J. steroid Biochem. Vol. 35, No. 2, pp. 219–229, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

BINDING, X-RAY AND NMR STUDIES OF THE THREE A-RING ISOMERS OF NATURAL ESTRADIOL

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(*Received* 17 *July* 1989)

Summary—The effect of the position of the phenolic hydroxyl on the conformations of the three A-ring isomers of estradiol, namely, estra-1,3,5(10)-trien-1,17β-diol (**10**), estra-1,3,5(10)-trien-2,17β-diol (**3**), and estra-1,3,5(10)-trien-4,17β-diol (**6**), has been analyzed by X-ray crystallography. The results of these analyses were correlated with the absorptions of the angular methyl groups in the [^{1}H]NMR spectra of these isomers and natural estradiol (**E2**). It was observed that the changes in chemical shift of protons at C18 corresponded to skeletal modifications in the steroid structure which changed the anisotropic effect of the hydroxyl group at C17.

Examination of the affinity of these A-ring isomers of **E2** for the estrogen receptor has shown the 2-hydroxylated isomer **3** to retain 1/5th the affinity of **E2** for its binding protein. The 1- and 4-hydroxylated derivatives (**10** and **6**, respectively) bound to a much lesser extent. The receptor affinities of these estrogen analogues may be related to the angle between the 18-methyl and the 17β-hydroxyl groups (or the dihedral angle between the planar A-ring and the angular C18 methyl) as well as the position of the A-ring hydroxyl group.

INTRODUCTION

Estrogens bind to a nuclear protein which possesses specificity based on recognition of ligand structure. The estrogen binding domain has been sequenced and shown to be important in deactivating DNA binding in the absence of ligand [1] and to contain a highly negative region important to transcription activation [2]. Furthermore, this receptor is known to be sensitive to the structure of both antiestrogens [3, 4] and estrogen metabolites [5, 6]. In fact, the resultant gene regulatory activity conveyed by the estrogen receptor complex is dependent, to some degree, on the structure of the ligand [7].

Although it is known that alterations of the estradiol-17β (**E2**) ligand, such as esterification of the 3-phenolic group, will prohibit binding to receptor [8], the addition of extra hydroxyl groups at C_2, C_4 or 16α [8, 9], an acetylenic group positioned at 17α [8], or a 16α iodine [10] do not inactivate the estrogen. Apparently, the estrogen-receptor interaction is tolerant of considerable alteration in the structure of the ligand. Nevertheless, a more definitive understanding of the influence of estradiol's structural elements on the binding to receptor and the transcriptional alteration they convey to the receptor complex would be forthcoming if only highly discriminatory changes in the structure of **E2** were introduced. Such distinguishing structural modifications can be discerned with X-ray diffraction.

The importance of X-ray diffraction as an analytical tool in the study of structure and function of steroids is well established [4, 6, 10]. Crystallographic studies of over 400 steroids [11, 12] have provided important comparative information on structure, preferred conformations and relative stabilities of steroid hormones.

Regional modifications of the phenolic OH described herein would be expected to produce changes in the skeletal structure of the steroidal rings due, possibly, to variations in the distribution of electronic density in the aromatic A-ring which affects the neighboring alicyclic carbon atoms. These structural modifications also can be detected by a non-destructive analytical method like NMR. Therefore, a correlation between crystallographic results and data from NMR is possible.

In this report we compare the effect of the position of the A-ring hydroxyl group on the conformation of the steroid as determined by X-ray crystallography and related to [^{1}H]NMR spectroscopy. The influence of the observed structural alterations on the affinity of the estrogen for its receptor is also reported.

EXPERIMENTAL

Synthesis

General methods. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus

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and are uncorrected. Elemental analyses were performed by M-H-W laboratories, Phoenix, Ariz. [^{1}H]NMR spectra were obtained with a Nicolet QE-300 FT spectrometer. Mass spectra were obtained with a Kratos MS-80 RFA.

2-Hydroxyestra-1,3,5(10)-trien-17-one (2). To a cold solution (5°C) of 2.5 g (2.3 mmol) of amino ketone **1** in 14 ml of concentrated H_2SO_4 and 25 ml of water, was added dropwise a cold (5°C) solution of 700 mg (9.8 mmol) of $NaNO_2$ in 12 ml of water, and the resulting mixture was stirred for 2 h in an ice-water bath. This solution was then added to a three-necked flask charged with a refluxing solution of 37 ml of concentrated H_2SO_4 and 25 ml of water and the mixture refluxed for 20 min. After cooling, the crude **2** was filtered and washed with water. The solid was dissolved in a mixture of ethyl acetate:hexanes (6:4) and passed through a plug of silica gel. Recrystallization from acetonitrile afforded 1.36 g (50%) of **2**, m.p. 200–202°C (Ref. [13], 202–204°C).

Estra-1,3,5(10)-triene-2,17β-diol (3). A mixture of 1.36 g (5.3 mmol) of **2** dissolved in 60 ml of isopropanol containing 2 g (50 mmol) of $NaBH_4$ was refluxed for 2 h. After neutralization with HCl, the solvent was removed under reduced pressure, and crude **3**, dissolved in a mixture of ethyl acetate:hexanes (6:4) was filtered through a plug of silica gel and the filtrate was evaporated to produce 1.23 g of a yellowish solid. Recrystallization from acetonitrile afforded 0.8 g (60%) of colorless crystals, m.p. 222–224°C (Ref. [13], 222–224°C).

4-Hydroxyestra-1,3,5(10)-triene-17-one (5). The latter was obtained in 48% yield from **4**, using the procedure described above for the preparation of **2**; m.p. 240°C(dec), [^{1}H]NMR (dioxane-d8) δ 0.800 (3H, s, 18-H), 6.60 (1H, d, J 7.8 Hz, 1-H), 6.70 (1H, d, J 7.8 Hz, 3-H), 6.91 (1H, t, J 7.8 Hz, 2-H). *Anal.* Calcd. for $C_{18}H_{22}O_{2.1/3}H_2O$: C, 78.26; H, 8.21. Found: C, 78.22; H, 8.34.

Estra-1,3,5(10)-triene-4,17β-diol (6). Following the procedure described above for the preparation of **3**, **6** was obtained in 65% yield as colorless crystals, m.p. 190–192°C (Ref. [14], 191–192°C).

1-(p-Nitrophenylazo) estra-1,3,5(10)-trien-17-one (7). To an ice-cooled stirred solution of 1.29 g (9.3 mmol) of *p*-nitroaniline in 10 ml of glacial acetic acid and 18 ml of 2 N hydrochloric acid was added a solution of 0.64 g (9.2 mmol) of sodium nitrite in 10 ml of water below the surface of the liquid and stirring was continued for 15 min at room temperature. The resulting solution was poured into a well stirred solution of 2.50 g (9.2 mmol) of **4** in 45 ml of glacial acetic acid, and stirring was continued for 15 min.

