

Toxicity of Pumiliotoxin 251D and Synthetic Analogs to the Cotton Pest *Heliothis virescens*

Thomas M. Bargar,* Renee M. Lett,[†] Peter L. Johnson, James E. Hunter, C. P. Chang,[‡]
Daniel J. Pernich, Mark R. Sabol, and Michael R. Dick

Insect Management Discovery Research, DowElanco Building 306, 9410 North Zionsville Road,
Indianapolis, Indiana 46268-1053

A series of 13 simplified analogs of frog skin derived pumiliotoxin indolizidine alkaloids was prepared and evaluated for their toxicity to the larvae of the important cotton pest *Heliothis virescens*. The alkyl side chain of pumiliotoxin 251D was replaced with a variety of substituents designed to influence or restrict its conformation and its ability to act as a site of metabolic detoxification. Significantly, a substituent in the *R* configuration at the C-2' carbon of the side chain was required for toxicity. Computational studies suggested that this substituent may control the active conformation of the side chain. No structural modification led to a significant improvement in toxicity over the natural product.

Keywords: *Pumiliotoxin; insecticide; Heliothis virescens; structure-activity*

Research directed at the isolation, characterization, pharmacological profiling, and total synthesis of the amphibian alkaloids has been remarkably fruitful. A number of these natural products have become established tools and leads for pharmaceutical discovery (Daly and Spande, 1986). Our attention was directed to the indolizidine pumiliotoxin alkaloids derived from the Panamanian frog *Dendrobates pumilio* by reports that pumiliotoxin B (1) (Figure 1) binds to an apparently unique site on the voltage-dependent sodium channel to cause an inhibition of channel inactivation in neuromuscular preparations (Gusovsky et al., 1992). Pumiliotoxin B derivatives have also been investigated for their potential as cardiotoxic agents (Daly et al., 1988). The sodium channel is an established target site for commercial insecticides (Narahashi, 1992); however, we are not aware of any published investigation of the toxicity of pumiliotoxins to insects. We report here a structure-activity study of several simplified synthetic derivatives of pumiliotoxin B against the important cotton pest *Heliothis virescens*.

Chemistry. We prepared pumiliotoxin analogs that varied in the side chain (R) using some simple modifications of the Overman synthesis (Overman and Sharp, 1988a,b) as outlined in Scheme 1. Epoxide 3 or 4 (as diastereomeric mixtures) (Overman et al., 1984) was allowed to react with diethylalanes derived from alkynes 5 in nonpolar solvents to provide alkynyl alcohols 6 and 8 or 7 and 9 in generally good yields. Usually, only the isomer with the desired stereochemistry at the C-1' center was isolated. In cases where both stereoisomers were produced, the desired isomer was almost always readily separated by flash chromatography on silica gel. An occasionally troublesome side product was identified as the chlorohydrin resulting from attack of chloride ion at the C-2' center of the epoxide. Its formation could

be prevented by removal of the lithium chloride byproduct from the alane-forming reaction by filtration through Celite prior to addition of the epoxide. When the nitrogen protecting group was CBZ (R = benzyl), cleavage was effected in a step prior to cyclization to give pyrrolidines 10. We found it more expedient in some cases to subject BOC derivatives 7 directly to the conditions of the alkyne-iminium ion cyclization, which provided expected vinyl iodides 11 and 12 in generally good yields. Isolation of these materials was best accomplished with precautions to minimize exposure to light. Treatment of the vinyl iodides 11 and 12 with *sec*-butyllithium followed by protonation of the resultant vinylolithium species afforded final products 13 and 14. Iodine-lithium exchange was best effected with *sec*-butyllithium since we found the rate of exchange using *n*-butyllithium to be quite variable. Separation of C-2' side-chain stereoisomers was accomplished at either the vinyl iodide or the final product stages. All pumiliotoxin derivatives were oils which darkened gradually when stored at ambient temperature; however, they appeared to be stable indefinitely at -20 °C.

The alkyne intermediates 5 were prepared using one of four methods (A-D) as described below. Alkynes 5a, 5d, and 5h were prepared via LAH reduction of allenic bromides 16, readily available from tertiary alkynols 15 as shown in Scheme 2 (method A) (Landor et al., 1966; Crandall et al., 1968). Alkyne 5b was obtained from the reaction of TMS-acetylene with 2-chloro-2-methylhexane Scheme 3 (method B) (Negishi and Baba, 1975). Alkynes 5e, 5f, and 5i were obtained using a convenient, one-pot adaptation of the alkyne synthesis depicted in Scheme 4 (method C) (Caporusso et al., 1987). Commercially available 3-butyn-2-ol was converted in quantitative yield to mesylate 17. (*Caution: differential scanning calorimetry indicated that 17 has the potential for rapid energy release on heating; therefore, we did not attempt distillation.*) Treatment of compound 17 with an equimolar mixture of LiI and CuI in THF resulted in an 85:15 mixture of 18 and 19, respectively. This mixture was cooled to -70 °C and treated in situ with the appropriate Grignard reagent to provide a mixture of desired alkyne 5 and unwanted allene isomer 20. Alkynes were favored by at least a 3:1 ratio. We found

* Author to whom correspondence should be addressed.

[†] Present address: DuPont Agricultural Products, Stine-Haskell Research Center, P.O. Box 30, Newark, DE 19714.

[‡] Present address: 613 Nantucket Ct., Walnut Creek, CA 94598.

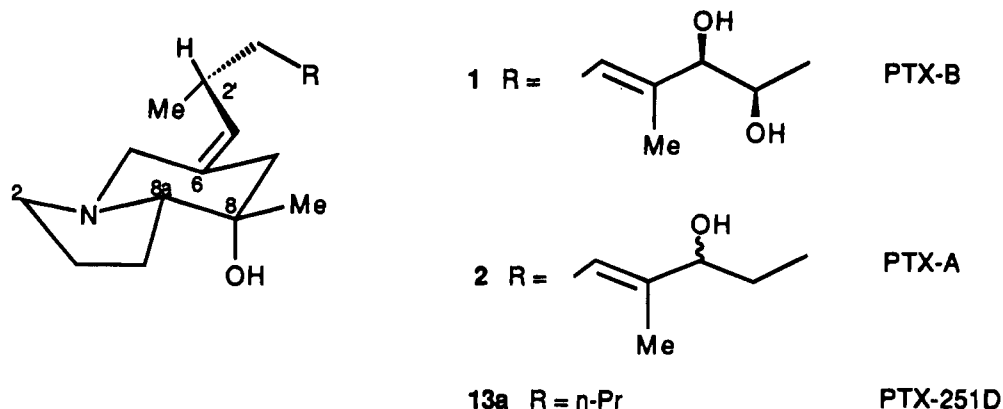
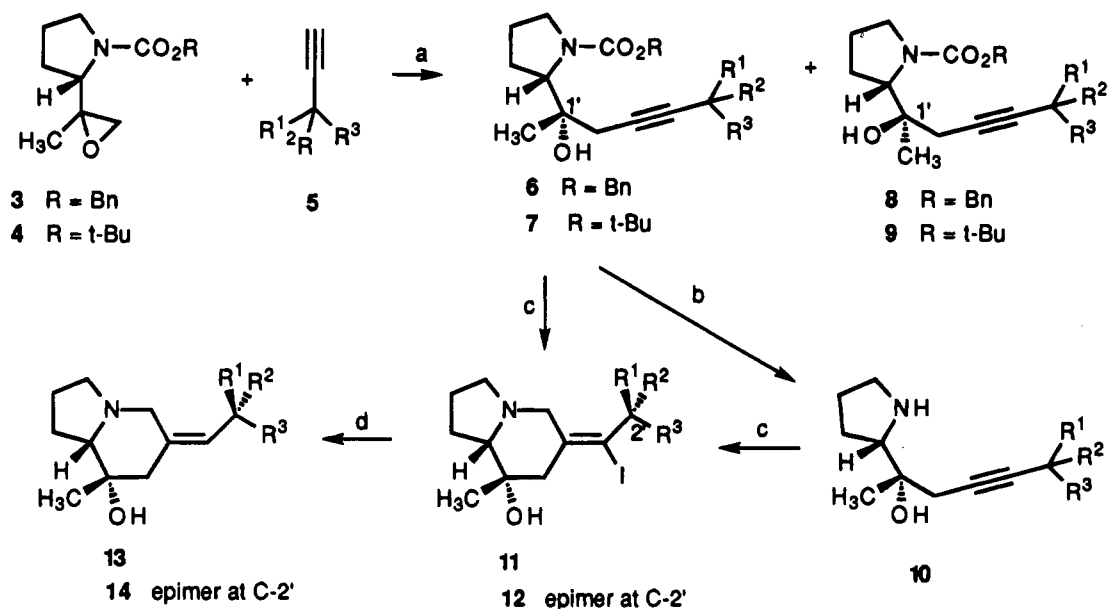


