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PUMILIOTOXINS: MAGNETIC RESONANCE SPECTRAL ASSIGNMENTS AND STRUCTURAL DEFINITION OF PUMILIOTOXINS A AND B AND RELATED ALLOPUMILIOTOXINS

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Abstract - Magnetic resonance spectral assignments for carbon-13 and protons of pumiliotoxin A, B and 251D and of related allopumiliotoxins (7-hydroxy congeners) are presented. Pumiliotoxin A and its 7-hydroxy congener allopumiliotoxin 323B can occur as pairs of 15-hydroxy epimers in dendrobatid frogs. Allopumiliotoxin 323B' and 323B'' and allopumiliotoxin 267A (the 7-hydroxy congener of 251D) have 7-axial hydroxy moieties. Allopumiliotoxin 339A and 339B are, respectively, the 7-axial-hydroxy and 7-equatorial-hydroxy congeners of pumiliotoxin B. Formation of ring phenyl boronides occurs only with allopumiliotoxin 339B which has the 7-equatorial hydroxy cis to the 8-axial hydroxy of the indolizidine ring.

Pumiliotoxins, first isolated from the Panamanian poison frog Dendrobates pumilio, belong to two structural classes: Pumiliotoxin A and B are 8-hydroxy-8-methyl-6-ylidene-1-azabicyclo[4.3.0]-nonanes, while pumiliotoxin C is a 2,5-dialkyl-cis-decahydroquinoline.[1,2] Dendrobatid alkaloids of the former type have been grouped into a pumiliotoxin A-class while related congeners with an apparent 7-hydroxy substituent have been placed in a subclass, the allopumiliotoxins.[3,4] The absolute configuration of a pumiliotoxin 251D was defined by x-ray crystallographic analysis.[5] This enantiomer has been synthesized from L-proline and the HCl salt shown to be dextrorotatory.[6] The structures of pumiliotoxin A and B were deduced from magnetic resonance studies to be as in Fig. 1 for pumiliotoxin A and pumiliotoxin B. The position of the additional hydroxyl group in closely related allopumiliotoxins was deduced from mass spectral studies to be at position 7 (Fig. 1). Preliminary carbon-13 nmr spectra of allopumiliotoxin 323B' and 323B", which occur as trace constituents in skin extracts of Dendrobates auratus, suggested that these materials were epimeric at C-7,[7] but repetition of these studies on more generous quantities shows that the chemical shifts of the carbon atoms of the indolizidine ring and its substituents are, in fact, remarkably similar. We describe here studies indicating that these two allopumiliotoxins differ only in the hydroxyl configuration at C-15, while the two allopumiliotoxins 339A and 339B are epimeric at C-7. Pumiliotoxin A, like allopumiliotoxin 323B, has been found to consist of two isomers which appear to be epimeric at C-15.

Dedication: We would like to dedicate this paper to Dr. Ulrich Weiss who over the years has contributed so much to science and to the development of younger colleagues at the National Institutes of Health. Dr. Weiss celebrated his seventy-fifth birthday in January, 1983.

