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Conformational Analysis of a Model for the trans-Fused FGH Ether Rings in Brevetoxin A

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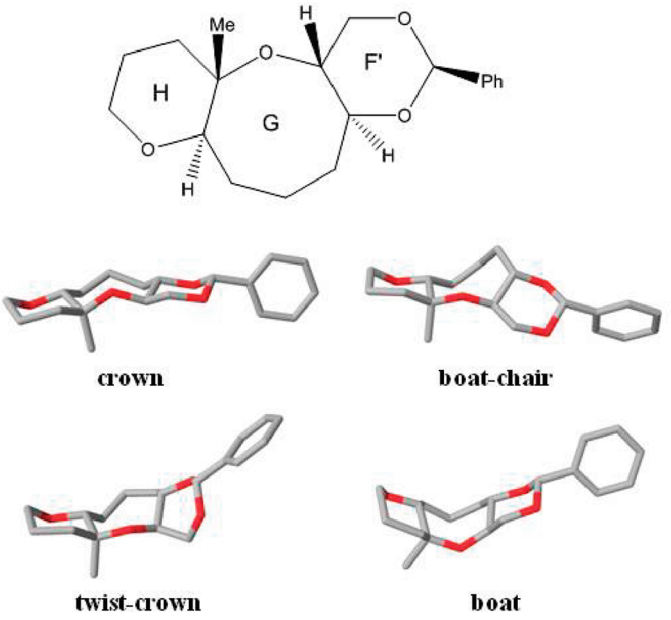

We have applied the Low Mode:Monte Carlo (LM:MC) conformational search method to an investigation of the low energy structures for 3*R*,4a*S*,7a*R*,11a*S*,12a*R*)-11a-methyl-3-phenyldecahydro-1*H*-[1,3]dioxino[5,4-*b*]pyrano[2,3-*g*]oxocine, **3**, a model for the FGH rings in brevetoxin A, a potent marine toxin. Searches performed using a variety of classical force field-solvent combinations yielded ensembles containing between 30 and 60 structures with the central G-ring adopting the crown, twist-crown, boat-chair, and boat conformations. The lowest energy structure depended on the force field-solvent treatment, and the twist-crown and boat-chair conformers were typically lowest in energy. B3LYP/6-31G** geometry optimizations using the SCRF continuum solvent model confirmed these structures but indicated that the crown was the energetically preferred conformer with the boat-chair lying within 1.4 kcal/mol.

Introduction

Brevetoxins are a class of polycyclic marine ethers and neurotoxins produced by the "red tide" dinoflagellate *Karenia brevis* (past taxonomy included *Gymnodinium breve* or *Ptychodiscus brevis*). These molecules are potent biological agents that have been identified as the causative agent of neurotoxic shellfish poisoning.[1] Brevetoxins function by binding to orphan receptor "site 5" on voltage-gated sodium ion channels, inducing prolonged ion channel opening times at lower membrane potentials.[2] This binding results in symptoms nearly identical to the human muscle

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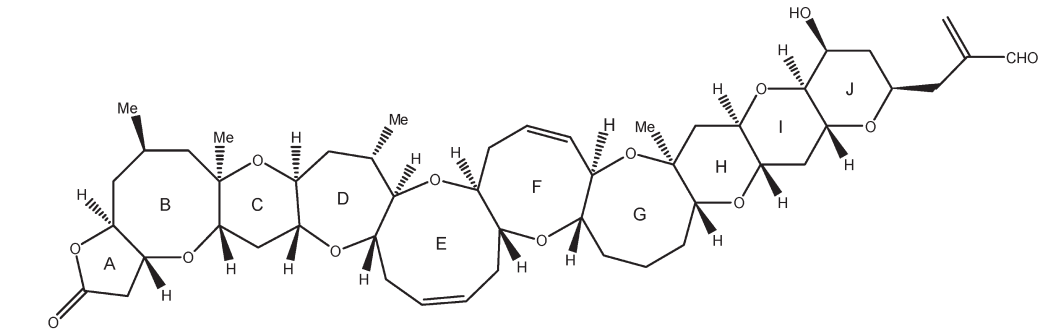
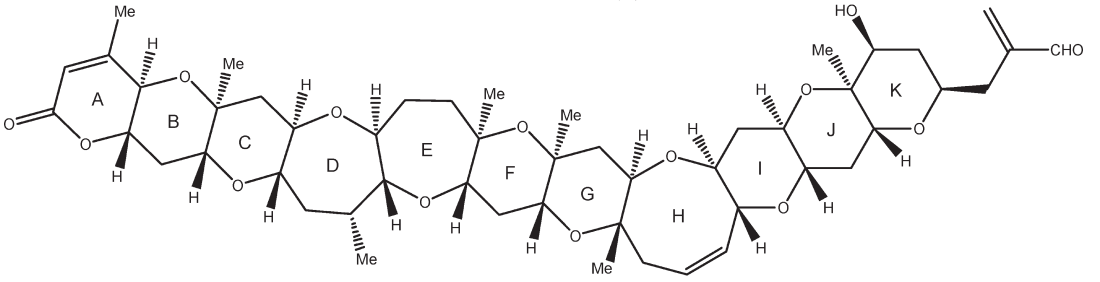


