



The conformations of 17 β -estradiol (E2) and 17 α -estradiol as determined by solution NMR

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ABSTRACT

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

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1. 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 bloodstream⁵ signaling to a number of tissues including breast, ovaries, uterus, and brain.⁶ Estrogens initiate rapid non-genomic 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 17 β -estradiol (E2) but not by the 17 α -estradiol isomer (Fig. 1).^{14,15} Recently, the structure of the estrogen hormone 17 β -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 17 β -estradiol (E2) as well as 17 α -estradiol by NMR and restrained molecular dynamics cal-

culations. Our data clearly demonstrate that for both E2 and the anomeric antiestrogen 17 α -estradiol there is only one conformation that exists in solution.

2. Results and discussion

We first assigned the proton resonances of 17 β -estradiol (E2) as well as 17 α -estradiol by analysis of their 1D ¹H, ¹³C, and 2D COSY, HSQC, and NOESY spectra recorded on a Bruker AVANCE/II 700 MHz 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 β} , H₈, H_{15 β} , and H_{7 α} for E2 that were between 1.19

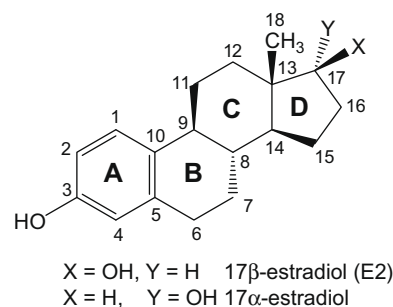


Figure 1. The hormone 17 β -estradiol (E2) and the isomeric antiestrogen 17 α -estradiol.

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

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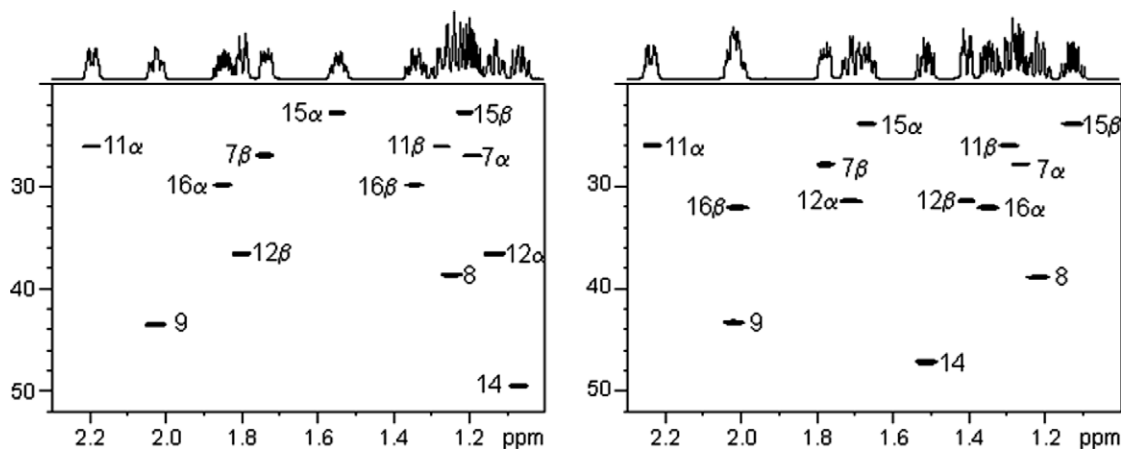


Figure 2. ^1H - ^{13}C HSQC spectra of 17 β -estradiol (E2, left) and 17 α -estradiol (right).