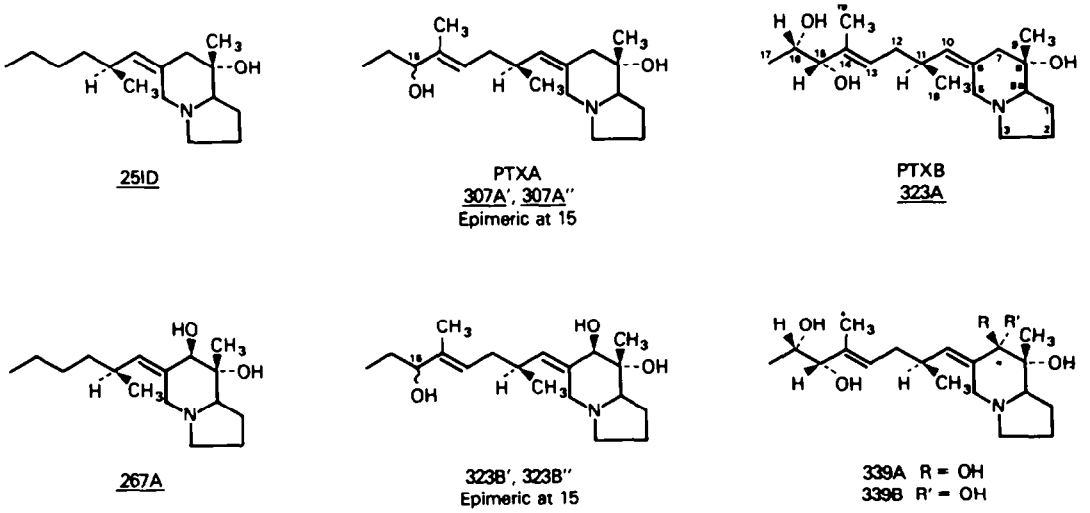

Figure 1

Structures of pumiliotoxin 251D, pumiliotoxin A (PTX-A, 307A', 307A"), pumiliotoxin B (PTX-B, 323A) and allopumiliotoxins (267A, 323B', 323B", 339A and 339B). Two isomers of pumiliotoxin A have now been isolated (307A' and 307A" see text) and appear to be epimeric at C-15. Allopumiliotoxins 323B' and B" both have an axial 7-hydroxy group and appear to be epimeric at C-15 (see text). Pumiliotoxin 339A has an axial 7-hydroxy group, while pumiliotoxin 339B has an equatorial 7-hydroxy group (see text). The absolute configurations are known only for 251D and 323A.

Pumiliotoxin A and B: Magnetic Resonance Assignments.

Prior to detailed investigation of the structures of the allopumiliotoxins, the assignments of the characteristic shifts of the individual carbon and protons of the parent compounds pumiliotoxin A and B had to be firmly established. Extensive double resonance studies performed on pumiliotoxin A and B led to the assignments for carbon-13 and protons given in Table 1 and 2. Certain proton resonances[5] have had to be reassigned (vide infra). Similarly, certain proton resonances of pumiliotoxin 251D have been reassigned (Table 3). Proton magnetic resonance spectra of pumiliotoxin A and B are given in Fig. 2.

For pumiliotoxin A, irradiation of the broad peak at 1.73 ppm (H-1 and H-2) results in the separation of the broad peak at 3.05 into a broad doublet with separation of 10 Hz, characteristic of a geminal coupling. This resonance is thus identified as arising from the C-3 methylene group, coupled to the closely coupled methylene groups of C-1 and C-2. Single proton spin decoupling of the ^{13}C spectra described below show that the proton responsible for the geminal coupling occurs at 2.20 ppm. The resonance at 3.05 ppm is assigned to the equatorial proton and that at 2.20 to the axial proton. The substantial difference in the chemical shifts of these protons is characteristic of the methylene groups adjacent to the nitrogen atom of transfused indolizidines, with the axial proton shifted to higher field by the influence of the nitrogen lone pair.[8] The doublets at 3.76 (earlier attributed to 8a) and 2.32 ppm (J = 11.4 Hz) are similarly identified as those of the C-5 methylene group. In carbon-13 spectra observed under conditions of single proton decoupling, the resonances at 54.6 and 53.3 ppm, triplets in coupled spectra, are recognized as those of C-3 and C-5 both by their characteristic chemical shifts, and by their appearance as doubled doublets in off resonance spectra. Because of the substantial difference in the chemical shifts of the attached protons, the effective decoupling field and residual couplings are different. Proton irradiation at either 3.05 or 2.20 ppm converts the resonance at 54.6 to a clean doublet, identifying it as that from C-3; similarly, irradiation at 2.32 and 3.76 converts the signal at 53.3 (C-5) to a doublet. The correct chemical shift of the proton of C-8a is located at 1.95 ppm by irradiation at this frequency which converts the doublet at 71.7 ppm to a singlet; the triplet at 35.6 (C-12) is similarly affected because of the coincidence of the proton chemical shifts of these two groups. The high field position of the H-8a again reflects the influence of the lone electron pairs in a trans indolizidine. Irradiation of 2.11 ppm converts the triplet at 48.9 (C-7) to a singlet.

