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Chemical Modulation of Activity in Steroidal Estrogens

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CONTENTS

I. INTRODUCTION

Estradiol (E2, **1**, Figure 1), the most important human estrogen, forms part of a family of molecules that share the basic cyclopentaneperhydrophenanthrene structure, **2**, and requires preliminary binding to receptor to elicit response.

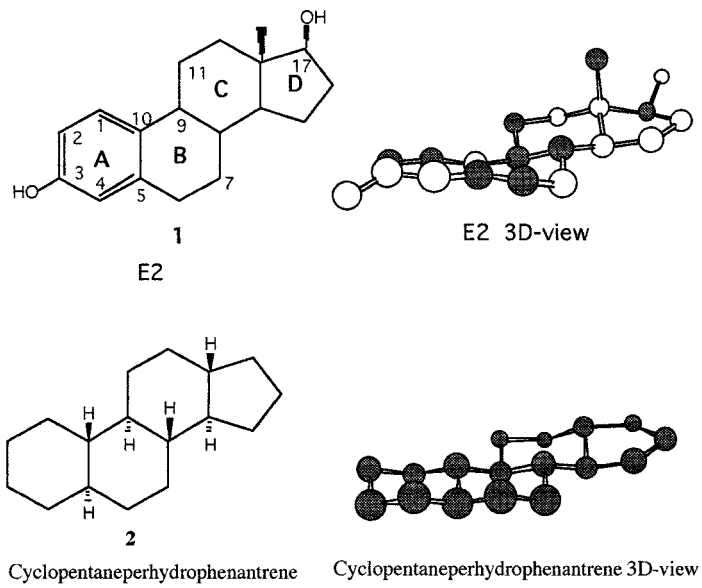

Figure 1 Structures of E2, **1**, and cyclopentaneperhydrophenantrene, **2**. The structure of E2 was taken from X-ray data (see Reference 9). Model **2** was generated using the Chem 3D plus program (Cambridge Scientific Co., Cambridge, MA, 1990) and optimized using the MM2 method within the same program.

Aromatization of **2** to form the estradiol molecule **1** causes a flattening in ring A (Figure 1) and a concomitant increase in electron-density around the same ring. It is commonly believed that electron-rich π-clouds contribute to the interaction of aromatic structures with proteins. Among the aromatic-protein interplays, the reaction commonly referred to as hydrogen bonding to π-clouds has been

demonstrated between benzene and water.[1] In addition, lipophilic-lipophilic interactions, cation-π interactions and aromatic self-association have been invoked as involved in the activity of some structures of natural and synthetic origin.[2] Furthermore, the planarity of ring A in E2 changes the tridimensional configuration of rings B, C, and D from the all alicyclic **2**. The latter effect not only changes the relative position of the hydroxyl group at C-17, but the alteration of the aliphatic rings may have an influence in the initial estrogen binding and, thus, be in part responsible for some of the physiological activities of E2. Two chemical factors can then be considered of importance for activity in estradiol and related molecules: 1) functional groups, type, and regiochemistry; and 2) hydrophobic or skeleton geometrical structure.

The importance of the functional groups and, to some degree, the polycyclic structure in the activity of natural estradiol has long been recognized. The phenolic substituent is suggested to be involved in the initial binding, whereas the aliphatic alcohol and the lipophilic cyclic backbone are assumed to control the degree of further interaction/response.[3]

A number of studies have involved the replacement of aliphatic hydrogens by functional groups. These studies have produced a picture of the importance of the α and β faces of the steroid in activity. Other studies have shown that the introduction of additional hydroxyl groups at positions 2 and 4 of E2 do not inactivate the estrogen.[4]

Replacement of a functional group in estradiol has a direct effect on activity primarily by modification of the binding affinity of the steroid to receptor. In addition, depending on the electronic characteristics of the replaced group, a more or less pronounced alteration of the geometrical structure of the aliphatic rings will occur. This departure from the skeletal conformation of E2 may have an additional influence on the binding ability of the modified steroid to the estrogen receptor, as well as other phenomena such as selectivity, toxicity, and gene induction. Thus, an understanding of the structure-activity relationship when small conformational changes in estrogens take place is important in the design of new estrogens and antiestrogens.

This chapter explores the effects that some simple chemical modifications of the basic structure of estradiol can have on estrogenic activity. Relevant are the electronic inductive effects of substituents on the aromatic A-ring and their influence on alicyclic structure as seen by X-ray crystallography. The inductively created skeletal conformations are compared with those caused by direct substitution on some aliphatic rings. The attempt is to establish a preliminary basis for structural prediction using molecular modeling, and the consequent correlation with some biological results such as gene induction, as well as the preeminent binding to the estrogen receptor.

A. THE ESTROGEN RECEPTOR

The estrogen receptor belongs to a large group of binding domains — the steroid receptor superfamily — whose members share some basic minimum requirements for affinity to the steroids. These regulatory proteins comprise, among others, the receptors for gluco- and mineralocorticoids, estrogen and progesterone, androgens, retinoic acid, thyroid hormone, vitamin D3 and even the oncogene v-*erb*.[5] On average, a target cell contains about 10,000 steroid receptors, each of which reversibly binds steroidal hormones with great affinity (K_a~10^9). These receptors are polypeptide chains of about 800 amino acids long that constitute less than 0.01% of the total protein in the cell.[6]

Members of the steroid receptor family are structured in a similar manner, with a variable N-terminal domain involved in modulation, a conserved cysteine-rich central DNA domain, and a well-conserved C-terminal domain believed to be involved in ligand binding[7] (Figure 2).

