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The Conformations of 17β-Estradiol (E2) and 17α-Estradiol as Determined by Solution NMR

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Abstract

The conformational structures of the hormone 17β-estradiol (E2) and the epimeric 17α-estradiol determined by solution NMR spectroscopy and restrained molecular dynamics calculations found a single low energy conformation.

Keywords

17β-estradiol; E2; 17α-estradiol; NMR; conformation; molecular modeling

Introduction

Estrogens are gonadal steroidal hormones that have important roles in reproductive function.[1,2] These amphipathic steroids are quite insoluble in an aqueous environment,[3,4] but circulate via the bloodstream[5] signaling to a number of tissues including breast, ovaries, uterus, and brain.[6] Estrogens initiate rapid nongenomic signaling events at cell membranes,[2,7-10] readily diffuse across membranes interacting with nuclear estrogen receptors that regulate gene expression,[6,11,12] and also act at mitochondrial membranes.[13] Estrogen receptors are activated by the hormone 17β-estradiol (E2) but not by the 17α-estradiol isomer (Figure 1).[14,15] Recently, the structure of the estrogen hormone 17β-estradiol (E2) as determined by NMR and X-ray was reviewed.[16] In addition to the low energy C-ring chair conformation that corresponded well to crystal structures, a second conformation with a twisted boat C-ring was proposed for E2 in dimethylsulfoxide solution.[16]

In conjunction with our ongoing studies of steroids interacting with phospholipid bilayer model membranes,[17,18] we have also characterized the solution structures of 17β-estradiol (E2) as well as 17α-estradiol by NMR and restrained molecular dynamics calculations. Our data clearly demonstrate that for both E2 and the anomeric antiestrogen 17α-estradiol, there is only one conformation that exists in solution.

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Results and discussion

We first assigned the proton resonances of 17β-estradiol (E2) as well as 17α-estradiol by analysis of their 1D ^{1}H-, ^{13}C-, and 2D COSY, HSQC, and NOESY spectra recorded on a Bruker AVANCE*II* 700MHz NMR spectrometer (see supplementary data). All spectra were referenced to the DMSO-d_6 multiplets at 2.47 ppm and 39.50 ppm. The 2D HSQC experiment was particularly useful for distinguishing resonances of $H_{11\beta}$, H_8, $H_{15\beta}$ and $H_{7\alpha}$ for E2 that were between 1.19 and 1.27 ppm (see Figure 2). We have also, for the first time, distinguished the two benzylic geminal 6α and 6β protons by analyzing the *J* couplings of this rather complicated ABXY pattern. Our analysis was further confirmed by comparing the ^{1}H- spectrum of E2-6β-*d* (Figure 3) which was synthesized in our lab according to a previously reported procedure.[19]

The observed proton chemical shifts, carbon chemical shifts, and the NOE intensities of key crosspeaks are tabulated for E2 and 17α-estradiol in Tables 1 and 2, respectively. Their observed *J*-couplings are listed in Table 3. Strong trans coplanar diaxial couplings were observed for $J_{6\beta,7\alpha}$, $J_{7\alpha,8}$, $J_{8,9}$, $J_{8,14}$, $J_{9,11\beta}$, $J_{11\beta,12\alpha}$, and $J_{14,15\beta}$. The chair conformation of the C-ring was clearly evidenced by the characteristic large $J_{8,9}$, $J_{8,14}$, $J_{9,11\beta}$, and $J_{11\beta,12\alpha}$ couplings. The C-18 methyl group gave NOEs with H_8, $H_{11\beta}$, $H_{12\beta}$, $H_{15\beta}$ and $H_{16\beta}$ for both 17β-estradiol (E2) and 17α-estradiol, and NOE of the C-18 methyl with H_{17} for 17α-estradiol was also observed (Figure 4). We did not, however, observe the reported[16] NOE between $H_{12\beta}$ and $H_{15\beta}$ for E2 which was due to their mis-assignment of the proton resonances. Interestingly, the NOESY of H_1 with $H_{11\alpha}$ in the plane of the aromatic ring gave a negative NOE, an unusal effect.[20,21] This aromatic H_1 proton had the expected positive NOEs with H_9 and $H_{11\beta}$, below and above the plane, analogous to H_4 NOEs with $H_{6\alpha}$ and $H_{6\beta}$.

