10% HCl to pH 4 and then loaded onto a Dowex 50-W (H^+) column (1.0 × 15 cm). The column was washed with 50 mL of H_2O and 75 mL of 2 N NH_3. Those fractions containing **7** were combined and reduced in vacuo. The product was further purified by thick-layer chromatography (1.0 mm) on Avicel F plates eluting with EtOH–H_2O (3:1). The product was isolated as a hydrochloride salt in 84% yield: for R_f values, see Table III; ^{1}H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.15 (2 s, 2 H, H-2 and H-8), 4.5–4.8 (m, 1 H, H-1′), 4.25–4.5 (m, 1 H, H-2′), 3.75–3.95 (m, 1 H, H-3′), 3.2–3.45 (m, 1 H, H-α), 3.0 (s, $NHCH_3$), 2.55–2.8 (d, 2 H, 2 H-5′), 2.5 (t, 2 H, 2 H-γ), 1.6–2.3 (several overlapping m, 5 H, 2 H-6′, H-4′ and 2 H-β); mass spectrum (DCI-CH_4), *m/e* 397 (MH^+), 353 ($MH^+ - CO_2$), 296 ($MH^+ - CH_2CH_2CH(NH_2)CO_2H$), 262 ($M - SCH_2CH_2CH(NH_2)CO_2H$), 150 ($N^6$-methyladenine H^+). Anal. ($C_{16}H_{24}N_6O_4S \cdot {}^7/_2$ HCl·EtOH) C, H, N.

C-S-3-DeazaAdoHcy (8). C-S-3-DeazaAdoHcy (8) was prepared in 84% yield according to the procedure of Montgomery et al.[11] mp 194–220 °C (220 °C < dec); for R_f values, see Table III; ^{1}H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.15 (s, 1 H, H-8), 7.6 (d, 1 H, H-2), 6.8 (d, 1 H, H-3), 4.3–4.65 (m, 1 H, H-1′), 4.05–4.3 (m, 1 H, H-2′), 3.7–3.85 (m, 1 H, H-3′), 3.2–3.25 (m, 1 H, H-α), 2.55–2.8 (d, 2 H, 2 H-5′), 2.5 (t, 2 H, 2 H-γ), 1.5–2.35 (several overlapping m, 5 H, 2 H-6′, H-4′; and 2 H-β). Anal. ($C_{16}H_{23}N_5O_4 \cdot 3H_2O$) C, H, N.

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Registry No. 1, 57816-79-2; **2**, 94800-44-9; **3**, 94800-45-0; **4**, 94842-38-3; **5**, 57884-84-1; **6**, 94800-46-1; **7**, 94820-19-6; **8**, 85647-47-8; **9**, 94842-39-4; **10**, 94800-47-2; 11, 94800-48-3; D-Hcy, 6027-14-1; *S*-benzyl-L-homocysteine, 7689-60-3; DL-homocysteine thiolactone hydrochloride, 6038-19-3; catechol *O*-methyltransferase, 9012-25-3; phenylethanolamine *N*-methyltransferase, 9037-68-7; histamine *N*-methyltransferase, 9029-80-5.

A New Class of Cardiotonic Agents: Structure–Activity Correlations for Natural and Synthetic Analogues of the Alkaloid Pumiliotoxin B (8-Hydroxy-8-methyl-6-alkylidene-1-azabicyclo[4.3.0]nonanes)

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Pumiliotoxin B (PTX-B, 6-(6′,7′-dihydroxy-2′,5′-dimethyl-(*E*)-4′-octenylidene)-8-hydroxy-8-methyl-1-azabicyclo-[4.3.0]nonane) increases the force of contractures of spontaneously beating guinea pig atrial strips by 3- to 5-fold with half-maximal effects at about 3 μM and increases rates of atrial contractions by 2- to 3-fold with half-maximal effects at about 6 μM. The presence of an axial 7-hydroxy substituent (PTX 339A) decreases the efficacy but not the potency of PTX-B as a positive inotropic agent while having only slight effects on activity as a positive chronotropic agent. The presence of an equatorial 7-hydroxy substituent (PTX 339B) greatly decreases efficacy and potency of PTX-B as a positive chronotropic and inotropic agent. Pumiliotoxin A which lacks the side-chain 7′-hydroxy group of PTX-B causes only a 2-fold increase in force of contracture at 54 μM while having minimal effects on rate. The presence of an axial 7-hydroxy substituent (PTX 323B′ and 323B″, epimeric at the 6′-hydroxy) markedly enhances positive inotropic and chronotropic effects of PTX-A. Another congener, PTX 251D with a 6-(2′-methylhexylidene) side chain, and a synthetic analogue with a 6-(6′-heptenylidene) side chain are cardiac depressants. Both lack hydroxyl groups in the side chain. The presence of an ω-1 hydroxy group in the side chain of PTX 251D yields an alkaloid (267C) with weak positive inotropic effects and minimal chronotropic effects. The presence of an axial 7-hydroxy group in the indolizidine ring of PTX 251D results in a compound (PTX 267A) with very weak positive inotropic effects while retaining the negative chronotropic effects of PTX 251D. A synthetic analogue with a 6-(7′-hydroxyheptylidene) side chain is a cardiac depressant even though it contains a side-chain hydroxyl corresponding in position to the 7′-hydroxyl of the side chain of PTX-B. The positive chronotropic and inotropic effects of pumiliotoxin B are reversed only by relatively high concentrations of the calcium channel blockers nifedipine and verapamil, suggesting that pumiliotoxin B may owe its cardiotonic activities to effects on internal mobilization of calcium.

