# Brevetoxin B: Chemical Modifications, Synaptosome Binding, Toxicity, and an Unexpected Conformational Effect

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Five naturally occurring brevetoxins and seven synthetically modified brevetoxins were examined for their affinity for site 5 of the voltage-gated sodium channel and their toxicity to mosquito fish, *Gambusia affinis*. All but three of the toxins studied still retained some affinity for their receptor site ($IC_{50}$'s in the range of 1–100 nM). Compound **7**, having all three carbon–carbon double bonds reduced, is almost 3 orders of magnitude less strongly bound than **4**, which has only two carbon–carbon bonds reduced. This large effect resulting from H-ring reduction was unexpected, due to the similarity of this region of the molecule to the corresponding region of brevetoxin-A, which has a fully saturated eight-membered G-ring and is the most strongly bound toxin of those studied. Conformational analysis revealed that the unsaturated H-ring of brevetoxin B favors the boat–chair conformation as does the saturated G-ring of brevetoxin A. Upon reduction, the H-ring of brevetoxin B shifts to a crown conformation. This subtle change in conformational preference induces a significant change in the gross shape of the molecule, which we believe is responsible for the loss of binding affinity and toxicity.

The normal voltage-gated function of sodium channel proteins is affected by a number of neurotoxins, which act at specific sites, numbered 1–7.[1] These sites are known generically as orphan receptors, since they are specific receptors that bind xenobiotics and produce demonstrable pharmacological effects, but for which there is no known endogenous ligand.[2] The brevetoxins (Figure 1),[3] produced by the Florida "red tide" dinoflagellate *Gymnodinium breve* (*Ptychodiscus brevis*),[4] bind to site 5 in a 1:1 stoichiometry.[5] The results of their binding are as follows: (i) a shift of the activation voltage for channel opening to more negative values (*i.e.*, channels are opened at normal resting potential) and (ii) inhibition of the inactivation of opened channels, resulting in persistent activation or prolonged open times.[6] Additionally, ciguatoxin[7] binds to site 5 in a competitive fashion.[8] This unique

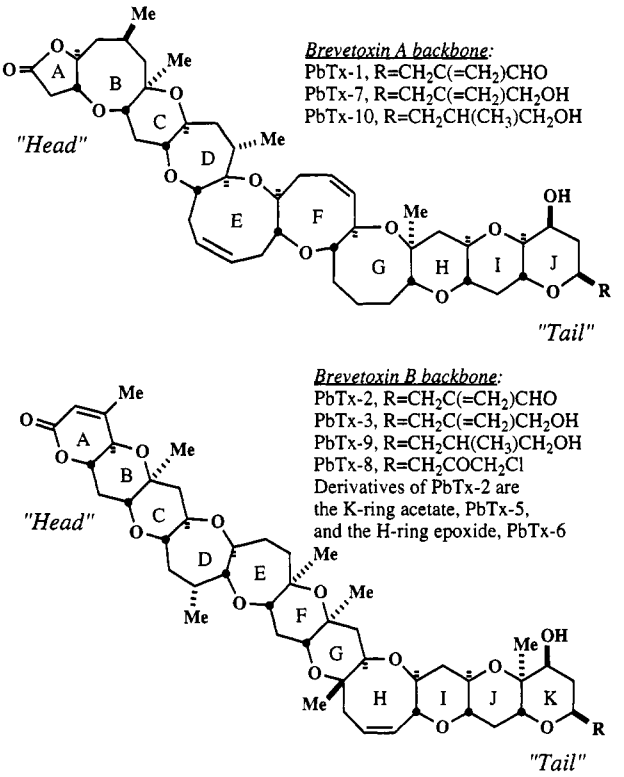


**Figure 1.**

activity has precipitated intense interest in the synthesis,[9] biosynthesis,[10] and pharmacology[11] of these toxins.

In an effort to identify the common pharmacophore of these structurally diverse toxins, we have completed a

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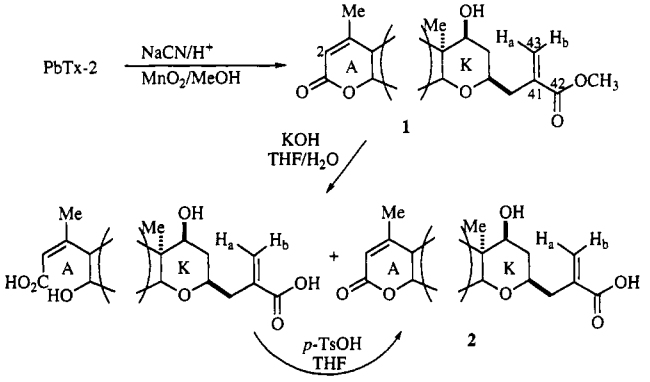
**Scheme 1**



conformational analysis of the brevetoxin B[12] and A[13] backbones and have identified noteworthy similarities in the gross shape of the two structural types.[13] In order to help develop a receptor–ligand model for their binding to site 5, we now report the chemical modification of brevetoxin B and the biological activity of these derivatives relative to five naturally occuring toxins. Two bioassays were used: competetive displacement of tritiated PbTx-3 (see Figure 1 for nomenclature) bound to rat brain synaptosomes (expressed as $IC_{50}$, the inhibitor concentration necessary to displace half of the radiolabeled toxin specifically bound to synaptosomes) and toxicity to mosquito fish, *Gambusia affinis* ($LC_{50}$, the concentration necessary to kill half a population of fish).