The resulting red solution of 4-amino-1-(*p*-nitrophenylazo) estra-1,3,5(10)-trien-17-one was cooled to 0–5°C and a solution of 0.64 g (9.2 mmol) of sodium nitrite in 10 ml of water was added below the surface of the liquid and stirring was continued for 15 min at room temperature. To this solution, 110 ml of hypophosphorous acid 25% were added at once and stirring was continued for 24 h. The precipitate was filtered, dissolved in methylene chloride washed with water and filtered through a plug of silical gel. Column chromatography using hexanes:ethyl acetate (8:2) gave 1.1 g (30%) of pure **7**, m.p. 175–177°C, [^{1}H]NMR(dioxane-d8) δ 0.918 (3H, s, 18H), 7.20 (1H, d, J 7.2 Hz, 4-H), 7.25 (1H, t, J 6.6 Hz, 3-H), 7.44 (1H, d, J 7.2 Hz, 2-H), 7.97 (2H, d, J 8.4 Hz, 2′-H), 8.43 (2H, d, J 8.4 Hz, 3′-H). *Anal.* Calcd. for $C_{24}H_{25}N_3O_3$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.27; H, 6.82; N, 10.04.

1-Amino-estra-1,3,5(10)-trien-17-one (8). To a stirred suspension of 5 g of zinc dust in 20 ml of acetic acid was added, in portions, a solution of 900 mg (2.23 mmol) of **7** in 20 ml of methylene chloride, and stirring was continued for 1 h. The zinc was filtered off and the solution concentrated under vacuum. The solid residue was dissolved in ethyl acetate:hexanes (1:1) and the solution filtered through a plug of silica gel to give, after concentration, 380 mg (95%) of crude **8**. Column chromatography on silica gel with hexanes:ethyl acetate (8:2) as the eluent afforded an analytical sample, m.p. 186–188°C, [^{1}H]NMR(dioxane-d8) δ 0.908 (3H, s, 18-H), 6.35 (1H, d, J 2.4 Hz, 4-H), 6.38 (1H, d, J 2.4 Hz, 2-H), 6.77 (1H, t, J 7.8 Hz, 3-H). *Anal.* Calcd. for $C_{18}H_{23}NO$: C, 80.24; H, 8.61; N, 5.20. Found: C, 80.12; H, 8.69; N, 4.99.

1-Amino-estra-1,3,5(10)-trien-17β-ol (9). Following the procedure described above for the preparation of **3**, **9** was obtained in 62% yield as pale yellow crystals, m.p. 135–137°C, [^{1}H]NMR(dioxane-d8) δ 0.800 (3H, s, 18-H), 6.35 (1H, d, J 2.4 Hz, 4-H), 6.38 (1H, d, J 2.4 Hz, 2-H), 6.77 (1H, t, J 7.8 Hz, 3-H). Molecular ion expected 271.1935; found 271.1930. *Anal.* Calcd. for $C_{18}H_{25}NO$: C, 79.66; H, 9.28; N, 5.16. Found: C, 79.64; H, 9.30; N, 5.29.

Estra-1,3,5(10)-triene-1,17β-diol (10). To an ice-cooled stirred solution of 380 mg (1.41 mmol) of **9** in 10 ml of 40% sulfuric acid and 6 ml of acetic acid were added beneath the surface, a solution of 115 mg (1.41 mmol) of sodium nitrite in 5 ml of water, and stirring was continued at room temperature for 1 h. A solution of 40% sulfuric acid (10 ml) was added and the mixture refluxed for 30 min. The solution was cooled and the solid was collected and recrystallized from acetonitrile to produce 260 mg (68%) of **10** as yellowish crystals. m.p. 175–178°C, [^{1}H]NMR(dioxane-d8) δ 0.768 (3H, s, 18-H), 6.38 (1H, d, J 7.8 Hz, 4-H), 6.53 (1H, d, J 7.8 Hz, 2-H), 6.83 (1H, t, J 7.8 Hz, 3-H). *Anal.* Calcd. for $C_{18}H_{22}O_{2.1/2}H_2O$: C, 76.87; H, 8.90. Found: C, 76.62; H, 9.04.

X-ray analysis

General methods. Reflections were collected on a Nicolet R3 diffractometer equipped with a MoKα

radiation ($\lambda = 0.71073$ Å) and a graphite monochromator. The structures were solved by direct methods [15] and refined in a full matrix with the programs of SHELX-76 [16]. All non-hydrogen atoms were refined anisotropically. No correction for absorption was made.

*Estra-1,3,5(10)-trien-1,17β-diol (**10**).* Suitable crystals were grown from acetone. A colorless fragment of a single crystal with dimensions $\cong 0.2 \times 0.4 \times 0.4$ mm was used in the study and coated with epoxy to prevent solvent evaporation. One equivalent of acetone was present in the lattice. A total of 3973 reflections, including a complete set of Friedel pairs, were collected; of these 2124 were observed with $I \geqslant 2.5\,\sigma(I)$. Abbreviated crystal data are as follows: **10**·acetone, $C_{21}H_{30}O_3$, f.w. 330.47 amu, space group $P2_1$, $a = 6.256(2)$ Å, $b = 12.505(5)$ Å, $c = 12.200(3)$ Å, $\beta = 102.57(2)°$, $V = 931.5(5)$ Å^3, $Z = 2$, $F_{000} = 360\,e^-$, $\mu = 0.72$ cm^{-1}, density(calc) = 1.175 gcm^{-1}, 2θ range 6–50°, $\theta/2\theta$ scans. Hydrogen atoms were placed in calculated positions and held fixed. No hydrogen atoms were placed in the two hydroxyl positions. A systematic empirical correction for secondary extinction was included ($F_{corr} = F(1 - 0.0001\,XF^2/\sin\theta)$, where X is refined to 0.00525). Final conventional and weighted R-values are 0.058 and 0.059 respectively.

*Estra-1,3,5(10)-trien-2,17β-diol (**3**).* Suitable crystals were grown from acetone. A pale yellow fragment of a single crystal with dimensions $\cong 0.12 \times 0.32 \times 0.16$ mm was used in the study. A total of 2536 reflections, including a complete set of Friedel pairs, were collected; of these 870 were observed with $\lambda \geqslant 2.5\,\sigma(I)$. Abbreviated crystal data are as follows: $C_{18}H_{24}O_2$, f.w. 272.38 amu, space group $P2_12_12_1$, $a = 6.150(3)$ Å, $b = 12.130(4)$ Å, $c = 20.193(7)$ Å, $V = 1506(1)$ Å^3, $Z = 4$, $F_{000} = 592\,e^-$, $\mu = 0.71$ cm^{-1}, density(calc) = 1.197 gcm^{-1}, 2θ range 6–45°, $\theta/2\theta$ scans. Hydrogen atoms were placed in calculated positions and held fixed. No hydrogen atoms were placed in the two hydroxyl positions. Final conventional and weighted R-values are 0.064 and 0.028 respectively.

