Tetrahedron Vol 43, No. 3, pp. 643 to 652, 1987
Printed in Great Britain

0040-4020/87 $3.00+.00

NEW CLASSES OF AMIDINE, INDOLIZIDINE AND QUINOLIZIDINE ALKALOIDS FROM A POISON-FROG, DENDROBATES PUMILIO (DENDROBATIDAE)*

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(*Received in USA 29 October* 1986)

Abstract - Skin extracts from the Panamanian poison-frog Dendrobates pumilio afforded three major alkaloids of known structure, namely the 6-ylidene-8-hydroxy-8-methylindolizidines pumiliotoxin A and B and the cis-decahydroquinoline pumiliotoxin C. Other alkaloids include congeners 307F and 321 of pumiliotoxin A, allopumiliotoxins 267A, 323B and 339A, a 7-alkylidene-9-hydroxy-9-methylquinolizidine 223G, homologous to the pumiliotoxin-A class of dendrobatid alkaloids, a 5-alkynyl-8-methylindolizidine 205A, a 5-alkenyl-8-methylindolizidine 235B, a 3,6,8-trimethyl-12-azatricyclo[7.2.1.$0^{5,12}$]-2-dodecene 205B, a 6-hydroxy analog (211A) of the cis-decahydroquinoline pumiliotoxin C, and three tricyclic amidines 222, 236, 252. The allopumiliotoxins 323B and 339A are 7-axial-hydroxy congeners of pumiliotoxin A and B, respectively, and allopumiliotoxin 267A is the 7-axial-hydroxy analog of pumiliotoxin 251D. Structures are proposed for 307F, 321, 223G, 205A, 235B, 205B, 211A, 222, 236 and 252, based primarily on mass and nuclear magnetic resonance spectral analysis.

Neotropical poison-frogs of the family Dendrobatidae contain a remarkable diversity of alkaloids. Over two hundred have been detected and grouped into various classes of "dendrobatid alkaloids" including the steroidal batrachotoxins and the simpler histrionicotoxins, gephyrotoxins and pumiliotoxins (1-3). The first of the simpler dendrobatid alkaloids were isolated from a Panamanian poison-frog, Dendrobates pumilio and were named pumiliotoxin A, B and C (4, see Figure 1, 307A, 323A and 195A). This nomenclature proved unfortunate, since pumiliotoxin C (195A) ultimately proved to belong to a different structural class than pumiliotoxins A and B and was relatively non-toxic (5). The gross structures of pumiliotoxin A (307A) and pumiliotoxin B (323A) were deduced in 1980 (6), followed by full structural definition in 1984 (7). Both have now been synthesized (8,9).

It was apparent from the initial studies that dendrobatid frogs would afford a large number of alkaloids and a code system of nomenclature was introduced in 1978 (1). Dendrobatid alkaloids were designated by molecular weight in boldface type (see above) with an added letter (or letters) to identify alkaloids. Many have been as yet characterized only by combined gas chromatography-mass spectrometry (chemical ionization with NH_3 and ND_3 and high resolution electron impact/chemical ionization) of alkaloid mixtures before and after perhydrogenation (3).

Various populations of Dendrobates pumilio, which are extremely variable in color, size and behavior (10), contain, based on gas chromatography-mass spectrometry, a total of about one hundred different alkaloids, representing nearly all of structural classes found in dendrobatid frogs (3). Further extracts of one population of Dendrobates pumilio have now afforded several of these alkaloids in quantities sufficient for structural definition by nu-

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Dedicated to Bernhard Witkop on the occasion of his seventieth birthday. The investigation of the structures and biological activity of frog alkaloids is only one of the many research areas that Dr. Witkop has fostered and nurtured during his long and fruitful scientific career.

clear magnetic resonance spectroscopy. The structures of the alkaloids isolated from Dendrobates pumilio are shown in Figures 1-2, while the amounts isolated are documented in Table 1.