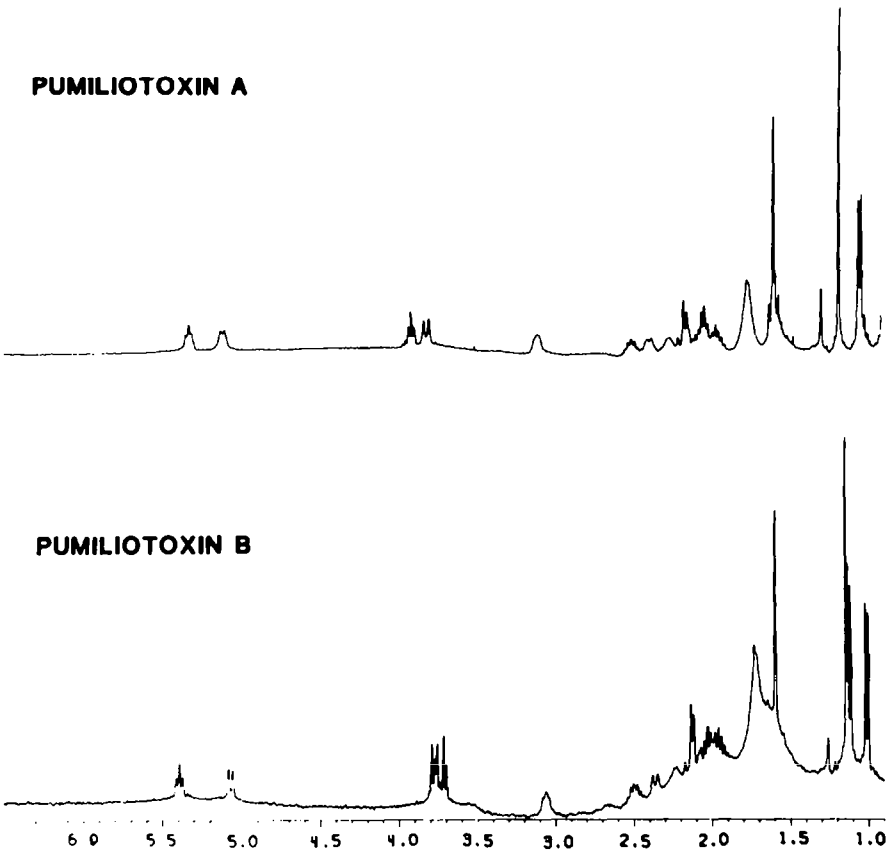

Figure 2

Proton magnetic resonance spectra (360 mHz) of Pumiliotoxin A and Pumiliotoxin B (solvent $CDCl_3$).

Table 1. Carbon 13-magnetic resonance assignments for pumiliotoxins and allopumiliotoxins, (see Figure 1 for numbering). Values are in δ units (solvent $CDCl_3$).

Position	PTX-A' 307A'	PTX-A'' 307A'	PTX-B 323A	Allopumiliotoxins 323B'	323B''	339A	339B
1	23.3	23.3	23.2	22.7	22.5	22.7	23.7
2	21.2	21.2	21.2	21.2	21.0	21.2	21.8
3	54.6	54.6	54.5	54.3	54.0	54.3	54.5
5	53.3	53.3	53.1	49.3	48.7	49.3	51.6
6	130.3	130.4	130.2	133.8	132.4	133.8	133.0
7	48.9	48.9	48.7	80.8	80.4	80.8	76.5
8	68.4	68.4	68.5	70.4	70.6	70.4	71.6
8a	71.7	71.7	71.6	65.2	65.7	65.2	70.6
9	24.3	24.3	24.3	20.6	20.8	20.6	20.4
10	133.8	133.8	133.8	137.1	138.7	137.1	132.0
11	32.6	32.6	32.4	32.6	32.6	32.8	32.4
12	35.6	35.6	35.5	34.4	35.1	35.5	35.5
13	125.0	125.0	127.3	125.6	124.0	127.2	127.3
14	137.7	137.7	135.2	137.6	138.0	135.1	135.4
15	79.6	79.5	82.8	80.1	78.8	82.1	82.6
16	27.8	27.8	68.8	27.8	27.8	68.3	68.8
17	10.2	10.2	19.0	10.2	10.3	19.3	19.0
18	21.3	21.4	21.2	21.0	21.0	21.2	21.4
19	11.4	11.4	12.1	12.4	11.0	12.5	12.4

Table 2. Proton magnetic resonance assignments (100 MHz) for pumiliotoxins and allopumiliotoxins. Values are in δ units (solvent $CDCl_3$) with m = multiplet, s = singlet, d = doublet, t = triplet, qn = quintet (see Figure 1 for numbering). Coupling constants (J in Hz) are in parentheses.