*Estra-1,3,5(10)-trien-4,17β-diol (**6**).* Suitable crystals were grown from acetonitrile. A colorless cubic crystal with maximum dimension $\cong 0.5$ mm was used in the study and sealed in a thin walled capillary with mother liquor. One equivalent of acetonitrile was present in the lattice. A total of 1954 reflections were collected; of these 1427 were observed with $\lambda \geqslant 2.5\,\sigma(I)$. Abbreviated crystal data are as follows: **6**·acetonitrile, $C_{20}H_{27}N_1O_2$, f.w. 313.44 amu, space group $P2_12_12_1$, $a = 7.4380(8)$ Å, $b = 12.283(2)$ Å, $c = 19.972(6)$ Å, $V = 1824.7(7)$ Å^3, $Z = 4$, $F_{000} = 680\,e^-$, $\mu = 0.68$ cm^{-1}, density(calc) = 1.141 gcm^{-1}, 2θ range 6–50°, $\theta/2\theta$ scans. Hydrogen atoms were placed in a combination of observed and calculated positions and held fixed. Final conventional and weighted R-values are 0.065 and 0.066 respectively.

Receptor binding studies

MCF-7 cells were grown to confluence in minimal essential medium with Hank's basic salt solution, 10% calf serum, antibiotics and insulin as described previously [17]. Following homogenization, the 100,000 ***g*** supernatant was collected as a source of estrogen receptor [18]. Cytosolic estrogen receptor assays were carried out according to classical Scatchard [19] and competitive binding methods [20] utilizing dextran-coated charcoal (DCC). The incubations (in triplicate) carried out to generate a Scatchard plot contained 0.4 ml cytosol and [2,4,6-^{3}H]estradiol-17β (100 Ci/mmol, New England Nuclear, Boston, Mass.) over a concentration range of 0.1–1.5 nM with and without 200-fold unlabeled estradiol-17β. Competitive binding assays (also in triplicate) contained the same volume of cytosol with 1.5 nM tritiated estradiol and five different concentrations of the estradiol analogue being tested. Concentrations selected were 1.5, 3.0, 30, 300 and 3,000 nM. The tubes were incubated overnight at 4°C before treatment with DCC and measurement of the radioactivity in the DCC treated supernatant [18].

RESULTS

The synthetic approach to the title steroids, estra-1,3,5(10)-trien-1,17β- (**10**), 2,17β- (**3**), and 4,17β- (**6**) diols, which is illustrated in Fig. 1, involved in each case, the replacement by OH of a diazonium cation derived, in turn, from either a percursory aminoestra-1,3,5(10)-trien-17-one (**1** and **4**) [14, 21] or 17β-ol (**9**). The preparation of **3** and **6** by essentially the same route has been previously described [14]. However, the prior work included the conversion of the diols to diacetates to achieve complete purification. In the present work it was found that the additional steps of esterification/saponification may be avoided by simply recrystallizing the diols from acetonitrile, following column chromatography.

The synthesis of 1-aminoestra-1,3,5(10)-trien-17β-ol (**9**), the precursor of **10**, utilized the approach employed by Bernstein and coworkers [22] for the preparation of 1-aminoestrone. Thus, the reaction of *p*-nitrobenzenediazonium chloride with 4-aminoestra-1,3,5(10)-trien-17-one (**4**) followed by diazotization and treatment with hypophosphorus acid provided 1-(*p*-nitrophenylazo) estra-1,3,5(10)-trien-17-one (**7**) in 30% yield. Reductive cleavage of the azo function in **7** with zinc-acetic acid at room temperature afforded 1-aminoestra-1,3,5(10)-trien-17-one (**8**) in high yield. Reduction of the ketone function in **8** to the 17β-diol derivative (**9**) was effected in 62% yield with sodium borohydride. Replacement of the diazonium cation generated from **9** by OH produced **10** in 68% yield.

Crystallographic data revealed a significant intermolecular hydrogen bonding network in the lattices of all 3 isomers examined (see Table 3 for compara-

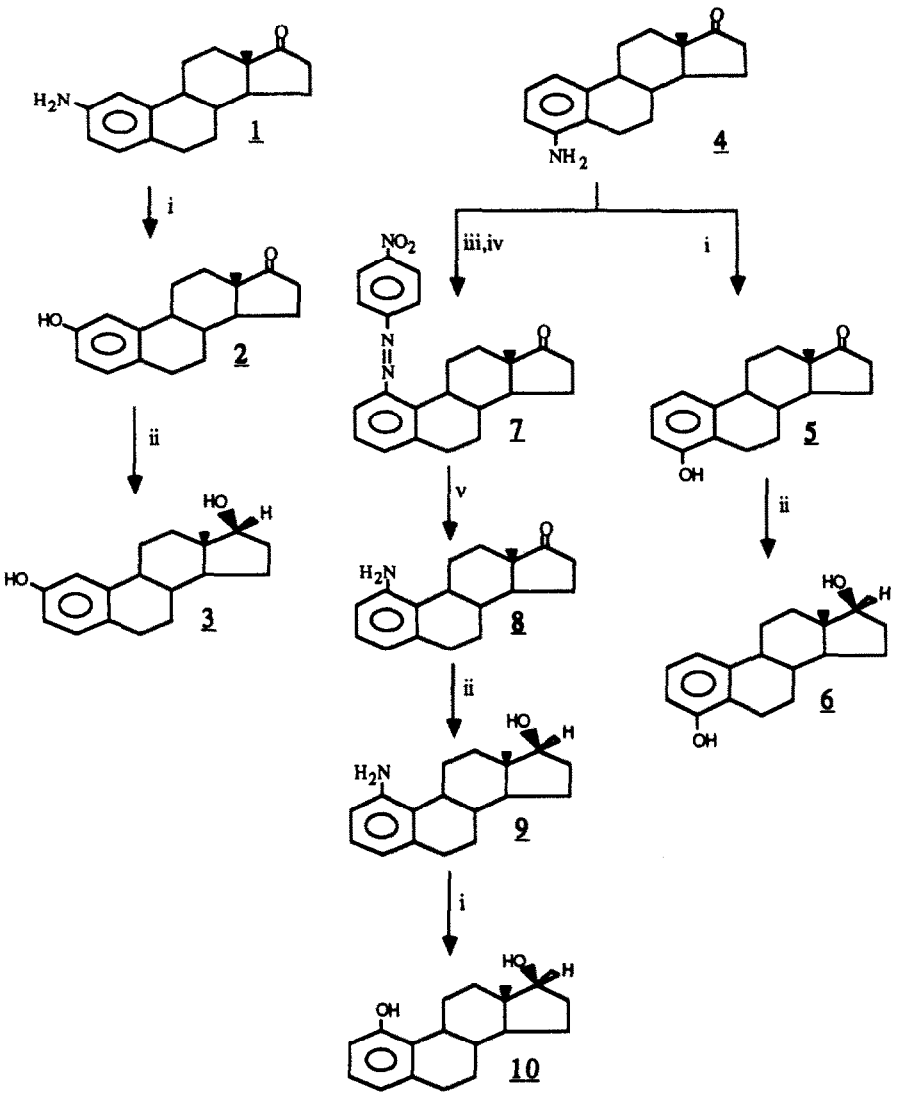

Fig. 1. Schematic pathway for the synthesis of estra-1,3,5(10)-trien-1,17β-(**10**), 2,17β-(**3**) and 4,17β-(**6**) diols.

tive values). Isomer **3** crystallizes without solvent molecules and shows close contacts between hydroxyl groups of adjacent molecules. Two distinct hydrogen bonds are discernible, O17-O2 at 2.799 Å and O17-O2 at 2.677 Å with different hydrogen atoms involved in each case. The other two isomers, **10** and **6**, crystallize with associated solvent molecules and these participate in hydrogen bonding. Close O-O contacts for **10** are 2.693 Å for O1(H)-O17 involving the hydrogen atom on the phenolic OH and 2.746 Å for O(ac)-O17(H) involving the oxygen atom of an acetone molecule. For **6**, O-O contact for O4(H)-O17 involving the hydrogen atom on the phenolic OH is 2.774 Å with a distance of 1.766 Å for O17-H(O4), and N-O17(H) is 2.970 Å involving the nitrogen atom of an acetonitrile solvent molecule, with a distance of 1.943 Å for N-H(O17).