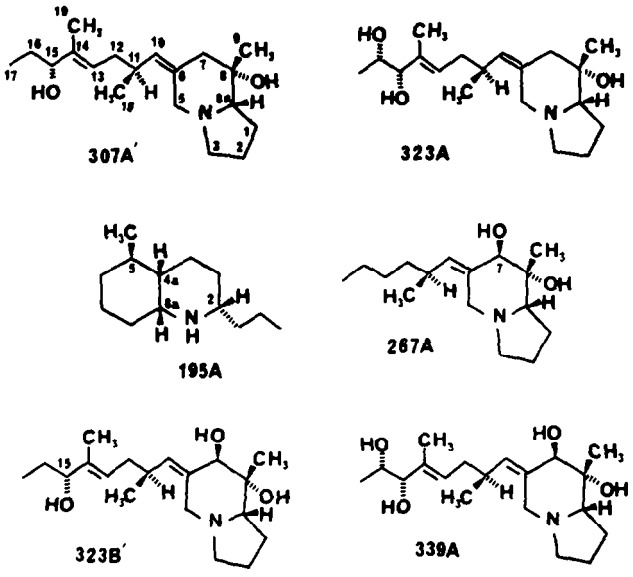


Figure 1. Structures of alkaloids previously isolated from Dendrobates pumilio: Pumiliotoxin A (307A'); pumiliotoxin B (323A); "pumiliotoxin" C (195A); allopumiliotoxin 267A; allopumiliotoxin 323B'; and allopumiliotoxin 339A. The major natural isomer of pumiliotoxin A (307A') has the configuration shown at C-15, while the lesser isomer (307A") is epimeric at C-15. The configuration of 323B' at C-15 is unknown, but presumably is the same as that of pumiliotoxin B (323A) and the major isomer (307A') of pumiliotoxin A.

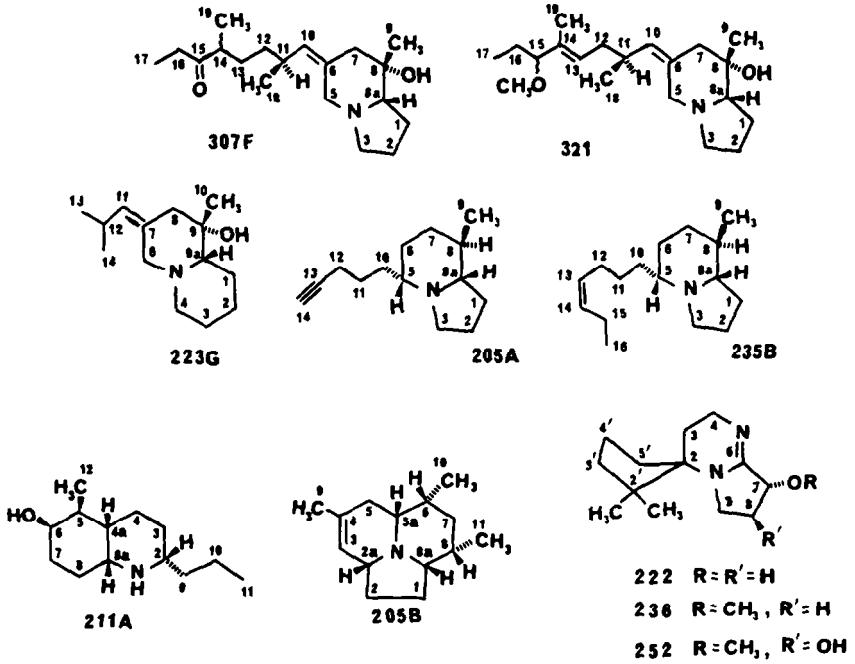


Figure 2. Structures proposed for alkaloids from Dendrobates pumilio. Pumiliotoxin 307F; pumiliotoxin 321; homopumiliotoxin 223G; indolizidine 205A; indolizidine 235B; decahydroquinoline 211A; azatricyclododecene 205B and amidines 222, 236 and 252.

Table 1. Isolation of alkaloids from skin extracts of Dendrobates pumilio.[a]

Alkaloid	Amount (mg)
Pumiliotoxin-A class	
307A' + A"	70
307F	57
321	80
323A	320
Allopumiliotoxins	
267A	----[b]
323B	11
339A	35
Homopumiliotoxins	
223G	8
Decahydroquinolines	
195A	80
211A	15
Indolizidines	
205A	16
235B	130
Azatricyclododecenes	
205B	15
Amidines	
222	18
236	63
252	37

[a]Extracts were prepared from skins of 2540 frogs collected on Isla Bastimentos, Bocas, Panama, and fractionated as described in EXPERIMENTAL.
[b]Allopumiliotoxin 267A was not isolated from this sample but was obtained in small quantity (4 mg) from another earlier sample of 1080 skins. Alkaloids 307F, 321, 211A and 235B were not isolated from the earlier sample, wh le the amidine alkaloids 222, 236 and 252 were isolated in much smaller amounts; 2, 3 and 9 mg, respectively.