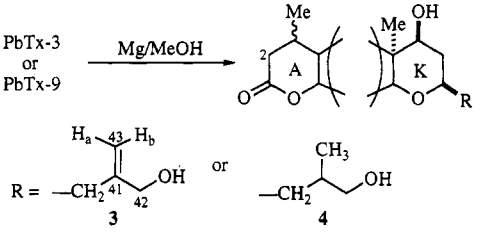
## Results and Discussion

**Synthetic Modifications.** The most readily available toxins (from culture) are PbTx-2 and PbTx-3, both of which have a brevetoxin B backbone. Examination of Figure 1 reveals three potential sites for chemical modification: the A-ring $\alpha,\beta$-unsaturated lactone, the H-ring double bond, and the K-ring side chain (the K-ring hydroxyl is axial and quite unreactive).

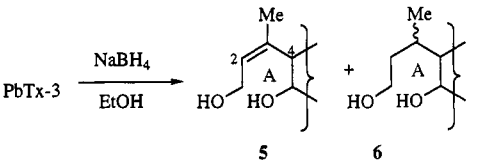
As shown in Scheme 1, PbTx-2 was oxidized at C-42 to the corresponding methyl ester (1), via the cyanohydrin, according to the procedure described by Corey.[14] Treatment of 1 with base resulted in the hydrolysis of the ester at C-42 (2) and partial hydrolysis of the A-ring lactone. Treatment of this mixture with a catalytic amount of *p*-TsOH in THF provided 2.

The C-2, C-3 double bonds of PbTx-3 and PbTx-9 were selectively reduced with Mg in methanol following Hudlicky's procedure,[15] (Scheme 2) to provide 3 (from PbTx-3) and 4 (from PbTx-9) as ~3:1 (inseparable) mixtures of diastereomers.

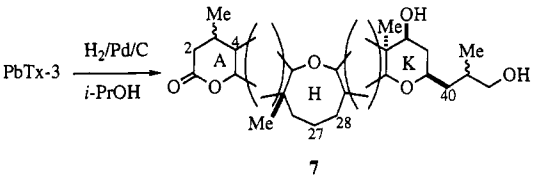
**Scheme 2**



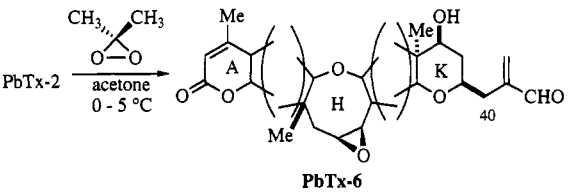
**Scheme 3**



**Scheme 4**



**Scheme 5**



Reduction of the A-ring lactone of PbTx-3 with sodium borohydride in ethanol provided a mixture of two diols, 5 and 6 (Scheme 3). The minor product (5) retained the C-2, C-3 double bond. We presume that 6 is a ~1:1 mixture of diastereomers.

Finally, all the double bonds of PbTx-3 were reduced catalytically to provide 7 as a mixture of epimers at C-3 and C-41 (Scheme 4). It is interesting to note that 4 may also be prepared by the catalytic reduction of PbTx-3, using less catalyst. Apparently, the H-ring double bond is significantly less reactive than the other two.

Although PbTx-6 is naturally occurring,[16] we find it more convenient to prepare it from PbTx-2 by oxidation with dimethyldioxirane,[17] as shown in Scheme 5.

**Bioassay.** Inhibition curves showing the displacement of tritiated PbTx-3 by competitor ligands (PbTx-1, PbTx-2, PbTx-3, PbTx-6, PbTx-9, and 1-7) are shown in Figures 2 and 3. Figures 4 and 5 show the linear regions of the log–logit plots[18] of these data. From the latter, the $IC_{50}$ values summarized in Table 1 were calculated. When sufficient quantities were available, $LC_{50}$ data were recorded as well.

For the five natural toxins, a *statistically* significant difference (within 95% confidence limits) in $IC_{50}$ was recorded for all except PbTx-2 and PbTx-9. All natural toxins have $IC_{50}$ values in the range of 1–50 nM, suggesting