In isomers **10** and **6** ring **B** is in a half-chair conformation with a 2-fold rotational axis through the C7-C8 and C5-C10 bonds. However, the asymmetry parameter (ΔC_2) [23] calculated from the torsion angles for this symmetry is larger for isomer **10** ($\Delta C_2 = 4.3°$) than for **6** ($\Delta C_2 = 3.5°$). Ring C has a chair conformation in both isomers, but again the asymmetry parameter for the three mirror planes (ΔCs) shows a significant difference ($\Delta Cs = 2.8°$ for

10 and ΔCs = 4.7° for **6**). Moreover, ring D is an envelope in both isomers with C13 the out-of-plane atom and ΔCs = 5.6° for **10** and ΔCm = 8.6° for **6**.

Ring B in isomer **3** is flattened in a sofa conformation with C8 as the out-of-plane atom. Ring C is distorted between chair and half-chair conformations. The predominant symmetry is a 2-fold axis passing through bonds C9-C11 and C13-C14. Ring D is, as in isomers **10** and **6**, an envelope with C13 as the out-of-plane atom (ΔCs = 2.8°).

Table 1 lists atomic positional parameters for steroids **10**, **3** and **6**. Figure 2 depicts isomers **10**, **3**, **6** and **E2** viewed parallel to the plane of the aromatic ring (ring A). The dihedral angle between the plane of ring A and the best least squares plane of rings B, C and D describes a bow in the molecule. These dihedral angles are 17.1(4)° for **10**, 19.2(6)° for **3** and 14.2(2)° for **6**. For comparison, **E2** has a bowing angle of 15.8°.

Nonbonding interatomic distances between the steroids substituents map the outline of the molecules. Numerical values for the interatomic distances C18-O17 and O-O17 (where O represents the phenolic hydroxyl), and the torsional angles C18-C13-C17-O17 and C1-C10-C13-C18, including values for **E2**, are shown in Table 2. Figures 3 and 4 show bond lengths, bond angles and torsion angles for steroids **10**, **3** and **6**. The numbers represent little variation with the reported values for **E2** [24]. The largest deviation of an angle from the **E2** value occurs at O17-C17-C13 which is 111.9° for **10** and 117.3° for **E2**. However, this position is involved in hydrogen bonding (*vide supra*) and this change could be entirely due to packing constraints. The absolute configuration (17β) was not determined crystallographically for the prepared isomers but rather was assigned to agree with the natural estradiol.

Data are presented in Table 3 from experiments to examine the binding of the estradiol 17β A-ring hydroxyl analogues to cytosolic estrogen receptor. Relative to estradiol-17β, estratriene-1,3,5(10)-2, 17β diol **3** displayed 1/5th the affinity for receptor whereas the 1- and 4-hydroxylated derivatives (**10** and **6** respectively) bound to a much lesser extent.

DISCUSSION

Proton NMR analysis of **E2** and its three A-ring regional isomers revealed some variations that can be correlated to structural features found in the crystallographic data. A basic assumption of little structural change of the four isomers upon going from the solid state to solution is made based on the known rigidity of the steroidal framework. A prominent characteristic change in the [^{1}H]NMR involves the angular methyl group at position 18. A value of 0.726 ppm was observed for this group in **E2**; an identical value was observed for the 4-hydroxy isomer (**6**), but a downfield shift was observed for 2-hydroxy isomer (**3**) with a value of 0.732 ppm, and a more pronounced

Table 1. Atomic positional parameters for 1,3,5(10)-Estratienes-1 (**10**), 4 (**6**), and 2 (**3**), 17β-diols

	(10)			(6)			(3)		
Atom	*x*	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*
C1	0.8379(8)	0.11700	0.4426(4)	−0.7245(7)	−0.0826(4)	−0.6517(3)	−0.288(1)	−0.0325(8)	−0.5701(4)
C2	1.0189(8)	0.0494(5)	0.4589(4)	−0.7553(7)	−0.1959(4)	−0.6453(3)	−0.191(2)	0.0261(8)	−0.5169(4)
C3	1.0874(8)	0.0070(5)	0.3691(5)	−0.6129(8)	−0.2660(4)	−0.6469(3)	−0.002(2)	−0.0171(8)	−0.4867(4)
C4	0.9711(9)	0.0310(5)	0.2609(4)	−0.4419(7)	−0.2290(4)	−0.6554(3)	0.086(2)	−0.1143(7)	−0.5125(5)
C5	0.7921(7)	0.0997(5)	0.2436(4)	−0.4070(7)	−0.1170(4)	−0.6641(3)	−0.006(2)	−0.1705(7)	−0.5643(4)
C6	0.6662(9)	0.1169(5)	0.1238(4)	−0.2146(7)	−0.0807(4)	−0.6775(3)	0.110(1)	−0.2712(7)	−0.5894(4)
C7	0.4384(8)	0.1616(5)	0.1179(3)	−0.2037(8)	0.0349(4)	−0.7028(3)	−0.003(2)	−0.3298(7)	−0.6475(4)
C8	0.4590(7)	0.2595(4)	0.1939(3)	−0.3192(7)	0.1093(4)	−0.6572(3)	−0.110(1)	−0.2452(7)	−0.6948(4)
C9	0.5350(7)	0.2248(4)	0.3178(3)	−0.5184(7)	0.0789(4)	−0.6688(3)	−0.294(1)	−0.1869(7)	−0.6550(4)
C10	0.7272(7)	0.1481(5)	0.3351(3)	−0.5500(7)	−0.0439(4)	−0.6615(3)	−0.192(2)	−0.1297(8)	−0.5936(4)
C11	0.5772(7)	0.3267(5)	0.3911(3)	−0.6405(8)	0.1469(4)	−0.6232(3)	−0.438(1)	−0.1109(7)	−0.6974(4)
C12	0.3718(7)	0.3967(5)	0.3762(3)	−0.6061(7)	0.2714(4)	−0.6312(3)	−0.517(2)	−0.1663(7)	−0.7618(4)
C13	0.2883(7)	0.4283(5)	0.2533(3)	−0.4081(7)	0.3002(4)	−0.6219(3)	−0.330(1)	−0.2157(6)	−0.8003(4)
C14	0.2516(7)	0.3264(5)	0.1829(3)	−0.2965(6)	0.2279(4)	−0.6700(3)	−0.216(1)	−0.3010(7)	−0.7546(4)
C15	0.1253(8)	0.3657(5)	0.0664(4)	−0.1090(7)	0.2819(4)	−0.6679(4)	−0.063(2)	−0.3671(7)	−0.8024(4)
C16	−0.0117(7)	0.4600(5)	0.0937(4)	−0.1491(8)	0.4008(4)	−0.6597(4)	−0.187(2)	−0.3639(7)	−0.8701(4)
C17	0.0569(7)	0.4751(5)	0.2222(3)	−0.3519(8)	0.4110(4)	−0.6480(3)	−0.394(1)	−0.2937(7)	−0.8580(4)
C18	0.4506(7)	0.5083(5)	0.2160(4)	−0.3544(8)	0.2837(4)	−0.5473(3)	−0.173(1)	−0.1250(7)	−0.8276(4)
O1	0.7596(5)	0.1522(4)	0.5335(2)	−0.2956(5)	−0.2957(3)	−0.6568(2) O2	−0.284(1)	0.1258(5)	−0.4994(3)
O17	0.0507(5)	0.5838(4)	0.2588(2) O4	−0.3846(6)	0.5028(3)	−0.6034(2)	−0.4727(9)	−0.2384(4)	−0.9165(3)