Position	Pumiliotoxin A		Pumiliotoxin B		Allopumiliotoxins		
	307A'	307A"	323A	323B'	323B"	339A	339B
3 (eq)	~3.05	~3.05	~3.05	~3.10	~3.00	~3.05	~3.05
3 (ax)	~2.20	~2.20	~2.20	~2.35	~2.35	~2.35	~2.15
5 (eq)	$3.76^{d}(11.4)$	$3.77^{d}(11.4)$	$3.77^{d}(12.3)$	$3.57^{d}(12.3)$	$3.66^{d}(12.2)$	$3.57^{d}(12.2)$	$3.78^{d}(12.2)$
5 (ax)	$2.32^{d}(11.4)$	$2.32^{d}(11.4)$	$2.36^{d}(12.3)$	$2.68^{d}(12.3)$†	$2.81^{d}(12.2)$	$2.68^{d}(12.2)$†	$2.38^{d}(12.2)$
7	2.11^{s}	2.10^{s}	2.11^{s}	3.67^{s}	3.72^{s}	3.66^{s}	3.72^{s}
8a	~1.95	~1.95	~1.95	~2.45	——	~2.50	——
9	1.12^{s}	1.12^{s}	1.12^{s}	1.18^{s}	1.22^{s}	1.19^{s}	1.18^{s}
10	$5.07^{d}(9.8)$	$5.07^{d}(9.7)$	$5.06^{d}(9.5)$	$5.28^{d}(10)$*	$5.37^{d}(10.3)$	$5.24^{d}(10.3)$*	$5.51^{d}(10.2)$
11	~2.40	~2.40	~2.45	~2.50	~2.50	~2.45	~2.40
12	~2.00	~2.00	~1.95	~2.05	~2.05	~2.05	~2.10
13	$5.30^{t}(7.2)$	$5.29^{t}(7.1)$	$5.38^{t}(7.2)$	$5.24^{t}(\sim7)$	$5.32^{t}(\sim7)$	$5.31^{t}(7.3)$	$5.39^{t}(7.5)$
15	$3.88^{t}(7.3)$	$3.88^{t}(7.4)$	$\sim3.65^{d}$	$3.86^{t}(6.8)$	$3.88^{t}(6.4)$	$\sim3.65^{d}$	$\sim3.69^{d}$
16	$1.54^{qn}(7.3)$	$1.54^{qn}(7.4)$	$\sim3.74^{m}$	$1.54^{qn}(6.6)$	$1.56^{qn}(6.6)$	$\sim3.75^{m}$	$\sim3.78^{m}$
17	$0.84^{t}(7.3)$	$0.83^{t}(7.4)$	$1.09^{d}(5.9)$	$0.83^{t}(7.4)$	$0.87^{t}(7.4)$	$1.12^{d}(6.1)$	$1.12^{d}(5.9)$
18	$0.98^{d}(6.6)$	$0.99^{d}(6.5)$	$0.99^{d}(6.6)$	$1.03^{d}(6.5)$	$1.04^{d}(6.5)$	$1.05^{d}(6.5)$	$1.01^{d}(6.6)$
19	1.55**	1.56**	1.58**	1.54**	1.59**	1.55**	1.60**

*Long range coupling (1.5 Hz).
**Singlet peak with long-range couplings (1.2 Hz^{d} with 13-H, ~0.6 Hz^{t} with 12-H's). Sample size inadequate for assignment of H-8A in 323B" and 339B.

The $\Delta^{13,14}$ double bond has now been shown in the more stable E-configuration.[9] This was based primarily on nuclear-Overhauser effects, namely a marked intensity enhancement (14%) in the C-13 olefin proton by irradiation on the C-15 proton. The configuration of the side-chain hydroxyl groups in pumiliotoxin B has been also defined.[9] Comparison of the nmr spectra to those of model compounds indicates the threo configuration for the hydroxyl groups in pumiliotoxin B. The absolute configuration of these hydroxyl groups (R,R) has been defined by identification of the ozonolysis product of pumiliotoxin B with the authentic sample synthesized from (-) tartaric acid.[10]

Allopumiliotoxins.