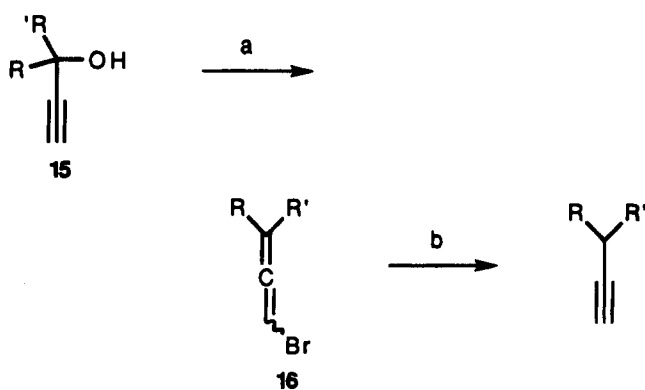
Figure 1. Structures of pumiliotoxins A, B, and 251D.

Scheme 1



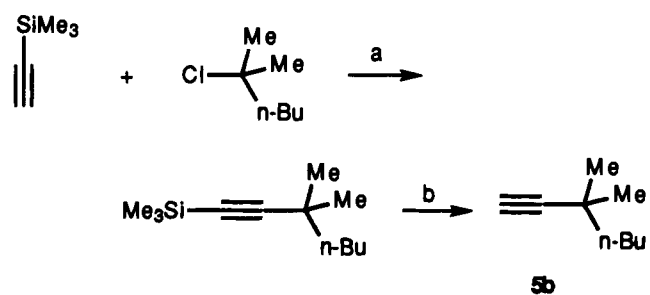
(a) $n\text{-BuLi}$, Et_2AlCl , PhCH_3 ; (b) $\text{Ba}(\text{OH})_2$, aq. DME, reflux; (c) $(\text{HCHO})_n$, CSA, NaI, aq. CH_3CN , 100°C ; (d) $s\text{-BuLi}$, Et_2O , -70°C , then CH_3OH .

Scheme 2. Method A



(a) HBr , CuBr , Cu powder. (b) LiAlH_4 , diglyme.

Scheme 3. Method B



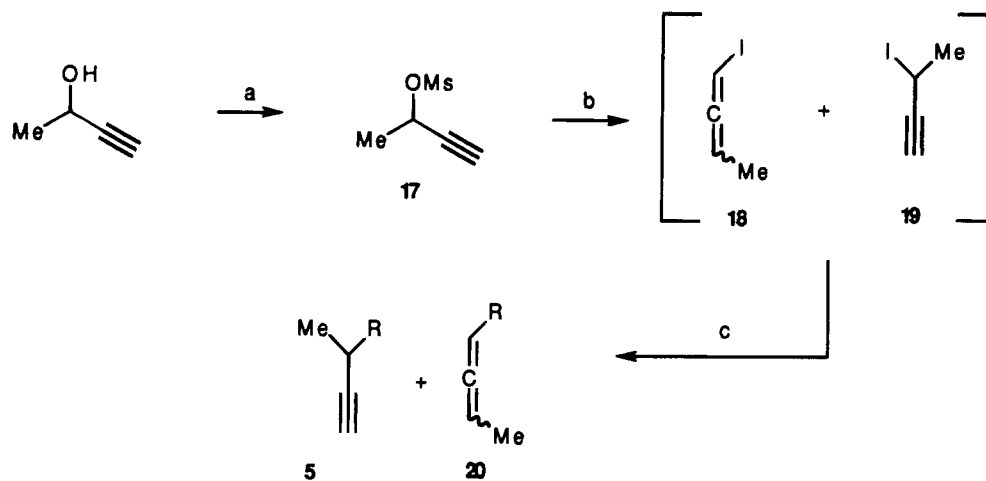
(a) $n\text{-BuLi}$, then $1/3$ equiv. AlCl_3 . (b) NaOMe , MeOH .

that separation of the allene was unnecessary and in some instances the alkyne/allene mixture was used in the next step (Scheme 1), where separation of the inert allene from the products was easily accomplished. Fluorinated alkyne **5j** was obtained using the procedure outlined in Scheme 5 (method D). Methallyl acetate was allowed to react with perfluorobutyl iodide to provide

the adduct **21** (Fuchikami and Ojima, 1984), which furnished alcohol **23** on reduction and hydrolysis. Swern oxidation of alcohol **23** gave the corresponding aldehyde which, without purification, was converted to alkyne **5j** via the intermediate dibromoolefin **24** (Van Hijfte et al., 1989; Corey and Fuchs, 1972). The remaining alkynes were available commercially.

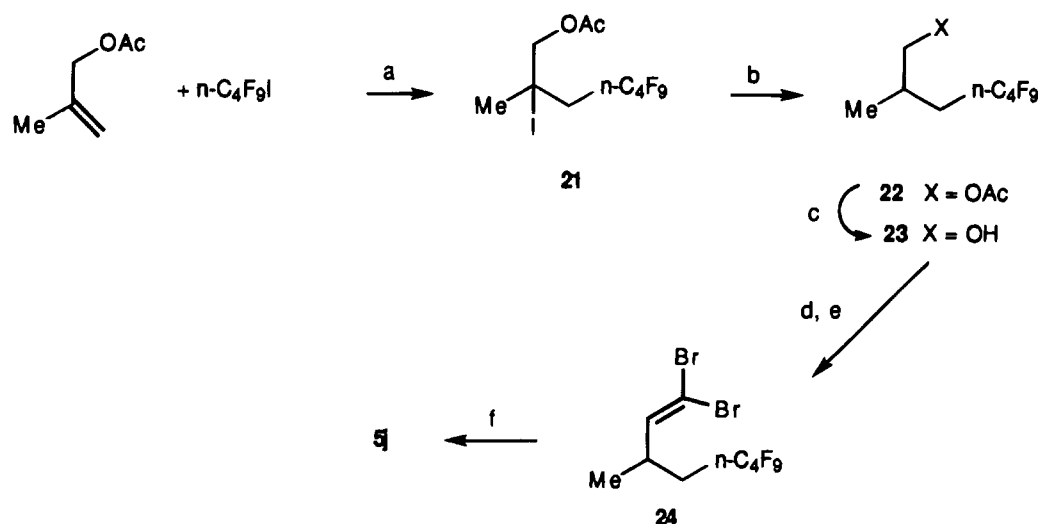
With alkynes **5** in hand, pumiliotoxin series **13** and **14** were obtained by employing Scheme 1. Pumiliotoxin

Scheme 4. Method C



(a) MsCl, Et₃N, CH₂Cl₂, -50°C; (b) 2 LiCuI₂, THF; (c) RMgBr, -70°C.

Scheme 5. Method D



(a) Fe₃(CO)₁₂, (cat.), pyridine (cat.), 60°C; (b) Bu₃SnH, AIBN (cat.), 60°C; (c) NaOH, MeOH, 35°C; (d) Swern oxidation; (e) CBr₄, Ph₃P, CH₂Cl₂; (f) Mg, THF, 65°C.