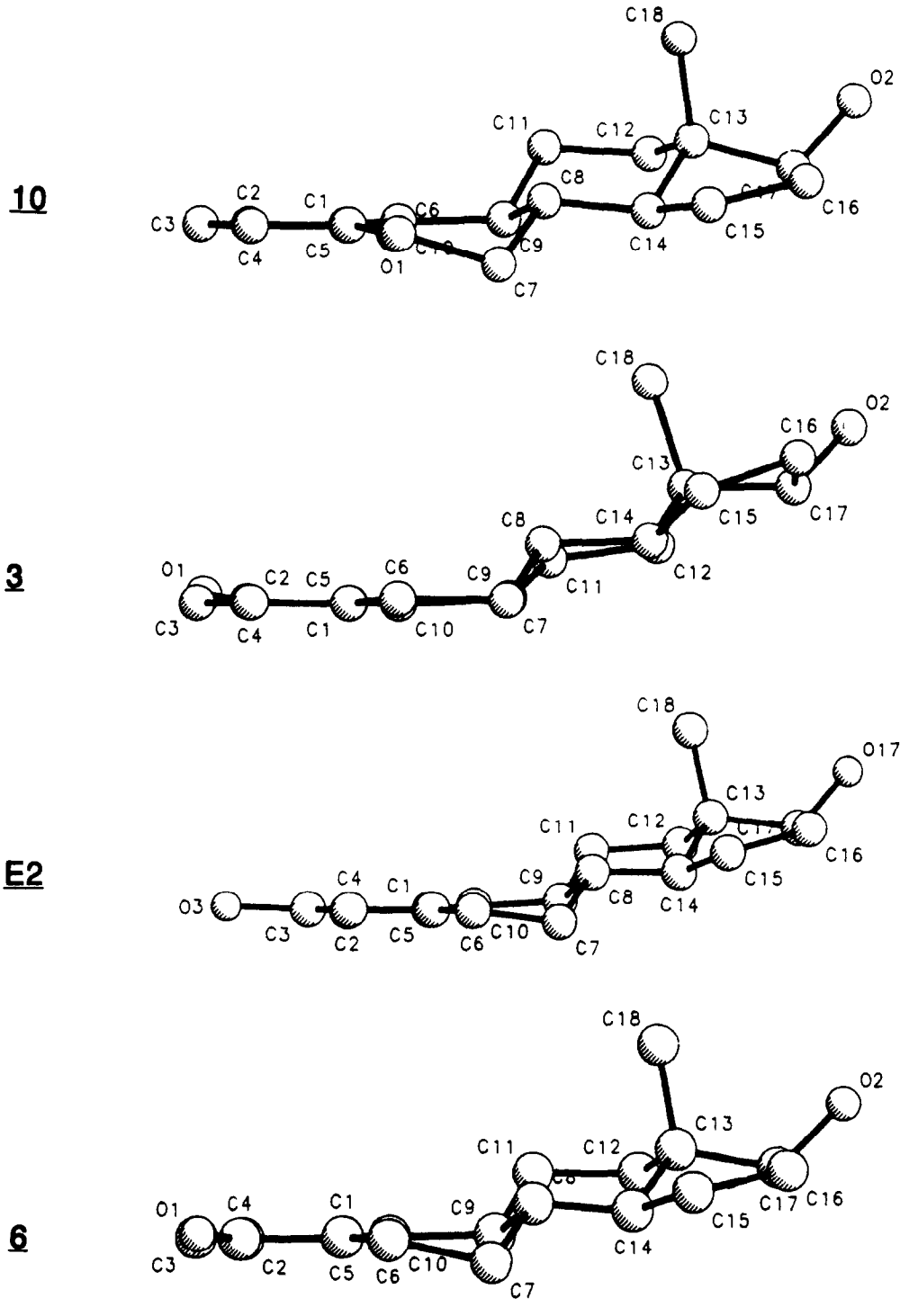

Fig. 2. Crystallographic conformations of estra-1,3,5(10)-trien-1,17β-(**10**), 2,17β-(**3**), 3,17β-(**E2**), and 4,17β-(**6**) diols viewed parallel to the aromatic ring.

effect for 1-hydroxy isomer (**10**) with a chemical shift of 0.768 ppm. The distant position and geometry of the aromatic hydroxyl group relative to C18 (5.943, 7.323, 7.458 and 8.51 Å for **10**, **3** and **6** and **E2**, respectively) preclude any consideration of through-space interaction. Therefore, the chemical shift of the latter must be ascribed, in the main, to interactions with the hydroxyl group at position 17. To explain these variations the interatomic distance and torsional angle between C18 and O17 must undergo changes and these parameters can only be modified, as described below, through structural variations of the steroidal skeleton.

The crystallographic study reveals that minimal skeletal variation has occurred in the 4-hydroxy derivative (**6**) with respect to **E2**, a fact that agrees

Table 2. Selected dihedral angles and interatomic distances for estradiol-17β (**E2**) and its three A-ring isomers

	<C18-C13-C17-O17°	C18-O17 Å	O-O17 Å	<C1-C10-C13-C18°
1-Hydroxyl (**10**)	−49.2	2.83	7.32	99.8
2-Hydroxyl (**3**)	−50.2	2.92	9.58	74.8
3-Hydroxyl (**E2**)	−46.6	2.87	10.93	88.0
4-Hydroxyl (**6**)	−43.3	2.92	9.89	90.2

with the [^{1}H]NMR result. The 2-hydroxy isomer (**3**), on the other hand, has undergone a structural modification with respect to **E2** that partially flattened ring B with a concomitant variation in rings C and D. This structural change produces the largest dihedral angle C18-C13-C17-O17 of the series (*vide supra*) that ultimately affects the chemical shift of protons in C18. Finally, estra-1,3,5(10)-trien-1,17β-diol (**10**) suffered modifications in rings B, C and D relative to the natural isomer, modifications that cause an increase in the dihedral angle C18-C13-C17-O17 and a reduction in the interatomic distance C18-O17, which is reflected in a more pronounced change in chemical shift for the angular methyl group in the series.