Two pairs of isomeric allopumiliotoxins were isolated from skin extracts of Dendrobates auratus. These compounds represent minor alkaloids in D. auratus. One pair had empirical formulae of $C_{19}H_{33}NO_3$ while the other pair had empirical formulae of $C_{19}H_{33}NO_4$. The mass spectra of the $C_{19}H_{33}NO_3$ pair are nearly identical as are the mass spectra of the $C_{19}H_{33}NO_4$ pair (see Experimental section). Mass spectral fragmentation patterns of these materials are characterized by a major fragmentation peak of m/z 182, corresponding to the indolizidine nucleus of the pumiliotoxin A series with an additional oxygen atom. A base peak at m/z 70 is retained, showing that the pyrrolidine ring does not contain the additional oxygen. In the absence of fragments corresponding to a hydroxymethyl system and in view of the stability of these materials to acid, the additional hydroxyl can only be a C-7. These inferences are consistent with the nuclear magnetic resonance spectra (vide infra). The existence of such pairs of allopumiliotoxin isomers in dendrobatid alkaloids was not recognized in previous studies.[3] The designation alkaloid 323B has thus undoubtedly been applied to both isomeric $C_{19}H_{33}NO_3$ compounds, and the designation alkaloid 339 to both isomeric $C_{19}H_{33}NO_4$ compounds. Alkaloid 339 was converted to a dimethylsilanate during gas chromatographic analysis and had been incorrectly designated as alkaloid 395 (see discussion in reference 3). The term allopumiliotoxin B applied by Daly et al.[3] to the $C_{19}H_{33}NO_3$ isomers proves inappropriate in light of their structures, since unlike pumiliotoxin B they do not contain a diol function in the side chain. It is suggested that an appropriate terminology for these isomeric allopumiliotoxins would be allopumiliotoxin 323B', 323B", 339A and 339B. Designation of the 323B pair and also the pair of pumiliotoxin A isomers (307A' and 307A") through the use of primes seems appropriate since these 15-hydroxy epimers appear to undergo interconversion (unpublished observations) while the 339 pair are readily separable and non-interconverting and hence are designated 339A and 339B.

Proton magnetic spectra of the allopumiliotoxins, as assigned through decoupling procedures, are shown in Table 2. In their spectra, large chemical-shift differences are observed between the two 3-C methylene protons (0.9 ppm) and between the two 5-C methylene protons (0.9 to 1.4 ppm). Thus, these allopumiliotoxins appear to have the trans fused indolizidine structure (vide supra). The proton magnetic resonance spectra of these allopumiliotoxins are in Fig. 3.

Treatment of allopumiliotoxin 339B with phenylboronic acid in chloroform at room temperature afforded a diboronide[9], characterized by a mass spectrum showing a parent ion at m/z 511 with an intense ion at m/z 268 corresponding to the indolizidine nucleus bearing a single boronide. Similar treatment of allopumiliotoxin 339A provided a monoboronide. The parent ion was at m/z 425. The clear implication that 339B bears a cis diol on the indolizidine ring while 339A bears a trans diaxial diol on the indolizidine ring is confirmed by comparison on the nuclear magnetic resonance spectra of the two compounds. Substitution of an equatorial hydroxyl at C-7 in 339B results in the downfield shift of the axial proton remaining from 2.12 to 3.68 ppm. The juxtaposition of the hydroxyl group with H-10 shifts the proton approximately 0.5 ppm downfield. The carbon-13 spectrum shows the large shift of C-7 anticipated, with smaller shifts of C-6, C-8 and C-9 (Table 1). With the exception of C-10, to be discussed later, all other carbon shifts are unchanged from those of pumiliotoxin B. The change of the hydroxyl configuration to axial in allopumiliotoxin 339A is accompanied by a downfield shift of the axial proton of the C-5 methylene group, and an upfield shift of H-10, each approximately 0.3 ppm. In the carbon-13 nmr spectrum, the presence of an axial substituent at C-7 produces the anticipated upfield shift of C-5 (2.3 ppm) and C-8a (5.4 ppm); relief of the interaction of the hydroxyl with C-10 produces a downfield shift of 5.1 ppm. Surprisingly, a downfield shift of C-7 of 4.3 ppm is observed; this

Table 3. Comparisons of ^{1}H- and ^{13}C-nmr spectra of allopumiliotoxin 267A and pumiliotoxin 251D* (solvent $CDCl_3$). Coupling constants in parentheses.

^{13}C-nmr spectra			^{1}H-nmr spectra		
Position	267A	251D	Position	267A	251D
1-C	22.8	23.4	3-H (eq)	3.10	3.09
2-C	21.3	21.2	3-H (ax)	2.24	2.24
3-C	54.3	53.3	5-H (eq)	3.61(12.1)	3.82(12)
5-C	49.0	54.7	5-H (ax)	2.72**(12.1)	2.36(12)
6-C	133.4	130.0	7-H	3.72	2.16
7-C	81.0	49.0	8a-H	——	1.96
8-C	70.3	68.4	9-CH_3	1.21	1.16
8a-C	65.3	71.8	10-H	5.34**(9.8)	5.07(10)
9-C	20.7	24.3	11-H	2.37	2.37
10-C	138.8	134.7	15-CH_3	0.88	0.89
11-C	32.0	32.1	18-CH_3	0.98(6.6)	0.98(7)
12-C	37.2	37.6			
13-C	29.8	29.8			
14-C	22.8	22.9			
15-C	14.2	14.2			
18-C	21.3	21.8			

*Values (ref. 5) have corrections in assignments for 3-H (ax) and 8a-H. **Peaks coupled to each other (1.5 Hz). Sample size inadequate for assignment of H-8a in 267A.