brevetoxin A (1)



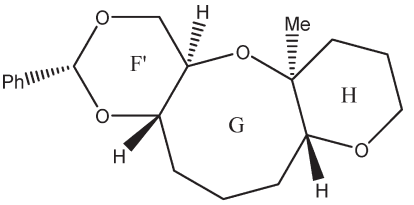
brevetoxin B (2)

FIGURE 1. Structures of brevetoxin A and B.

diseases *hyperkalemic periodic paralysis* and *paramytonia congenita*.[3]

The brevetoxin family is composed of brevetoxin A and brevetoxin B (Figure 1). The conformational flexibility of these ladder-like toxins is believed to play a role in their binding behavior and associated toxicity.[4] The crystal structure of brevetoxin A contains a central eight-membered ether G-ring in the boat-chair conformation, imparting a twist to the molecule that connects a relatively flat sheet of A−F rings to a perpendicularly oriented sheet of H−J rings.[1,5] A systematic analysis of the conformations of eight-membered hydrocarbon rings using a very early three-term implementation of a molecular mechanics formalism[6] led to the suggestion that the G-ring might undergo a slow interconversion between the boat-chair and crown conformation when brevetoxin A is in solution.[1] Previous modeling results using a conformational scan of gas-phase MM2* structures followed by reminimization in a continuum solvent have also suggested that the lowest energy structure of brevetoxin A adopts a boat-chair G-ring conformation.[3] The same methodological approach revealed that desaturating the H-ring in brevetoxin B causes the central H-ring to convert from the boat-chair to the crown conformation effecting a significant change in the overall molecular shape resulting in a large change in experimental binding affinity.[2]

In an effort to shed further light on the conformational behavior of these molecules, Shida and Tachibana synthesized a model of the trans-fused eight-membered ether ring region of brevetoxin A and studied the resulting NMR



3

FIGURE 2. Model for the FGH trans-fused ether ring of brevetoxin A.

behavior.[7] In their model, the oxocine F-ring of breveotoxin A was replaced with a phenyl dioxane (F′-ring) as shown in Figure 2. Using this model structure and variable temperature NMR, two conformations were clearly resolved with an 83:17 ratio at −90 °C, corresponding to a free energy difference of ca. 3.9 kJ/mol. An analysis using vicinal proton−proton coupling constants and structures obtained from molecular mechanics suggested that the major conformer contained a crown G-ring; however, this was in disagreement with their energetic results which favored the boat-chair conformer in all force field-solvent combinations except one (OPLS*-$CHCl_3$).[7]

We were interested in exploring further the nature of the disagreement between the molecular mechanics results and the conformational preferences obtained by NMR on the Shida−Tachibana model. Additionally, because of the importance of these flexible trans-fused ether rings in binding, we were interested in fully exploring the conformational behavior of **3** using new force fields and more efficient conformational searching methods. This will provide information about the molecular shape and solution phase behavior of brevetoxin A and perhaps provide further insight

(3) Rein, K. S.; Baden, D. G.; Gawley, R. E. *J. Org. Chem.* **1994**, *59*, 2101–2106.
(4) Gawley, R. E.; Rein, K. S.; Kinoshita, M.; Baden, D. G. *Toxicon* **1992**, *30*, 780–785.
(5) Van Duyne, G. A. *ACS Symp. Ser.* **1990**, *418*, 144–165.
(6) Hendrickson, J. B. *J. Am. Chem. Soc.* **1964**, *86*, 4854–4866.
(7) Shida, T.; Tachibana, K. *Tetrahedron Lett.* **2005**, *46*, 1855–1857.