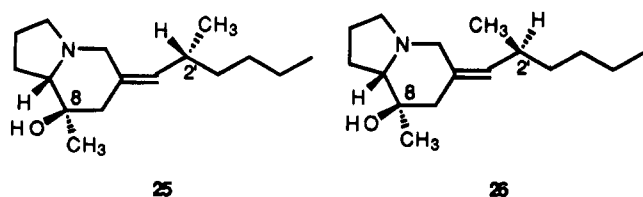
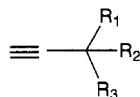


Figure 2. Structures of pumiliotoxin stereoisomers **25** and **26**.

251D stereoisomers **25** and **26** (Figure 2) were obtained by carrying "undesired" intermediate **8a** through the sequence.

Stereochemical Assignments. Pumiliotoxin 251D (**13a**) has stereogenic centers at C-8a, C-8, and C-2'. The center at C-8a, derived from L-proline, remained fixed for all analogs. Stereochemical assignments for the four pumiliotoxin 251D diastereomers **13a**, **14a**, **25**, and **26** were based on comparison with published NMR spectral data for isomers **13a** and **14a** (Overman et al., 1984). Compounds **13a** and **14a** were derived from alkyne **6a**, and compounds **25** and **26** were derived from alkyne **8a**. The most apparent difference between **13a** (2'*R* configuration) and **14a** (2'*S*) were proton chemical shifts

of 0.95 and 0.88 ppm for the C-2' methyl group, respectively. Compounds **25** and **26** exhibited C-2' methyl chemical shifts of 0.93 and 0.88 ppm and so were assigned the 2'*R* and 2'*S* configurations, respectively. The only significant difference between the C-8 epimers **25** and **26** versus **13a** and **14a** was that the signals at 3.06 and 3.04 ppm for **25** and **26**, attributed to H-2 α , were cleanly resolved triplets, while the signals at 3.03 and 3.02 for **13a** and **14a** were multiplets. Alkynols **6a** and **8a** also exhibited a similar spectral distinction in that the multiplet at approximately 4.1 ppm (attributed to the pyrrolidine methine proton) appeared as a broad hump, whereas the corresponding proton in **8a** appeared as a sharper, distorted triplet. In addition, the 1'-methyl group in **6a** appeared at 1.12 ppm, whereas the corresponding methyl group in **8a** occurred at 1.28 ppm. These NMR distinctions, although subtle, were consistent over all of the analogs prepared via epoxides **3** and **4**. Stereochemical assignments for all analogs were further supported by a consistent pattern of bioactivity in which analogs assigned the "natural" 8*S*,2'*R* configurations on the basis of NMR data were always more active than their diastereomers (*vide infra*). In addition,

Table 1. Alkyne Intermediates 5 Used in Pumiliotoxin Synthesis

alkyne	R ₁	R ₂	R ₃	bp (°C)	method
5a	H	Me	<i>n</i> -Bu	106–108 ^a	A
5b	Me	Me	<i>n</i> -Bu	102–118 ^b	B
5c	H	H	<i>n</i> -Bu		c
5d	H	Me	<i>n</i> -C ₆ H ₁₃	150–155	A
5e	H	Me	<i>n</i> -C ₈ H ₁₇	76–78/5 mmHg ^d	C
5f	H	Me	cyclo-C ₆ H ₁₁	56–62/5 mmHg ^e	C
5g	H	(CH ₂) ₅			c
5h	H	<i>n</i> -Bu	<i>n</i> -Bu	63–70/5 mmHg	A
5i	H	Me	CH ₂ CH ₂ <i>t</i> -Bu	135–145	C
5j	H	Me	CH ₂ <i>n</i> -C ₄ F ₉	100–110	D
5k	H	H	<i>n</i> -C ₆ H ₁₃		c

^a Okhlobystin et al. (1962). ^b Purity 75% by gas chromatography. ^c These alkynes were obtained from commercial sources. ^d 7:1 alkyne to allene ratio. See Materials and Methods. ^e 3:1 alkyne to allene ratio.

we observed that the C-2'*R* isomers always had a lower R_f on TLC than the corresponding C-2'*S* isomers.

MATERIALS AND METHODS

Reference samples of pumiliotoxins A, B, and 251D were obtained from Professor L. E. Overman (University of California, Irvine).

Synthesis. Nuclear magnetic resonance spectra were recorded on a Varian EM-390 (90 MHz), a Varian XL-200 (200 MHz), or a Bruker Aspect 3000 (400 MHz) spectrometer. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane unless otherwise indicated. Infrared spectra were obtained using either a Nicolet 5DXC-FT-IR or Beckman Acculab 3 spectrometer. Mass spectra were obtained using a Hewlett-Packard 5995 GC/MS. High-resolution mass spectra (HRMS) were obtained using facilities at the University of California, Berkeley. Combustion analyses were performed by the staff of the Molecular Spectroscopy Group at DowElanco and were within 0.4% of the theoretical values unless otherwise noted. Compounds for which elemental composition was determined by HRMS were at least 95% pure by GC and NMR unless otherwise indicated.

Method A: Preparation of Alkynes 5a, 5d, and 5h. The preparation of 3-*n*-butyl-1-heptyne (**5h**) is illustrative. A 500 mL, four-neck round-bottom flask equipped with an addition funnel, mechanical stirrer and thermometer was charged with 7.6 g (0.19 mol, 95%) of LiAlH₄, followed by 150 mL of dry THF. To this rapidly stirred suspension, at 0 °C, under N₂, was added dropwise 57.8 g of the allenic bromide **16h** (0.15 mol), while the temperature was kept below 10 °C. Allenic bromide **16h** was prepared from the corresponding tertiary alkynol using the literature method (Landor et al., 1966). The mixture was then allowed to warm to room temperature. The reaction was complete after 21 h (GC analysis). The reaction mixture was cooled to 0 °C and quenched by the careful dropwise addition of 100 mL of saturated NH₄Cl, and again the temperature was kept below 10 °C. The resultant mixture was filtered through Celite and washed with pentane, and the phases were separated. The aqueous phase was extracted with pentane (2 × 100 mL). The combined organics were washed with saturated NH₄Cl (2 × 100 mL), H₂O (1 × 100 mL), and saturated NaCl (1 × 100 mL), dried (K₂CO₃), filtered, and concentrated. Distillation gave 23.67 g of the desired product as a colorless liquid (62% yield): bp 63–70 °C (5 mmHg); ¹H NMR (CDCl₃) δ 2.36–2.24 (m, 1H, C≡CHCH₃), 2.03 (d, 1H, *J* = 2.4 Hz, C≡CH), 1.54–1.22 (m, 12H, 6 × CH₂), 0.90 (t, 6H, *J* = 7.2 Hz, 2 × CH₃); MS (EI, 70 eV) *m/z* 137 (M – CH₃, 1), 123 (1), 109 (11), 95 (90), 81 (100), 67 (72), 54 (60); IR (neat) cm⁻¹, 3313, 2958, 2932, 2873, 2860, 2113, 1467, 1459, 1379. Alkyne **5a** was prepared according to the same method used

to prepare **5h** with the exception that the reduction solvent was diglyme instead of THF and that the crude product was steam distilled directly from the water-quenched reaction mixture.