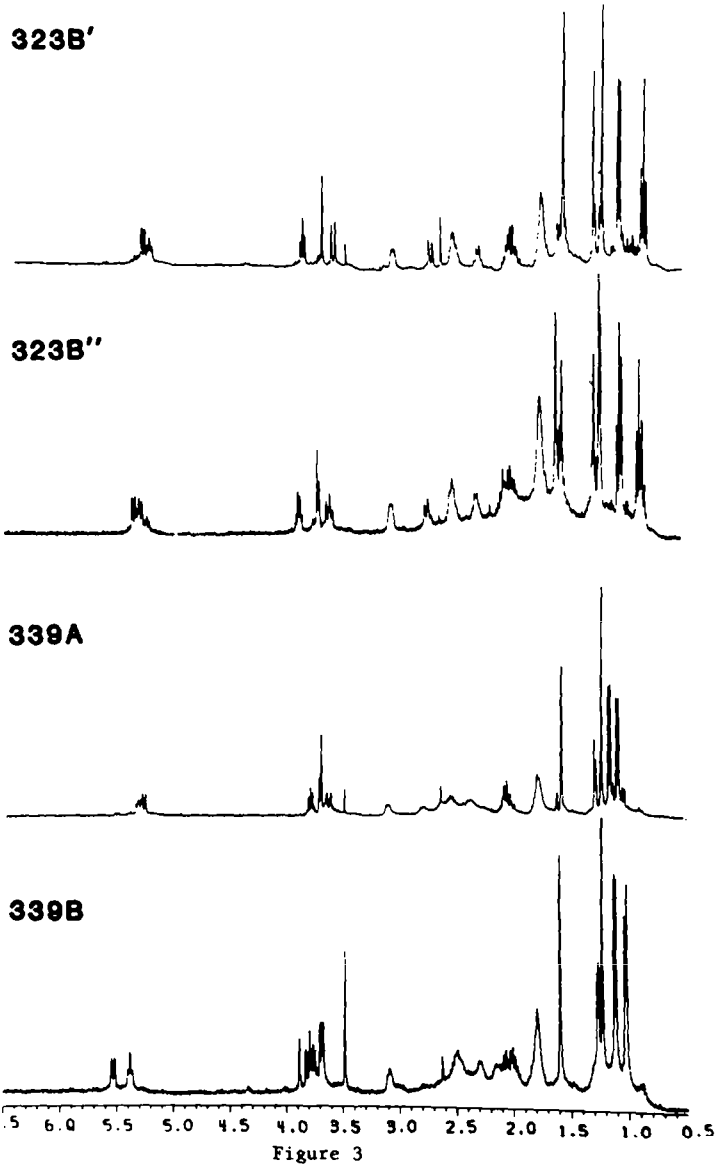

Figure 3

Proton magnetic resonance spectra (360 mHz) of allopumiliotoxins ($CDCl_3$).

may be explained as a result of the gem-substitution at C-8, which results in guache interactions involving the hydroxyl group in either configuration.

Allopumiliotoxins 323B' and 323B" fail to form boronides, or to react with periodic acid. The implication that the diol on the indolizidine ring is trans-diaxial is supported by the nmr spectra, for both proton and carbon-13 chemical shifts are nearly identical to those of 339A (Table 1, 2). The exceptions are, somewhat unaccountably, the carbon-13 chemical shifts of the 6,10 and 13 double bond carbons, which differ by approximately 1 ppm. These two alkaloids, therefore, appear likely to differ only at C-15. A more rigorous demonstration of this difference was precluded by the paucity of the available supplies.

Allopumiliotoxin 267A has been detected in many dendrobatid frogs[3] and has now been isolated in sufficient quanity from Dendrobates pumilio (vide infra) for detailed nuclear magnetic resonance spectral analysis. Comparison of the proton and carbon nuclear magnetic resonance spectra of allopumiliotoxin 267A with those of pumiliotoxin 251D (Table 3) and with those of other allopumiliotoxins (Tables 1 and 2) indicates that 267A has a 7-axial hydroxyl group as do allopumiliotoxins 323B', 323B" and 339A.