TABLE 1. Number of Low Energy Structures Found within 11.95 kcal/mol (50 kJ/mol) on Each Potential Energy Surface of the F′GH Model Compound of Brevetoxin A, 3, along with an Analysis of the Ring Conformations Found in the Global Minimum Structure on Each Surface

		ring conformations of global minimum		
force field (GBSA solvent)	no. of low energy structures	F-ring	G-ring	H-ring
OPLS2005, $CHCl_3$	42	twist-boat	twist-crown	chair
OPLS2005, H_2O	48	twist-boat	twist-crown	chair
AMBER*, $CHCl_3$	31	chair	boat-chair	chair
AMBER*, H_2O	40	chair	boat-chair	chair
MM2*, $CHCl_3$	58	twist-boat	twist-crown	chair
MM2*, H_2O	60	twist-boat	twist-crown	chair

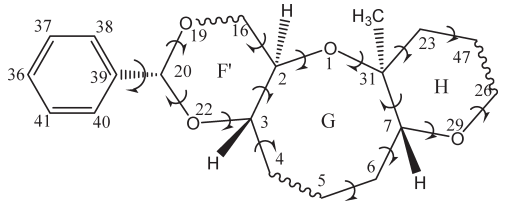


FIGURE 3. Degrees of freedom varied during the conformational searches. Arrows represent torsions that were allowed to vary, and the wavy lines indicate the bonds used to open rings for conformational interconversions.

regarding the process by which brevetoxins bind to their target receptor sites on sodium ion channels.

Results and Discussion

Conformational Analysis. We utilized the Low Mode: Monte Carlo (LM:MC) method to sample exhaustively the potential energy surfaces of **3** as constructed using three different force fields (AMBER*, MM2*, and OPLS2005) and two different solvent environments (GBSA/H_2O and GBSA/$CHCl_3$) for a total of six searches. We employed six different potential energy surfaces in order to compare with previous results[7] and to gauge the dependence of our results on the methodological approach. The details of each search, including convergence, can be found in the Supporting Information; a summary of the results appears in Table 1.

In contrast to the Shida−Tachibana results, we find significantly more low energy structures of **3** and more dependence of the G-ring conformation on the force field-solvent method employed. This dependence is also seen in the F′-ring conformations where we see chair and twist boat structures depending on the force field employed. We find that the lowest energy structures obtained are independent of the solvent treatment.[8] The OPLS2005 and MM2* surfaces contain a global minimum with a twist-boat F′-ring and a twist-crown G-ring (also referred to as crown-2 in previously published works[3]). On the AMBER* surfaces the G- and F′-rings adopt boat-chair and chair conformations, respectively. The H-ring in the global minimum energy structures on all six surfaces adopts a chair conformation.

(8) Global minimum structures from each force field−solvent pair were superimposed. rmsd values of heavy atom superimposition ranged from 0.0226 (nearly perfectly superimposable) to 0.4101. In the later case, the structures are the same but with variation in the phenyl substituent on the F′-ring. See Supporting Information for rmsd values and superimpositions of the most- and least-similar comparisons.

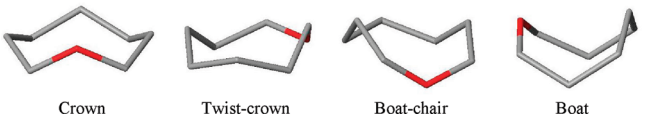


FIGURE 4. Representative structures of the four central G-ring conformations of **3**. Substituent atoms and rings have been removed for clarity.

The number of unique structures found on each surface is shown in Table 1. In this study, we are particularly interested in the flexibility of the G-ring because the conformation of this ring is thought to induce a significant change in the overall shape of brevetoxin A and this may play a role in the higher toxicity of brevetoxin A as compared to brevetoxin B.[2] In the ensemble of low energy structures determined here, the central G ring adopts one of four distinct conformations; crown, twist-crown, boat-chair, and boat. The representative structures for the central G-ring conformations are shown in Figure 4.