The steroid domains contain regions and subregions of variable lengths. Region I of the estrogen receptor, for example, is located within the DNA domain and contains 66–68 amino acids, 9 of which are cysteine units. Eight of these cysteine amino acids form two so-called zinc fingers, in which a zinc

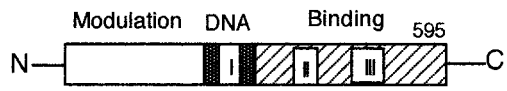

Figure 2 Simplified diagram of the domains and regions (in roman numerals) of the estrogen receptor. The N-terminal domain (modulation) is variable in length, the central DNA domain is conserved and rich in cysteine units, and the C-terminal domain is believed to be involved in ligand binding. The three regions depicted (I-III) are conserved through most of the receptors. The zinc finger subregion is located in region I and forms the basis of recognition of the steroid receptor family.

atom is coordinately bound to the cysteine residues. This zinc subregion forms the basis of recognition of the estrogen receptor from other transcription factors.

Steroid receptors inside the cell may occupy different locations but are mostly nuclear in nature. The glucocorticoid receptor is found in the cytoplasm and nucleus; estrogen, progesterone, and androgen receptors are weakly bound to the nucleus, whereas the thyroid hormone receptor is tightly bound to the nucleus.[8]

It is now well established that the steroid receptor forms a complex upon interaction with a steroid ligand, and that a transformation follows this interaction causing an increased affinity of the complex for chromatin. After ligand-receptor interaction, the mechanism of gene transcription is poorly understood, and models involving a stable initiation complex through enhancer intervention, or through modulation of the chromatin-DNA structure have been proposed.

Ligand binding to the estrogen receptor does not necessarily produce gene activation or transcription. This fact allows for the use of antiestrogenic therapy in estrogen-dependent tumors, since the antiestrogen binds competitively to the receptor but fails to induce transactivation in the hormone binding domain.

Binding to the estrogen receptor is favored by a variety of chemical structures, a fact that underscores the flexibility of these regulatory proteins. The estrogen receptor accepts, in addition to derivatives of the cyclopentaneperhydrophenantrene family, structures such as carotane sesquiterpenes (**3**), triarylethylenes, isoflavones, coumarins, arylindenes (**4**), etc.[9] (Figure 3). The correlation of these structures with binding activity and gene induction is poorly understood.

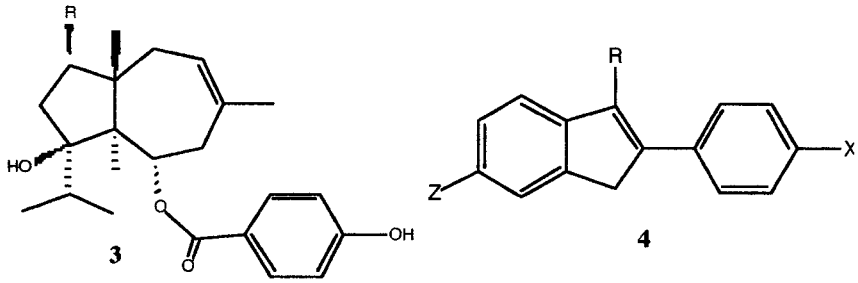

Figure 3 Structures of a carotane sesquiterpene (**3**) and an arylindene (**4**).

Interaction and post-binding activity of estrogens with the receptor is complex as indicated above. Some attempts to understand these mechanisms use theoretical models of structure-activity relationships (*vide infra*) based on computer-generated skeletal conformations, upon which steric (shape) and electrostatic (electronic) contributions are mathematically evaluated. This approach has produced fair to good correlations to receptor binding properties. However, in order to study the activities of other steroids activities, minor alterations in the structures of these steroids should be considered and this evaluation can depart from actual crystallographic data. Crystal structure and solution conformation as studied by nuclear magnetic resonance spectrometry (NMR) seem to correlate quite closely.[10] The analyses of minor conformational changes in the crystal structure, therefore, should provide information directly relevant to *in vivo* studies.

II. REGIOCHEMICALLY INDUCED SKELETAL CONFORMATIONS

Of importance in the understanding of the mechanism of action of estrogens and their modulation of activity, is the evaluation of changes in response caused by chemical modifications in the estradiol molecule. This type of study, as noted earlier, is facilitated by the nature of the steroid nucleus that undergoes substantial modifications in its tridimensional structure after minimal chemical modifications. Those variations in conformation can be analyzed by the use of X-ray crystallography, or, to an extent, by a molecular modeling approach based on crystallographic data when particular X-ray diffraction values are not available.

As mentioned previously, functional groups and skeletal geometric structure have a high degree of importance in estrogen function. Although the contribution of each to the modulation of estrogenic activity is not clear, some understanding of their roles can be obtained by studying the effects caused by small variations in the steroid structure. Positional changes of the aromatic hydroxyl group in natural

E2, for example, provide information not only about the role of the polar substituent itself, but also about the electronic inductive influence of such polar groups on the conformation of the alicyclic rings. These conformational changes may influence the binding to receptor and other further transcriptional effects. In addition, the inductively created conformational changes can be compared with the conformations directly produced by substituents on the alicyclic structure. The outcome of these studies is knowledge that can facilitate the design of estrogens or antiestrogens with specific action.

A. A-RING ESTRADIOL ISOMERS

Ring A of natural E2, **1**, behaves chemically as any other aromatic nucleus. Nucleophilic substitution such as nitration or bromination, for example, occurs *ortho* (positions 2 and 4, Figure 1) to the aromatic hydroxyl group. It follows that for any substitution in ring A a concomitant activation or deactivation of carbons 5 and 10 will take place. This change in electronic density around these quaternary centers causes a corresponding density change in the adjacent alicyclic carbons. The net effect is a conformational change in rings B, C, and D.