CONCLUSION

The present data clarify structures and/or spectral assignments of several pumiliotoxin A class alkaloids from dendrobatid frogs. These are pumiliotoxin A, of which two closely related 15-hydroxy epimers 307A' and 307A" have been detected, pumiliotoxin B, allopumiliotoxins 323B' and 323B", allopumiliotoxins 339A and 339B, and allopumiliotoxin 267A. The structure of pumiliotoxin 251D (11-C substituent = $CH_2CH_2CH_2CH_3$) has been proven by synthesis[4] and structures for pumiliotoxin 237A (11-C substituent = $CH_2CH_2CH_3$) and allopumiliotoxin 253 (11-C substituent = $CH_2CH_2CH_3$) have been proposed.[3] In view of the potent cardiotonic and myotonic activity of these alkaloids[14,15] and the marked dependence of their biological activity on potentiation of calcium excitation-contraction coupling and excitation-secretion coupling[15] elucidation of the remaining alkaloids of this class identified in dendrobatid frogs and investigation of their structure-activity correlates assumes a high significance. Allopumiliotoxins 323B', 323B", 339A and 339B have cardiotonic activity (unpublished results).

EXPERIMENTAL

High-resolution mass spectral data were obtained on JEOL D-300 mass spectrometer (electron impact, 70 eV). Gas chromatographic analyses were with a 2% OV-1 on Chromosorb WAW DMCS column with programing at 10 degrees per min from 150°C. Nuclear magnetic resonance spectra were obtained on JEOL FX-100 spectrometer. Proton spectra were determined at 99.60 MHz using a 16 K Fourier transform and 1 KHz spectra range for a digital resolution of 0.125 Hz and on a Nicolet NT360 at 360 MHz. Typically, free induction decays from a 45° pulse were collected at 6 sec intervals. Carbon-13 spectra were determined at 25.05 MHz using a 16 K or 8 K Fourier transform and 4 KHz spectra range for a digital resolution of 0.49 Hz or 0.98 Hz. Typically, 2,000 free induction decays from a 45° pulse were collected at 1.5 sec intervals to obtain a completely decoupled spectra. All values of chemical shifts and coupling constants are determined under the first order analysis.

The optical rotations of pumiliotoxin A and B are, respectively, $[\alpha]_D^{25}$ = +22.7° (c 1.0, CH_3OH) and $[\alpha]_D^{25}$ = +20.5° (c 1.0, CH_3OH). The rotation of pumiliotoxin B was independent of the source, namely skin extracts from either Dendrobates pumilio or D. auratus (vide infra). In chloroform the rotation of pumiliotoxin 307A' and 307A" are $[\alpha]_D^{25}$ = +14.3° (c 0.74, $CHCl_3$) and $[\alpha]_D^{25}$ +14.4° (c 0.52, $CHCl_3$), respectively, while the rotation of pumiliotoxin B is $[\alpha]_D^{25}$ +1.5° (c 1.0, $CHCl_3$). This should be compared to the weak levorotatory properties of the related (synthetic) pumiliotoxin 251D with an $[\alpha]_D^{25}$ = -3.1° (c 1.6, $CHCl_3$).[6] Synthetic 251D HCl has an $[\alpha]_D^{25}$ = +32° (c 0.5, CH_3OH).[6] Natural 251D HCl also is dextrorotatory but present quantities were insufficient for an accurate determination. An approximate $[\alpha]_D^{25}$ = +17° (c 0.15, CH_3OH) was obtained.

The rotations $[\alpha]_D^{25}$ of the allopumiliotoxins were as follows: 323B' +22.3° (c 1.0, CH_3OH); 323B" +55.0° (c 0.1, CH_3OH); 339A +29.4° (c 1.0, CH_3OH); 339B +4.5° (c 0.5, CH_3OH); 267A +24.7° (c 0.17, CH_3OH). It should be noted that the rotations of 323B" and 267A were obtained on dilute solutions. Allopumiliotoxin 339B with an equatorial 7-hydroxy group exhibits a much lower $[\alpha]_D^{25}$ than other allopumiliotoxins, all of which have axial 7-hydroxy groups.

Electron impact mass spectra for allopumiliotoxins, with the intensity relative to base peak set equal to 100 in parentheses, are as follows. Allopumiliotoxin 323B', m/z 323 (20), 306 (18), 288 (20), 276 (10), 262 (15), 222 (20), 210 (32), 209 (43), 192 (28), 182 (100), 114 (32), 70 (73). Allopumiliotoxin 323B", m/z 323 (15), 306 (22), 288 (17), 276 (10), 222 (21), 210 (27), 209 (39), 192 (27), 182 (100), 114 (25), 70 (70). Allopumiliotoxin 339A, m/z 339 (25), 322 (20), 321 (11), 304 (15), 295 (10), 294 (33), 222 (16), 210 (42), 209 (27), 192 (54), 182 (100), 114 (45), 70 (95). Allopumiliotoxin 339B, m/z 339 (25), 322 (18), 321 (10), 304 (12), 294 (23), 210 (40), 209 (25), 192 (29), 182 (92), 114 (47), 70 (100).