Method B: 3,3-Dimethyl-1-heptyne (5b). To a solution of 11.3 mL (80 mmol) of trimethylsilylacetylene in 40 mL of hexane in an ice bath under N₂ was added dropwise 32.0 mL of 2.5 M *n*-BuLi/hexane, while the temperature was kept below 15 °C. A white precipitate formed. After 15 min, 3.55 g (26.7 mmol) of powdered AlCl₃ was added all at once and the mixture was stirred in the ice bath for 1.5 h and then diluted with 150 mL of CH₂Cl₂. To this mixture was added 10.12 g (75 mmol) of 2-chloro-2-methylhexane dropwise over 15 min. The mixture became bright yellow and then faded to brown. The mixture was poured onto a mixture of ice and 1 N HCl and extracted with two portions of CH₂Cl₂. The CH₂Cl₂ solution was washed with saturated NaCl, dried over MgSO₄, and concentrated at 1 atm to about 12 g of a yellow liquid. Analysis by GC showed about 35% of the mixture was the desired product, 1-trimethylsilyl-3,3-dimethyl-1-heptyne. A small aliquot was bulb-to-bulb distilled (103 °C at 25 mmHg) for characterization: ¹H NMR (CDCl₃) δ 1.2–1.4 (m, 6H, 3 × CH₂), 1.15 (s, 6H, 2 × CH₃), 0.91 (distorted t, 3H, CH₃), 0.13 [s, 9H, (CH₃)₃Si]; MS (70 eV) *m/z* 196 (2, M⁺), 181 (35, M – CH₃), 140 (13, M – C₄H₈), 139 (18, M – C₄H₉), 123 (15, M – SiMe₃), 122 (22), 109 (11), 97 (79, Me₃Si^{≡+}), 73 (100, Me₃Si⁺). The crude silylacetylene was dissolved in 150 mL of CH₃OH and treated with 25 mL of a 25% solution of NaOCH₃ in CH₃OH for 2.5 h at 23 °C. The mixture was poured into water and twice extracted with pentane. The combined pentane layers were washed with water, dried over K₂CO₃, and distilled through a 15 cm Vigreux column. The fraction boiling 102–119 °C (1.13 g) was collected. Analysis by GC showed this material to be about 75% pure. ¹H NMR (CDCl₃) δ 2.07 (s, 1H H-C≡), 1.1–1.5 (m, 6H, 3 × CH₂), 1.16 (s, 6H, 2 × CH₃), 0.88 (distorted t, 3H, CH₃); MS (EI, 70 eV) *m/z* 109 (16, M – CH₃), 95 [18, [H₂C=C(CH₃)₂CH=CH₂]⁺], 82 (25), 81 (21), 68 (25), 67 (100, M – C₄H₉).

Method C: General Procedure for Preparation of Alkynes 5e, 5f, and 5i. 3-Butyn-2-yl methanesulfonate (**17**) was added to 2.2 equiv of LiCuI₂ under N₂ in THF at 20 °C. After 1 h, conversion to a 5:1 mixture of allenic and propargyl iodides was complete as monitored by gas chromatography. To the cooled mixture (–65 °C) was added 1.1 equiv of the appropriate Grignard reagent in THF. The mixture was then poured without prior warming into saturated NH₄Cl solution, allowed to warm to ambient temperature, filtered through Celite, and extracted with pentane. The products were isolated by distillation as alkyne–allene mixtures which predominate in desired alkyne.

Method D: Preparation of Alkyne 5j. This alkyne was obtained through the following sequence.

1-Acetoxy-2-methyl-4,4,5,5,6,6,7,7,7-nonafluoroheptane (22). A mixture of 16.42 g (35.70 mmol) of **21** (Fuchikami and Ojima, 1984) and 200 mg (1.22 mmol) of azobis(isobutyronitrile) under N₂ was heated in a 60 °C oil bath and treated dropwise, via a syringe pump, with 13 mL (48.33 mmol) of tributyltin hydride over 4 h. After the addition was complete, the desired product was distilled away from the crude reaction mixture (80–85 °C, 30 mmHg), giving 8.79 g (74%) of **22** as a clear, colorless oil: ¹H NMR (CDCl₃) δ 3.99 (m, 2H, H-1), 2.34 (m, 2H, H-3), 2.25–1.75 (m, 1H, H-2), 2.08 [s, 3H, C-1 (O)CCH₃], 1.12 (d, *J* = 6.2 Hz, 3H, C-2 CH₃); ¹⁹F NMR (90 MHz, CDCl₃, ref C₆F₆) δ 36.1 (m, 2F), 37.6 (m, 2F), 49.0 (m, 2F), 81.0 (tt, *J* = 11, 3 Hz, 3F); MS (EI, 70 eV) *m/z* 291 (4, M⁺ – 42), 273 (6), 73 (100), 61 (69); IR (neat) cm⁻¹ 1751, 1233, 1167, 1135, 1046, 718. Anal. Calcd for C₁₀H₁₁F₉O₂: C, 35.94; H, 3.32. Found: C, 35.58; H, 3.31.

2-Methyl-4,4,5,5,6,6,7,7,7-nonafluoroheptan-1-ol (23). A solution of 57.56 g (0.172 mol) of **22** in 110 mL of MeOH was treated with 30 mL of 10% NaOH. The mixture was heated in a 35 °C bath for 3 h, then taken up in 200 mL of H₂O, and extracted with pentane (2 × 100 mL). The combined organic phases were dried (MgSO₄) and concentrated in vacuo. The crude material was purified by fractional distillation (80–82 °C, 25 mmHg), giving 39.81 g (79%) of **23** as a colorless oil:

^1H NMR (CDCl_3) δ 3.56 (m, 2H, H-3), 2.54–1.73 (m, 3H, H-2 + H-3), 1.66 (s, 1H, OH), 1.10 (d, $J = 6.6$ Hz, 3H, CH_3); ^{19}F NMR (90 MHz, CDCl_3 , ref C_6F_6) δ 36.5 (m, 2F), 37.9 (m, 2F), 49.2 (m, 2F), 81.2 (tt, $J = 11$, 3 Hz, 3F); MS (EI, 70 eV) m/z 256 (100, $\text{M}^+ - 35$), 239 (76), 73 (53), 69 (39); IR (neat) cm^{-1} 3350, 1221, 1134, 1047. Anal. Calcd for $\text{C}_8\text{H}_9\text{F}_9\text{O}$: C, 32.89; H, 3.11. Found: C, 32.98; H, 3.10.

1,1-Dibromo-3-methyl-4,4,5,5,6,6,7,7,7-nonafluorooct-1-ene (24). A 2 L three-neck round-bottom flask equipped with an addition funnel and overhead stirrer was charged with 26 mL (0.298 mol) of oxalyl chloride and 500 mL of CH_2Cl_2 . The mixture was cooled in a dry ice/2-propanol bath, and 27 mL (0.382 mol) of DMSO in 50 mL of CH_2Cl_2 was added dropwise at such a rate that the internal reaction temperature was maintained below -62 °C. After 10 min, 34.87 g (0.119 mol) of alcohol **23** in 50 mL of CH_2Cl_2 was added at such a rate that the reaction temperature was kept below -70 °C. The mixture was stirred for an additional 20 min, and 130 mL (0.932 mol) of Et_3N was added. After 2 h, the mixture was warmed to ambient temperature, stirred for an additional 1 h, and then quenched with 200 mL of H_2O . The layers were separated, and the organic phase was washed with 10% HCl (200 mL) and dried (MgSO_4). The crude product mixture, which was concentrated from 1.2 L to 200 mL, was carried on to the next step.

An ice bath-cooled solution containing 79.0 g (0.237 mol) of CBr_4 in 350 mL of CH_2Cl_2 was treated with 127.55 g (0.487 mol) of triphenylphosphine in portions at such a rate that the internal temperature was maintained below 20 °C. The mixture was stirred for 10 min prior to the addition of the crude aldehyde prepared above. During the addition the reaction mixture was kept below 10 °C. After 3 h, the mixture was poured into 1 L of pentane and filtered. The precipitate was washed with pentane. The filtrate was concentrated in vacuo and taken up in the minimum amount of CH_2Cl_2 required to dissolve the remaining material, then poured into pentane, filtered, etc. The process was repeated three times. The crude dibromide was purified by vacuum distillation (bp 64 – 66 °C, 5 mmHg), yielding 35.23 g (66%) of **24** as a colorless oil: ^1H NMR (CDCl_3) δ 6.28 (d, $J = 9.3$ Hz, 1H, H-2), 3.01 (m, 1H, H-3), 2.13 (m, 2H, H-4), 1.17 (d, $J = 6.8$ Hz, 3H, C-3 CH_3); ^{19}F NMR (90 MHz, CDCl_3 , ref C_6F_6) δ 36.1 (m, 2F), 37.3 (m, 2F), 49.8 (m, 2F), 81.0 (tt, $J = 11$, 3 Hz, 3F); MS (EI, 70 eV) m/z 446 (49, M^+), 444 (100, M^+), 442 (50, M^+), 431 (24), 367 (91), 365 (95), 213 (76); IR (neat) cm^{-1} 1234, 1223, 1135. Anal. Calcd for $\text{C}_9\text{H}_7\text{Br}_2\text{F}_9$: C, 24.24; H, 1.58. Found: C, 24.24; H, 1.56.