Our NOE data in Tables 1 and 2 were used as distance restraints to determine the conformations of 17β-estradiol (E2) and 17α-estradiol, respectively. The calculations were performed using Macromodel in the Schrodinger software package. In order to fully sample the entire conformational space, stochastic dynamics simulations with NOE distance restraints were carried out at 1000K with a time step of 1.0 fs. Conformations were recorded every 250,000 steps for a total of 20 trajectories. Each conformer was further subject to conjugate gradient minimization until the maximum derivative was less than 0.05 kJ/mol. Figure 5a depicts the 20 conformers of 17β-estradiol (E2) and Figure 5b depicts the 20 conformers of 17α-estradiol. For each compound, all 20 conformers were closely superimposable over the entire backbone. The solution conformations of the anomeric steroids were in good agreement with reported X-ray structures[22-26] and included the following features. The aromatic A-ring was planar. The unsaturated B-ring had trans coplanar diaxial orientations for $H_{6\beta}$ with $H_{7\alpha}$ and for H_8 with H_9 and was a half-chair (Figure 5c). The C-ring was a chair conformation with trans coplanar diaxial orientations for $H_{11\beta}$ with H_9 and $H_{12\alpha}$ as well as for H_8 with H_9 and H_{14} (Figure 5d). Thus, the conformations of the ABC rings of both anomers were nearly identical. The five-membered D-rings were both C-13 β-envelopes with only 3° of distortion (C18-C13-C17 was 110.3° for E2 and 107.3° for the α-anomer, Figures 5e and 5f, respectively) due to the steric interaction between the C-18 methyl group and the C-17 hydroxyl group of E2.

The conformational structures of the hormone 17β-estradiol (E2) and the anomeric antiestrogen 17α-estradiol have now been clearly established in DMSO solution using a combination of NMR spectroscopy and restrained molecular dynamics calculations. We have found that there is only one low energy conformation for theses steroids with a chair conformation for their C-rings. We have also, for the first time, assigned all resonances including the two benzylic geminal $H_{6\alpha}$ and $H_{6\beta}$ protons. We are currently using these

structural details to study the interactions of these compounds with bicelle model membranes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

COSY	correlation spectroscopy
E2	17β-estradiol
HSQC	heteronuclear single quantum coherence
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser enhancement spectroscopy

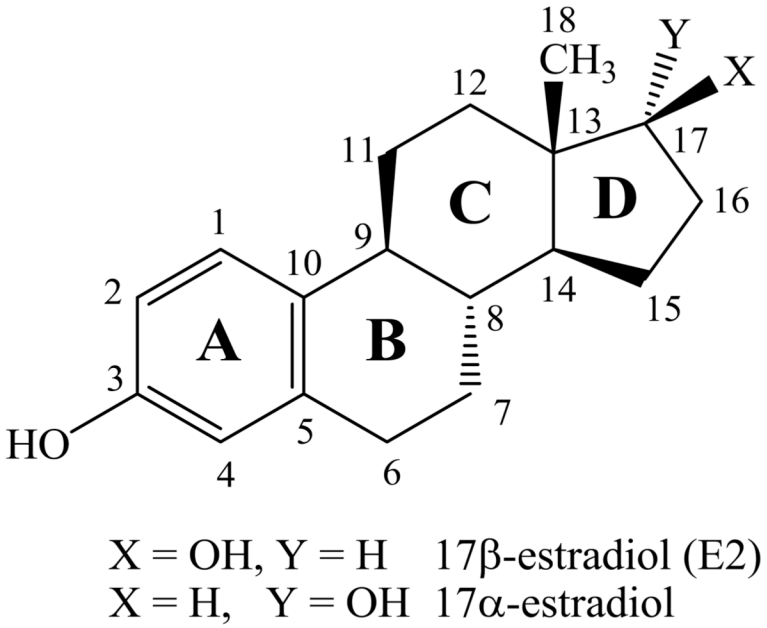


Figure 1.
The hormone 17β-estradiol (E2) and the isomeric antiestrogen 17α-estradiol.