On a semiquantitative scale, the sigma (σ) values of the Hammett equation for inductive effects can be used to assess electronically promoted variations.[11] Although the influence of an *ortho* orienter is not a reliable predictor in this equation, values for *meta* and *para* substituents have found favorable applications. Sigma values for *meta* (σ_m) and *para* (σ_p) inductive effects of a hydroxyl group (OH), for instance, are +0.12 and –0.37, respectively. A $+\sigma$ value denotes a substituent which withdraws electrons relative to hydrogen, whereas a $-\sigma$ value refers to substituents with electron-donating capacities. Implicit in these numbers is the fact that a negative σ value will produce an effect on aliphatic carbons connected to an aromatic ring through an alteration of hybridization by electronic shift. This effect, in theory, should be of opposite direction for positive σ values. Based on the above arguments, an increased electronic density will surround carbon 10 when the OH is located on carbon 3, or on carbon 5, when the hydroxyl group is located on carbon 2 (see Figure 1 for numbering). Given the steric constraints of carbon 9 (a tertiary carbon) in estradiol compared to carbon 6 (a secondary carbon), a more dramatic effect in structural change should be observed upon substitution on carbon 2. These changes are explained as the effect of electronic density shifted by movement of electrons through the backbone of the molecule.

The values of σ_p and σ_m include electrostatic (σ') as well as resonance effects. It has been shown[11] that for *meta* effectors,

$$\sigma_m - \sigma' = \pm 0.1$$

suggesting that electronic interactions are the major constituents of m-substituent effects. Thus, based on the given values, the σ_m effect on carbon 6 and remaining aliphatic backbone, when the OH substituent is on carbon 3, should be less influential than σ_p. However, since crystallographic data for an unsubstituted A-ring estradiol derivative is still forthcoming, conclusions on $+\sigma_m$ skeletal influence should be, at this point, interpreted with caution.

Depending on the size and polarity, substituents on carbon 1, by contrast, should cause skeletal alterations of rings B, C, and D, mainly by steric hindrance and through space electronic induction on C11 and its attached hydrogens. Substitutions on C4, on the contrary, should not create major changes in conformation of the alicyclic system since *ortho* inductive effects are normally minimal. In this regard, an early study on inhibition of adrenal estrogen sulfotransferase suggested that factors other than polar effects could be responsible for the activity of some 4-substituted estrogens.[12]

Changes in electronic density created at any carbon center will have a chain effect on adjacent carbons. The physically observable consequence is a change in conformation of the tricyclic system as seen by X-ray crystallography. When the crystal structure of the three A-ring isomers of natural estradiol are compared to the crystal structure of estradiol itself, the theoretical conclusions of electron density variations can be verified.[10] Figure 4 depicts X-ray-derived carbon backbones for the four A-ring estratriene-diol isomers.

Compared to natural estradiol, **1**, shifting the OH to the 4-position to produce **7** has a relatively small effect on the conformations of the alicyclic rings B, C, and D. This result is expected from theory since the main *para* effect has no incidence on any junction carbon, and polar *meta* and *ortho* effects are negligible. Moreover, the distance between the aromatic and aliphatic hydroxyls has shortened from 10.93 Å for **1** to 9.89 Å for **7**. It could be predicted on this basis that any physiological effect caused by **7** will be mandated by the position of the phenolic substituent. Since this position represents hindrance, the initial binding to the receptor should be hampered at the aromatic ring level, and could even disturb