3-Methyl-4,4,5,5,6,6,7,7,7-nonafluorooct-1-yne (5j). A 50 mL, three-neck round-bottom flask equipped with a reflux condenser and an overhead stirrer was charged with 390 mg (16.04 mmol) of Mg turnings and 5 mL of THF. The mixture was heated to reflux. Several crystals of I_2 were added, followed by a dropwise addition of 4.075 g (9.41 mmol) of **24** in 25 mL of THF. The mixture was heated to reflux for an additional 2 h and then was cooled and taken up in 50 mL of hexane. The crude product mixture was filtered through a pad of silica gel and washed with Et_2O . The product was isolated by fractional distillation (100–110 °C) giving 593 mg (23%) of **5j** as a clear colorless oil: ^1H NMR (CDCl_3) δ 2.95 (m, 1H, H-3), 2.57–2.00 (m, 2H, H-4), 2.13 (d, $J = 2.0$ Hz, 1H, H-1), 1.35 (d, $J = 6.9$ Hz, CH_3); ^{19}F NMR (90 MHz, CDCl_3 , ref C_6F_6) δ 36.0 (m, 2F), 37.2 (m, 2F), 49.0 (m, 2F), 80.8 (tt, $J = 11$, 3 Hz, 3F).

Pumiliotoxin Synthesis. The experimental procedures used in the sequence of Scheme 1 are illustrated by the preparation of pumiliotoxin 251D (**13a**). The synthesis of pumiliotoxin analog **13j** employed the BOC protected pyrrolidine **7j**, which obviated the need to isolate cyclization precursor **10j**.

Benzyl (S)-2-(1'-Hydroxy-1',5'-dimethyl-3'-nonyne)-1-pyrrolidinecarboxylate (6a) and (8a). To a solution of 3-methyl-1-heptyne (**5a**) (3.9 g, 35 mmol) in toluene (48 mL) at 0 °C was added *n*-butyllithium (12 mL of 2.5 M, 30 mmol) under a N_2 atmosphere. A gel formed, and the flask had to be shaken manually break up the gel. After 15 min at 0 °C, diethylaluminum chloride (17 mL of 1.8 M solution in toluene, 30 mmol)

was added. The gel was consumed and LiCl precipitated out, making the solution easier to stir. After 1 h at 0 °C, the epoxide **3** (4.0 g, 15 mmol) in toluene (8 mL) was added. The solution was allowed to stir for an additional 15 min at 0 °C and then saturated aqueous NH_4Cl was carefully added. The aqueous phase was separated and extracted with CH_2Cl_2 . The combined organic phases were dried (Na_2SO_4), concentrated (vacuum), and chromatographed on silica gel with 10% EtOAc /hexane. Separation of the product isomers gave 3.26 g of the 1'S isomer and 1.00 g of the 1'R isomer in 75% yield as pale yellow oils. **6a**: ^1H NMR (CDCl_3) δ 7.35 (s, 5H, Ph), 5.28 (br s, 1H, OH), 5.14 (s, 2H, CH_2Ph), 4.1–4.3 (m, 1H, H-2), 3.65–3.85 (m, 1H, H-5b), 3.2–3.4 (m, 1H, H-5a), 2.37 (br s, 2H, $\text{CH}_2\text{C}\equiv\text{C}$), 2.0–2.2 (m, 1H, H-5'), 1.6–2.0 (m, 4H, H-3 + H-4), 1.15–1.50 (m, 6H, $3 \times \text{CH}_2$), 1.13 (d, 3H, $J = 5.0$ Hz, CH_3CH), 1.12 (s, 3H, CH_3CO), 0.88 (t, 3H, $J = 6.5$ Hz, terminal CH_3). **8a**: ^1H NMR (CDCl_3) δ 7.35 (s, 5H, Ph), 5.5 (br s, 1H, OH), 5.14 (s, 2H, CH_2Ph), 3.9–4.1 (m, 1H, H-2), 3.65–3.85 (m, 1H, H-5b), 3.2–3.4 (m, 1H, H-5a), 2.35 (br s, 2H, H-2'), 2.0–2.2 (m, 1H, H-5'), 1.6–2.0 (m, 4H, H-3 + H-4), 1.1–1.5 (m, 6H, $3 \times \text{CH}_2$), 1.28 (s, 3H, CH_3CO), 1.11 (d, 3H, $J = 6.7$ Hz, $\text{CH}_3\text{-CH}$), 0.88 (t, 3H, $J = 6.0$ Hz, terminal CH_3).

(2S,2'S)-2-(2'-Pyrrolidinyl)-6-methyl-4-decyn-2-ol (10a). To the carbamate **6a** (2.0 g, 5.4 mmol) in 1,2-dimethoxyethane (112 mL) and water (76 mL) was added $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (8.5 g, 27 mmol). The solution was heated to reflux under N_2 for about 30 h. After the solution cooled to room temperature, CO_2 gas was bubbled through the reaction mixture for 45 min to precipitate Ba salts. The slurry was filtered through a pad of Celite and concentrated under vacuum. The resultant residue was taken up in CH_2Cl_2 , dried (Na_2SO_4), filtered, and concentrated again to remove the last traces of water. Chromatography on a plug of silica gel placed in a fritted glass funnel was accomplished by first eluting with 250 mL of Et_2O and second eluting with 350 mL of 2% $\text{NH}_4\text{OH}/18\%$ methanol in CHCl_3 . After concentration, the residue was again taken up in CH_2Cl_2 , dried (Na_2SO_4), and concentrated to remove the aqueous NH_4OH . The product was obtained as a colorless oil (1.00 g, 78% yield): ^1H NMR (CDCl_3) δ 3.31 (distorted t, 1H, H-2'), 2.8–3.1 (m, 3H, H-5' + NH), 2.3–2.5 (m, 1H, H-6), 2.31 (br s, 2H, $\text{CH}_2\text{C}\equiv\text{C}$), 1.6–1.9 (m, 4H, H-3' + H-4'), 1.2–1.5 (m, 6H, $3 \times \text{CH}_2$), 1.19 (s, 3H, C-2 CH_3), 1.11 (d, 3H, $J = 6.7$ Hz, C-6 CH_3), 0.88 (t, 3H, $J = 7.0$ Hz, terminal CH_3).

(8S,8aS)-8-Hydroxy-8-methyl-6(E)-[1'-iodo-2'(RS)-methylhexylidene]-1-azabicyclo[4.3.0]nonane (11a) and (12a). A glass tube fitted with a Kontes Teflon valve was charged with the amino alcohol **11a** (1.0 g, 4.2 mmol), camphorsulfonic acid (0.98 g, 4.2 mmol), NaI (6.3 g, 42 mmol), and paraformaldehyde (0.25 g, 8.4 mmol) in water (14 mL) and CH_3CN (4 mL). After a purge with N_2 , the tube, which contained a small stir bar for even mixing, was heated in an oil bath at 100 °C. After 3 h, the reaction was complete (determined by GC analysis) and the tube was cooled to room temperature. The reaction mixture was taken up in CH_2Cl_2 and saturated aqueous NaHCO_3 . The aqueous layer was separated and extracted with CH_2Cl_2 . The combined organic phases were dried (Na_2SO_4) and concentrated (vacuum), and the residue was taken up in Et_2O . The Et_2O solution was filtered through a plug of silica gel in a fritted glass funnel to remove darkly colored solid impurities. After concentration of the solvent, the product was obtained as a colorless oil (1.12 g, 71% yield) which turned yellow on standing. A portion of the crude product was separated into the C-2' diastereomers by flash chromatography on silica gel using 0.5% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ to elute first **12a** (2'S isomer), followed by **11a** (2'R isomer). **12a**: ^1H NMR (CDCl_3) δ 4.08 (dd, 1H, $J = 12.2$, 1.5 Hz H-5a), 3.00 (dd, 1H, $J = 14.4$, 1.7 Hz, H-2a), 2.64 (br s, 1H, OH), 2.48 (d, 1H, $J = 12.2$ Hz, H-5b), 2.1–2.4 (m, 3H, H-7 + H-2'), 2.04 (d, 1H, $J = 14.4$ Hz, H-5b), 2.02 (m, 1H, H-8a), 1.6–1.9 (m, 4H, H-3 + H-4), 1.0–1.5 (m, 6H, $3 \times \text{CH}_2$), 1.15 (s, 3H, C-8 CH_3), 0.92 (d, 3H, $J = 6.4$ Hz, C-2' CH_3), 0.88 (t, 3H, $J = 7.4$ Hz, terminal CH_3); MS (EI, 70 eV) m/z 377 (6, M^+), 320 (7), 250 (100), 84 (36), 83 (21), 79 (16), 77 (12), 70 (93), 55 (39); HRMS m/z 317.1215, calcd 317.1216 for $\text{C}_{16}\text{H}_{28}\text{INO}$. The ^1H NMR spectrum of the 2'R isomer **11a** was virtually identical to that of **12a** with the