The unique low energy structures of **3** in each ensemble were visualized and grouped according to their G-ring conformation (Table 2). Structures were initially grouped according to conformational categories originally identified in cyclooctane[6,9] and the G-ring in brevetoxin A.[3] The crown and boat conformations were easily identified and distinguished. Within the crown ensemble, a portion of the rings exhibited an asymmetrical twist, and these conformations were culled out and defined as twist-crown structures. The boat-chair conformations were the most difficult to distinguish, as this was the most structurally diverse category containing conformers with slight angle variations that made further divisions within the boat-chair category difficult. Thus, these were grouped together as the boat-chair ensemble. Again we see that solvent plays a relatively small role in the overall conformational behavior; in all cases, the global minimum structure does not change with different solvents, and in most cases the number of structures found for a given force field-solvent combination differs by only a few structures. There is some solvent dependent energetic reordering within ensembles in agreement with previous results.[3] The largest solvent effects occurred for AMBER*/$CHCl_3$ and AMBER*/H_2O, but even in this case the number of boat-chair conformations differs by only six structures. This indicates that changing the solvent system does not significantly change the conformational ensembles; however, the force field does affect the distribution of conformers

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TABLE 2. Number of Unique Structures in Each Molecular Mechanics Ensemble Grouped According to G-Ring Conformational Preference

conformation	OPLS2005 $CHCl_3$	OPLS2005 H_2O	MM2* $CHCl_3$	MM2* H_2O	AMBER* $CHCl_3$	AMBER* H_2O
boat-chair	23	25	24	27	11	17
crown	12	10	13	13	6	11
twist-crown	4	9	15	14	10	7
boat	3	4	6	6	4	5
total no. of structures	42	48	58	60	31	40

TABLE 3. Relative Molecular Mechanics Energies (kcal/mol) of 3 Containing the Four Possible G-Ring Conformations Using Different Force Fields and Solvent Models

conformation	OPLS2005 $CHCl_3$	OPLS2005 H_2O	AMBER* $CHCl_3$	AMBER* H_2O	MM2* $CHCl_3$	MM2* H_2O
twist-crown	0.000	0.000	3.581	2.673	0.000	0.000
crown	0.466	0.191	1.279	1.158	1.450	1.520
boat-chair	1.492	1.090	0.000	0.000	0.943	0.949
boat	6.828	5.968	6.377	5.988	4.243	4.159

(Table 2). OPL2005 and MM2* contain a larger number of structures where the G-ring adopts the boat-chair or symmetrical crown conformation, whereas the AMBER* surface contains a somewhat more even distribution across all four G-ring possibilities. In all cases, the force fields find the largest number of G-ring conformations for **3** in the boat-chair orientation and significantly fewer for the boat conformation.

Energetic Ordering of Molecular Mechanics Results

Using the various force fields (OPLS2005, MM2*, AMBER*) and solvent systems (GBSA-$CHCl_3$ and -H_2O), the energetic orderings of the lowest energy conformers of **3** displaying the four possible G-ring conformations were found to be similar although not exactly the same (Table 3). In all cases, the structure with the G-ring in the boat conformation is the highest in energy; 4−7 kcal/mol higher in energy than the global minimum energy G-ring twist-crown (OPLS2005 and MM2*) or boat-chair (AMBER*) orientation. With the OPLS2005 and MM2* force fields, we find that the twist-crown, crown, and boat-chair conformation are similar in energy and within approximately 1.5 kcal/mol of each other. In fact, using the OPLS2005 force field, the conformations of **3** containing crown and twist-crown conformations are almost isoenergetic, varying less than 0.2 or 0.5 kcal/mol on the GBSA H_2O or $CHCl_3$ surface, respectively. The AMBER* force field clearly favors the boat-chair G-ring conformation followed relatively closely in energetic terms by the crown; the twist-crown and boat conformers are significantly less stable on this surface.