Pumiliotoxin-A Class.

Pumiliotoxin A and B are the parent member of a class of 6-ylidene-8-hydroxy-8-methyl-indolizidines found in dendrobatid frogs (1). Two new members of this class, namely 307F and 321 were isolated from skin extracts of Dendrobates pumilio. Analysis of PMR (Figure 3) and ^{13}C NMR (Table 2) demonstrated that 307F was a 13,14-dihydro-15-keto analog of pumiliotoxin A, while 321 was 15-O-methyl ether of pumiliotoxin A (Figure 2). It appears likely that 321 is an artefact formed by reaction of methanol with pumiliotoxin A during fractionation or isolation. A small amount of the 15-O-methyl ether of allopumiliotoxin 323B (20 mg) was also isolated (mol. wt. 337, data not shown, see EXPERIMENTAL). Pumiliotoxin A and B do form O-methyl ethers with methanolic HCl (4). The PMR spectrum of 321 (Figure 3) has at least two OCH_3 singlets suggestive of the presence of both the 15R and 15S isomers. It also is uncertain whether 307F is an artefact formed by isomerization from pumiliotoxin A. It has similar gas chromatographic-mass spectral properties to another alkaloid, pumiliotoxin 307B, which also occurs in extracts of D. pumilio and other dendrobatid frogs, but which appears based on preliminary data to contain a cyclic ether function rather than a ketone in the side chain. Pumiliotoxin A isolated from Dendrobates pumilio (Table 1) was a mixture of 15R and 15S-epimers (7). Further properties of pumiliotoxins 307F and 321 are in EXPERIMENTAL.

Table 2. Carbon 13-magnetic resonance assignments for pumiliotoxins 307A' (see ref. 7), 307F and 321 (solvent $CDCl_3$).

Carbon Number	307A	307F	321
1	23.3	23.1	22.9
2	21,2	21.0	20.8
3	54.6	54.4	54.2
5	53.3	53.0	52.9
6	130.3	130.6	129.9
7	48.9	48.7	48.6
8	68.4	68.3	68.0
8a	71.7	71.6	71.4
9	24.3	24.2	24.0
10	133.8	132.6	133.5
11	32.6	27.9	32.2
12	35.6	20.3	35.2
13	125.0	34.8	126.9
14	137.7	46.6	134.5
15	79.6	213.6	89.0
16	27.8	48.3	26.0
17	10.2	14.5	10.0
18	21.3	21.5	21.0
19	11.4	15.9	10.2
CH_3O-	---	---	55.3