exception that the C-2' CH₃ group had a chemical shift of 1.00 ppm ($J = 6.4$ Hz).

(8*S*,8*aS*)-8-Hydroxy-8-methyl-6(*Z*)-[2'(*RS*)-methylhexylidene]-1-azabicyclo[4.3.0]nonane (**13a** and **14a**). A solution of the iodides **11a** and **12a** (0.98 g, 2.6 mmol) in Et₂O (10 mL) was cooled to -78 °C (dry ice/2-propanol bath) and treated with *n*-butyllithium (3.1 mL of 2.5 M, 7.8 mmol) under N₂. The bath was allowed to slowly warm to -20 °C and then recooled to -78 °C. Methanol (0.5 mL) was added to protonate the anionic species, and the reaction mixture was allowed to warm to room temperature. Methylene chloride and saturated aqueous NaCl were added and the two layers separated. After extraction of the aqueous phase with CH₂Cl₂, the combined organic phases were dried (Na₂SO₄) and concentrated (vacuum). Flash chromatography (silica gel, eluting with 0.03% NH₄OH/0.6% CH₃OH/CHCl₃) separated the two diastereomers, which were further purified by bulb-to-bulb distillation (100–110 °C/0.5 mmHg) to yield **13a** (lower *R_f* isomer, 0.28 g) and **14a** (0.27 g) in 84% yield. ¹H NMR for **3** (CDCl₃) δ 5.01 (d, 1H, $J = 9.4$ Hz, H-1'), 3.76 (d, 1H, $J = 11.8$ Hz, H-5α), 2.95–3.10 (m, 1H, H-2α), 2.64 (s, 1H, OH), 2.2–2.4 (m, 1H, H-2'), 2.32 (d, 1H, $J = 11.8$ Hz, H-5β), 2.1–2.2 (m, 1H, H-2β), 2.11 (s, 2H, H-7), 1.9–2.0 (m, 1H, H-8a), 1.6–1.8 (m, 4H, H-3 + H-4), 1.1–1.4 (m, 6H, 3 × CH₂), 1.11 (s, 3H, C-8 CH₃), 0.95 (d, 3H, $J = 6.6$ Hz, C-2' CH₃), 0.85 (t, 3H, $J = 6.7$ Hz, terminal CH₃); MS (EI, 70 eV) *m/z* 251 (6, M⁺), 250 (3), 236 (2), 208 (7), 206 (4), 194 (8), 176 (4), 166 (58), 112 (11), 84 (17), 72 (18), 70 (100), 55 (15); HRMS (EI) *m/z* 251.2255, calcd 251.2249 for C₁₆H₂₉NO. The ¹H NMR spectrum of **13a** was virtually the same as that for **14a** with the exception that the chemical shift of the C-2' methyl was at 0.88 ppm ($J = 6.7$ Hz); HRMS (EI) *m/z* 251.2255, calcd 251.2249 for C₁₆H₂₉NO.

(8*R*,8*aS*)-8-Hydroxy-8-methyl-6(*Z*)-[2'(*RS*)-methylhexylidene]-1-azabicyclo[4.3.0]nonane (**25** and **26**). These compounds were obtained as oils from **8a** by the same reaction sequence used to prepare **13a** and **14a**. Separation of C-2' diastereomers was effected by flash chromatography on silica gel, eluting with 0.1% NH₄OH/1% CH₃OH/CHCl₃. Compound **25** eluted first: ¹H NMR (CDCl₃) δ 4.94 (d, 1H, $J = 9.8$ Hz, H-1'), 3.73 (d, 1H, $J = 12.1$ Hz, H-5α), 2.32 (d, 1H, $J = 12.1$ Hz, H-5β), 2.2–2.5 (m, 1H, H-2'), 1.95–2.2 (m, 4H, H-7 + H-8a + H-2β), 1.4–1.9 (m, 5H, OH + H-3 + H-4), 1.0–1.4 (m, 6H, 3 × CH₂), 1.12 (s, 3H, C-8 CH₃), 0.93 (d, 3H, $J = 6.6$ Hz, C-2' CH₃), 0.85 (t, 3H, $J = 6.6$ Hz, terminal CH₃); MS (EI, 70 eV) *m/z* 251 (5, M⁺), 194 (7), 166 (49), 123 (9), 84 (19), 70 (100), 55 (15); HRMS (EI) *m/z* 251.2239, calcd 251.224 for C₁₆H₂₉NO. Compound **26** had virtually the same ¹H NMR spectrum as **25** with the exception that the chemical shift of the C-2' methyl occurred at 0.88 ppm; HRMS (EI) *m/z* 251.2237.

Computational Methods. Conformational searching utilized version 2.1 of the Biograf software package (Biodesign, Inc., Pasadena, CA) using the MMP2 force field. This is an implementation of Allinger's MM2 force field (Sprague et al., 1987), which has been shown to be very reliable with hydrocarbon-like structures. The major difference in the MMP2 implementation is that the electrostatic contribution to the energy function is calculated from atomic point charges rather than from bond dipoles. This difference should be small when applied to the nonpolar alkyl chain conformations in pumiliotoxin analogs. Starting geometries for conformational searches were obtained via a two-step procedure. First, molecular mechanics minimization (using the MMP2 force field) of the crystal structure of pumiliotoxin 251D (Daly et al., 1980) gave a geometry very close to that of the crystal structure. The proper alkyl chain was then reconstructed using standard symmetrical bond lengths and angles for the atoms C-2' and beyond. Atomic charges used were derived using Mulliken charges from a single-point calculation using the AM1 semiempirical molecular orbital method and adjusted by averaging the charges between pairs of methylene protons and between the three methyl protons in the alkyl chain region only to reduce the artificial asymmetry inherent in a charge calculation from a single geometry. The conformational search was done at angle increments of 1° for the C-1'–C-2' bond of interest and at 120° increments for the three torsional angles between C-3' and C-6'. A fixed-valence search strategy was

Table 2. Injection Toxicity of Pumiliotoxin Analogs to *H. virescens*

compd	ED ₅₀ ^a	LD ₅₀ ^b
13a (PTX-251D)	0.01	0.15
13b	0.07	0.47
13c	0.23	2.4
13d	0.006	0.17
13e	0.17	0.77
13f	0.15	1.1
13g	>10	>10
13h	0.03	0.58
13i	0.04	1.1
13j	0.03	0.32
13k	0.07	10
14a	0.03	1.8
25	0.76	5.6
26	>10	>10

^a Dose (in μg/larva) at which 50% of insects exhibited convulsions. ^b Dose (in μg/larva) that caused 50% mortality after 48 h.

employed; that is, the conformational energy was not minimized further at each point.