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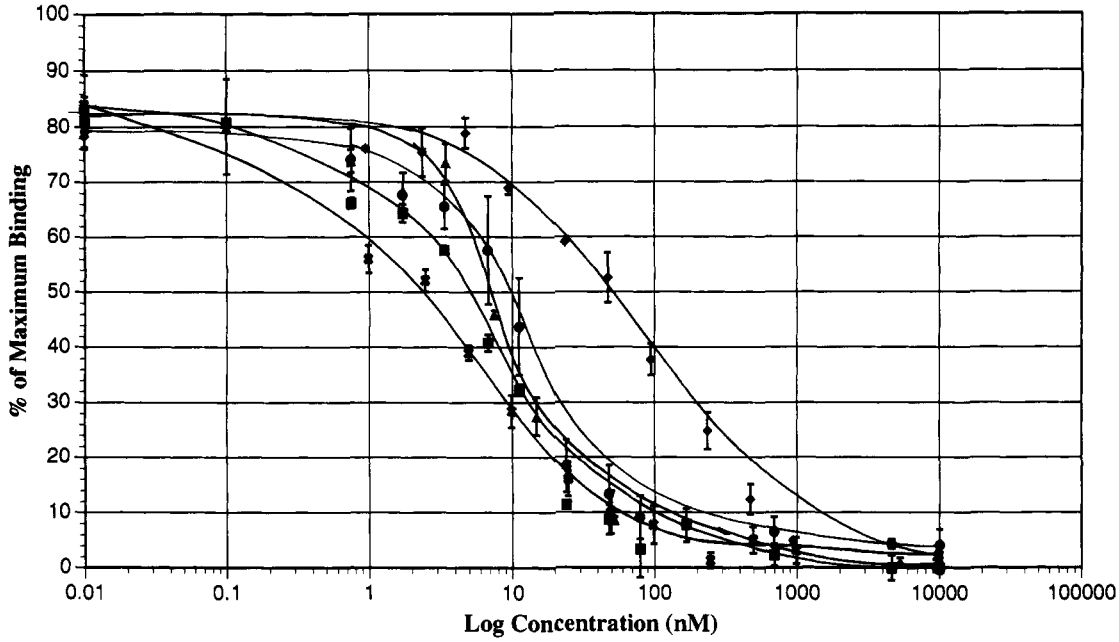


**Figure 2.** Inhibition of bindings of [$^3$H]-PbTx-3 to site 5 of the voltage-gated sodium channel by the native toxins, PbTx-1 (×), PbTx-2 (▲), PbTx-3 (■), PbTx-6 (◆), and PbTx-9 (●). Points represent the mean of triplicate determinations. Error bars represent one standard deviation. Where no error bars are seen, bars are within the symbol.

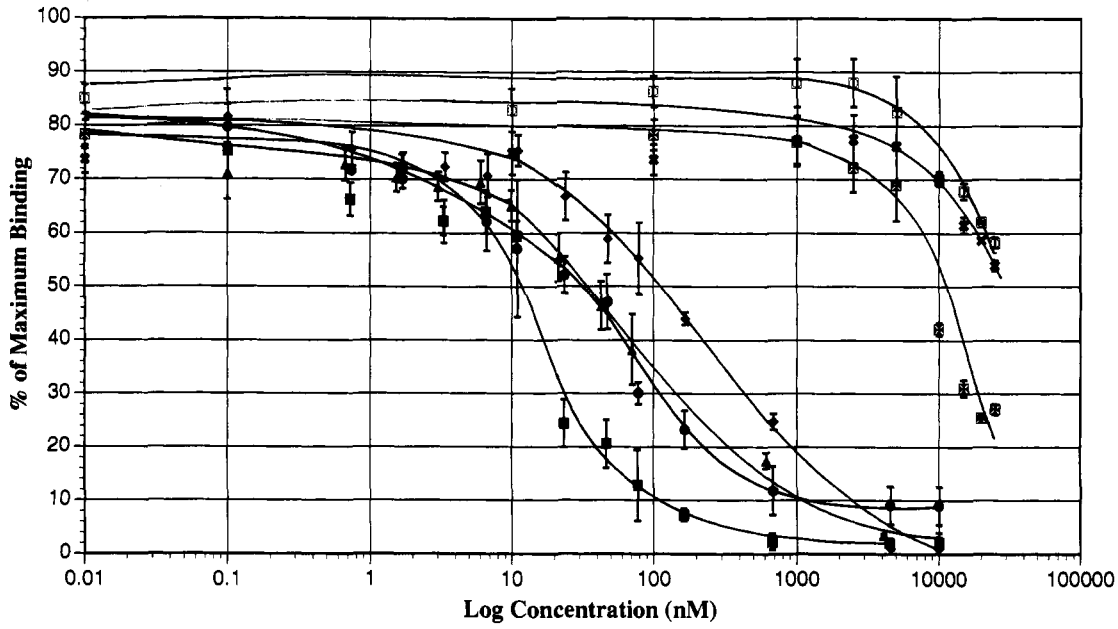


**Figure 3.** Inhibition of binding of [$^3$H]-PbTx-3 to site 5 of the voltage-gated sodium channel by the derivative toxins, 1 (■), 2 (●), 3 (▲), 4 (◆), 5 (×), 6 (□), and 7 (⊠). Points represent the mean of triplicate determinations. Error bars represent one standard deviation. Where no error bars are seen, bars are within the symbol.

relatively small differences in binding energies. It is significant that, among the native toxins having the brevetoxin B backbone, all (including the H-ring epoxide, PbTx-6) have very similar binding affinities, independent of the side chain.

