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A Note on the Conformational Flexibility of the Antiestrogenic Drug Tamoxifen: Preferred Conformations in the Free State and Bound to the Protein Calmodulin

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Abstract

The conformational properties of the antiestrogenic drug tamoxifen, a triphenylbut-1-ene derivative, have been studied using molecular mechanics. Four distinct conformers have been identified, and the energy barriers between them have been established. The orientation of the ethyl group substitutent has been examined in particular, since the lowest-energy conformers have this group orientated 180° away from its position in the crystal structures of tamoxifen and its derivatives. These differences have implications for the interactions of tamoxifen with the calcium-binding protein calmodulin; relevant results from a molecular-modelling study of this protein–drug complex are presented.

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Introduction

The *trans*-triphenylbut-1-ene compound tamoxifen (Fig. 1) has established clinically useful anticancer activity (Jordan, Fritz & Gottardis, 1987) with its binding to the estrogen receptor believed responsible for its action against hormone-positive human breast cancer. There is, however, increasing evidence that the drug acts on other macromolecular targets as well. In the course of molecular-modelling studies in this laboratory on structure–activity relationships of tamoxifen and its derivatives and their interactions with non-estrogenic receptors, especially the calcium-binding protein calmodulin (Rowlands, Parr, McCague, Jarman & Goddard, 1990), it has become necessary to establish the conformational flexibility and energetics of tamoxifen itself. Several crystallographic studies in the tamoxifen series have been

reported; on tamoxifen itself (Kilbourn & Owston, 1970; Precigoux, Hospital, Leroy, Delbarre & Roques, 1979), and on a number of derivatives (for example, Kuroda, Cutbush, Neidle & Leung, 1985; Cutbush, Neidle, Foster & Leclercq, 1982; Camerman, Chan & Camerman, 1980). All show a propeller conformation for the three phenyl rings in the tamoxifen ethene system with the dihedral angles between them being in the 50–60° range. This paper systematically examines the conformational properties of tamoxifen by means of molecular mechanics, in the light of these crystallographic results.

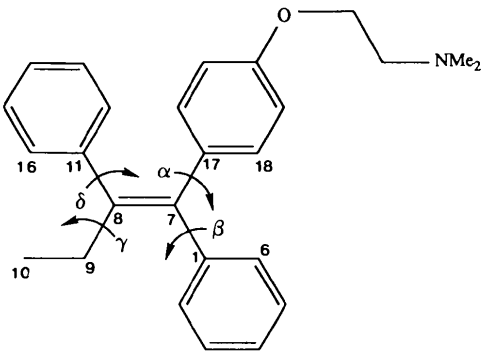

Fig. 1. The chemical structure of tamoxifen, indicating the conformational angles varied in this study.

Methods

Molecular-mechanics calculations were performed with the program *MMP2*(85) (available from the Quantum Chemistry Program Exchange) running on a VAX 11/751 computer. Interactive molecular-modelling displays and manipulations were performed with the program *GEMINI* 1.02 (CRC Biomolecular Structure Unit) running on an IRIS 3130 workstation. Initial atomic coordinates for *trans*-tamoxifen were obtained from our previous crystallographic analyses (Kuroda *et al.*, 1985).

Results

Initial minimization

In order to reduce computational time the alkylamino side chain on tamoxifen was replaced by a single H atom, since there was no reason to believe that this side chain would influence the conformation of the central region of the molecule. The molecular structure was subjected to an initial minimization using *MMP2*(85). An r.m.s. shift of 0.141 Å for all atoms was observed for the minimized structure which indicated that the crystal structure is an energetically favourable one, close to a minimum energy position.

Conformational search

Fig. 1 shows the four torsion angles α, β, γ and δ which define local minima in conformational space. A two-dimensional conformational search for all possible low-energy conformations was performed by driving each of the four torsion angles, in two sets (α and β; γ and δ) through 10° intervals using the dihedral driver in the program, with full energy minimization after each adjustment. Each angle was driven through a full 360° rotation. A two-dimensional matrix was thus obtained for each set of torsion angles, containing the final steric energy values for each conformation. Contour plots (Fig. 2) were constructed from this data. These show two