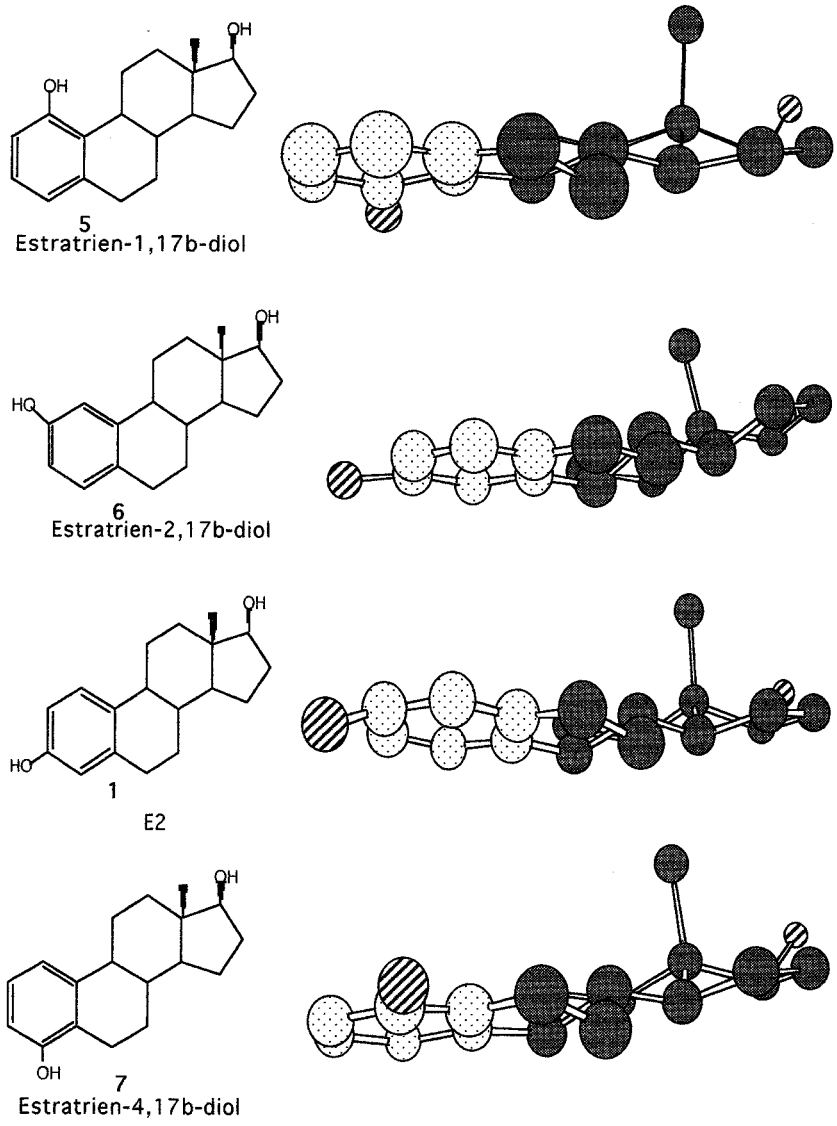

Figure 4 X-ray structure of the A-ring isomers of E2 (semiparallel view from aromatic carbon 4). Aromatic and heteroatoms are depicted in different shade for simplicity.

the binding attributed to the 17β-alcohol. However, it should be noted that, although estrogen receptor allows large flexibility in binders, some minimum requirements are essential; thus, minor changes to key molecular areas may have a dramatic effect in estrogen activity.

The apparent lack of skeletal modification brought about by substituents on the 4-position of the aromatic ring is again displayed in the crystal structure of some 4-nitro estrogens. The nitro derivatives of E2, **8** and **9** (Figure 5), had little variation in structure when compared to natural E2.[13] However, in these systems the inductive effect attributed to the nitro group (σ_m = +.710, σ_p = +.778) on carbon 4 is not fully operative since the nitro oxygens are positioned antiperiplanar to the aromatic ring (see X-ray structures in Figure 5), a position that precludes effective resonance with the nucleus. In addition, due to differences in inductive effect between nitro and hydroxyl groups, different skeletal variations are observed between compounds **7** and **8** (Figures 4 and 5).

Relocation of the aromatic 3-hydroxyl group of E2 to positions 1 or 2 to generate isomers **5** and **6**, respectively, produces substantial changes in rings B, C, and D. The powerful inductive effect on carbon 5 of isomer **6** causes a partial flattening of ring B. By a chain sequence, this flattening modifies rings

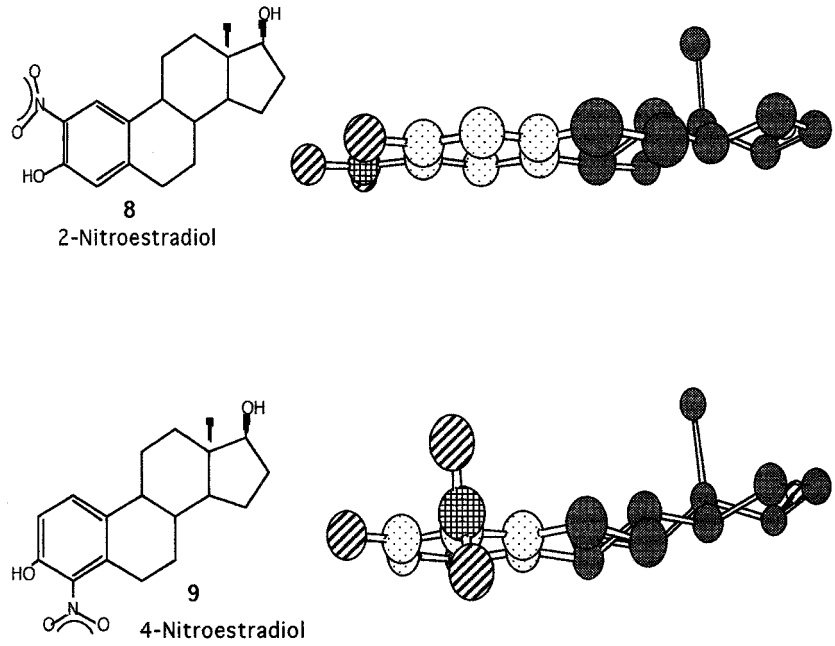

Figure 5 X-ray structure of nitro-isomers of E2. Structures are shown on a semiparallel view with the benzene ring seen from carbon 4. Aromatic and heteroatoms are depicted in different shade for simplicity.

C and D, and increases the dihedral angle between the angular methyl group (C18) and the aliphatic hydroxyl group (O17) to its largest value of the A-ring estradiol isomers (50.2º).

Isomer **5** undergoes a different variation in its alicyclic rings. Instead of a partial flattening of ring B by leveling carbon 7, a further distortion of this ring occurs with the formation of a twisted boat conformation. The chain sequence that follows on rings C and D causes an increase in the C18..O17 dihedral angle (49.2º) by comparison with E2 (46.6º).

The introduction of a nitro group at the 2 position of E2 to form **9** represents, along with its isomer **8**, an interesting case in which theoretical calculations fail to predict crystallographic data. The position of the oxygens in the nitro group at C-4 of **8**, as indicated previously, is antiperiplanar or perpendicular to the aromatic ring. This geometry prevents the alignment of lone pair orbitals of oxygen with aromatic orbitals with a concomitant decrease in conjugation. The inductively created conformational effect of the nitro group here is minimal, and the only possible effect of this group should be steric. In **9**, on the other hand, nitro oxygens are in the same plane as the aromatic ring; thus, conjugation is fully operative. The result is a substantial batochromic effect in the UV absorption of **9** compared to **8**, and a flattening of the aliphatic rings compared to E2.