Although these differences are statistically significant, they reflect small differences in binding energies ($\Delta\Delta G$). Because these differences in binding energies are so small, we do not offer any specific explanation for the observed effects. A similarly small difference in binding is observed for methyl ester 1, which has an $IC_{50}$ indistinguishable from that of PbTx-9. However, the corresponding acid, 2, is bound less tightly and is much less toxic, an effect that is not unexpected due to the overall hydrophobic nature of the natural toxins.

Reductive cleavage of the A-ring lactone completely eliminates the binding and toxicity. Since compounds 5 and 6 have suffered several changes (i.e., carbonyl removal, additional rotational freedom, etc.), it is difficult to identify which of these changes have caused this effect. Other chemical modifications of the A-ring are planned to resolve this question and will be reported in due course. Some preliminary insight into the effect of the A-ring can be gained by comparing PbTx-3 with compound 3 and PbTx-9 with compound 4. For both 3 and 4, a mixture of diastereomers at C-3 was obtained (3:1). On the basis of mechanistic considerations (radical and carbanionic intermediates), we presume that the major isomer has the methyl group equatorial. This configuration fairly closely resembles the natural toxins. It appears, therefore, that

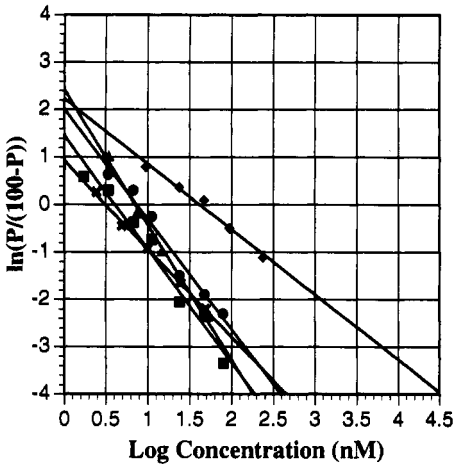


**Figure 4.** Log–logit plots of inhibition data for the native toxins PbTx-1 (×), PbTx-2 (▲), PbTx-3 (■), PbTx-6 (◆), and PbTx-9 (●).

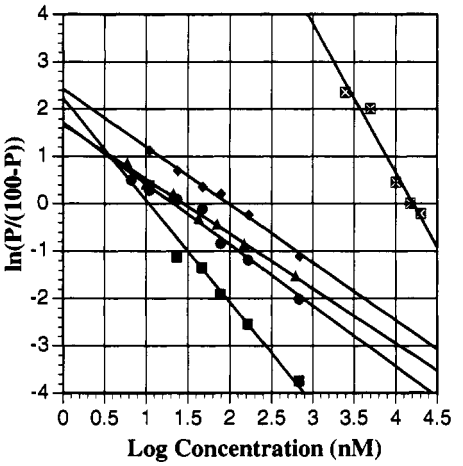


**Figure 5.** Log–logit plots of inhibition data for the derivative toxins 1 (■), 2 (●), 3 (▲), 4 (◆), and 7 (⊠).

**Table 1. Binding and Icthyotoxicity Data for Native Brevetoxins and Derivatives**

| compd | $IC_{50}$[a] (nM) | range | $LC_{50}$ (nM) | ($\sigma_{logm}$)[b] |
|---|---|---|---|---|
| PbTx-1 | 3.10 | 2.9–3.4 | 2.57 | (0.348) |
| PbTx-2 | 6.89 | 6.36–7.93 | 14.3 | (0.492) |
| PbTx-3 | 4.07 | 2.87–5.77 | 15.8 | (0) |
| PbTx-6 | 42.0 | 34.2–51.8 | 77.7 | (0.492) |
| PbTx-9 | 7.39 | 5.92–9.21 | 31.4 | (0) |
| 1 | 10.8 | 7.92–14.8 | 56.3 | (0) |
| 2 | 21.3 | 15.12–29.9 | 616 | (0.203) |
| 3 | 28.2 | 23.8–33.4 | 499 | (0.203) |
| 4 | 96.8 | 84.5–111 | 1860 | (0.492) |
| 5 | >25 000 | >25 000 | >2 μM | |
| 6 | >25 000 | >25 000 | >2 μM | |
| 7 | 17 000 | 13 200–21 900 | >2 μM | |

[a] Measured against 1.78 nM [$^3$H]-PbTx-3. [b] The range of values for $IC_{50}$ may be calculated, within 95% confidence limits, according to the equation described by Weil,[31] log $LC_{50}$ = log $m$ ± $\sigma_{logm}$.

the reduction in binding affinity caused by the A-ring double bond reduction is primarily an electronic effect, possibly caused by a decrease in basicity of the carbonyl oxygen upon reduction. Such an effect would be consistent with the presence of a hydrogen bond donor in this region of the site 5 receptor.[13]

Compound 7, having all three carbon–carbon double bonds reduced, is almost 3 orders of magnitude less strongly bound than 4, which has only two carbon–carbon bonds reduced. This large effect resulting from H-ring reduction