From the numerical values for the distance between C18 and O17 and the torsional angle C18-C13-C17-O17, shown in Table 2, it is apparent that a decrease in the interatomic distance of C18-O17 and/or an increase in numerical value of the torsional angle will make the electronegative O17 more effective in deshielding the protons in C18.

An important finding which emerged from the crystallographic studies is the relative constancy of the O17 position with respect to the aromatic plane in all four ring-A isomers. This invariance in relation to the position of the angular methyl group at C18 is indicated, in two dimensions, in Fig. 5. The relative position of C18 can be correlated with the twist through the steroid ring system represented by the torsion angle C1-C10-C13-C18 (Table 2) as shown in Fig. 5. This feature may be of relevance in the binding of the estrogens to biological receptors and may well be a consequence of the position and nature of the substituent in the aromatic ring. Thus, the close skeletal similarity of the 4-hydroxy isomer (structure **6**) relative to **E2** would imply greater affinity than is the case. Apparently, the poor affinity of this compound must derive from the position of the phenolic OH (see Table 3).

In summary, structural variations in the steroid's skeleton are reflected in the chemical shift of the angular methyl group due to changes in the position of the distal O17. This change in chemical shift can be used to provide limited guidance to assess the structural changes in a given estrogen with respect to **E2**.

Two relatively strong interatomic hydrogen bonds (*vide supra*) exist in isomer **3**, linking the phenolic (O2) and aliphatic (O17) hydroxyl groups in a lattice in which both units act as donor and/or acceptor. The hydrogen bonding of **E2** incorporates solvent of crystallization in which the O3 and O17 atoms act as hydrogen donors and acceptors (average distances are 2.70 and 2.76 Å, from Ref. [6], Table 3). In isomers **6** and **10** the O4 and O1 in each molecule acts as a hydrogen bond donor for O17, and O17 as the hydrogen donor for the solvent molecules. The hydrogen bond distances for isomer **6** are longer than for **E2** (Table 3), which may be correlated as suggested by Wawrzak *et al.* [6] to its poor binding to estrogen receptor (Table 3). The values for the hydrogen bond distances of isomers **10** and **3** are shorter than for **E2** (Table 3), an indication of stronger bonds. However, **10** and **3** exhibit reduced binding affinities compared to **E2** (Table 3); therefore, a direct correlation between binding affinities and hydrogen bonding strengths in the cases under study is not obvious.

Data from competitive binding studies have shown the aromatic A-ring of estrogens to be important in the binding to receptor [4, 7, 8]. However, the polycyclic structure itself possesses little affinity for the estrogen binding protein [7]. Since the ligand 3-hydroxyestra-1,3,5(10)-triene displays half the K_a of estradiol-17β [7], it would appear that the 3-phenolic hydroxyl group is most important in estrogen's interaction with receptor. On the other hand, the 17β-hydroxyl group, although necessary in providing the proper orientation for the binding of estradiol-17β to receptor, contributes much less to the affinity of estra-1,3,5(10)-triene to the estrogen receptor (K_a estra-1,3,5(10)-triene-17β-ol is 0.08 that of estradiol 17β, Ref. [7]). However, there is evidence, based on the impotence of estra-1,3,5(10)-trien-17-one as a ligand and on the ineffectiveness of a 17α- and a 16α-hydroxyl group on the K_a when added to 3-hydroxyestra-1,3,5(10)-triene [7], which suggests that the 17β-hydroxy participates in hydrogen bonding to a group on the receptor which lies above ring D. Furthermore, in the absence of the 3-phenolic hydroxyl group, estra-1,3,5(10)-triene-17β-ol is fully capable of bringing about the induction of progesterone receptor in MCF-7 cells [5]. Although less active than **E2** at equal concentrations, this monohydroxy estratriene was somewhat more potent in gene stimulation than the phenolic 3-hydroxyestra-1,3,5(10)-triene [5]. Estra-1,3,5(10)-triene, however, is inactive in this tissue culture system [25]. Therefore, when considering an estrogen's binding to its receptor and the response elicited by the receptor-ligand interaction, the contribution of the 17β-hydroxyl group must be considered.

The 1-hydroxyl isomer (**10**) bound to the receptor 1/500th as tightly as the natural estradiol-17β. Other

