. 6). The energy required to produce these "E_2 like" conformations of 9β, 11-oxo-estrogens are quite different (transition energy from 11K9β-I to -III or -IV = 11.5 or 5.5 kcal/mol, respectively, Fig. 9). Under *in vivo* conditions, interaction of 11K9β with receptor may involve a transformation to the 11K9β-IV conformation. On the other hand, the conversion of 11K9β-I to -III may not be feasible due to the high potential energy of the transition state (Fig. 8).

A similar case in point is represented by the diethylstilbestrol (DES) metabolite Z-pseudo-diethylstilbestrol (ZPD), an estrogen that crystallographic determinations have shown to exist in a bent conformation. Nevertheless, this ligand has been characterized as having high affinity for the estrogen receptor [14]. The postulated mechanism for receptor–ZPD interaction involves a transition of this molecule to a slightly higher energy conformation that is geometrically similar to the potent estrogen DES [37]. The observation that ZPD has much less uterotrophic activity than other estrogens with similar receptor affinity [38, 39] may be an indication that the conformational strain involved in ZPD's receptor interaction interferes with subsequent transactivation.

Thus, it is conceivable that estrogen ligands which can exist in a number of conformations may be converted to a preferred geometry by binding within the specific site of receptor. Once bound, the extent of estrogenic response elicited by a particular ligand could depend on the degree which the bound conformation mimics that of E_2, as well as, the electronic properties of additional functional groups [34, 36, 40–42]. Furthermore, ligands which possess an elevated potential energy of transformation to a preferred conformation would have lower affinity and may be expected to subsequently induce aberrant receptor mediated transactivation [36, 43, 44].

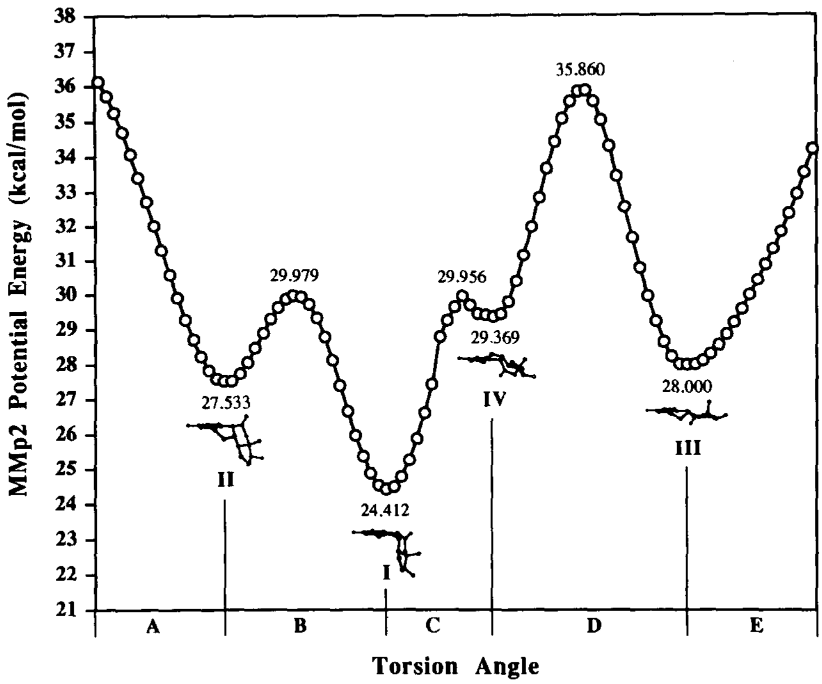

Fig. 9. Conformational energy surface of 11K9β. Predicted conformations are designated by I, II, III and IV (see Fig. 6). Torsion angles and increments used in dihedral driving experiments were: A, C9-C11-C12-C13 from −49.7° (II) to 32.7° (III); B, C5-C6-C7-C8 from −44.3° (I) to 55.5° (II); C, C9-C11-C12-C13 from −54.0° (I) to 13.2° (IV); D, C5-C6-C7-C8 from −63.4° (IV) to 57.0° (III); E, C9-C11-C12-C13 from 32.7° (III) to −49.7° (II). Potential energy (kcal/mol) of conformational minima and barriers are indicated.

The possibility exists that the interaction of an estrogen with its receptor may not be a strict "lock and key" mechanism [45], but rather involves significant strain on the ligand which could result in conformational alterations essential to the transactivation function of the complex. In such case, molecular modeling of steroid ligands may provide considerable insight into the activity of estrogens where receptor affinity is not related to receptor activation.