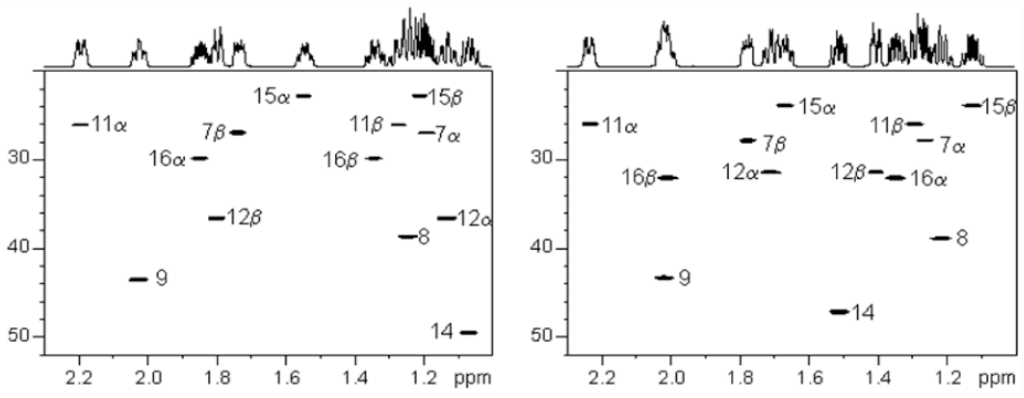


Figure 2.
^{1}H-^{13}C HSQC spectra of 17β-estradiol (E2, left) and 17α-estradiol (right).

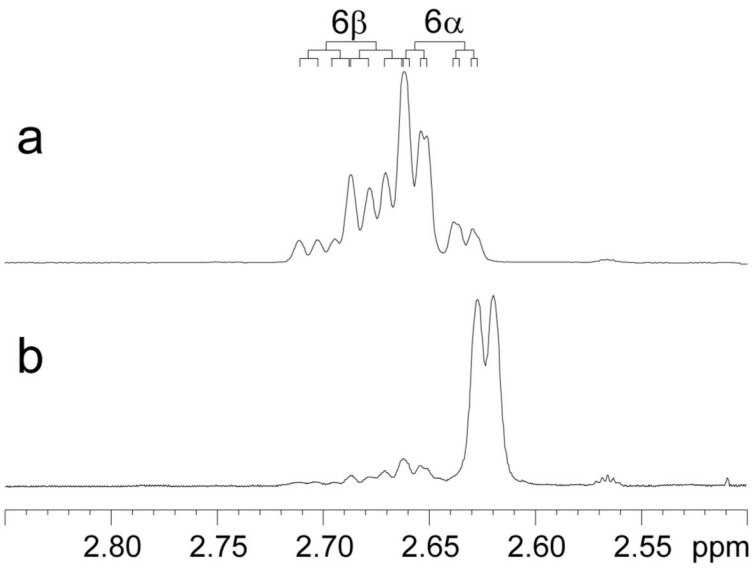


Figure 3.

^{1}H NMR of E2 (**a**) and E2-6β-*d* (**b**) in DMSO-d_6 solution. (**a**) The benzylic C-6 protons of E2 appeared as an ABXY pattern with the $H_{6\beta}$ (*ddd*) downfield of the $H_{6\alpha}$ (*ddd*). (**b**) The deuterated analog, which contained approximately 10% non-deuterated E2, appeared as a broadened doublet with a 0.03 ppm upfield shift due to the isotope effect.

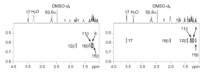


Figure 4.
NOESY spectra of 17β-estradiol (E2, left) and 17α-estradiol (right).