An alkaloid, pumiliotoxin B (PTX-B, Figure 1), from the neotropical frog *Dendrobates pumilio*,[1,2] has marked myotonic[3,4] and cardiotonic[4] activity. In nerve-striated muscle preparations, PTX-B (1–30 μM) markedly increased both direct and indirect elicited twitch.[3] It was markedly more potent (10-fold) in potentiating contractures with isolated muscle fibers. PTX-B had no effect on Na^+, K^+, or Cl^- conductances[3] nor on Na^+-K^+-ATPase but was reported at high concentrations to inhibit Ca^{2+}-ATPase of sarcoplasmic reticulum.[5] It was proposed that PTX-B in some manner facilitated or enhanced stimulus-evoked release of calcium in both nerve and muscle.[3] In atria, PTX-B (1.5–7.5 μM) caused marked positive inotropic and chronotropic effects which were readily reversible and not blocked by a β-antagonist.[4] The present paper concerns structure–activity correlations for this class of alkaloids with respect to positive inotropic and chronotropic effects in spontaneously beating guinea pig atria.

† National Institutes of Health.
‡ University of California.

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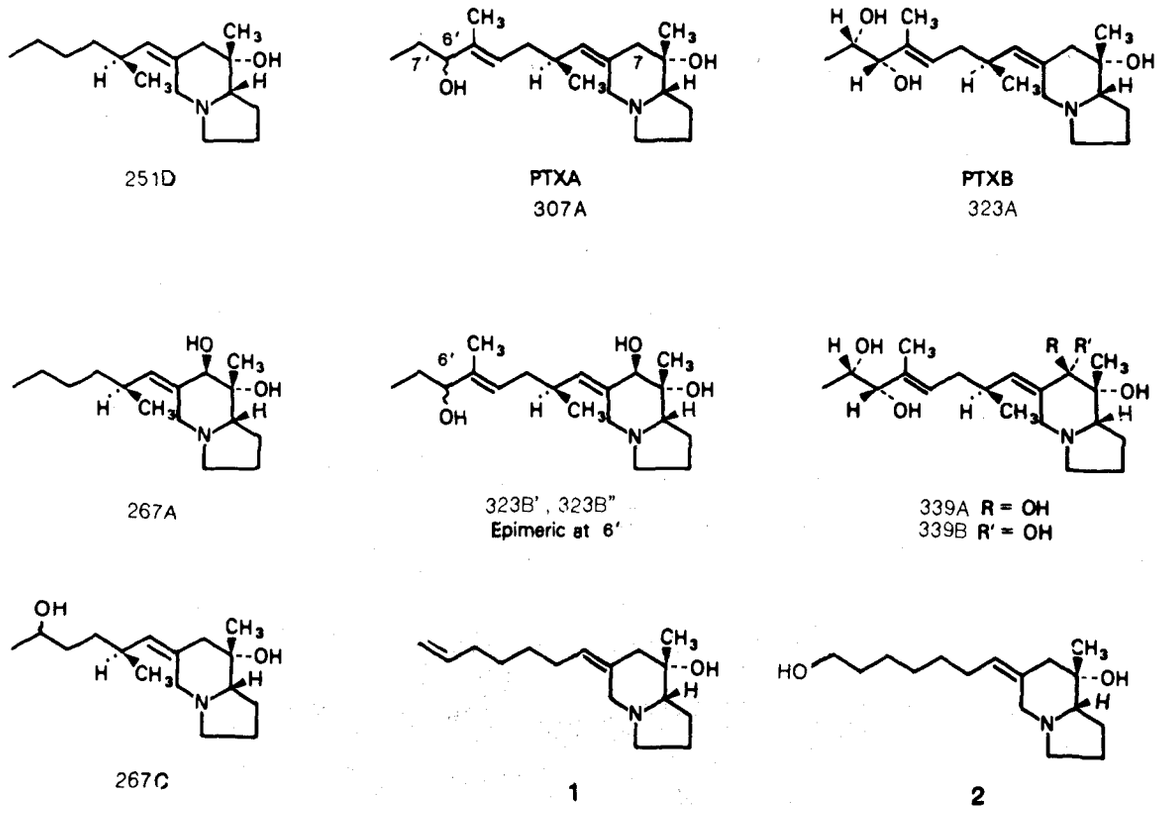
Scheme I