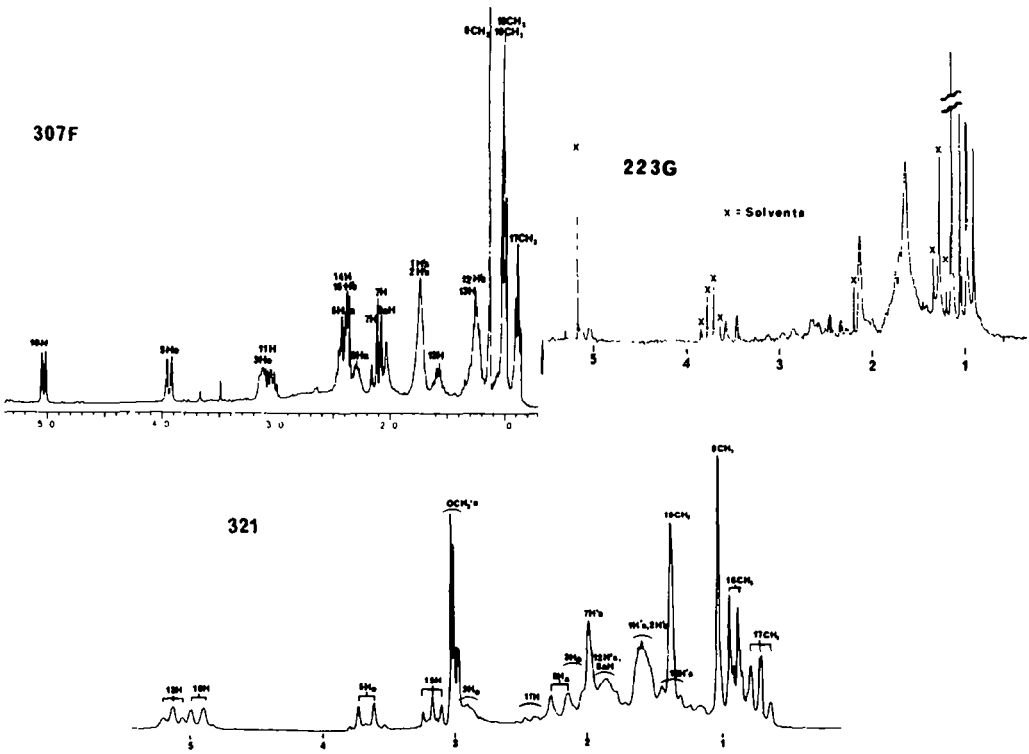


Figure 3. Proton magnetic resonance spectra ($CDCl_3$) of pumiliotoxins 307F (300 MH_z) and 321 (100 MH_z) and homopumiliotoxin 223G (100 MH_z). Chemical shifts in δ.

Allopumiliotoxins.

An allopumiliotoxin subclass of the pumiliotoxin-A class of dendrobatid alkaloids has been proposed (1). The structures of allopumiliotoxins 267A, 323B and 339A (Figure 1) have been previously defined (7).

Homopumiliotoxins.

One of the alkaloids of Dendrobates pumilio, namely 223G was remarkable in affording a mass spectral base peak at m/z 84 ($C_5H_{10}N^+$) rather than the m/z 70 ($C_4H_8N^+$) base peak characteristic of the pumiliotoxin-A class (see EXPERIMENTAL). It also exhibited a major fragment ion at m/z 180 ($C_{11}H_{18}NO^+$) instead of the m/z 166 ($C_{10}H_{16}NO^+$) fragment ion characteristic of the pumiliotoxin-A class. Only small amounts of 223G were available. A tentative structure for 223G (Figure 2) was indicated by PMR (Figure 3). Further spectral studies are in progress. The structure was compatible with the mass spectra and suggested the possible existence of a new class of dendrobatid alkaloids. Such 7-ylidene-9-hydroxy-9-methylquinolizidines could logically be called homopumiliotoxins. Whether other such quinolizidines occur in skin extracts of dendrobatid frogs is as yet uncertain.

Decahydroquinolines.

Pumiliotoxin C (195A) (Figure 1) had remained the only clearly defined member of cis-decahydroquinoline class of dendrobatid alkaloids since being described in 1969 (5). One trace alkaloid from D. pumilio, namely 211A, has now been shown by PMR (Figure 4) and ^{13}C-NMR spectral analysis (Table 3) to be the axial 6-hydroxy derivative of pumiliotoxin C. Recently, two additional members of the decahydroquinoline class have been isolated from Dendrobates histrionicus and were found to be trans-isomers unlike 195A and 211A, which are cis-isomers: alkaloid 219A is 2,5-diallyl-trans-decahydroquinoline and 243A is 2-allyl-5-pent-2-en-4-ynyl-trans-decahydroquinoline (11).

Table 3. Carbon 13-magnetic resonance spectral assignments for cis-decahydroquinolines 195A (pumiliotoxin C) and 211A (solvent $CDCl_3$). Assignments were confirmed by 2D-NMR analysis.

Carbon Number	195A	211A
2	57.8	57.8
3	27.3	26.9
4	27.1	26.7
4a	42.7	34.9
5	27.4	31.1
6	36.0	72.3
7	21.3	26.8
8	33.5	28.9
8a	56.1	55.8
9	39.7	39.3
10	19.1	19.1
11	14.2	14.3
12	19.9	15.8