The molecular mechanics ensembles contain four possible G-ring orientations (vide supra) which when combined with (1) various orientations of the phenyl ring relative to the fused ring system, (2) the F′- and H-ring flexing between the twist-boat and chair conformations, and (3) variability in surface roughness, leads to 31−60 low energy structures in each ensemble (Table 2). This is significantly more low energy structures than reported previously for **3** by Shida and Tachibana, who identified only the crown and boat-chair conformations within the same energy window (11.95 kcal/mol or 50 kJ/mol) and on the same or similar surfaces as used in this report, albeit using older versions of the software. Furthermore, in almost all of the cases, the Shida−Tachibana force field analysis identified the boat-chair as the lowest energy conformation, in disagreement with their NMR results, which indicated a 3.9 kJ/mol free energy preference for the crown conformation.[7] In our hands, using OPLS2005 and MM2, we see lowest energy structures adopting a G-ring twist-crown conformation. We also obtain low energy ensembles containing a significant number of crown, twist-crown, and boat-chair G-ring orientations, in addition to a small number of boat conformations. It is not surprising that the results obtained using the newer force fields and solvent models are different. In particular, the OPLS2005 force field parameters have been improved in a variety of ways, including a refitting to reproduce high level quantum mechanical conformational energies.[12]

In computational studies of the full brevetoxin A molecule, Rein reported 48 conformations on the MM2* vacuum surface, which when reminimized using GBSA H_2O or $CHCl_3$ resulted in energetic reorderings but no new minima.[3] Within this ensemble of structures, they identified five G-ring conformations: the crown, twist-crown, boat-chair, and boat structures along with a second, higher energy boat-chair conformation. The lowest energy structure contained a boat-chair G-ring. With these results, coupled with experimental binding affinities and a comparison with brevetoxin B low energy conformers, Rein et al. concluded that binding to site 5 on the sodium ion channel is most likely to occur when the central G-ring in brevetoxin A adopts the boat-chair conformation.[2]

To summarize our conformational findings, after 55000 search steps, we find significantly more low energy G-ring conformations on each surface of **3** than Shida−Tachibana

(10) The size of brevetoxin A precluded a full conformational analysis with all available force fields. The OPLS2005 force field was chosen as it is one of the newer force fields which has been shown to be compatible with the GBSA model. In addition, the lowest energy structure for **3** on this force field contains a twist-crown G-ring although the crown and boat-chair conformations are not too much higher in energy. Fifty-five thousand LM:MC steps were performed. Full details of the conformational search for brevetoxin A can be found in the Supporting Information.

(11) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.

(12) Kaminski, G.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. L. *J. Phys. Chem.* **2001**, *105*, 6474–6487.

TABLE 4. Relative Energies (kcal/mol) of the Lowest Possible G-Ring Conformations of 3 Obtained Using B3LYP/6-31G in the Presence of SCRF-PBF CCl_4 and H_2O**

	CCl_4	H_2O
crown	0.00	0.00
boat-chair	1.35	1.38
twist-crown	5.10	6.01
boat	5.95	5.87

and we find in general slightly fewer conformers within the same energy window as reported by Rein et al. on the larger brevetoxin. The boat-chair was the lowest energy conformer of **3** using the AMBER force field, and with the OPLS2005 and MM2 force fields, the boat-chair is at most 1.49 kcal/mol higher than the lowest energy twist-crown conformation. The central G-ring exhibits preferences for the "twist-crown" and "boat-chair" conformations, in agreement with previous reports on the low energy conformations of brevetoxin A and favorable conformations of eight-membered ring systems.[3,7,9]

Quantum Minimizations

To more definitively determine the most energetically favorable structure of the G-ring in **3**, we subjected 24 structures obtained by sampling on the classical surfaces to a B3LYP/6-31G** geometry optimization in the presence of a continuum solvent (SCRF-PBF(H_2O or CCl_4)) (Table 4). Starting structures were chosen by selecting the lowest energy structure containing each of the four possible G-ring conformations from each ensemble. The resulting geometry optimized quantum structures were then superimposed onto the molecular mechanics starting structures to determine how much the geometries differ, if at all. Superimposition using all heavy atoms resulted in root mean square deviations (rmsd) ranging from 0.85 to 0.06 Å. A summary of the rmsd values may be found in the Supporting Information. By superimposition and visual inspection, the molecular mechanics minima are shown to correspond closely to the quantum mechanical structures; however, the energetic orderings are different.