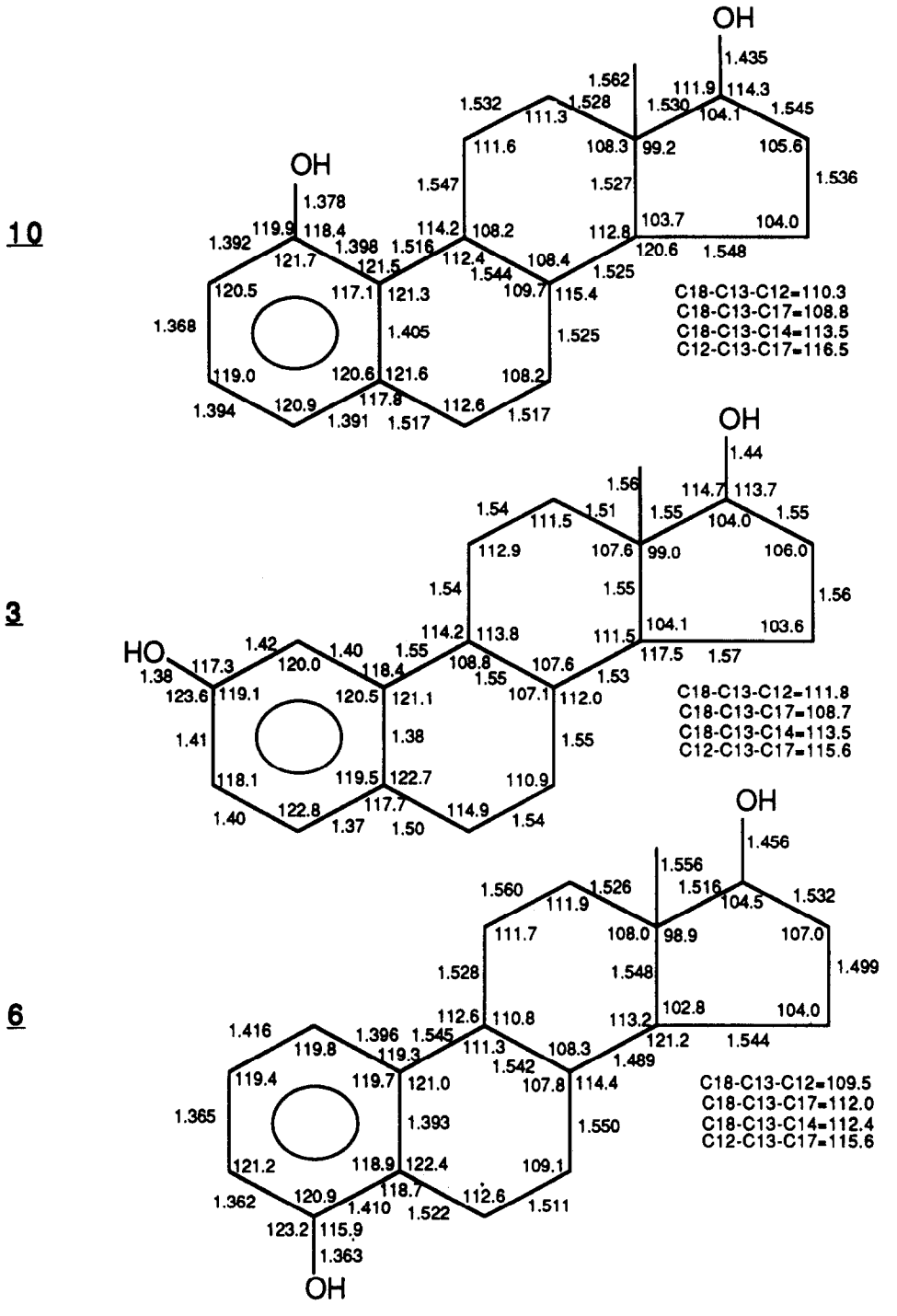

Fig. 3. Bond distances (Å) and bond angles (°) of estra-1,3,5(10)-trien-1,17β-(**10**), 2,17β-(**3**) and 4,17β-(**6**) diols. Esd's for the bond lengths are $\cong$0.005–0.007 Å in **10**; $\cong$0.010 Å in **3**; $\cong$0.006–0.008 Å in **6**. Bond angles have esd's of $\cong$0.3–0.4° in **10**; 0.7–0.8° in **3**; and 0.4–0.5° in **6**.

than the repositioning of the A-ring hydroxyl group, this molecule displayed the greatest dihedral angle variation between C1-C10-C13-C18 (Table 3 and Fig. 2), possibly reducing the availability of the α-side of this estrogen to the receptor protein. In addition, the distance between the 17β-hydroxyl group and the C18 methyl is the smallest of all these estra-1,3,5(10)-trien-diols (Fig. 5), thereby interfering with the proximity of the receptor protein above ring D and the ease of hydrogen bonding to O17.

In the case of estra-1,3,5(10)-trien-2,17β-diol (**3**), the affinity for estrogen receptor is only decreased to 18% that of **E2**. This appreciable binding, in part, results from the lack of significant alteration in the orientation (relative to the plane of the A-ring) of the B-, C- and D-rings (Table 3 and Fig. 2), thereby,

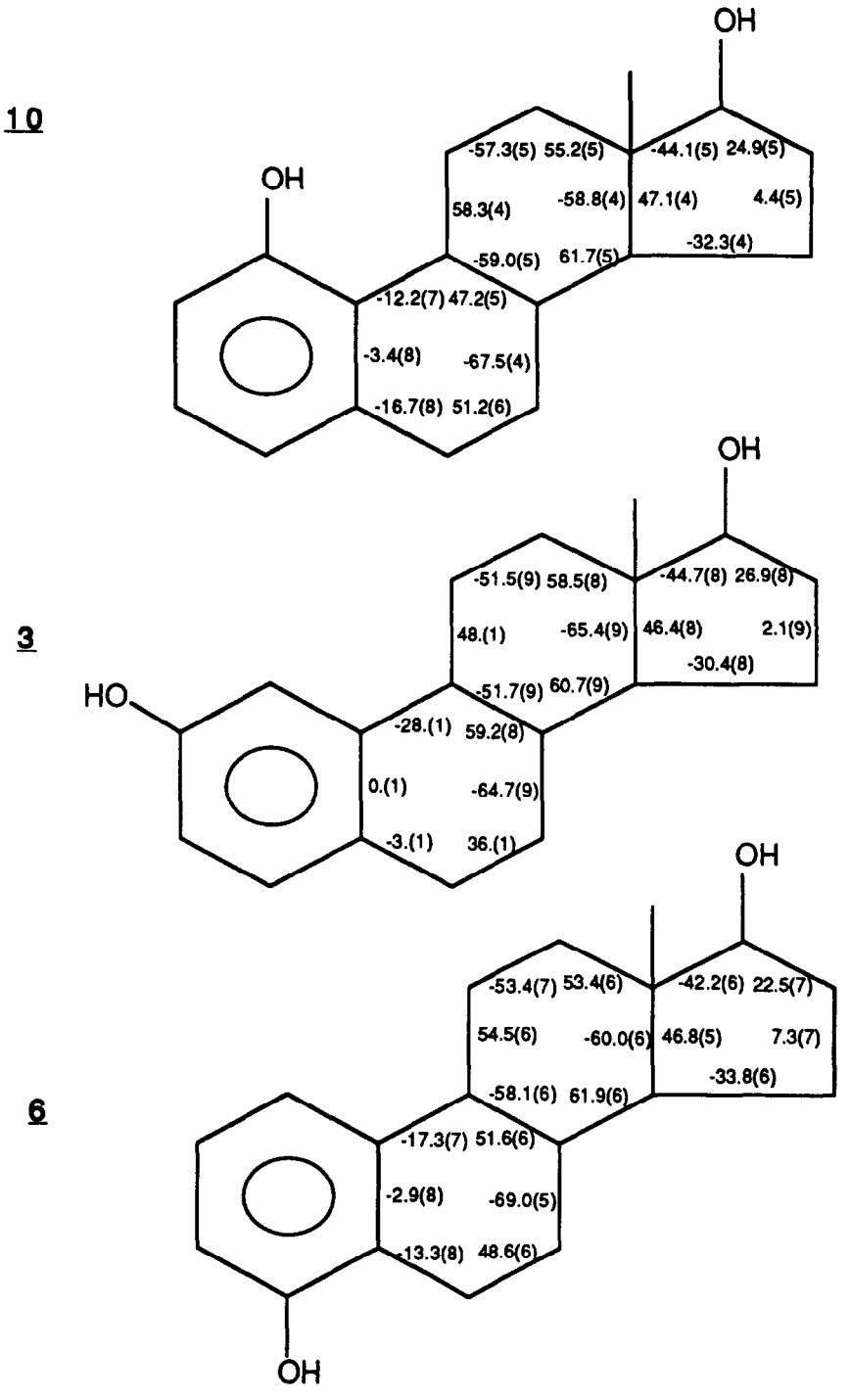

Fig. 4. Torsion angles (°) of estra-1,3,5(10)-trien-1,17β-(**10**), 2,17β-(**3**) and 4,17β-(**6**) diols.

allowing adequate interaction between the estrogen and the binding protein. In addition, the increased dihedral angle between the 18-methyl and the 17β-hydroxyl groups (Table 2 and Fig. 5) facilitates the hydrogen bonding to an area of the receptor which exists above ring D. The repositioning of the phenolic hydroxyl from C3 to C2, although associated with decreased affinity, did not prohibit binding.