Toxicity Studies. *Heliothis virescens* (Tobacco Budworm, TBW) Bioassays. The insecticidal activity of these compounds was evaluated in TBW by both injection assays and topical applications. For injection bioassays, the test materials were dissolved in DMSO and diluted to the appropriate concentration. A 0.5 mL aliquot of the tested solution was injected into the hemolymph of fifth instar TBW (55–65 mg body weight) with a 10 mL Hamilton syringe. For topical bioassays, the test materials were dissolved in acetone. A 1 mL aliquot was applied to the dorsum of third instar (25–35 mg) TBW. Treated larvae (10 per dose) in both tests were held in plastic Petri dishes with ample diet. Anesthesia was avoided to permit observation of initial effects of the compounds. The dose–response data were subjected to probit analysis for ED₅₀ and LD₅₀ calculations. Mortalities were corrected by Abbott's method if control mortalities exceeded 10% (Abbott, 1925).

RESULTS AND DISCUSSION

Toxicity data for pumiliotoxin (PTX) analogs are listed in Table 2. We focused on LD₅₀ determinations in tobacco budworm 48 h after injection of the compounds as our primary determinant of activity. The endpoint of this test (death) is dependent on both the test compound's intrinsic activity as well as the ability of the insect to reduce its concentration at the target site through the detoxification processes of metabolism and elimination. As an indicator of the potential importance of these detoxification processes relative to a measure of the test compound's "intrinsic" activity, we also determined an ED₅₀—the dose at which 50% of the insects exhibited convulsive symptoms immediately after injection of the test compound. The convulsant symptoms were consistent with the expected neurotoxic mode of action of pumiliotoxins. The more active analogs caused rapid onset of symptoms upon topical application, a desirable feature for commercial uses. That the pumiliotoxins are fast acting and topically active is not surprising, given the speculation that they have evolved as defensive secretions which render the frogs, having neither fangs nor stingers to inject toxins, distasteful to potential enemies (Daly and Spande, 1986). That pumiliotoxins have good insecticidal activity could not be predicted. By comparison with other insecticides, PTX-251D (**13a**) was about 100-fold less active than the commercial pyrethroid fenvalerate in the injection assay and about 30-fold less active in the topical assay. While small sample size was a constraint to making a precise, quantitative comparison, PTX-

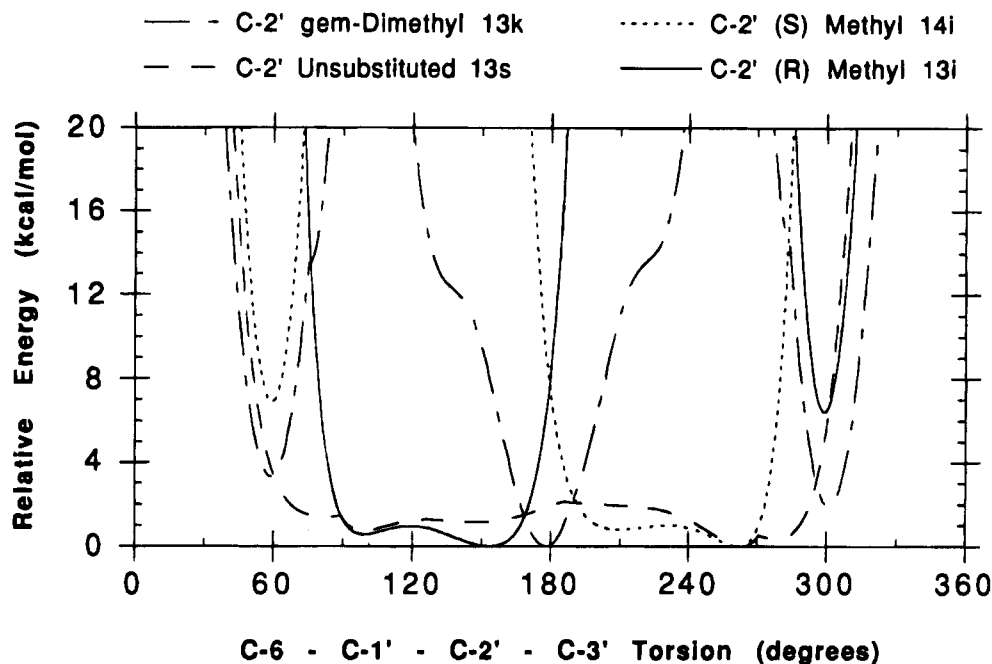


Figure 3. Plot of relative energy of best conformation vs C-6-C-1'-C-2'-C-3' torsion angle for each of four analogs with all possible hydrogen or methyl substitutions at C-2' methylene.

251D was significantly (ca. 20-fold) more active than the other natural toxins, PTX-A and PTX-B, in the injection assay.

We approached analog synthesis initially from the standpoint of trying to identify structural features necessary for biological activity. Both stereocenters at C-8 and C-2' were found to be important, comparing **13a** with **25** and **13a** with **14a**. The C-2' (*S*) diastereomers were also found to be consistently less active than the natural C-2' (*R*) diastereomers (comparing compounds **13a** with **14a**, **13i** with **14i**, and **13d** with **14d**). Compound **26**, "unnatural" at both C-8 and C-2', had no detectable activity. Alkyl substitution at C-2' was critical for activity. When the branch at C-2' was eliminated, activity was significantly reduced, as shown by comparison of compounds **13c** with **13a** and **13k** with **13d**. Doubly branched analog **13b** had an intermediate level of activity. Inspection of molecular models and the results of the computational study described below suggested that an alkyl substituent at C-2' inhibits rotation about the C-1'-C-2' bond due to crowding of the C-2' substituent against the equatorial proton on C-5, anchoring the butyl group of **13a** in a preferred spatial orientation with respect to the ring system. Presumably, this preferred orientation would not be energetically favorable in the case of the C-2' (*S*) diastereomer **14a**, **14d**, or **14i**.

Guided by the observations that a C-2' substituent is crucial for insecticidal activity, we attempted to deduce a putative active conformation around the C-1'-C-2' bond using computational techniques. Such an active conformation would be one available to the lead compound **13a** and the C-2' *gem*-dimethyl analog **13b** but unavailable to the C-2' epimer **14a** and the C-2' unsubstituted analog **13c**. The results of a conformational search are compiled in Figure 3. For each of the four analogs, the relative energy of the best conformation from among the C-3'-C-6 torsions is plotted against the C-1'-C-2' torsional angle. It is apparent from the figure that a fixed-valence scan using the MMP2 force field (see Materials and Methods) greatly overestimated the energy barriers between accessible states. This is

to be expected when using a relatively "hard" force field parametrized for free-valence conformational searching in a fixed-valence method where one would normally employ a "softer" field. We fully optimized the stationary points on the surface and a few additional select points. While the barrier heights lowered considerably, the relative energy ordering of the conformations did not change. Inspection of Figure 1 suggested two possible active conformations at 165° and 300° around the C-6-C-1'-C-2'-C-3' torsion angle that are energetically accessible to both the C-2' (*R*) methyl lead **13a** and the C-2' *gem*-dimethyl analog **13b** but prohibitively energetically costly for the C-2' (*S*) analog **14a**. The C-2' unsubstituted analog **13c** may show reduced activity for entropic reasons since it can access nearly all conformations available to the other analogs. The two active conformation candidates are depicted as stereodrawings in Figure 4, showing the alkyl chain in extended conformation. The information at hand does not allow us to distinguish between these two possibilities for the active conformation or to establish a preferred conformation for the alkyl chain. Distinguishing between the two models may require construction and evaluation of rigid cyclic analogs designed to mimic each possibility.

Extending the length of the side chain also led to a decrease in activity, as evident by comparison of the homologous series **13a**, **13d**, and **13e** (butyl to hexyl to octyl). While **13a** and **13d** were approximately equipotent, lengthening the side chain of PTX-251D by more than two methylene units caused a rapid decrease in all activities (compounds **13e** and **13i**). Reducing the conformational flexibility of the side chain by introducing rings (compounds **13f** and **13g**) greatly diminished or eliminated activity.