B. SOME ALICYCLIC-SUBSTITUTED ESTRADIOLS

Effects on activity by substitution of rings B, C, or D of E2 have been reported in the literature. Alterations to the D-ring have involved substitution mostly by halogens and some hydroxyl groups.[3] Ring B substitutions, on the other hand, have centered on C7, where α-carboxyamidoalkyl groups have produced compounds with remarkable antiestrogenic activity.[14,15] However, crystallographic data on such compounds is not available. Ring C substitutions have involved mostly modifications at C11. An apparent predominance of face activity has been registered for these compounds, with the C11β substitution being the most active. The preference for C11 substitution is based on its close proximity to the aromatic C1 which can be influenced via steric interaction and through space electronic induction. The effect can be so pronounced that substitution of a carbonyl in C11 to produce 11-keto estradiol shifts the balance of stability for the 9β-isomer by at least 2 kcal.[16,17] Due to the conformation of the B-ring, substitutions on the α-face on C11 have a more dramatic structural effect than those on the β-face. Those skeletal variations have been described in detail for the two hydroxylated analogs 11α- and 11β-hydroxyestradiols.[16] Of interest at this point is the relationship that these skeletal variations, promoted by face location, can have with variations caused by groups in other locations, particularly the A-ring. Comparison with

the E2 isomers **5** and **6** reveals some common features. Hydroxyl substitution at C11α causes an upward bend of rings B, C, and D with a reduction in distance between the aromatic and the aliphatic hydroxyl groups, from 10.9 Å for E2 to 10.8 Å for 11α-hydroxylestradiol. The 11β-hydroxylestradiol, on the other hand, relaxes those rings somewhat causing an increase of this distance to 11.0 Å. Those changes in relative distance between polar groups do not reveal, however, the alterations in the hydrophobic alicyclic carbons. Comparisons by superimposition with isomers **5** and **6** (Figure 6), shows that substitution on the α-face of C11 has the same general effect as transposing the hydroxyl groups from C3 in E2 to C2 in **6**. Not surprisingly, however, is the fact that substitution on the β-face of C11 has a similar effect as the relocation of the hydroxyl group from C3 in E2 to C1 (Figure 6). These results signal once more the importance of the aromatic ring in influencing the structural conformations of the rest of the steroid molecule. This conclusion bears some importance given the fact that chemical replacements on the aromatic A-ring are relatively simple to perform compared to modifications on the alicyclic backbone. If skeletal alterations on those rings can be achieved by electronic induction via ring A, then the effect of the hydrophobic aliphatic region on binding and post-binding activation can be more adequately assessed.

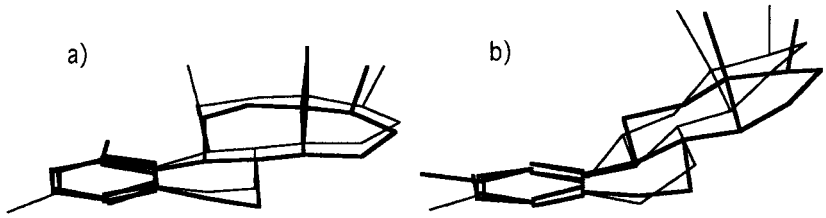

Figure 6 Overlay of (a) 1-hydroxyestratrien-17β-ol, **5**, and 11β–hydroxyestradiol, and (b) 2-hydroxyestratrien-17β-ol, **6**, and 11α-hydroxyestradiol. Fit was performed using the 6 carbons of the aromatic rings through the program Chem 3D plus 3.0 (Cambridge Scientific Co., Cambridge, MA, 1990).

III. ACTIVITY MODULATION AND STRUCTURAL MODIFICATION

With the realization in the late nineteenth century that cells in multicellular organisms are controlled partly by outside molecules, a great deal of information has been gained at the micromolecular level on the so-called hormones of Bayless and Starling.[18] The mechanism of hormone activity on preexisting cellular reactions has begun to unfold in the past 30 years. The three main groups of hormones, polypeptides, mono-amino acid derivatives, and steroids, share some common features, the most prominent of which is binding to specific receptors of their effector cells. Whereas the receptors for polypeptides and certain amino acid hormones are, due to their polarity, located on the cell membrane, receptors for the highly lipophilic steroid hormones are found largely in the nucleus, which they can reach through passive diffusion. The interaction between steroid hormone and receptor generates a complex that interacts with particular genomic loci involved in turning on transcription mechanisms of appropriate genes. Best understood is the glucocorticoid receptor with its five binding regions, studied in the mouse mammary tumor virus (MMTV).

The complexity of the estrogen receptor (ER), on the other hand, has made it difficult to map its interaction sites and mechanisms of action. However, the use of structurally modified E2 molecules has given some clues to the requirements of the ER for the initial binding, as well as specific effects on gene induction.

A. RECEPTOR BINDING

Competitive binding assays using tritiated E2 have been used as a guideline for estrogen activity, and continue to be employed as a predictive tool in molecular modeling studies.[19] Since this chapter deals with some of the recent reports, the reader is referred to tables in previous publications for values on relative binding affinities (RBA)[3,19] of various estrogen analogs.