Isolation of Alkaloids from a Crude Base of Dendrobates auratus.

Methanolic extracts from 300 skins of Dendrobates auratus (Isla Taboga, Panama) were prepared and partitioned between aqueous methanol/chloroform (see ref. 5 for details of Methodology). Alkaloids were then extracted from the chloroform phase into 0.1 N HCl. After adjusting pH to 10 with aqueous ammonia, the aqueous layer was extracted first with hexane and then with chloroform. The chloroform layer was evaporated in vacuo to dryness to afford 130 mg of alkaloids. Preparative chromatography on a reversed phase silica gel column (Merck, prepacked Lobar column, RP-8, size B) with a mixed solvent of tetrahydrofuran:dioxane:triethylamine:water (30:5:1:70) yielded four fractions as monitored by refractive index. Pumiliotoxin B (35 mg) eluted in fraction 2 in almost pure state. Fraction 3 and 4 (13 mg and 32 mg, respectively) were rechromatographed on the RP-8 column separately with acetone:water:triethylamine (40:60:1) to afford allopumiliotoxins 323B' and 323B" (25 mg and 6 mg, respectively). Fraction 1 (36 mg) was rechromatographed on a RP-2 column (20 mm diameter x 350 mm) with a mixed solvent of acetone: water:triethylamine (30:70:1) to yield allopumiliotoxins 339A and 339B (10 mg and 7 mg, respectively). Thin layer chromatography (silica gel, $HCCl_3$:CH_3OH, 9:1) does not separate 323B' from 323B" (R_f 0.20), while readily separating 339A (R_f 0.07) from 339B (R_f 0.12). Gas chromatography does not readily separate these isomers. Other alkaloids isolated from D. auratus will be the subject of separate reports.

Isolation of Alkaloids from a Crude Base of Dendrobates pumilio.

Methanolic extract from 1080 skins of Dendrobates pumilio were prepared and partitioned between aqueous methanol/chloroform. Alkaloids were then extracted from the chloroform phase into 0.1 N HCl. After adjusting pH to >10 with aqueous ammonia, the aqueous layer was extracted with hexane and then with chloroform. The hexane layer was evaporated in vacuo to dryness to afford 0.26 g of alkaloids (Extract I). Evaporation of the chloroform layer gave 0.18 g of alkaloids (Extract II).

Extract I mainly consists of pumiliotoxin A's, pumiliotoxin C and many minor congeners. Preparative chromatography of Extract I on a DIOL-column (Merck, prepacked Lobar column, size B) with a mixed solvent of hexane:chloroform:triethylamine (80:20:1) yielded two main fractions as monitored by refractive index. Pumiliotoxin A's (40 mg) eluted in the second fraction which was repeatedly chromatographied on the same column to separate pure pumiliotoxin 307A' (10 mg) and 307A" (7 mg). Neither thin-layer chromatography (silica gel $HCCl_3$:CH_3OH, 9:1) nor gas chromatography separate pumiliotoxin 307A' from 307A". The first fraction (55 mg) was rechromatographed on the DIOL-column with hexane containing 0.5% of triethylamine to yield pure pumiliotoxin C (195A, 30 mg), an alkaloid 205 ($C_{14}H_{23}N$, 6 mg), a previously undetected alkaloid with a molecular weight of 223 ($C_{14}H_{25}NO$, 2 mg). During the first chromatography on the DIOL-column, additional minor alkaloids 252 ($C_{14}H_{24}N_2O_2$, 9 mg), 267A ($C_{16}H_{29}NO_2$, 4 mg), 236 ($C_{14}H_{24}N_2O$, 3 mg) and 222 ($C_{13}H_{22}N_2O$, 2 mg) were isolated. The structures of the amidine alkaloids (252, 236 and 222) and of the $C_{14}H_{25}NO$ alkaloid will be the subject of other reports.

Separation of Extract II was carried out under the same method applied for the separation of the D. auratus alkaloids described above. Chromatography on a reversed phase silica gel column (RP-8, Merck) yielded pumiliotoxin B (323A, 113 mg), allopumiliotoxin 339A (14 mg) and 323B' (3 mg). Allopumiliotoxin 323B" and 339B were not obtained from the D. pumilio crude extract.

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