The 4-hydroxyl group had little effect either on the orientation of the B-, C- and D-rings or on the angular methyl and the D-ring hydroxyl group (Figs 2 and 5). Yet, this unnatural phenolic substitution decreased the affinity of the estrogen ligand for receptor more than 1000-fold. Apparently, the steric requirements of the 4-hydroxyl group are not tolerated well by the estrogen receptor, since in its absence (i.e. estratrien-17β-ol), the ligand binds more tightly (rel. $K_a = 0.08$, Ref. [7]).

These observations are intriguing when one considers the opposite binding effects reported for 2- and

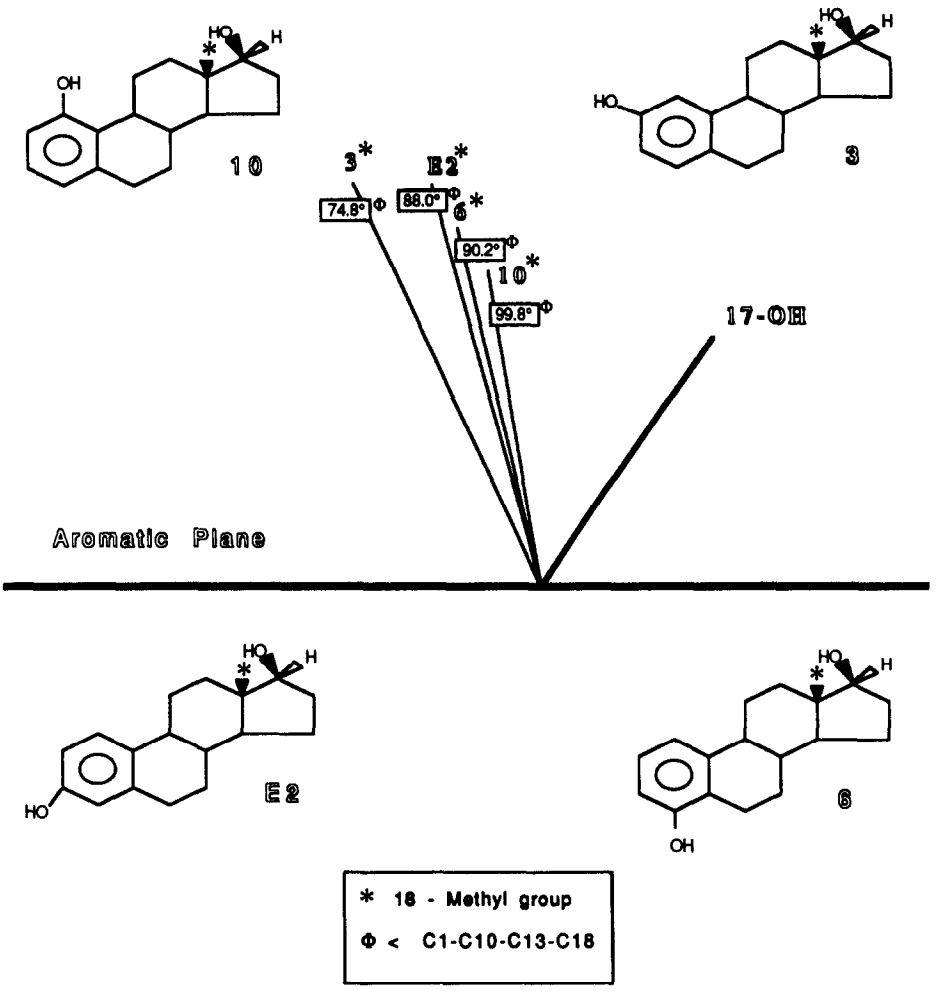

Fig. 5. Two-dimensional view of relative position of the 18-methyl group with respect to the aromatic plane and the aliphatic β-hydroxyl (O17) group of estra-1,3,5(10)-trien-1,17β-(**10**), 2,17β-(**3**), 3,17β-(**E2**), and 4,17β-(**6**) diols. The values for the torsion angle C1-C10-C13-C18 are included.

4- hydroxyl groups on **E2** [6, 9, 26]. While both substitutions diminished the affinity of the steroid for receptor, this effect is greater for 2- than for 4-hydroxylation. The information provided by the present data offers the opportunity to interpret the results of the experiments with catechol estrogens in a new light. In the absence of a 3-phenolic hydroxyl, the 2,17β-diol binds to estrogen receptor with an affinity over 200-fold greater than the 4,17β-diol (Table 3). This observation does not concur with the suggestion that the 2-hydroxyl group mimics the missing 3-hydroxy and in doing so places the B-, C- and D-rings in a vastly divergent orientation for adequate hydrogen bonding of the O17 [6]. Likewise the poor binding of isomer **6** does not indicate that the 4-hydroxy is well tolerated by the receptor as was the case of the catechol 1,3,5(10)-estratriene-3,4,17β-triol [6].

An added element which is expressed in the case of the 2- and 4-hydroxy isomers **3** and **6**, is the effect

Table 3. Hydrogen bond distances and relative binding affinity of various A-ring hydroxylated estratrien-17β ols

Compound	O-O17[a] (Å)	O17-A[b] (Å)	$K_a \times 10^9\ M^{-1}$	Relative binding affinity ($\times 10^3$)
1-Hydroxyl (**10**)	2.693	2.746	0.096	2
2-Hydroxyl (**3**)[c]	2.677[d]	2.799[d]	0.6	180
3-Hydroxyl (**E2**)[c]	2.70	2.76	3.0	1000
4-Hydroxyl (**6**)[c]	2.774	2.970	0.0024	0.8

[a]O refers to phenolic oxygen. [b]A refers to acetone for isomer **10**, to O2 for isomer **3**, to O3 for **E2**, and to acetonitrile for isomer **6**. [c]Binding data for these estrogen analogues have been published previously from this laboratory [7]. [d]A precise determination of the hydrogens' positions was not possible. The correspondence of the values is arbitrary. [e]Average values taken from Ref. [6].

which the relocation of the natural O3 to the 2- or 4-position had on the B-, C- and D-rings of the estrogen molecule (Fig. 2). Unlike the O3 or O4 substituent, the O2 brought about a more planar ring A/ring B junction resulting in the sofa form in ring B and ultimately in a greater dihedral angle C18-C13-C17-O17 between the C18 methyl and O17 (Table 2). The result is a shift from this symmetrical 7α, 8β half chair conformation to the 8β-sofa form in the molecule with O2 substitution which facilitates binding [6]. In addition, as described above, the wider angle in this molecule between the C18 methyl and O17 aids hydrogen bond formation with receptor above ring D.

Acknowledgements—These investigations were supported in part by NIH grants Nos CA-44771 and CA-37387, and an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. The diffractometer used herein was purchased through an NSF equipment grant to Wayne State University.

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