It has been determined that effective binding requires the presence of the polar hydroxyl groups at C3 and C17. Thus, the estratriene molecule has an RBA which is too low to be measured (<0.5 relative to 1000 for E2). The 17β-hydroxyl group in estratrien-17β-ol, improves the affinity (RBA = 110) but it is not as effective as the phenolic OH group in 3-hydroxyestratriene (RBA = 793). Aside from these straightforward relationships, the effect on binding of other groups, alone or in combination with hydroxyl

substituents, is not completely clear. A combination of steric and electrostatic interferences may be involved in the variations in binding affinity relative to E2. In estrone, for instance, where the 17β-OH of E2 has been replaced by a keto substituent, the RBA decreases to 222. However, if the stereochemistry of the 17β-OH is changed to the 17α-enantiomer the comparative decrease in activity is much lower (RBA = 802), whereas an added 16α-OH in E2 reduces the RBA value to 130.

Table 1 displays RBA values for the three estradiol regioisomers. The repositioning of the aromatic hydroxyl to C1 in isomer **5** causes a distortion of aliphatic rings B, C, and D, and a probably steric hindrance to the binding of the aromatic ring alone, giving the lowest RBA value of the three isomers (RBA = 5). Isomer **6**, despite a pronounced flattening of the alicyclic region, has an RBA of 706 confirming that the C2-C3 region of the steroid molecule is binding tolerant, as was shown previously for the corresponding cathecol estrogen, 2-hydroxyestradiol.[20] Estratriene-4,17-diol (**7**), by contrast, has undergone little structural change with respect to E2; however, its binding is poor (RBA = 67), signaling perhaps the influence of steric hindrance for perfect fitting as in the case of **5**.

Table 1 Binding Affinities and Gene Inductive Effects of Estradiol Analogs at 10^{-8} *M*

	RBA	%pS2	%PgR	%Cath D	%-pA
Estradiol (**1**)	1000	80[a]	100	80[a]	100
1-Hydroxyestratriene 17β-ol (**5**)	5	90	50	100	28
2-Hydroxyestratriene 17β-ol (**6**)	706	70	37	9	13
4-Hydroxyestratriene 17β-ol (**7**)	67	0	2	0	0
2-Nitroestradiol (**8**)	1	8	3	0	0
4-Nitroestradiol (**9**)	39	85	42	78	20
11β-Hydroxyestradiol	17	—	—	—	—
11β-Hydroxyestradiol	3	—	—	—	—
11-Ketoestradiol	1	—	—	—	—
9 (11)-Dehydroestradiol	196	—	—	—	—
2-Bromoestradiol	12[b]	—	—	—	—
4-Bromoestradiol	50[b]	—	—	—	—

[a] These values are 100% at 10^{-10} *M*.

[b] Data from Reference 19.

The effect of introduction of other groups into the E2 molecule, in general has a deleterious effect on binding. A nitro substituent in C-2 (analog **9**) completely hinders binding (RBA = 1), while the effect is less drastic but still evident when the nitro group is in position 4 (RBA = 39 for analog **8**). Apparently less steric hindrance occurs in the latter case.

Introduction of a substituent at the C-11 position also causes a virtual elimination of receptor binding with 11α-hydroxyestradiol and 11-ketoestradiol analogs possessing little affinity, and 11β-hydroxyestradiol displaying a slight binding activity (RBA = 17). The introduction of an unsaturation at C9-C11 yields an analog with 20% the binding affinity of estradiol, despite the fact that a severe flattening of rings B, C, and D has occurred in this molecule.[16] Apparently, the electrostatic influence of an endocyclic double bond does not prohibit binding. This assertion correlates with the fact that an exocyclic double bond at C11 has proven to have a strong binding affinity.[21]

From the results obtained it is apparent that receptor binding is strongly dependent upon the presence of an aromatic hydroxyl group and the conformation of the hydrophobic portion of the molecule. When the skeletal conformation of an analog approximates that of E2, as in **7**, binding is subject to steric factors. It is also evident that the RBA of substituted estradiols is more susceptible to steric and/or electrostatic effects when the substitution is on C2 rather than on C4, which explains the difference between **8** and **9**. Following this rational, a 4-bromo estradiol should have a better RBA than its 2-bromo counterpart. In point of fact 4BrE2 has an RBA of 50, whereas 2BrE2 has a value of 12.[19]

B. GENE INDUCTION

Within the cell, receptor binding is followed by "processing" of the endoplasmic reticulum (ER) complex. This last event, defined as a 50% loss of detectable receptor, was originally conceived essential for E2 activity. More recently it has been shown that nuclear processing is the result of a downregulation of ER mRNA by E2. A direct correlation of this phenomenon with gene induction has not been demonstrated.[22]

Gene regulation by modified estrogens has been shown to be independent of the affinity of the ligand for receptor.[22] This important observation, which may be related to the kinetic stability of the produced complex, leaves the door open to the evaluation of gene activity and ligand structure. A small number of gene induction processes and their relation to estrogen structure have been studied. Among those, the stimulation of progesterone synthesis (PgR), induction of pS2 and cathepsin D (Cath D) genes, as well as tissue plasminogen activator (t-pA) mRNA have received special attention.

The pS2 gene is expressed in several tissues, but its regulation by estrogen has been reported in ER positive breast cancer cells. In addition, this gene is also regulated by other growth factors involved in E2 response. Although the pS2 protein shares some similarities with growth factors, its physiological role remains unknown.

The lysosomal aspartyl protease, Cath D, on the other hand, has been strongly associated with cancer prognosis. Levels of the Cath D mRNA which are found in normal tissues, are 8–50 times higher in breast cancer cells, and are stimulated by E2.[23]

Similarly, elevated levels of fibrinolytic activity in human tissues have been attributed to a serine protease, tissue plasminogen activator (t-pA), which converts the serum zymogen plasminogen to the active nonspecific protease plasmin, and is controlled by estrogen via a receptor-mediated mechanism.