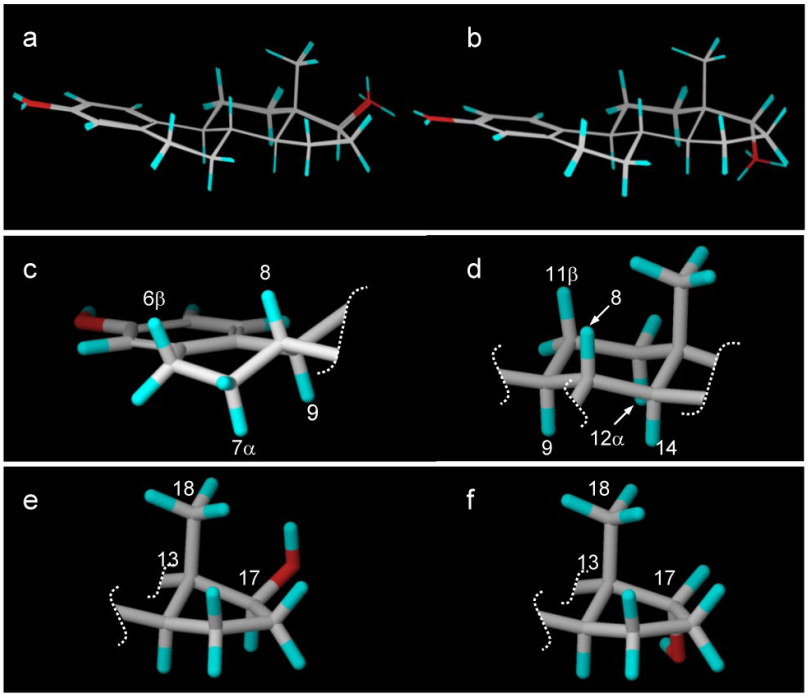


Figure 5.

(a) The 20 low-energy conformers of 17β-estradiol (E2). (b) The 20 low-energy conformers of 17α-estradiol. (c) The unsaturated B-ring half-chair showing trans coplanar diaxial orientations for $H_{6\beta}$ with $H_{7\alpha}$ and for H_8 with H_9. (d) The C-ring chair with trans coplanar diaxial orientations for $H_{11\beta}$ with H_9 and $H_{12\alpha}$ as well as for H_8 with H_9 and H_{14}. (e) The D-ring for 17β-estradiol (E2). (f) The D-ring for 17α-estradiol.

Table 1

17β-Estradiol (E2)

Position	Chemical Shift (ppm)		^{1}H-^{1}H NOEs*
	δ_H	δ_C	
1	7.00	126.0	2(m); 9 (w); 11β(w)
2	6.47	112.7	1(m)
3	—	154.8	
4	6.39	114.9	6α(m); 6β(w)
5	—	137.1	
6α	2.65	29.1	4(m); 7α(w)
6β	2.68		4(w); 7β(w); 8(w)
7α	1.19	26.9	6α(w); 7β(s); 9(m)
7β	1.74		6β(w); 7α(s); 8(m)
8	1.24	38.7	7β(m); 18(m)
9	2.03	43.5	7α(m); 11α(w); 14(w)
10	—	130.4	
11α	2.19	26.0	9(w); 11β(s); 12α(w)
11β	1.27		11α(s); 18(m)
12α	1.13	36.5	11α(w); 12β(s); 14(w); 17(w)
12β	1.80		12α(s);18(w)
13	—	42.8	
14	1.07	49.5	9(w); 12α(w); 15α(m)
15α	1.55	22.7	14(m); 15β(s); 16α(w); 16β(w)
15β	1.21		15α(s); 16α(w); 18(m)
16α	1.85	29.8	15α(w); 15β(w); 16β(s); 17(m)
16β	1.34		15α(w); 16α(s); 17(w); 18(w)
17	3.48	80.0	12α(w);16α(m); 16β(w)
18	0.63	11.2	8(m); 11β(m); 12β(w); 15β(m); 16β(w)

*NOE intensities were categorized as “strong” (s), “medium” (m) and “weak” (w), and were converted into upper limit distance constraints of 2.7, 3.5, and 5.0 Å in the molecular dynamics calculations, respectively.

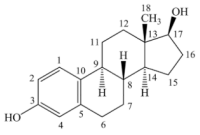
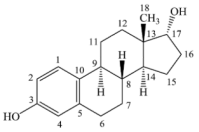
Table 2