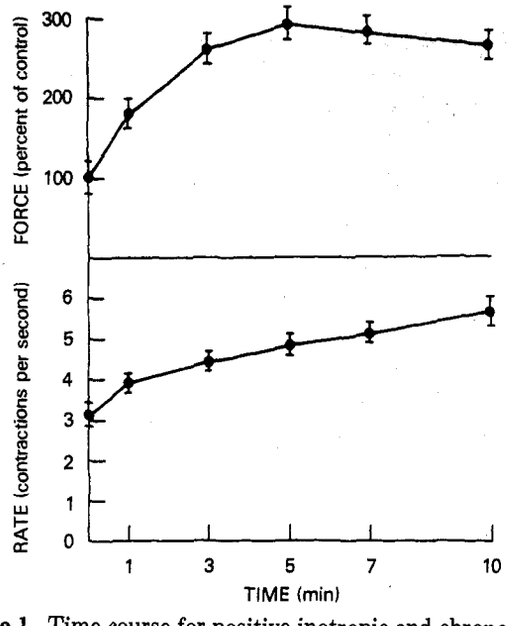


Figure 1. Time course for positive inotropic and chronotropic effects of pumiliotoxin B. Data ± SEM from eight experiments with isolated guinea pig atria.

Results

Pumiliotoxin B at 6 μM caused a time-dependent 3-fold increase in the force and a 2-fold increase in the rate of contractions of guinea pig atrial preparation (Figure 1). The positive chronotropic and inotropic effects of pumiliotoxin B were sustained without arrthymias over at least 30 min (data not shown). Structure–activity comparisons were done after 10-min exposure to each concentration of the various analogs (Scheme I), a time point at which effects usually appeared to have stabilized.

PTX-B caused the greatest increase in both force and rate of contractions of any of the analogues tested (Figure 2). The potentiation of force by PTX-B ($EC_{50} \sim 3$ μM) maximized at about 6 μM and was decreased at 18 μM, while the enhancement of rate maximized at 18 μM. At higher concentrations (54 μM) the force decreased further while the rate remained similar to that at 18 μM (data not shown). The allo (7-hydroxy) analogues of PTX-B, namely, PTX 339A with an axial 7-hydroxy substituent and PTX 339B with an equatorial 7-hydroxy substituent,[6] showed quite different activities. PTX 339A was nearly as potent as PTX-B with respect to force and rate of contraction but was much less efficacious with respect to force. In contrast PTX 339B was much less potent than PTX-B.

Pumiliotoxin A (PTX-A) was much less potent than PTX-B as a positive inotropic agent ($EC_{50} > 30$ μM) and had minimal effects on rate of contractions. It should be noted that pumiliotoxin A has now been shown to consist of a mixture of nearly equivalent amounts of two 6'-hydroxy epimers.[6] The allo (7-hydroxy) pumiliotoxin analogue of PTX-A has axial 7-hydroxy substituents and again both 6'-hydroxy epimers occur naturally.[6] The two 6'-hydroxy epimers (323B' and 323B") were tested with atria. One of these (323B') was much more potent and efficacious as a positive inotropic agent than PTX-A itself. The other 6'-hydroxy epimer (323B") was similar in activity as an inotropic agent to PTX-A. Both 323B' and 323B" were much more efficacious as positive chronotropic agents than PTX-A.

Pumiliotoxin 251D, which has no side-chain hydroxyl group, was a cardiac depressant. Its allo (7-hydroxy) analogue 267A, which has an axial 7-hydroxy moiety,[6] also reduced rate of contractions but caused no reduction in force. Indeed a slight augmentation in force occurred at high concentrations. A ω-1 hydroxy analogue of pumiliotoxin 251D, namely, 267C,[7] had positive inotropic activity,

(6) Tokuyama, T.; Daly, J. W.; Highet, R. J. *Tetrahedron* **1984**, *40*, 1183.