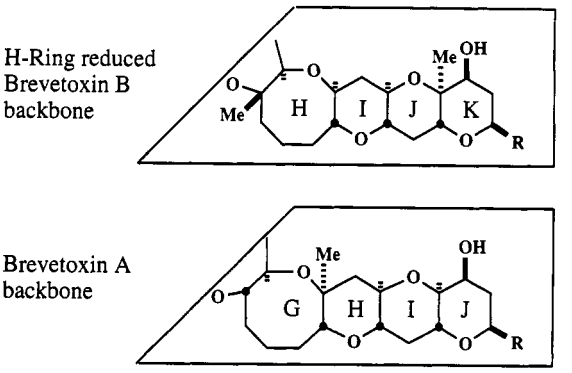


**Figure 6.**

was totally unexpected, due to the similarity of this region of the molecule to the corresponding region of brevetoxin A (Figure 6), which is the *most strongly* bound toxin of those studied. A comparison of the preferred confromation of the H-ring of brevetoxin B[12] and the G-ring of brevetoxin A[13] with the preferred conformation of the H-ring of 7 revealed a probable explanation.

Specifically, the H-ring of brevetoxin B (PbTx-2) favors the boat–chair illustrated in Figure 7 by 4.5 kcal/mol,[12b] while the corresponding (saturated!) G-ring of brevetoxin A also favors the illustrated (Figure 7) boat–chair by about 3 kcal/mol, depending on the conformation of the F-ring.[13,19] Additionally, the H-ring of PbTx-6 (the epoxide) favors a similar boat–chair by >5 kcal/mol.[20,21] In brevetoxin A, the preference for the boat–chair has been attributed to the presence of the angular methyl at the G–H ring fusion.[19b]

In contrast, modeling of the H-ring of 7[21] *reveals a preference for the crown* conformation, which is favored over the lowest boat–chair by 2.8 kcal/mol. This is consistent with Van Duyne's conclusion regarding the G-ring of brevetoxin A preference being dependent on the presence of the brevetoxin A angular methyl (G–H ring fusion) that destabilizes the crown.[19b] The corresponding position of the brevetoxin B backbone has no methyl. In the brevetoxin B backbone, the H-ring boat–chair is preferred because of the torsional constraint imposed by the double bond or the epoxide.

The effect of this conformational shift is illustrated in Figure 7. Figure 7 (top) illustrates the preferred boat–chair conformations of PbTx-2 (blue), PbTx-6 (purple), and PbTx-1 (white). The preferred crown conformation of the H-ring of 7 is shown in green. For all four, only the bonds to the adjacent G-ring (or F-ring of PbTx-1) are shown, and all are oriented such that the "tails" of the two backbones (*i.e.*, the I–K-rings of the brevetoxin B backbone and the H–J-rings of the brevetoxin A backbone) would

(19) (a) This is true for five of six brevetoxin A conformations that differ only in the G-ring. In ref 13, see conformational pairs *a/g, b/n, d/p, e/t, c/i,* and *f/x*). Van Duyne previously noted this preference for two conformations of brevetoxin A as well, although with a smaller $\Delta E$: (b) Van Duyne, G. A. *Marine Toxins: Origin, Structrre, and Molecular Pharmacology;* ACS Symposium Series 418; American Chemical Society: Washington, DC, 1990; pp 144–165.

(20) The higher energy conformation of the PbTx-2 H-ring is another boat–chair, which is inaccessible to PbTx-6 because of a severe transannular interaction betweeen the epoxide and an angular methyl. In the brevetoxin-A backbone, higher energy conformations include both crown and boat–chair conformations (see ref 13).

(21) Modeling calculations were done using the Macromodel program: Mohamade, 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.