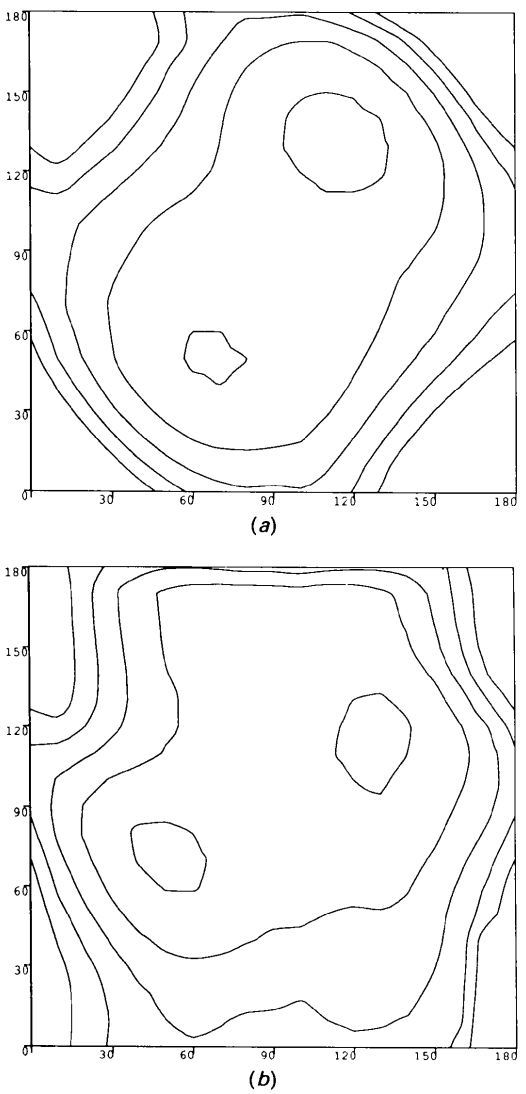

Fig. 2. Energy contour plots, with contours drawn at 2 kcal mol^{-1} intervals (1 kcal mol^{-1} = 4.1868 kJ mol^{-1}). (*a*) For angles α (vertical) and β (horizontal). (*b*) For angles γ (vertical) and δ (horizontal).

symmetry-related energy minima for each set of torsion angles. On the basis of this information, it was possible to construct eight conformers which correspond to all possible unique combinations of favoured torsion angles. Energy minimization resulted in these eight reducing to four distinct conformers, analysis of which showed that these correspond to two distinct low-energy forms, together with their mirror images. Table 1 gives the final steric energies and torsion angles for the four conformers and Figs. 3(*a–d*) show their structures.

In order to check that the four local energy minima obtained for tamoxifen were correct, and that the whole of conformational space had been explored, a four-dimensional conformational search using a modified version of *MMP*2 (D. Burke, personal communication), was performed. The modified program allowed the four torsion angles α, β, γ and δ to be driven simultaneously by the dihedral driver. α, β and δ were driven through 180° in 30° increments (due to the twofold symmetry of the phenyl rings), while γ was driven through 360° in 30° increments. A scan on all generated conformers produced 14 possible local minima which were then subject to full molecular-mechanics minimization using *MMP*2. This resulted in these 14 minima being reduced to the four (*i.e.* two mirror images) low-energy conformers previously predicted by the two-dimensional conformational searches.

Table 1. *Dihedral angles and final steric energies for the four low-energy conformers*

Data from the crystal structure analysis and its initial minimization is included for comparison. The conformers $(1)_m$ and $(2)_m$ are the mirror images of (1) and (2) respectively. 1 kcal mol^{-1} = 4.1868 kJ mol^{-1}.

	Dihedral angle (°)				Energy
Conformer	α	β	γ	δ	(kcal mol^{-1})
Crystal structure	50	64	−120	58	
Initial geometry optimization	48	59	−118	49	−3.99
(1)	47	66	72	47	−5.48
$(1)_m$	−45	−63	−71	48	−5.47
(2)	47	62	−118	49	−5.22
$(2)_m$	−45	−60	118	−49	−5.20

Barrier to rotation about the ethyl group

In order to investigate the energy barrier to rotation about the ethyl group, a conformational search was performed by driving the γ dihedral angle through 360° at 10° intervals, with full energy minimization at each point (Fig. 4). This shows two minima, at −5.48 kcal mol^{-1} and −5.20 kcal mol^{-1}, separated by 180°. The barrier between them is over 2 kcal mol^{-1}.

Discussion

This study has shown that the difference between the two non-mirror-image conformers is solely due to two distinct orientations of the ethyl group (angle γ),