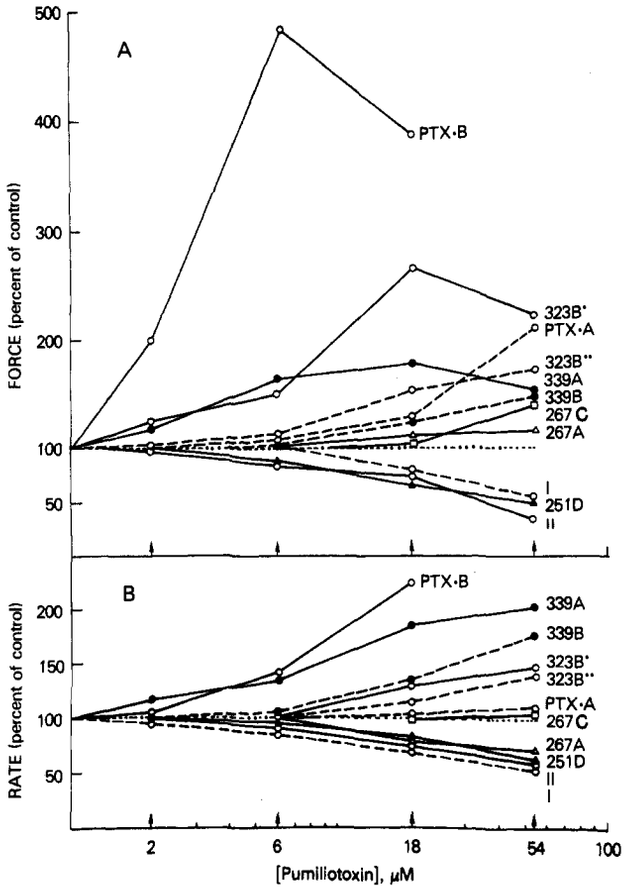


Figure 2. Effects of pumiliotoxin B and various analogues on (A) force and (B) rate of contractures of isolated guinea pig atria. The following compounds were tested: PTX-B (n = 12) (O–O), PTX-A (n = 3) (O--O), allo-PTX 339A (n = 4) (●–●), allo-PTX 339B (n = 3) (●--●), allo-PTX 323B′ (n = 3) (O–O), allo-PTX 323B″ (O--O); PTX 267C (n = 1) (□–□), allo-PTX 267A (n = 2) (△–△); PTX 251D (n = 1) (▲—▲); compound **1** (n = 3) (O--O); compound **2** (n = 3) (O–O).

but like PTX-A had nearly no effect on rate.

The two synthetic compounds tested differ from PTX-A and PTX-B in having an unbranched side chain. These were 6-(6′-heptenylidene)-8-hydroxy-8-methyl-1-azabicyclo[4.3.0]nonane (**1**) without a side-chain hydroxyl group and 6-(7′-hydroxyheptylidene)-8-hydroxy-8-methyl-1-azabicyclo[4.3.0]nonane (**2**) with a side-chain hydroxyl group that is distant from the indolizidine ring system by seven carbons as is the farthest hydroxyl group in PTX-B. Both **1** and **2** were cardiac depressants. The former compound (**1**) was shown to antagonize the cardiac stimulant effects of PTX-B (Figure 3).

The mechanism of cardiotonic action of PTX-B was briefly investigated. PTX-B (100 μM) has no effect on heart adenylate cyclase or phosphodiesterase activity (data not shown). The cardiotonic effects of PTX-B were not readily antagonized by calcium channel blockers such as nifedipine or verapamil, which are very potent cardiac depressants in control atria. About 10- to 20-fold higher concentrations of the calcium antagonists were required to depress the cardiotonic effects of PTX-B than to affect control atria (Table I).

Discussion

Structurally, PTX-B represents a new class of cardiotonic agents. Its effects on rate and force of contracture

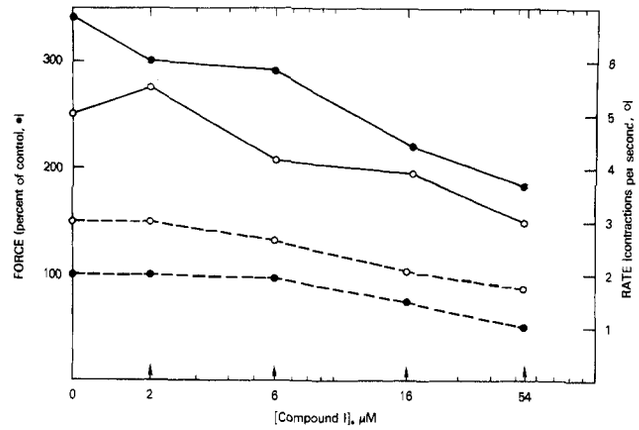


Figure 3. Negative inotropic (●) and negative chronotropic (O) effects of compound 1 in absence (dotted lines) and presence of 6 μM PTX-B (solid lines).

Table I. Effect of Calcium Channel Antagonists on Force and Rate of Guinea Pig Atrial Contractions[a]

	IC_{50}, μM			
	force		rate	
antagonist	control	PTX-B	control	PTX-B
nifedipine	0.4	6	0.6	12
verapamil	0.8	10	0.7	16

[a] Concentrations of nifedipine and verapamil required to reduce force and rate by 50% or more were estimated in two or three experiments in control atria and in atria stimulated by 6 μM PTX-B.