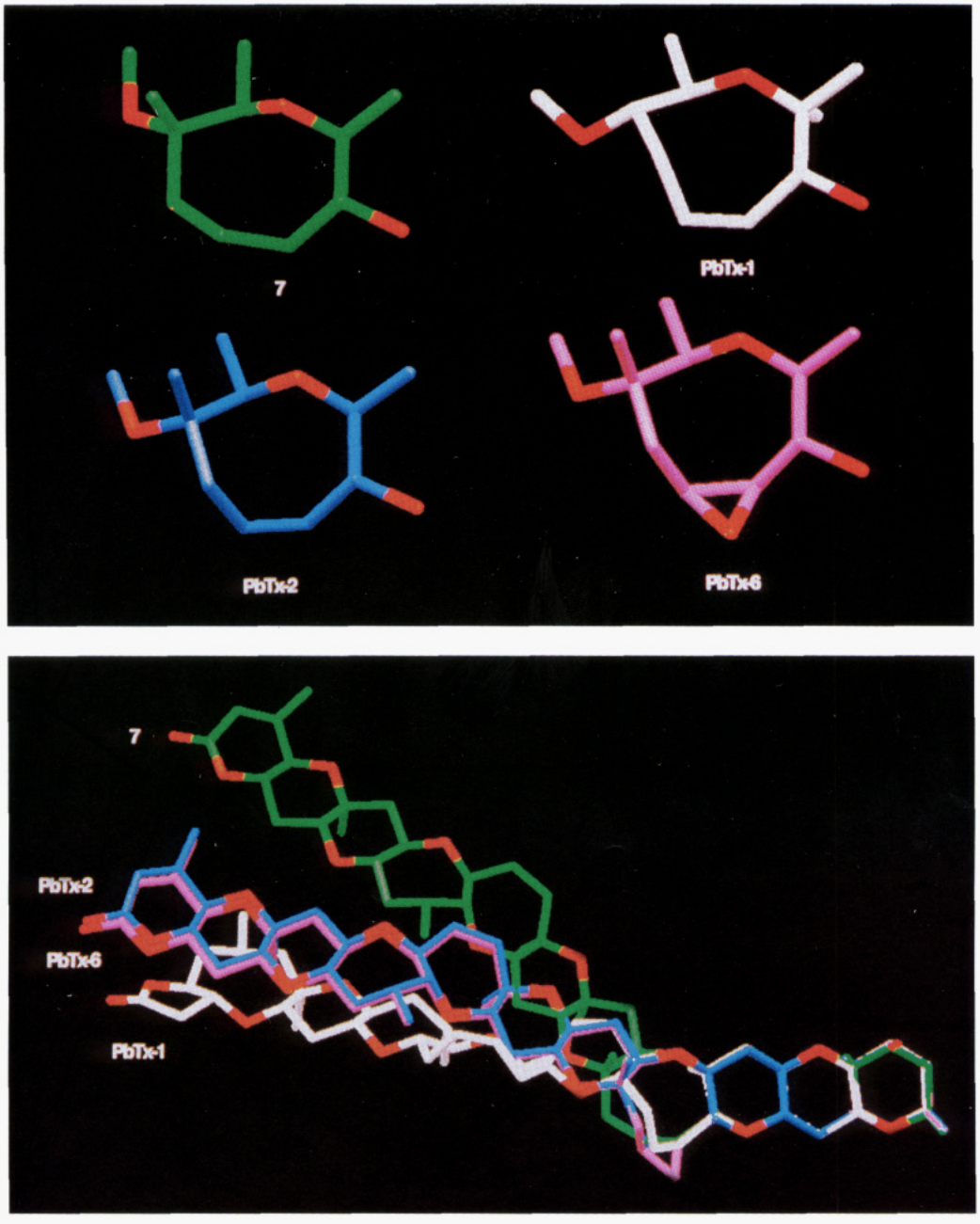


**Figure 7.** PbTx-1 (white), PbTx-2 (blue), PbTx-6 (pink), and **7** (green) in MM2*[21]-minimized conformations. (Top) the H-rings of PbTx-2, PbTx-6, and **7**, and the G-ring of PbTx-1 in their global minimum energy conformations. (Bottom) full backbone structures (*i.e.*, no side chains), with the tails of the two backbones superimposed. The conformations illustrated for the A–F rings of PbTx-1 and the A–G rings of PbTx-2, PbTx-6, and **7** correspond to the X-ray crystal structures.

be superimposed. Note that for the three brevetoxin B derivatives, the G–H angular methyl is in essentially the same position in both the crown and boat–chair conformations, suggesting that a change in the position of this methyl is not responsible for the change in binding affinity between **4** and **7**. On the other hand, note the similarity of the direction of the bonds to the G-ring for PbTx-2 (blue) and PbTx-6 (purple), and to the F-ring of PbTx-1 (white), but the clockwise shift of the corresponding bonds for **7** (green). A superimposition of the four (whole) backbones is shown in Figure 7 (bottom), where the consequence of this subtle conformational change can be seen. Whereas the lactone carbonyls of PbTx-2 (blue), PbTx-6 (purple), and PbTx-1 (white) are <3 Å apart (consistent with a similar shape in the binding site for the two backbones[13]) the carbonyl of **7** is >7 Å away from that of PbTx-2.

Because of the close structural similarities between the tails of the brevetoxin A and B backbones (see Figure 1), we believe that these regions of the toxin molecules occupy the same region of the binding site. From the results reported herein, we conclude that the geometric requirements of this region of the binding site are rather strict and that the conformation of the G-ring of bound brevetoxin A and the H-ring of brevetoxin B is the boat–chair. In the preceding paper in this issue, we compare the conformations of the two brevetoxin backbones and offer a hypothesis regarding the shape of the bound toxins.

## Experimental Section

**General.** All solvents used were HPLC grade. Brevetoxins were purified from laboratory cultures of *Ptychodiscus brevis* by a combination of chloroform/methanol extraction and TLC.[11b,22,23]

Starting materials (PbTx-2, -3, and -9) and products were routinely purified by reversed phase HPLC (85% isocratic methanol) using a Microsorb-MV, C-18 column (5 μm, 25-cm bed) and monitored by UV at 215 or 195 nM and/or refractive index. $^1$H NMR spectra were recorded in $CDCl_3$ ($CHCl_3$ internal standard) at 400 MHz. Mass spectra were run in either DCI or FAB mode. High-resolution mass spectra were obtained from the mass spectrometry facility at the University of California, Riverside.