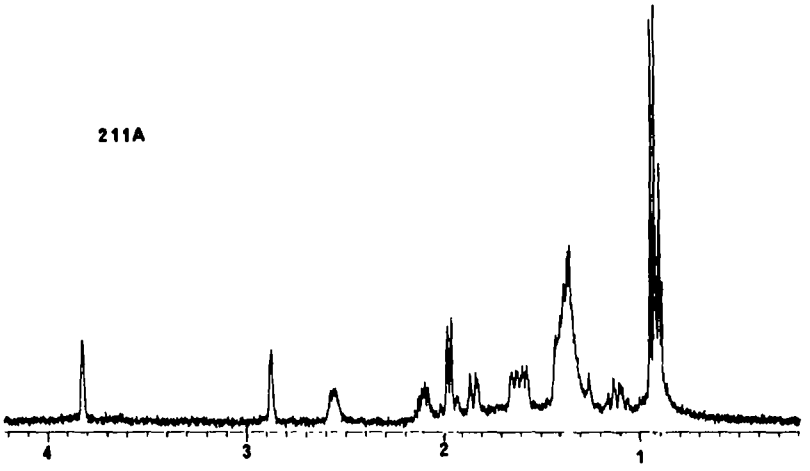


Figure 4. Proton magnetic resonance spectra ($CDCl_3$) of decahydroquinoline 211A (400 MH_z).

Indolizidines.

Earlier studies had suggested the presence of 3,5-disubstituted indolizidines, at that time termed gephyrotoxins, in skin extracts of dendrobatid frogs (1). The structures of three of these (223AB, 239AB, 239CD) have been elucidated (12,13). A structure for a fourth (195B) has now been proposed (11). Alkaloids 205A and 235B isolated from Dendrobates pumilio (Table 1) proved on ^{13}C NMR (Table 4) and PMR (Figure 5) analysis to be 5-substituted 8-methylindolizidines with 205A containing an equatorial 5-(pent-5-ynyl) substituent and 235B containing an equatorial 5-(hept-4,5-cis-enyl) substituent (Figure 2). Comparison of the ^{13}C chemical shifts of the sidechain of indolizidine 235B to those of cis 2- and 3-octenes (Table 5) confirms the sidechain structural assignment. It appears likely that a number of such 5-substituted 8-methylindolizidines occur in dendrobatid frogs (see ref. 3). One of these, namely 207A, has been isolated from Dendrobates speciosus (14). It was shown to be a 5-(pent-5-enyl) congener of indolizidine 205A. The mass spectra of these indolizidines are dominated by a base peak at m/z 138 (see EXPERIMENTAL).

Azatricyclododecenes.

One trace alkaloid (205B, $C_{13}H_{25}N$) from D. pumilio was isolated and shown by PMR (Figure 5) and ^{13}C NMR spectral analysis (see below) to be a novel 3,6,8-trimethyl-12-azatricyclo-[7.2.1.$0^{5,12}$]-2-dodecene. The structure is shown in Figure 2 (note numbering differs from IUPAC numbering above). The carbon-13 resonance assignments ($CDCl_3$, ppm) are as follows: C-1 29.3; C-2 28.5; C-2a 58.2; C-3 125.6; C-4 129.6; C-5 28.5; C-5a 56.6; C-6 32.7; C-7 35.6; C-8 32.5; C-8a 60.6; C-9 23.5; C-10 20.2; C-11 18.8. In a Cosy-spectrum cross peaks indicating couplings between 5aH and 6H, 8H and 8aH were scarce, but long range CH couplings for 6C (5 Hz), 7C (5 Hz) and 10C (4.5 Hz) were observed on irradiation of 5aH. This irradiation caused a 6% NOE effect on the 10-methyl group. The mass spectra and other properties (1 double bond, no exchangeable hydrogens) were compatible with the proposed structure of 205B. However, a Δ 4,5-structure could not be rigorously excluded by NMR analysis.

Table 4. Carbon 13-magnetic resonance spectral assignments for indolizidines 205A and 235B (solvent $CDCl_3$). *Values may be interchanged within the column.

Carbon	205A	235B
1	28.9t	29.1t
2	20.4t	20.4t
3	51.6t	51.8t
5	63.2d	63.4d
6	31.3t	31.3t
7	33.3t*	33.7t
8	36.0d	36.5d
8a	71.6d	71.3d
9	18.9q	18.8q
10	33.5t*	34.2t
11	24.8t	25.9t
12	18.8t	27.4t
13	84.4s	128.9d
14	68.6d	131.7d
15	----	20.5t
16	----	14.3q

Table 5. Comparison of carbon 13-magnetic resonance peaks of the side chain of indolizidine 235B to cis-2- and 3-octenes (ref. 15).