The symmetric crown conformation is the lowest energy structure of **3** in either CCl_4 or H_2O, lying slightly more than 1 kcal/mol lower than the boat-chair conformation. This is in agreement with the experimental NMR results obtained previously by Shida and Tachibana and indicates that the force fields are overstabilizing the twist-crown (OPLS2005 and MM2*) or boat-chair (AMBER*) conformation. The literature suggests that the methyl substituent between the G- and H-rings in brevetoxin A destabilizes the crown conformation; however, our model **3** contains this group and the crown conformation is in fact the most stable structure.[1,5] Quantum mechanically, the twist-crown and boat conformations lie 5−6 kcal/mol higher in energy than the crown. We were surprised to see the twist-crown conformer so high in energy; however, a visual comparison of the crown and twist-crown structures shows that the twist-crown G-ring imparts a somewhat more highly strained, twisted-chair conformation in the F′-ring relative to the symmetric orientations of each ring in the lowest energy crown structure (Figure 5).

While the symmetric crown is not the lowest energy structure in any of the force field results, it is not energetically far from the lowest energy conformation. In fact, using OPLS2005/$CHCl_3$, the symmetric crown lies 0.466 kcal/mol above the asymmetric crown, and using OPLS2005/H_2O, that difference decreases to 0.191 kcal/mol. The boat-chair conformation is determined by QM to be approximately 1 kcal/mol higher in energy, and this is very similar to the relative energetic orderings found on the OPLS2005 and MM2* force field surfaces. Similarly, relative energies for the boat conformation found on the quantum mechanical surfaces agree well with its corresponding energetic position on all force field surfaces.

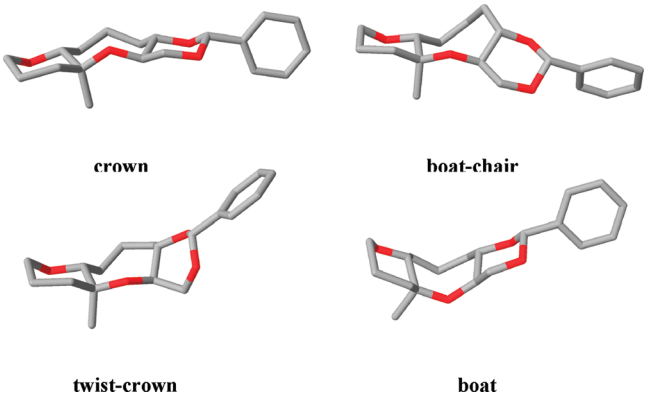

FIGURE 5. B3LYP/6-31G**/SCRF-PBF H_2O geometry optimized structures of **3**.

The similarities in the quantum mechanical energies for the four conformations of **3** suggest that the previous NMR experiments contained all four conformations but that it was not possible to distinguish the crown from twist-crown or the boat-chair from boat conformations. This could be due to similarities in the observed vicinal proton−proton coupling constants or a rapid interconversion between the crown ↔ twist-crown and boat ↔ boat-chair structures along with a slow interconversion between the crown and boat groups resulting in an average NMR conformation for each group. Of course, the enthalpic analysis performed here does not include entropic effects that are present in experimental results. Free energy simulations are underway in our laboratory to examine this effect.

The picture that is emerging for **3** is of a relatively flexible molecule with distinct conformers having similar energies. The global minimum structure adopts a chair, crown, chair orientation for the F′, G, and H rings, respectively. However, this result is inconsistent with the results of Rein et al., which indicate that the lowest energy conformation for brevetoxin A has a boat-chair G-ring conformation. To gain insight into these differences, we performed conformational searches on the full brevetoxin A molecule using the OPLS2005 force field with GBSA $CHCl_3$ and H_2O.[10] The results indicate that in either solvent, the lowest energy structure adopts a G-ring boat-chair orientation, as do 9 of the 10 structures in each low energy ensemble. The crown G-ring conformation appears as the eighth and tenth structure at 1.93 and 2.06 kcal/mol in $CHCl_3$ and H_2O, respectively. The global minimum energy structure found in each ensemble was confirmed by quantum mechanical optimization at the B3LYP/6-31G** SCRF-PBF H_2O and CCl_4 level of theory. These results suggest that the molecular environment plays a vital role in determining the conformational preferences for the G-ring in

brevetoxin A. It is likely that a system larger than **3**, perhaps including the effects of the flexible seven-membered F- and nine-membered E-rings in brevetoxin A is needed in order to adequately model the dynamical behavior of this interesting marine toxin.