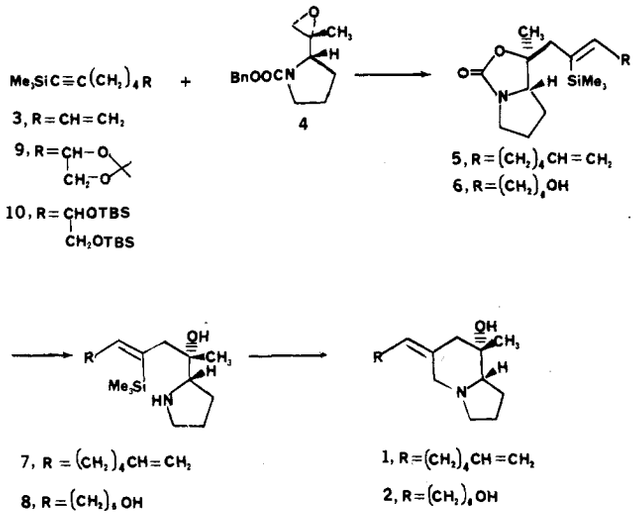
of isolated atria preparations are remarkably dependent on the nature of the side chain. Thus, PTX-A which lacks only the 7′-hydroxy group of PTX-B has virtually no effect on heart rate and is much less potent and efficacious with respect to augmenting force of contracture. The allo series (alkaloids with a 7-hydroxy group in the indolizidine ring) of alkaloids are active. Indeed the 7(a)-hydroxy-PTX-B (339A) is nearly as potent as PTX-B although less efficacious with respect to augmenting force. The 7(e)-hydroxy-PTX-B (339B) is significantly less active. Most naturally occuring allopumiliotoxins have an axial 7-hydroxy moiety.[6] In the allo series, the lack of a side-chain 7′-hydroxy group at 339A might be expected in analogy to the relative activities of PTX-B and PTX-A to yield alkaloids with low cardiotonic activity. The natural allopumiliotoxin corresponding to PTX-A exists as a pair of 6′-hydroxy epimers.[6] One of these (323B′) is quite active with respect to force, while the other (323B″) is less active. Both compounds are less active than 339A with respect to rate. It would appear that the side-chain structural requirements for activity are influenced by alterations (addition of an axial 7-hydroxy moiety) in the indolizidine ring.

Only a limited number of natural alkaloids are available for analysis of structure–activity correlations and most are available in small quantities, particularly 323B′, 267C, 267A, and 251D. The alkaloid PTX 251D differs from PTX-A and PTX-B in having a simple methylhexylidene side chain. Remarkably, it is a cardiac depressant rather than a cardiac stimulant. The corresponding allo compound 267A with an axial 7-hydroxy group has a slight stimulatory effect on force but remains depressant with respect to rate. A recently isolated side-chain ω-1 hydroxy analogue of PTX 251D, namely, 267C,[7] has weak stimulant effects on force and virtually no effect on rate.

Attempts at structure–activity correlations for PTX-B and its analogues are complicated by a number of factors. First, the augmentations are time dependent and at higher

(7) Daly, J. W.; Highet, R. J.; Myers, C. W. *Toxicon* **1984**, *22*, 905.

Scheme II



concentrations the alkaloids can elicit first the augmentation, followed by a reduction in force. Whether this is due to a phenomenon, possibly partial depletion of critical stores of calcium, similar to that proposed for striated muscle[3] is uncertain. The fact that certain PTX-B analogues have cardiac depressant effects introduces a further complication, namely, that the response to any of these alkaloids may be a composite of both stimulatory and inhibitory actions on cardiac function.

Systematic synthesis of various structural analogues of PTX-B are required to determine optimum requirements for activity. Initial syntheses were designed to provide analogues with hydroxyl substituents at the terminal carbons of a simple hexylidene side chain. The synthetic approach which was utilized is shown in Scheme II and followed directly from the strategy recently employed to prepare pumiliotoxin 251D[8,9] and pumiliotoxin B[8] in enantiomerically pure forms. Unfortunately, initial attempts to introduce directly a diol side chain were thwarted by our inability to cleanly hydroaluminate protected silylalkynediols such as **9** and **10**. Very recently alternate hydroalumination conditions, which will likely obviate this problem, have been developed.[10] The synthetic analogues employed in this study were assembled from 1-(trimethylsilyl)octen-1-yne (**3**) and the enantiomerically pure epoxide **4**[8,9] as outlined in Scheme I. The terminal hydroxyl group was introduced by selective hydroboration of dienyl carbamate **5** with 9-borabicyclo[3.3.1]nonane.[11]

The two synthetic compounds (**1** and **2**) were found to both be cardiac depressants. This indicates either that both the 6′-hydroxy and 7′-hydroxy substituents of the side chain are critical for activity or that other structural entities such as the 4′,5′-double bond are critical. Further synthetic analogues are being prepared. Compound **1** was found to be capable of antagonizing the cardiotonic effects of PTX-B (Figure 3), but no conclusions as to whether this is competitive at a single site or noncompetitive are possible.

In striated nerve muscle preparations, PTX-B appears to in some manner facilitate stimulus-evoked release of calcium from internal storage sites.[3] Possible mechanisms could include (a) enhancement of influx of sodium or calcium ions, thereby increasing sodium–calcium or calcium–calcium exchange, (b) direct release of calcium from sarcoplasmic reticulum, (c) facilitation of evoked release of calcium from sarcoplasmic reticulum, of (d) augmentation of effects of calcium on contractile responses. The proposed involvement of inhibition of Ca^{2+}-ATPase which occurred at higher concentrations of PTX-B no longer appears relevant since it was due to an as yet unidentified and physiologically unnecessary impurity in earlier PTX-B samples.