During the observation period, the larvae showed a tendency to recover from convulsions caused by low doses of test compounds. For all compounds, significantly higher doses were required to produce mortality (ratio of LD₅₀ to ED₅₀). At the end of the observation period, insects were either dead or recovered from convulsions. By analogy with pyrethroid insecticides, it seemed reasonable that metabolism of the toxin could

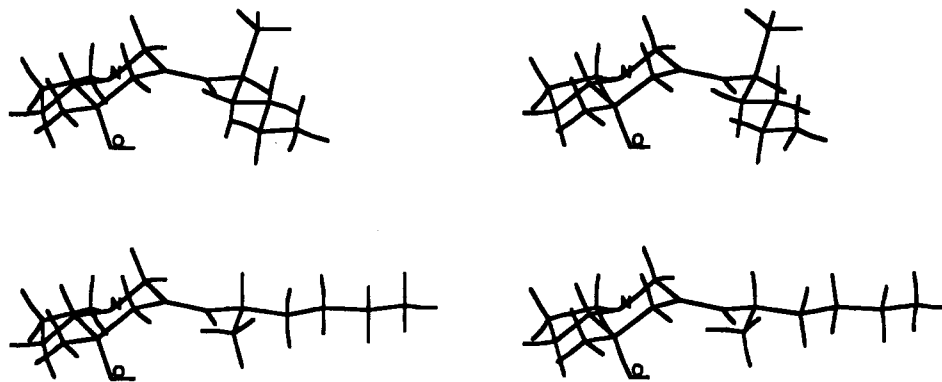


Figure 4. Crossed stereoviews of possible active conformations with C-6-C-1'-C-2'-C-3' torsion 300° (top) and 165° (bottom).

play a role in this recovery (Ruijt, 1985). Because ω -1 hydroxylation of the side chain of **13a** was observed in an in vitro P-450 enzymatic oxidation system (unpublished observations of Dr. Joel Sheets of this department), we prepared terminal branched compound **13i** and perfluorinated analog **13j** in an attempt to block this potential detoxification pathway. Compound **13i** appeared to be more readily detoxified than **13a**, and compound **13j** was only marginally less readily detoxified (comparison of the ratios LD₅₀/ED₅₀). Therefore, we have no evidence to suggest that ω -1 hydroxylation is a significant detoxification pathway in vivo.

By synthesizing derivatives of the pumiliotoxin indolizidine ring system, we were able to define many of the structure requirements for toxicity; however, no simple analog was more toxic than the lead compound **13a** in the TBW injection assay. We found that the natural stereochemistry of pumiliotoxin 251D was important for activity and that a substituent at the C-2' position was required for toxicity, leading to the hypothesis that toxicity is dependent on a particular side chain conformation. The pumiliotoxin analogs may serve as models for design of a new class of sodium channel-binding insecticides. Once the requirements of the putative receptor site are understood, it should be possible to design alternative novel carbon frameworks to express the key functionality in the optimum binding orientation.

ACKNOWLEDGMENT

We thank L. E. Overman for providing reference samples of pumiliotoxins A, B, and 251D and some experimental details prior to publication. We also thank M. J. Ricks and G. A. Bradfish of this department for providing expert advice and Sue Schoonover for technical assistance with the bioassays.

LITERATURE CITED

- Abbott, W. S. A Method for Computing the Effectiveness of an Insecticide. *J. Econ. Entomol.* **1925**, *18*, 265-267.
- Caporusso, A. M.; Polizzi, C.; Lardicci, L. Reactions of 3-Alkyl- and 3,3-Dialkyl-1-bromoallenes with Organocuprates: Effects of the Nature of the Cuprate Reagent on the Regio- and Stereoselectivity. *Tetrahedron Lett.* **1987**, *28*, 6073.
- Corey, E. J.; Fuchs, P. L. A Synthetic Method for Formyl to Ethenyl Conversion. *Tetrahedron Lett.* **1972**, *13*, 3769.
- Crandall, J. K.; Deanna, J. K.; Kohne, J. Allenes by Lithium Aluminum Hydride Reduction of Propargyl Derivatives. *J. Org. Chem.* **1968**, *33*, 3655.
- Daly, J. W.; Spande, T. F. Amphibian Alkaloids: Chemistry, Pharmacology, and Biology. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New York, 1986; Vol. 4, Chapter 1.
- Daly, J. W.; Tokuyama, T.; Fujiwara, T.; Highet, R. J.; Karle, I. L. A New Class of Indolizidine Alkaloids from the Poison Frog, *Dendrobates tricolor*. X-ray Analysis of 8-Hydroxy-8-methyl-6-(2'-methylhexylidene)-1-azabicyclo[4.3.0]nonane. *J. Am. Chem. Soc.* **1980**, *102*, 830.
- Daly, J. W.; McNeal, E.; Gusovsky, F.; Ito, F.; Overman, L. E. Pumiliotoxin Alkaloids: Relationship of Cardiotoxic Activity to Sodium Channel Activity and Phosphatidylinositol Turnover. *J. Med. Chem.* **1988**, *31*, 477.
- Fuchikami, T.; Ojima, I. Transition-Metal Complex Catalyzed Polyfluoroalkylation. I. Facile Addition of Polyfluoroalkyl Halides to Carbon-Carbon Multiple Bonds. *Tetrahedron Lett.* **1984**, *25*, 303.
- Gusovsky, F.; Padgett, W. L.; Creveling, C. R.; Daly, J. W. Interaction of Pumiliotoxin B with an "Alkaloid-Binding Domain" of the Voltage-Dependent Sodium Channel. *Mol. Pharmacol.* **1992**, *42*, 1104 and references cited therein.
- Landor, S. R.; Patel, A. N.; Whiter, P. F.; Greaves, P. M. The Preparation of 3-Alkyl- and 3,3-Dialkyl-1-bromoallenes. *J. Chem. Soc. C* **1966**, 1223.
- Narahashi, T. Nerve Membrane Sodium Channels as Targets of Insecticides. *Trends Pharmacol. Sci.* **1992**, *13*, 236-241.
- Negishi, E.; Baba, S. A Convenient Method for the Tertiary Alkyl-Alkynyl Coupling via Organoalanes. *J. Am. Chem. Soc.* **1975**, *97*, 7385.
- Okhlobystin, O. Yu.; Bregadze, V. I.; Punomarenko, A. A.; Zalcharkin, L. I. *Chem. Abstr.* **1962**, *57*, 14919a.
- Overman, L. E.; Sharp, M. J. Enantioselective Total Synthesis of the Pumiliotoxin A Alkaloids via Reductive Iminium Ion-Alkyne Cyclizations. Total Synthesis of (+)-Pumiliotoxin A. *Tetrahedron Lett.* **1988a**, *29*, 901.
- Overman, L. E.; Sharp, M. J. Nucleophile-Promoted Electrophilic Cyclization Reactions of Alkynes. *J. Am. Chem. Soc.* **1988b**, *110*, 612.
- Overman, L. E.; Bell, K. L.; Ito, F. Enantioselective Total Synthesis of Pumiliotoxin B and Pumiliotoxin 251D. A General Entry to the Pumiliotoxin A Alkaloids via Stereospecific Iminium Ion-Vinylsilane Cyclizations. *J. Am. Chem. Soc.* **1984**, *106*, 4192.
- Ruijt, G. S. F. Pyrethroids. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*; Kerkut, G. A., Gilbert, L. I., Eds.; Pergamon Press: Oxford, U.K., 1985; Vol. 12, Chapter 7.
- Sprague, J. T.; Tai, J. C.; Yuh, Y.; Allinger, N. L. The MMP2 Computational Method. *J. Comput. Chem.* **1987**, *8*, 581-603.
- Van Hijfte, L.; Kolb, M.; Witz, P. A Practical Procedure for the Conversion of Aldehydes to Terminal Alkynes by a One Carbon Homologation. *Tetrahedron Lett.* **1989**, *30*, 3655.

Received for review September 19, 1994. Accepted January 27, 1995.*

JF9405259

* Abstract published in *Advance ACS Abstracts*, March 1, 1995.