Table 1 shows the effect of ring A isomers of E2 and nitro analogs **8** and **9** on gene induction. Although limited in scope, these initial results confirm the lack of correlation of gene stimulation with binding affinity. However, these values may have an association with estrogen structure and/or electronic isopotential around the backbone of the ligand. At a concentration of 10^{-8} *M*, C1 isomer **5** and 4-nitro analog **9** displayed similar and high level of gene induction of the six compounds tested; with pS2 and Cath D values comparable to E2, PgR values reduced to roughly half, and tpA to one fifth those of E2. These results are of interest when one considers that the binding is very low for **9** and poor for **5**.

Values for C2 isomer **6**, by contrast, show a level of gene activity which appears to correlate with a high level of binding to receptor. The reduced values for Cath D and t-pA, however, may indicate the effect of subtle structural differences in the skeleton backbone caused by the shift in electronic induction and negative isopotential.

The results obtained for the C4 isomer **7** and the 2-nitroanalog **8**, are worth further consideration. While the repositioning of the hydroxyl group to the C4 location to produce **7** caused very little skeletal change, overall gene induction was turned off despite the fact that receptor binding was better than for isomer **5**. When one compares these results between **5** and **6**, electronic inductive effects appear to play a role. In isomer **7** this inductive effect withdraws electrons from C10 (σ_m = +.121). Although the same could be said of isomer **5** with similar inductive effect on C5, the steric influence of C11 may shift the electron-pairs in oxygen, preventing effective conjugation. By contrast, inductive effects on isomer **6** have no interference causing electronic accumulation on C5 (σ_p = +.370) and withdrawal at C10 (σ_m = +.121). Since sigma values are additive, a net increase in electronic density (σ_{total} = –.259) accumulates along the C5-C10 bond, should be similar in nature but different in orientation with the one produced by the 3-OH in E2, and it is responsible for the large skeleton variation seen in **6**.

Nitro analog **8** was complementary in its effects with insignificant inductive values for pS2 and pgR, and virtually no binding. These contrasting results with isomer **9** could be linked to the very strong inductive effect that withdraws electrons from C5 (σ_p = +.778) and C-10 (σ_m = +.710) in **8** but causes very little electronic effect in **9** due to the fact that steric hindrance at C4 causes a rotation of the nitro oxygens to a position antiperiplanar for induction through conjugation.

Based on the above discussion, gene induction seems to be more sensitive to changes in electronic density throughout the steroid molecule, whereas binding by and large depends more on presence and orientation of hydroxyl groups. The electrostatic induction created by polar groups in the aromatic ring produces a negative isopotential whose orientation and size seems to be related to the gene inductive activity of the steroid. For instance, maximum PgR activity is observed when an electronegative isopotential extends beyond the A-ring between carbons 3 and 4, and it is lowest when the isopotential is oriented over the junction C4-C5.[20] In addition, Cath D and pS2 induction is produced by similar levels on estratriene 17β-ol (Cath D = 75%, pS2 = 50%) and estratriene-3-ol (Cath D = 90%, pS2 = 60%) at 10^{-8} *M* concentrations.[23] These latter inductions can be partly explained by the innate isopotential values of the aromatic ring. Electronegative isopotentials for E2 isomers are shown in Figure 7.

Since isopotential clouds are intimately linked to the electronic induction of the substituents, and this induction creates alterations in the steroid skeleton, a closer look at the fit between those modified estrogens and E2 may yield another visual method of interpretation of binding and gene induction.

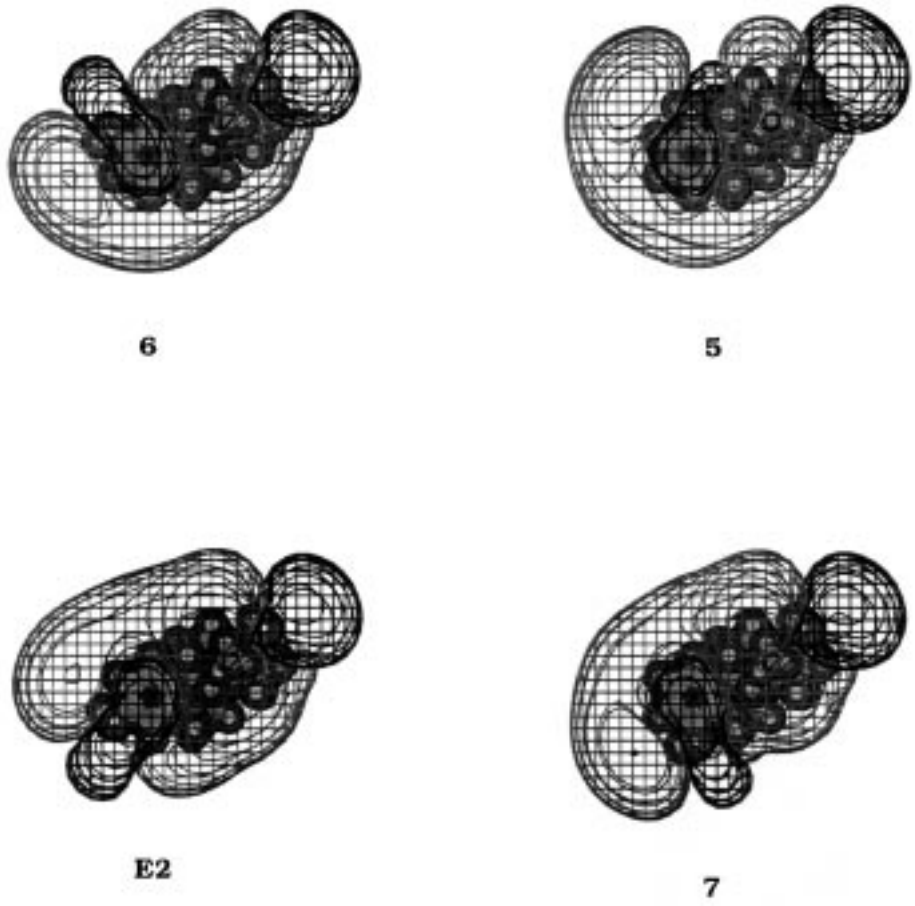

Figure 7 Electronegative isopotentials for A-ring isomers of E2. Dark lines represent negative charge accumulation and correspond to the location of polar groups.