The cardiotonic effects of this class of alkaloids are undoubtedly due to mechanisms similar to those that pertain in striated muscle. However, several other possible mechanisms for the cardiotonic effects of these alkaloids can be eliminated. Thus, PTX-B is unlikely to function as a cardiotonic agent through inhibition of Na^+-K^+-ATPase since unlike ouabain it has no effects on this enzyme.[5] The effects of PTX-B in heart are not blocked by a β-antagonist, practolol,[4] and PTX-B was found at 100 μM to have no significant effects on cardiac adenylate cyclase (data not shown). Thus, PTX-B does not owe its cardiotonic effects to activation of adenylate cyclase either indirectly through β-receptors, as is the case for the cardiotonic effects of isoproterenol, or directly at the enzyme, as is the case for the cardiotonic effects of forskolin. PTX-B was found at 100 μM to have no significant effects on cardiac phosphodiesterase (data now shown). Thus, PTX-B does not owe its cardiotonic effects to blockade of degradation of cyclic AMP as is the case for the cardiotonic effects of theophylline. The effects of PTX-B on heart are difficult to reverse with calcium channel blockers such as nifedipine or verapamil, indicating that contraction and pacemaker activity are now less dependent on influx of external calcium. The data suggest that PTX-B in some way enhances availability of internal calcium, as was the case for striated muscle.[3]

Experimental Section

Natural Pumiliotoxins. Isolation and characterization of the naturally occurring (allo)pumiliotoxins (PTX-B, PTX-A, 323B′, 323B″, 339A, 339B, 251D, 267A, 267C) have been described.[2,6,7]

Synthetic Analogues. General experimental details for the syntheses of pumiliotoxins were described recently.[8]

(1*S*,7a*S*)-Tetrahydro-1-methyl-1-[2-(trimethylsilyl)-2-(*Z*),8-nonadienyl]-1*H*,3*H*-pyrrolo[1,2-*c*]oxazol-3-one (5). Neat *i*-Bu_2AlH (0.18 mL, 1.0 mmol)[12] was added dropwise at 23 °C to a solution of 1-(trimethylsilyl)-8-hexen-1-yne (**3**; 180 mg, 1.00 mmol; prepared in 60% yield from the reaction of [2-(trimethylsilyl)ethynyl]lithium[13] with the tosylate of 5-hexen-1-ol at 23 °C in a 2:1 mixture of 1,2-dimethoxyethane and hexamethylphosphoramide] and dry ether (1 mL). The resulting solution was heated at reflux for 1 h and cooled to 23 °C. MeLi (0.68 mL of a 1.47 M solution in ether) and dry THF (4 mL) were then added dropwise. After 0.5 h, Et_3N (0.14 mL, 1.0 mmol) and epoxide **4**[8] (130 mg, 0.50 mmol) were added, and the resulting solution was heated at 50 °C for 48 h under an Ar atmosphere. After cooling to 23 °C, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with ether (three times, 20 mL). After drying ($MgSO_4$), the concentrated organic extracts were purified by flash chromatography on silica gel (4:1 hexane–ethyl acetate) to give 98 mg (59%) of **5** as a colorless oil: 99% pure by capillary GC analysis;[14] ^1H NMR (250 MHz, $CDCl_3$) δ 6.08 (t, J = 7.4 Hz, SiC=CH), 5.9–5.7 (m, HC=C), 5.0–4.9 (m, C=CH_2), 3.6–3.5 (m, NCH_2), 3.2–3.0 (m, NCH), 2.47 (app AB q, J = 13.6 Hz, $\Delta\nu$ = 53 Hz, CH_2(Si)C=C), 2.2–1.4 (br m, 8 H), 1.41 (m, 4 H), 1.29 (s, Me), 0.17 (s, $SiMe_3$); ^{13}C NMR (63 MHz, $CDCl_3$)

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(14) A 15-m SE-30 silica capillary column (4000 plates/m) was used for this analysis.

δ 160.6, 149.7, 138.9, 133.6, 114.5, 82.6, 67.7, 48.5, 45.5, 33.8, 32.6, 29.4, 28.8, 26.9, 26.0, 21.8, 9.6; MS (isobutane, CI) 336 (MH), 186, 140, 139, 114, 96, 73; high-resolution MS (70 eV) 335.2283 (335.2280 calcd for $C_{19}H_{33}NO_2Si$); IR (film) 1760, 1645, 1609 cm^{-1}; $[\alpha]^{25}_D$ −33.8° (c 0.95, $CHCl_3$).