**Oxidation of C-42 of PbTx-2 to Ester 1.** PbTx-2 (4.8 mg, 5.37 μM) was oxidized with activated $MnO_2$,[24] to the corresponding methyl ester 1, via the cyanohydrin, according to the procedure described by Corey.[14] The reaction mixture was filtered through Celite and concentrated in vacuo. The residue was taken up in water (15 mL) and extracted with ether (3 × 15 mL). The ether phase was evaporated in vacuo and the residue purified by HPLC to yield 3.624 mg (73%) of the desired product. The $^1$H NMR is shown in the supplementary material. DCI MS ($NH_3$): 925 (M + 1), 942 (M + $NH_4$), 906 (M − $H_2O$). (Attempts to obtain a high-resolution mass by FAB and DCI were unsuccessful.)

**Hydrolysis of Methyl Ester 1 To Provide Carboxylic Acid 2.** Methyl ester 1 (3.759 mg, 4.068 μM) was dissolved in 2 mL of THF/$H_2O$ (50:50). An aqueous solution of KOH (0.4 mL, 10 mg/mL) was added, and the reaction mixture was stirred at ambient temperature for 2 days. Water (1 mL) was added and the mixture extracted with ether (3 × 2 mL). The aqueous phase was acidified with 10% HCl and extracted with ethyl acetate (3 × 2 mL) and the organic phase evaporated in vacuo. This residue consisted of a mixture of two products. On the basis of NMR data, these two products appear to be the C-42 carboxylic acid with an intact A-ring lactone and the C-42 carboxylic acid with a hydrolyzed A-ring lactone. The residue was taken up in THF (1 mL), and a catalytic amount of *p*-toluenesulfonic acid was added. The mixture was stirred for 1 h and then evaporated in vacuo. The residue was taken up in water (1 mL) and extracted with ethyl acetate (3 × 2 mL). Evaporation of the solvent in vacuo yielded 3.469 mg (93%) of the crude product. Purification of the residue by HPLC provided 1.019 mg (28%) of **2**. $^1$H NMR: see supplementary material. DCI MS($NH_3$): 911 (M + 1), 929 (M + $NH_4$), 892 (M − $H_2O$). (Attempts to obtain a high-resolution mass by FAB and DCI were unsuccessful.)

**Reduction of the C-2, C-3 Double Bond of PbTx-3 to Provide 3.** Following the procedure of Hudlicky,[15] PbTx-3 (4.00 mg, 4.46 μM) and Mg (approximately 200 mg, 99.98% from Timminco metals, Haley, Ontario, Canada) were dried in vacuo over $P_2O_5$ for 18 h. Methanol (3 mL freshly distilled from $CaH_2$) was added and the mixture stirred at room temperature (cooling in an ice bath was required) under nitrogen for 2 h. HCl (10%, 10 mL) was added to dissolve the magnesium methoxide and the remaining magnesium. The reaction mixture was concentrated to approximately 5 mL and extracted with ether (3 × 15 mL). The ether phase was evaporated in vacuo and the residue purified by HPLC. The isolated material consisted of the desired product with the C-2, C-3 double bond reduced and a second product in which the double bond was reduced and the lactone opened to the methyl ester, as evidenced by mass spectral and NMR data. This mixture was taken up in THF, and a small amount of *p*-TsOH acid was added. The mixture was stirred for 1 h and then evaporated in vacuo. The residue was taken up in water (1 mL) and extracted with ethyl acetate (3 × 2 mL). Evaporation in vacuo yielded 3.256 mg (81%) of **3**. The $^1$H NMR spectrum is shown in the supplementary material. DCI MS($NH_3$): 899 (M + 1), 916 (M + $NH_4$), 880 (M − $H_2O$). HRMS (DCI): calcd for $C_{50}H_{74}O_{14}$ ($MH^+$) 899.5156, found 899.5128.

**Reduction of the C-2, C-3 Double Bond of PbTx-9 To Provide 4.** PbTx-9 (7.49 mg, 8.34 μM) was reduced according to the procedure described for the preparation of **3** to yield 1.645 mg (22%) of **4**. The $^1$H NMR spectrum is shown in the supplementary material. DCI MS($NH_3$): 901 (M + 1), 918 (M + $NH_4$), 882 (M − $H_2O$). HRMS (DCI): calcd for $C_{50}H_{76}O_{14}$ ($MH^+$) 901.5313, found 901.5323.

**Sodium Borohydride Reduction of PbTx-3 To Form 5 and 6.** PbTx-3 (3.451 mg, 3.85 μM) was dissolved in 2.5 mL of EtOH. A large excess of $NaBH_4$ (5 mg) was added in one portion. The reaction mixture was stirred at ambient temperature for 18 h. The excess $NaBH_4$ was decomposed by the careful addition of 10% HCl. The reaction mixture was concentrated in vacuo to 1 mL and extracted with $CH_2Cl_2$ (3 × 2 mL). The combined organic phases were then evaporated to dryness, and the residue was purified by HPLC. Two peaks were collected from the HPLC. The first peak was the minor product **5**, 0.755 mg (22%), and the second peak was the major product **6**, 1.042 mg (30%). Compound **5**. $^1$H NMR: see supplementary material. DCI MS($NH_3$): 880, 729. FAB MS (*m*-nitrobenzyl alcohol matrix): 901 (M + 1). HRMS (FAB): calcd for $C_{50}H_{76}O_{14}$ ($MH^+$) 901.5313, found 901.5324. Compound **6**. $^1$H NMR: see supplementary material. FAB MS (*m*-nitrobenzyl alcohol matrix): 903 (M + 1), 766, 731. HRMS (FAB): calcd for $C_{50}H_{78}O_{14}$ ($MH^+$) 903.5470, found 903.5418.