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APPENDIX A

Supplementary Table 1. Comparison of modeled structures to X-ray structures: individual ring matching

Estrogen X-Ray structure	Optimization method	RMS ring match[a] A-ring	B-ring	C-ring	D-ring
Estratrien-3,17β-diol					
E_2-H_2O	MMP2	0.0206	0.0159	0.0197	0.0173
	AM1	0.0261	0.0281	0.0226	0.0332
	PM3	0.0274	0.0207	0.0184	0.0313
E_2-propanol	MMP2	0.0160	0.0111	0.0183	0.0105
	AM1	0.0214	0.0330	0.0239	0.0335
	PM3	0.0217	0.0185	0.0217	0.0289
E_2-urea	MMP2	0.0291	0.0260	0.0250	0.0168
	AM1	0.0288	0.0330	0.0348	0.0452
	PM3	0.0290	0.0176	0.0328	0.0413
Estratriene-1,17β-diol					
(1OH)	MMP2	0.0196	0.0080	0.0219	0.0224
	AM1	0.0124	0.0221	0.0288	0.0438
	PM3	0.0156	0.0257	0.0264	0.0425
Estratriene-2,17β-diol					
(2OH)	MMP2	0.0224	0.0512	0.0252	0.0331
	AM1	0.0205	0.0383	0.0208	0.0540
	PM3	0.0192	0.0587	0.0368	0.0514
Estratrien-3,11α,17β-triol					
(11αOH)	MMP2	0.0197	0.0910	0.0228	0.0139
	AM1	0.0090	0.0526	0.0351	0.0577
	PM3	0.0114	0.1000	0.0389	0.0522
Estratrien-3,11β,17β-triol					
(11βOH)	MMP2	0.0203	0.0291	0.0282	0.0278
	AM1	0.0189	0.0443	0.0432	0.0483
	PM3	0.0174	0.0224	0.0320	0.0433
9β-Estratrien-3,17β-diol-11-one					
(11K9β)	MMP2	0.0220	0.0084	0.0189	0.0251
	AM1	0.0197	0.0302	0.0413	0.0534
	PM3	0.0187	0.0168	0.0348	0.0500

[a]RMS ring match is the root mean square fit of all carbon atoms comprising a particular ring in the model compared to the corresponding ring atoms in that estrogen's X-ray structure.

APPENDIX B

Supplementary Table 2. Comparison of modeled structures to each other: overall molecular shape

Estrogen analog and optimization method	RMS MATCH and optimization method[a] AM1	PM3
Estratrien-3,17β-diol (E_2)		
MMP2	0.0937	0.0774
AM1		0.0979
Estratriene-1,17β-diol (1OH)		
MMP2	0.0877	0.0889
AM1		0.678
Estratriene-2,17β-diol (2OH)		
MMP2	0.0908	0.0911
AM1		0.1025
Estratrien-3,11α,17β-triol (11αOH)		
MMP2	0.1204	0.0786
AM1		0.0676
Estratrien-3,11β,17β-triol (11βOH)		
MMP2	0.1318	0.0767
AM1		0.1436
9β-Estratrien-3,17β-diol-11-one (11K9β)		
MMP2	0.1827	0.1002
AM1		0.1160

[a]RMS MATCH is the root mean square fit of the C and O atoms in one modeled structure to the corresponding atoms of a structure of the same estrogen produced by another method.

APPENDIX C

Supplementary Table 3. Ring conformations of estrogens

Estrogen	Conformer	A-Ring	B-Ring	C-Ring	D-Ring
Estratrien-3,17β-diol					
(E_2)	X-ray[a]	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	I	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	II	Planar	Boat	Chair	13β-Envelope
Estratrien-1,17β-diol					
(1OH)	X-ray	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	I	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	II	Planar	Boat	Chair	13β-Envelope
Estratrien-2,17β-diol					
(2OH)	X-ray	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	I	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	II	Planar	Boat	Chair	13β-Envelope
Estratrien-3,11α,17β-triol					
(11αOH)	X-ray	Planar	Boat	Chair	13β-Envelope
	I	Planar	8β-Sofa	Chair	13β-Envelope
	II	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	III	Planar	Boat	Chair	13β-Envelope
Estratrien-3,11β,17β-triol					
(11β-OH)	X-ray	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	I	Planar	7α,8β-Half-chair	Chair	13β-Envelope
	II	Planar	Boat	Chair	13β-Envelope
9β-Estratrien-3,17β-diol-11-one					
(11K9β)	X-ray	Planar	7β,8α-Half-chair	Chair	13β-Envelope
	I	Planar	7β,8α-Half-chair	Chair	13β-Envelope
	II	Planar	Boat	Chair	13β-Envelope
	III	Planar	7α-Sofa	Twist	13β-Envelope
	IV	Planar	7β,8α-Half-chair	Twist	13β-Envelope

[a]E_2–H_2O, E_2–propanol and E_2–urea X-ray structures of E_2 have identical ring conformations.