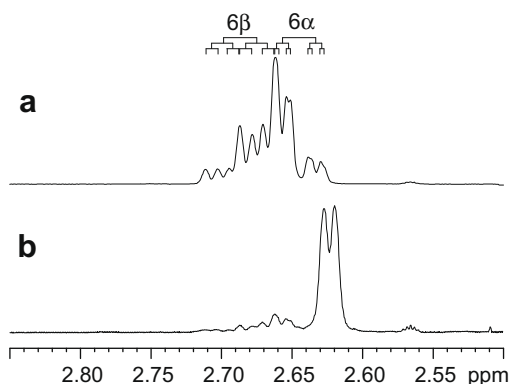


Figure 3. ^1H NMR of E2 (a) and E2-6 β -d (b) in $\text{DMSO}-d_6$ solution. (a) The benzylic C-6 protons of E2 appeared as an ABXY pattern with the $\text{H}_{6\beta}$ (*ddd*) downfield of the $\text{H}_{6\alpha}$ (*ddd*). (b) The deuterated analog, which contained approximately 10% non-deuterated E2, appeared as a broadened doublet with a 0.03-ppm upfield shift due to the isotope effect.

and 1.27 ppm (see Fig. 2). We have also, for the first time, distinguished the two benzylic geminal 6 α and 6 β protons by analyzing the J -couplings of this rather complicated ABXY pattern. Our analysis was further confirmed by comparing the ^1H spectrum of E2-6 β -d (Fig. 3) which was synthesized in our laboratory 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 17 α -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 , $\text{H}_{11\beta}$, $\text{H}_{12\beta}$, $\text{H}_{15\beta}$, and $\text{H}_{16\beta}$ for both 17 β -estradiol (E2) and 17 α -estradiol, and NOE of the C-18 methyl with H_{17} for 17 α -estradiol was also observed (Fig. 4). We did not, however, observe the reported¹⁶ NOE between $\text{H}_{12\beta}$ and $\text{H}_{15\beta}$ for E2 which was due to their mis-assignment of the proton resonances. Interestingly, the NOESY of H_1 with $\text{H}_{11\alpha}$ in the plane of the aromatic ring gave a negative NOE, an unusual effect.^{20,21} This aromatic H_1 proton had the expected positive NOEs with H_9 and $\text{H}_{11\beta}$, below and above the plane, analogous to H_4 NOEs with $\text{H}_{6\alpha}$ and $\text{H}_{6\beta}$.

Our NOE data in Tables 1 and 2 were used as distance restraints to determine the conformations of 17 β -estradiol (E2) and

Table 1
17 β -Estradiol (E2)

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

^a 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, respectively, in the molecular dynamics calculations.

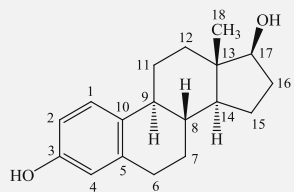
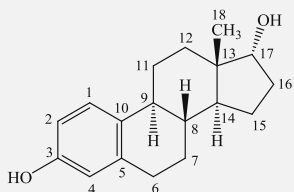
17 α -estradiol, respectively. The calculations were performed using MacroModel in the Schrodinger software package. In order to fully sample the entire conformational space, stochastic dynamics simulations with NOE distance restraints were carried out at 1000 K with a time step of 1.0 fs. Conformations were recorded every 250,000 steps for a total of 20 trajectories. Each conformer was further subjected to conjugate gradient minimization until the maximum derivative was less than 0.05 kJ/mol. Figure 5a depicts the 20 conformers of 17 β -estradiol (E2) and Figure 5b depicts the 20 conformers of 17 α -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 the 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 $\text{H}_{6\beta}$ with $\text{H}_{7\alpha}$ and for H_8 with H_9 and was a half-chair (Fig. 5c). The C-ring

Table 2
17 α -Estradiol

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

^a 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, respectively, in the molecular dynamics calculations.