**Catalytic Reduction of PbTx-3 to Yield 7.** PbTx-3 (1.8 mg, 2.00 μM) was dissolved in *i*-PrOH (1 mL). Acetic acid (50 μL) and a catalytic amount of 10% Pd on activated carbon were added. The reaction mixture was stirred at ambient temperature under an atmosphere of $H_2$ for 24 h. The suspension was filtered through Celite and concentrated in vacuo to provide 0.986 mg (54%) of **7** which was not purified further. $^1$H NMR: see supplementary material. DCI MS ($NH_3$): 903 (M + 1), 920 (M + $NH_4$), 894 (M − $H_2O$). HRMS (DCI): calcd for $C_{50}H_{78}O_{14}$ ($MH^+$) 903.5470, found 903.5444.

**Epoxidation of the C-27, C-28 Double Bond of PbTx-2 To Provide PbTx-6.** Dimethyldioxirane was generated in a distillation apparatus, connected to a dry ice condenser, according to the procedure described by Adam[17] for a small-scale preparation. The receiving flask was charged with PbTx-2 (2.33 mg) in 5.0 mL of acetone and was cooled in an ice/salt bath. The reaction was monitored by HPLC. When all of the PbTx-2 was consumed, the acetone was evaporated in vacuo, and the residue taken up in 1.0 mL of methanol and purified by HPLC to provide 2.25 mg (95%) of PbTx-6. DCIMS ($NH_3$): 911 (M + 1), 928 (M + $NH_4$), 893 (M − $H_2O$). $^1$H and $^{13}$C NMR were identical to that reported by Shimizu.[16]

**Preparation of [$^3$H]PbTx-3.** Tritiated PbTx-3 was prepared at a specific activity of 12–15 Ci/mmol by reductive tritiation of PbTx-2 using cerium chloride–sodium borotritiide.[23,25] Specific activity was calculated using HPLC against toxin standards and liquid scintillation counting standardized to a tritium quench curve.

**Synaptosome Binding Experiments.** Synaptosomes were prepared by the method described by Dodd et al.[26] from male Sprague–Dawley rats (200–250 g). Total binding was measured using a rapid centrifugation technique described previously.[27] Nonspecific binding was measured in identical incubation tubes by adding 10 μM unlabeled PbTx-3 to completely displace all specifically bound radioactive toxin (radioactivity that cannot be displaced by excess ligand is nonspecific).[28] Specific binding was calculated by subtracting nonspecific binding from total binding. All binding experiments were performed in standard binding medium (SBM) consisting of 50 mM HEPES (pH 7.4), 130 mM choline chloride, 5.5 mM glucose, 0.8 mM magnesium sulfate, 5.4 mM potassium chloride, 1 mg/mL of bovine serum albumin, and 0.01% Emulphor EL-620, a nonionic detergent used as an emulsifier. The latter was required to solubilize the high concentration of competitor toxins. A suspension of synaptosomes (40–80 μg of total protein in 100 μL of SBM) was added to microfuge tubes containing [$^3$H]PbTx-3 (1.78 nM) and competitor (12 different concentrations ranging from 0.5 to 25

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$\mu$M) for a total final volume of 1.0 mL. After being mixed and incubated at 4 °C for 1 h, the tubes were centrifuged for 3 min at 15000*g*. Supernatant solutions were aspirated, and the remaining pellet was rapidly rinsed with 0.5 mL of cold (0 °C) SBM. The pellets were transferred to liquid scintillation vials, and bound radioactivity was counted on a Beckman LS 1801 liquid scintillation counter in 3.0 mL of Ecolume (ICN Biomedicals, Costa Mesa, CA).

**Icthyotoxicity Studies.** Fish bioassays were conducted according to the previously described method[23] using female mosquito fish, *Gambusia affinis*. *Gambusia* were equilibrated in a holding tank for a minimum of 1 week. Bioassays were conducted in triplicate at a minimum of six different concentrations of toxin (plus controls with no toxin) in 20 mL of water (artificial seawater was diluted to match the osmotic pressure of the holding tank, 30–40 mosmol, as measured on a Wescor 5100C vapor pressure osmometer). Toxins were added in 150 $\mu$L of ethanol. Mortality was assessed after 24 h. Death was defined as cessation of opercular movement (i.e., respiratory arrest). $LC_{50}$ values were calculated according to the method described by Weil.[29]

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**Supplementary Material Available:** 400-MHz $^1$H NMR spectra of compounds 1–7 (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm edition of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.