17α-Estradiol

Position	Chemical Shift (ppm)		^{1}H-^{1}H NOEs*
	δ_H	δ_C	
1	7.02	126.0	2(m); 9(w); 11β(w)
2	6.46	112.6	1(m)
3	—	154.8	
4	6.39	114.8	6α(m); 6β(w)
5	—	137.1	
6α	2.65	29.2	4(m); 7α(w)
6β	2.68		4(w); 7β(w); 8(w)
7α	1.26	27.8	6α(w); 7β(s); 9(m)
7β	1.78		6β(w); 7α(s); 8(m)
8	1.22	38.8	7β(m); 18(m)
9	2.02	43.3	7α(m); 11α(w); 12α(w); 14(w)
10	—	130.4	
11α	2.23	26.0	9(w); 11β(s); 12α(w)
11β	1.29		11α(s); 18(m)
12α	1.71	31.4	9(w); 11α(w); 12β(s); 14(m)
12β	1.40		12α(s); 17(w); 18(w)
13	—	45.0	
14	1.51	47.2	9(w); 12α(m); 15α(m)
15α	1.67	23.8	14(m); 15β(s); 16α(w); 16β(w)
15β	1.12		15α(s); 16α(w); 18(w)
16α	1.34	32.0	15α(w); 15β(w); 16β(s); 17(w)
16β	2.01		15α(w); 16α(s); 17(m); 18(w)
17	3.54	77.9	12β(w); 16α(w); 16β(m); 18(m)
18	0.58	16.9	8(m); 11β(m); 12β(w); 15β(w); 16β(w); 17(m)

*NOE intensities were categorized as “strong” (s), “medium” (m) and “weak” (w), and were converted into upper limit distance constraints of 2.7, 3.5, and 5.0 Å in the molecular dynamics calculations, respectively.

Table 3
Observed coupling constants for 17β- and 17α-estradiol

 17β-estradiol (E2)	*J* (Hz)	 17α-estradiol	*J* (Hz)
$J_{1,2}$	8.5	$J_{1,2}$	8.4
$J_{2,4}$	2.7	$J_{2,4}$	2.6
$J_{6\alpha,6\beta}$	17.1	$J_{6\alpha,6\beta}$	17.1
$J_{6\alpha,7\alpha}$	6.3	$J_{6\alpha,7\alpha}$	6.3
$J_{6\alpha,7\beta}$	2.4	$J_{6\alpha,7\beta}$	2.4
$J_{6\beta,7\alpha}$	11.6	$J_{6\beta,7\alpha}$	11.3
$J_{6\beta,7\beta}$	6.1	$J_{6\beta,7\beta}$	6.2
$J_{7\alpha,7\beta}$	12.3	$J_{7\alpha,7\beta}$	12.6
$J_{7\alpha,8}$	12.0	$J_{7\alpha,8}$	12.0
$J_{7\beta,8}$	2.3	$J_{7\beta,8}$	2.5
$J_{8,9}$	11.2	$J_{8,9}$	11.2
$J_{8,14}$	12.4	$J_{8,14}$	12.2
$J_{9,11\alpha}$	4.3	$J_{9,11\alpha}$	4.3
$J_{9,11\beta}$	11.2	$J_{9,11\beta}$	11.0
$J_{11\alpha,11\beta}$	13.5	$J_{11\alpha,11\beta}$	13.4
$J_{11\alpha,12\alpha}$	4.1	$J_{11\alpha,12\alpha}$	4.3
$J_{11\alpha,12\beta}$	2.9	$J_{11\alpha,12\beta}$	2.7
$J_{11\beta,12\alpha}$	12.8	$J_{11\beta,12\alpha}$	13.2
$J_{11\beta,12\beta}$	3.8	$J_{11\beta,12\beta}$	4.0
$J_{12\alpha,12\beta}$	12.7	$J_{12\alpha,12\beta}$	13.0
$J_{14,15\alpha}$	7.4	$J_{14,15\alpha}$	7.4
$J_{14,15\beta}$	10.8	$J_{14,15\beta}$	10.8
$J_{15\alpha,15\beta}$	12.1	$J_{15\alpha,15\beta}$	12.1
$J_{15\alpha,16\alpha}$	9.4	$J_{15\alpha,16\alpha}$	9.5
$J_{15\alpha,16\beta}$	3.4	$J_{15\alpha,16\beta}$	2.9
$J_{15\beta,16\alpha}$	5.8	$J_{15\beta,16\alpha}$	6.6
$J_{15\beta,16\beta}$	11.5	$J_{15\beta,16\beta}$	12.2
$J_{16\alpha,16\beta}$	13.4	$J_{16\alpha,16\beta}$	14.3
$J_{16\alpha,17\alpha}$	8.9	$J_{16\alpha,17\beta}$	0.0
$J_{16\beta,17\alpha}$	8.4	$J_{16\beta,17\beta}$	5.8