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

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

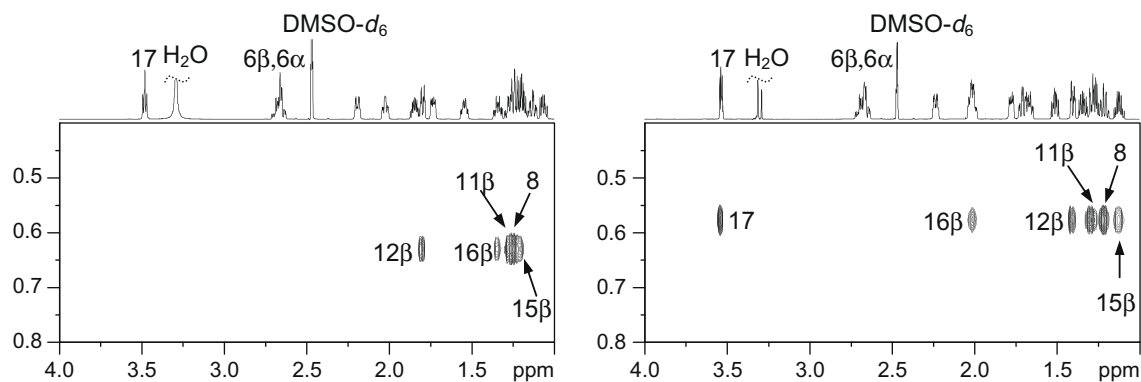


Figure 4. NOESY spectra of 17 β -estradiol (E2, left) and 17 α -estradiol (right).

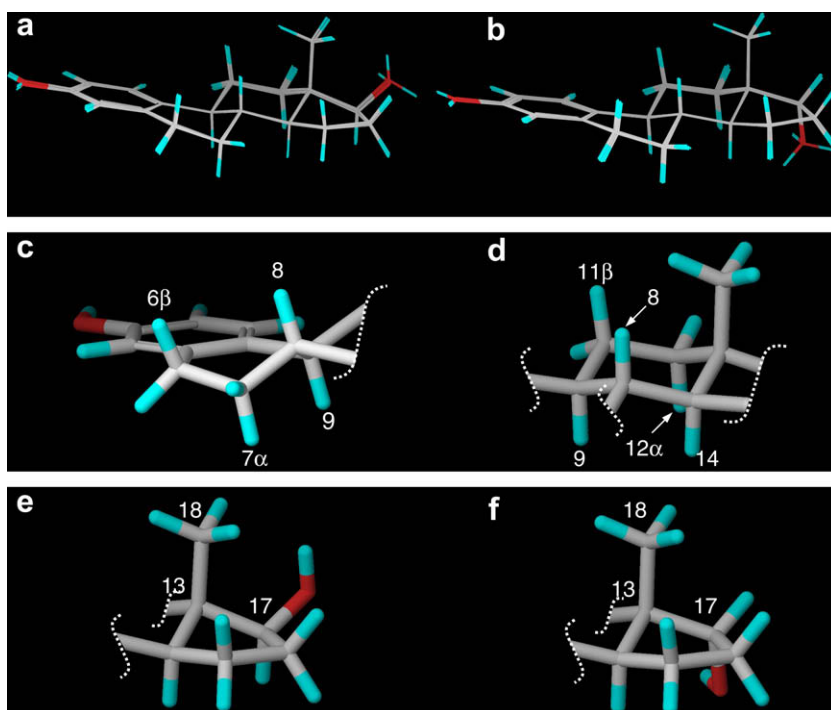


Figure 5. (a) The 20 low energy conformers of 17 β -estradiol (E2). (b) The 20 low energy conformers of 17 α -estradiol. (c) The unsaturated B-ring half-chair showing trans coplanar diaxial orientations for H_{6 β} with H_{7 α} and for H₈ with H₉. (d) The C-ring chair with trans coplanar diaxial orientations for H_{11 β} with H₉ and H_{12 α} as well as for H₈ with H₉ and H₁₄. (e) The D-ring for 17 β -estradiol (E2). (f) The D-ring for 17 α -estradiol.

was a chair conformation with trans coplanar diaxial orientations for H_{11 β} with H₉ and H_{12 α} as well as for H₈ with H₉ and H₁₄ (Fig. 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, Fig. 5e and f, 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 17 β -estradiol (E2) and the anomeric antiestrogen 17 α -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 these 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 α} and H_{6 β} protons. We are currently using these structural details to study the interactions of these compounds with bicelle model membranes.

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Supplementary data

Supplementary data (¹H and ¹³C NMR, HSQC, COSY, and NOESY spectra for 17 β -estradiol (E2) and 17 α -estradiol in DMSO-*d*₆ solutions) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.077.

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