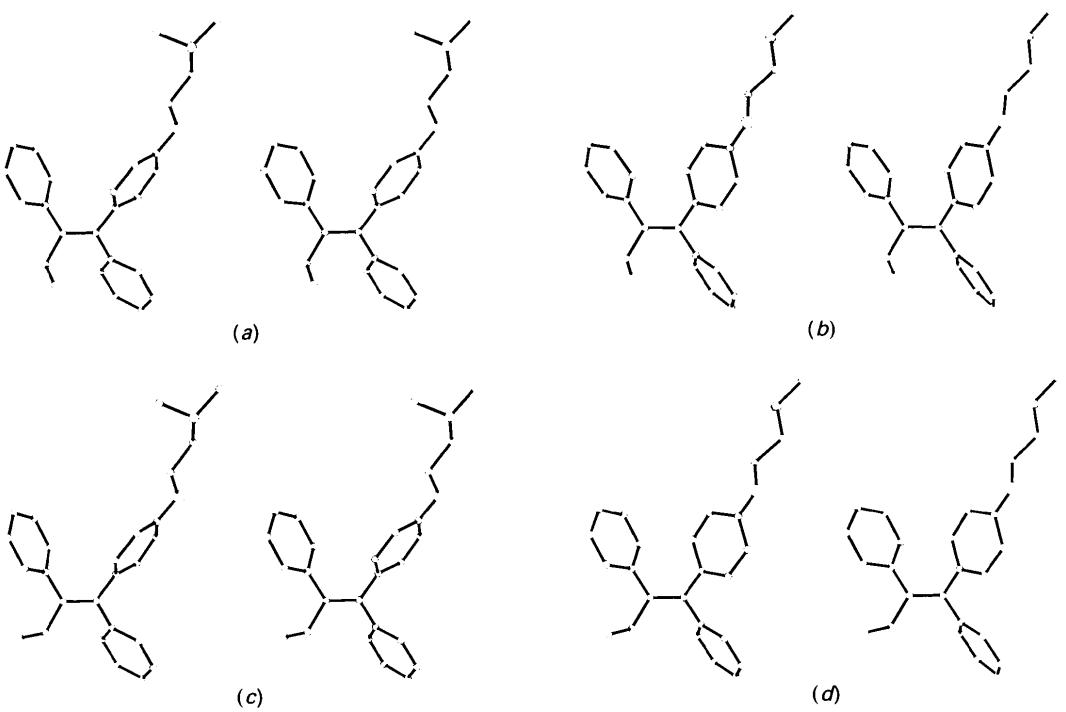

Fig. 3. Stereoplots of the four low-energy conformers. Plots (*a*)–(*d*) correspond to (1), $(1)_m$, (2) and $(2)_m$ respectively.

separated by 180°, with the crystal structures all adopting the conformation shown by conformer (2) in Table 1. The energy difference between the two conformers is unlikely to be significant. The energy plot of the ethyl group rotation (Fig. 3) also shows that both barriers to rotation are low, *ca* 4.3 and 2.0 kcal mol^{-1}. Thus, the ethyl group in tamoxifen has to be considered to be highly flexible, rather than fixed in space as suggested from a consideration of the crystallographic data on triphenylbut-1-enes (Duax & Griffin, 1987). In contrast the barrier to interconversion of the mirror-image propeller conformers [(1), $(1)_m$ and (2), $(2)_m$] has been previously estimated to be much higher, *ca* 16–18 kcal mol^{-1} (Kaftory, Biali & Rappaport, 1985). Energy calculations reported here show that all four major conformers have similar energies, and so all should be considered as candidates for binding to a macromolecular site.

All of these conformational possibilities have been taken into account in a molecular-modelling study by us of tamoxifen interacting with the hydrophobic clefts of calmodulin. The structure of this protein has recently been determined to 2.2 Å resolution (Babu, Bugg & Cook, 1988). This has been used by us in conjunction with the *AMBER* molecular-mechanics force-field (Weiner, Kollman, Nguyen & Case, 1986) to calculate binding enthalpies. The detailed results will be reported elsewhere (K. J. Edwards, C. A. Laughton & S. Neidle, to be published). Table 2 summarizes the calculated enthalpies, for binding sites on both N and C domains of the protein. The C-terminus domain has a more specific and better-defined hydrophobic binding-site geometry. It has a significantly larger binding enthalpy [for conformer (1)], than for any of the conformers in the N-terminus site. This result is consistent with the experimental finding of two classes of binding sites, with marked differences in tamoxifen affinity (Lopes, Vale & Carvalho, 1990), having dissociation constants of 6 nM and 9 μM. We conclude that the precise conformation of the ethyl group, as well as a particular propeller conformation, are important determinants of optimal binding to calmodulin. Considerations based solely on the crystal structures of tamoxifen itself would have been misleading since they are at variance with its preferred conformation (1) in the drug–protein complex.

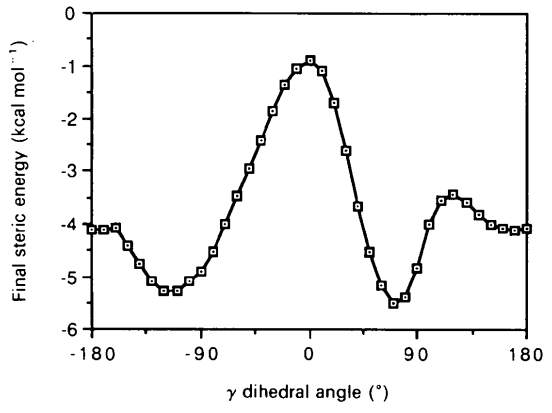

Fig. 4. Conformational energy plot showing the variation in angle γ with calculated energy, sampled at 10° intervals.

Table 2. *Calculated binding enthalpies* (kcal mol^{-1}) *for the interactions of the four tamoxifen conformers with the highest-affinity hydrophobic binding sites on calmodulin*

In the initial minimizations (*A*), the drug structure was fully minimized whilst the protein was kept fixed. In (*B*), the side chains of the protein were also minimized. 1 kcal mol^{-1} = 4.1868 kJ mol^{-1}.

	$\Delta E_{binding}$	
Model	*A*	*B*
Site (I) (C terminus)		
(1)	−20.3	−32.8
$(1)_m$	−18.2	−21.4
(2)	−17.5	−13.1
$(2)_m$	−13.5	−14.5
Site (II) (N terminus)		
(1)	−19.0	−9.2
$(1)_m$	−23.5	−16.0
(2)	−19.7	−18.9
$(2)_m$	−19.3	−13.1

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