Overlay of estrogen isomers and analogs with E2 using the aromatic ring as a proven fit[19] is shown in Figure 8. Although isomer **5** has undergone drastic B, C, and D transformations, its overall fit is colinear and cospatial (that is, similar geometric boundaries) with E2. This colinearity, first, gives more weight to the location of the aromatic OH, which serves to explain the poor binding affinity of the ligand. Secondly, since gene induction is independent of binding, the close fit in the hydrophobic alicyclic backbone also may be responsible for the effective gene inductive properties of this isomer.

Close fit analysis of the C4 isomer of E2, **7**, provides a contrasting view with **5**. Whereas **5** displays a modified structure of rings B, C, and D while maintaining its co-spatial linearity with E2, **7** has little alteration in its overall conformation with respect to E2; that is, **7** is colinear with E2. However, this compound is not cospatial with E2 and its overall alicyclic backbone protrudes upward with respect to E2 (Figure 8). This suggests that some binding is possible, as is observed (RBA = 67), but gene induction should be poor. Similar visual analysis of close fit may explain the general inactivity of nitro analog **8** and the gene inductive properties of its isomer **9**, as well as the good binding and adequate gene stimulation of isomer **6**.

Of particular importance in this close-fit analysis is the predominance of superimposition of ring B, particularly C7, as a key element in the overlay-activity profile. Figure 8 shows that those compounds displaying gene induction properties have a close fit at C7 relative to E2. These results, although preliminary, have some significance when one considers that pure antiestrogens have been produced by replacement of the 7α-hydrogen with amidoalkyl groups.[14]

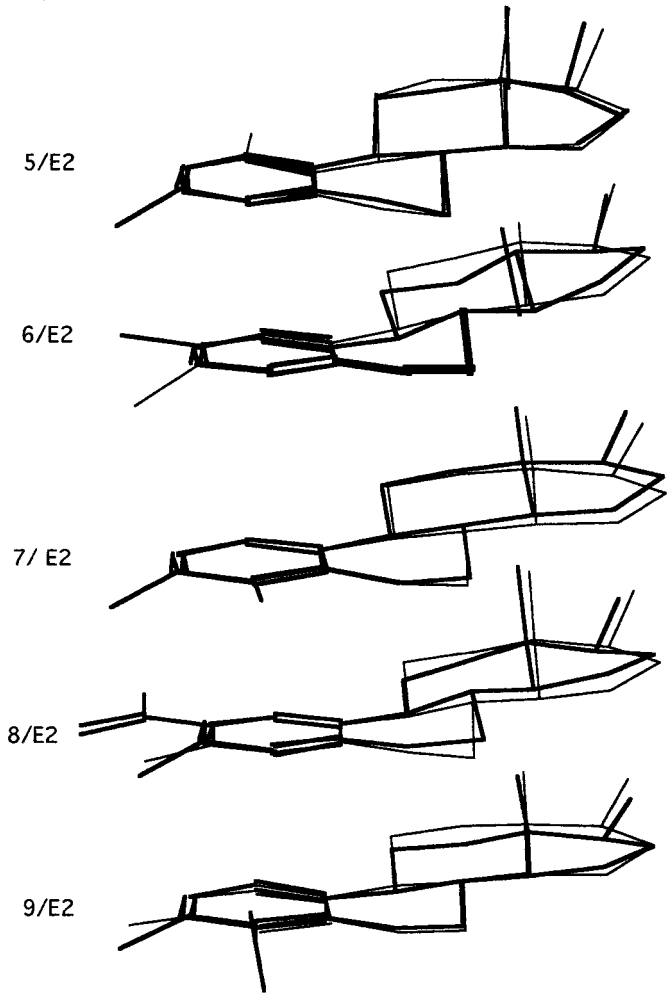

Figure 8 Overlay of crystal structures of E2 with A-ring isomers and nitro-analogs. Fit was performed using the 6 carbons of the aromatic rings.

IV. PERSPECTIVE

Preliminary evidence shows that modification of the skeletal conformation of estrogens bears importance in binding to the ER and in the general modulation of activity. Although the steroid molecule is remarkably rigid compared to noncyclic structures, its interactions with the receptor for binding, and with the steroid domains for gene induction, seem to be highly susceptible to orientation of electronegative isopotential, and/or minor skeletal modifications in its alicyclic backbone. The conformations of that hydrophobic portion of the molecule are, in turn, amenable to alterations through electronic induction by substituents in the flat aromatic A-ring, alterations that are comparable to those caused by regional substituents on the aliphatic rings. Evidence for the achievement of those skeletal changes has been presented in this chapter, and although the scope of the effects of those modifications is incomplete, it serves to establish a pattern for further studies.

The ultimate goal of these studies is to achieve enough refinement for the theoretical calculation of activity using molecular modeling. Initial strides in that direction have been made by obtaining models that conform to prediction for binding[19] and for selectivity of appropriate computational methods.[25] A more adequate theoretical model, however, would require experimental results in conjunction with crystallographic analyses.

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