Conclusion

We have performed a conformational analysis of a model for the trans-fused FGH ether rings in brevetoxin A. The molecular behavior and energies were examined using the mixed Low Mode:Monte Carlo conformational search technique to exhaustively sample the potential energy surface using several force fields and solvent systems. The resulting low energy structures were divided according to their conformations and further analyzed in terms of their structures and energies. It is found that within the 50 kJ/mol (11.95 kcal/mol) energy gap from the lowest energy conformation, there exist approximately 30−60 other conformations. In this ensemble of structures, the central G-ring can adopt crown, twist-crown, boat-chair, and boat orientations. Quantum mechanical calculations revealed that the lowest energy conformations found in the various conformational searches are shown to be minima on the corresponding QM surface. The central G-ring in brevetoxin A plays a role in active site binding and in this model compound the G-ring exhibits preferences for "crown" and "boat-chair" conformations. This is consistent with previous findings describing the need for the G-ring in brevetoxin A to adopt the boat-chair conformation in order to bind to the ion channel active site. Calculations including solvation indicated that solvent does not play a major role in the conformational preferences.

Methods

The conformational ensembles generated in this study were calculated using the MacroModel V9.1 suite of software[11] programs running on 3.2 GHz Athlons under the Red Hat 9 operating system. The OPLS2005,[12] AMBER*,[13] and MM2*[14] force fields, as implemented in MacroModel v9.1, were used in this study because they contained the fewest low quality torsion parameters for **3**. (Complete analysis for each force field may be found in the Supporting Information.) Solvent effects were investigated using the generalized Born/surface area (GB/SA) continuum model[15−20] for H_2O and $CHCl_3$.

The Low Mode (LM) method[21,22] was used in a 1:1 combination[23] with the Monte Carlo (MC) method[24] to explore the potential energy surfaces of **3**. Each MC conformational search step varied a random number of torsional degrees of freedom between a minimum of two and a maximum of 16, where 16 is the total number of variable torsion angles as shown in Figure 3. LM frequencies corresponding to the 10 lowest eigenvectors were explored. The total traveling distance for each step was selected randomly between 3 and 6 Å. Interconversion of ring structures was enabled using the ring−opening method of Still.[9] The *wavy* bonds in Figure 3 were used for opening each ring during the conformational search. Starting structure chirality was preserved throughout the search process. Searches were run in multiple blocks of 5000 LM:MC steps until they had reached convergence. Convergence was judged by monitoring the (1) energy of the most stable structure, (2) number of times this structure was visited, and (3) number of unique conformations found within 11.95 kcal/mol of the lowest energy minimum. Unique conformations were determined by superimposition of all heavy (non-hydrogen) atoms as well as reflection and/or rotation of the atom-numbering scheme. Structures were considered to be duplicates and rejected if the maximum interatomic distance was 0.25 Å or less following optimal rms superposition. Structures that were found in previous searches were used to seed subsequent searches. Searches utilized the usage-directed structure selection method[24] that identifies the least-used structure from among all known conformations and then uses this structure as the starting point for each new search. This insures that a variety of different starting structures from different regions of the potential energy surface are used to begin each search. During the conformational search, all structures were subjected to 1500 steps of the truncated Newton conjugate gradient (TNCG)[25] minimization method to within a derivative convergence criterion of 0.01 kJ $Å^{-1}$ mol^{-1}. In most cases, this led to large ensembles with many structures not completely minimized. Various tests demonstrated that the most efficient method for obtaining fully minimized ensembles was to complete the search and apply a second set of 2500 minimization steps to the resulting ensemble.

Quantum mechanical calculations were performed using the Jaguar v7.0 suite of software programs in order to confirm the structures obtained from the force-field based conformational search results. The B3LYP functional and 6-31G** basis set, as implemented in Jaguar v7.0, were used. Solvent effects were included at each step of the geometry optimization using a self-consistent reaction field method with a standard Poisson−Boltzmann[26−28] solvent model for H_2O and CCl_4.

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Supporting Information Available: Force field parameter analysis, LM:MC conformational search results and superimposition of force field and quantum minimized structures for **3**, Cartesian coordinates for low energy quantum minimized conformations of **3**, conformational search details for brevetoxin A. This material is available free of charge via the Internet at http://pubs.acs.org.