(2*S*)-2-[(1*S*,3*Z*)-1-Hydroxy-1-methyl-3-(trimethylsilyl)-3,9-decadienyl]pyrrolidine (7). A carefully degassed solution of **5** (230 mg, 0.69 mmol), KOH (770 mg, 12 mmol), EtOH (3.3 mL), and H_2O (0.9 mL) was heated at 90 °C for 24 h. After cooling to 23 °C, the reaction was concentrated, CH_2Cl_2 (20 mL) was added, and the organic layer was washed several times with H_2O (~10 mL) and then dried (K_2CO_3). Concentration and purification of the residue by flash chromatography (silica gel, 10:10:1 CH_2Cl_2–MeOH–12 N NH_4OH) gave 124 mg (70%) of **7** as a chromatographically homogeneous colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 5.99 (t, J = 7.0 Hz, SiC=CH), 5.82 (m, C=CHC), 5.9–5.7 (m, C=CH_2) 3.1–2.8 (m, NH, NCH, C*H*HN), 2.15 (app AB q, J = 12.3 Hz, $\Delta\nu$ = 83.2 Hz, CH_2(Si)C=C), 1.00 (s, Me), 0.16 (s, $SiMe_3$); ^{13}C NMR (23 MHz, C_6D_6) δ 147.7 (d), 139.4 (d), 137.2 (s), 115.1 (t), 73.4 (s), 67.2 (d), 49.6 (t), 47.4 (t), 34.5 (t), 33.2 (t), 30.4 (t), 29.5 (t), 27.1 (t), 23.2 (d); MS (isobutane CI) 311, 310 (MH), 130, 114, 70; high-resolution MS (70 eV) 309.2490 (309.2488 calcd for $C_{18}H_{35}NOSi$); IR ($CHCl_3$) 3400, 1645, 1605 cm^{-1}; $[\alpha]^{25}_D$ −35.0° (c 1.02, MeOH).

(6*Z*,8*S*,8a*S*)-8-Hydroxy-6-(6-heptenylidene)-8-methyloctahydroindolizidine (1). A mixture of **7** (48 mg, 0.16 mmol), paraformaldehyde (23 mg, 0.78 mmol), and EtOH (2 mL) was heated under an Ar atmosphere at reflux for 1 h. After cooling to 23 °C, camphorsulfonic acid (19 mg, 0.08 mmol) was added and the resulting mixture was heated at reflux for 24 h. After cooling to 23 °C, HCl (~3 drops, 6 N) was added and the reaction mixture was concentrated. The residue was partitioned between CH_2Cl_2 (20 mL) and NaOH (5 mL, 1 M) and the organic layer was dried (K_2CO_3) and concentrated by distillation (50 °C, atmosphere pressure). Purification of the residue on silica gel (95:5:0.1 CH_2Cl_2–MeOH–12 N NH_4OH) gave 31 mg (78%) of **1** as a colorless liquid: 98% pure by capillary GC analysis;[14] ^1H NMR (250 MHz, $CDCl_3$) δ 5.9–5.7 (m, CH_2=C*H*), 5.26 (app t, J = 7.5 Hz, CH_2=C*H*), 5.1–4.9 (m, C=CH_2), 3.80 (d, J = 12.1 Hz, H-5α), 3.1–3.0 (m, H-3α), 2.65 (s, OH), 2.35 (d, J = 11.8 Hz, H-5β, NCH_2C=C), 2.15 (app d, J = 7.0 Hz, H-3β), 2.2–1.9 (m, 7 H), 1.8–1.6 (m, 5 H), 1.5–1.3 (m, 4 H), 1.12 (s, Me); ^{13}C NMR (63 MHz, $CDCl_3$) δ 139.1, 131.8, 127.8, 114.4, 71.9, 68.5, 54.7, 52.9, 49.0, 33.8, 29.5, 28.6, 27.4, 24.4, 23.4, 21.3; MS (EI, 70 eV) 249 (M, 5%), 166 (22%), 84 (32%), 71 (24%), 70 (100%); high-resolution MS (70 eV) 249.2089 (249.2089 calcd for $C_{16}H_{37}NO$); $[\alpha]^{25}_D$ +34.3° (c 1.15, $CHCl_3$); HCl salt $[\alpha]^{25}_D$ +26.7° (c, 3.77, $CHCl_3$).

(1*S*,7a*S*)-Tetrahydro-1-methyl-1-[8-hydroxy-2-(trimethylsilyl)-2(*Z*)-nonenyl]-1*H*,3*H*-pyrrolo[1,2-*c*]oxazol-3-one (6). Following the general procedure of Brown,[11] **5** (130 mg, 0.37 mmol) was selectively hydroborated at 23 °C in THF (1 mL) with 9-borabicyclo[3.3.1]nonane (94 mg, 0.76 mmol, freshly recrystallized from 1,2-dimethoxyethane). After 18 h, oxidative workup[11] followed by purification of the crude product on silica gel (EtOAc) gave 62 mg (47%) of **6** as a colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 6.08 (t, J = 7.4 Hz, HC=C), 3.7–3.5 (m, NCH_2, OCH_2), 3.2–3.1 (m, 1 H), 2.45 (app AB q, J = 13.8 Hz, $\Delta\nu$ = 51.6 Hz, CH_2(Si)C=C), 1.27 (s, Me), 0.15 (s, $SiMe_3$); ^{13}C NMR (63 MHz, $CDCl_3$) 160.9, 149.9, 133.4, 82.7, 67.7, 62.9, 48.5, 45.6, 32.9, 32.7, 29.9, 29.3, 26.9, 26.0, 25.8, 21.9, 1.2; MS (isobutane CI) 354 (MH), 186, 139, 114, 73, 71, 70; high-resolution MS (70 eV) 353.2388 (353.2383 calcd for $C_{19}H_{35}NO_3Si$); IR (film) 3450, 1749, 1609 cm^{-1}; $[\alpha]^{25}_D$ −34.0 (c 3.25, $CHCl_3$).

(2*S*)-2-[(1*S*,3*Z*)-1,10-Dihydroxy-1-methyl-3-(trimethylsilyl)-3-decenyl]pyrrolidine (8). Hydrolysis of **6** as described for the preparation of **7**, followed by purification of the crude product on silica gel (10:10:1 CH_2Cl_2–MeOH–12 N NH_4OH) gave **8** (57% yield) as a chromatographically homogeneous colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 5.98 (t, J = 7.2 Hz, HC=C), 3.60 (app t, J = 6.5 Hz, OCH_2), 3.1 (m, 6 H), 2.18 (app AB q, J = 13.0 Hz, $\Delta\nu$ = 75.3 Hz, CH_2(Si)C=C), 1.17 (s, Me), 0.14 (s, $SiMe_3$); ^{13}C NMR (63 MHz, $CDCl_3$) 148.0, 135.8, 73.0, 66.6, 63.1, 48.3, 46.9, 32.9, 32.7, 30.1, 29.4, 26.6, 26.2, 25.9. 22.9, 1.0; MS (isobutane CI) 328 (MH); IR (film) 3400, 1609 cm^{-1}; $[\alpha]^{25}_D$ −30.2 (c 0.825, MeOH).

(6*Z*,8*S*,8a*S*)-8-Hydroxy-6-(7-hydroxyheptylidene)-8-methyloctahydroindolizidine (2). Iminium ion–vinylsilane cyclization of **8** was accomplished as described for the preparation of **1** and gave, after chromatographic purification (silica gel, 90:10:1 CH_2Cl_2–MeOH–12 N NH_4OH), **2** (60% yield) as a chromatographically homogeneous colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 5.26 (app t, J = 7.2 Hz, CH=C), 3.80 (d, J = 11.8 Hz, H-5a), 3.63 (app t, J = 6.4 Hz, CH_2O), 3.1–3.0 (m, H-3α) 2.7–2.6 (br s, OH), 2.34 (app d, J = 11.9 Hz, H-5β), 2.3–1.9 (m, 6 H), 1.8–1.7 (m, 5 H), 1.4–1.2 (m, 8 H), 1.13 (s, Me); MS (EI, 70 eV), 267 (m, 15%), 166 (34%), 84 (31%), 71 (32%), 70 (100%); MS (isobutane CI) 268 (MH), 267, 266, 166, 84, 71, 70; high-resolution MS (EI) 267.2189 (267.2199 calcd for $C_{16}H_{29}NO_2$); IR (CHC_2Cl_2) 3605, 3450, 1650 cm^{-1}; $[\alpha]^{25}_D$ +5.0 (c 0.12, $CHCl_3$).

Biological Effects. Atrial strips were prepared from male 250–300-g Hartley strain guinea pigs and were suspended at 37 °C in a 20-mL organ bath with Tyrod's solution aerated with a 95:5 mixture of O_2–CO_2 gas.[15] After equilibration for at least 1 h, cumulative dose–response curves for effects on rate and force of spontaneous contractions were determined by serial additions of aliquots of the alkaloid in methanol with at least 10 min between additions.

Adenylate cyclase activity in a membrane preparation from guinea pig heart was assayed essentially as described.[16] Bovine cyclic AMP phosphodiesterase (Sigma Chemical Corp.) was assayed with 1 and 50 μM cyclic AMP essentially as described.[17] PTX-B was tested with these preparations at 100 μM.

Registry No. **1**, 94596-72-2; **2**, 94596-78-8; **3**, 91657-05-5; **4**, 77733-65-4; **5**, 94596-73-3; **6**, 94596-74-4; **7**, 94596-75-5; **8**, 94596-76-6; PTX-B, 67016-65-3; PTX-A, 67054-00-6; allo-PTX 323B′, 67255-99-6; allo-PTX 323B″, 92216-55-2; allo-PTX 339A, 92216-56-3; allo-PTX 339B, 91550-04-8; PTX-251D, 73376-35-9; allo-PTX 267A, 73376-38-2; PTX-267C, 94596-77-7; 9-borabicyclo[3.3.1]nonane, 280-64-8.

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