	Carbon Number in Octenes or (235B)							
	1 (16)	2 (15)	3 (14)	4 (13)	5 (12)	6 (11)	7 (10)	8 (5)
cis-2-octene	11.5	122.5	130.0	26.1	28.7	30.9	21.9	13.0
cis-3-octene	13.3	19.7	130.6	128.4	26.1	30.8	21.6	12.9
Indolizidine 235B	14.3	20.5	131.7	128.9	27.4	25.9	34.2	63.4

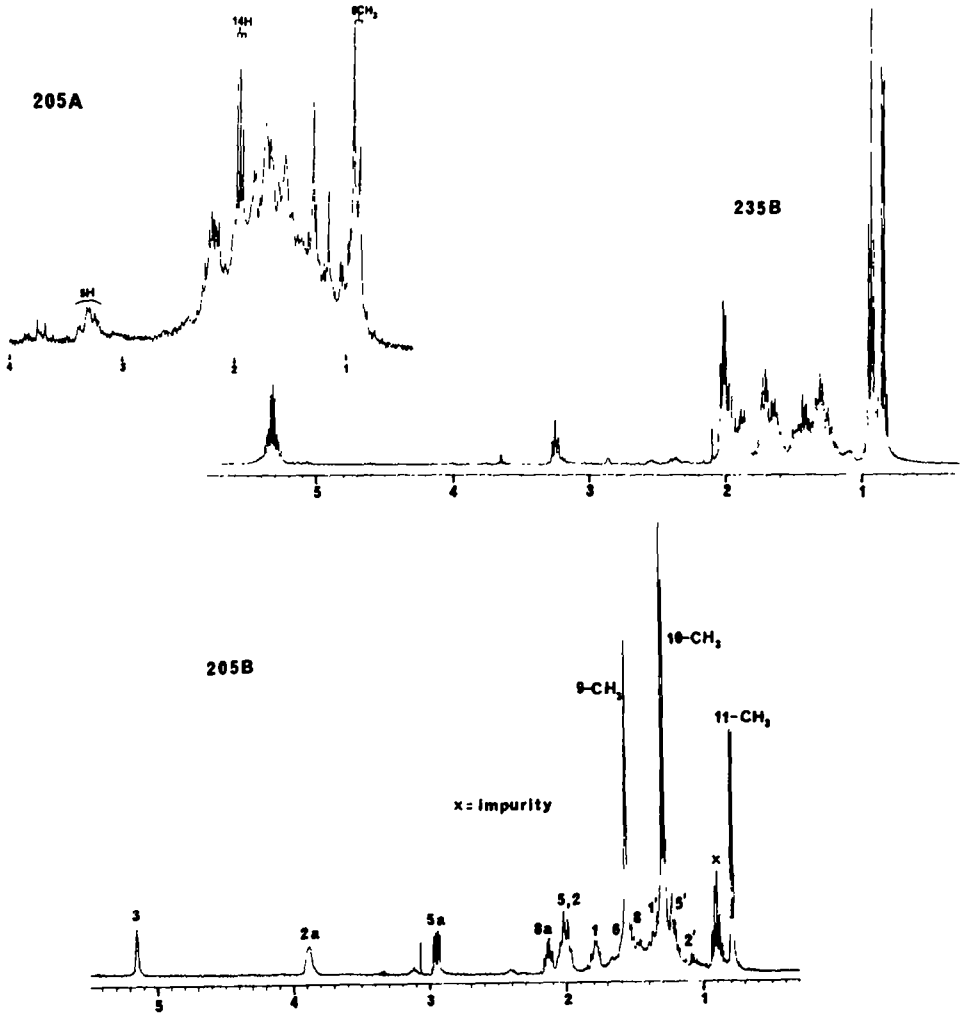


Figure 5. Proton magnetic resonance spectra ($CDCl_3$) of indolizidine 205A (100 MH_z) and 235B (400 MH_z) and azatricyclododecene 205B (400 MH_z). Chemical shifts in δ.

Such alkaloids with a tricyclic 6,6,5 ring system have not apparently been found in nature. But alkaloid 205B is similar in structure to alkaloids from lady bug beetles, which differ in having a tricyclic 6,6,6 ring system (16-18). Indeed, one insect alkaloid, namely hippocasine, has the same $-CH=CCH_3-$ moiety as in 205B, but lacks the other two methyl groups of 205B

Amidines.

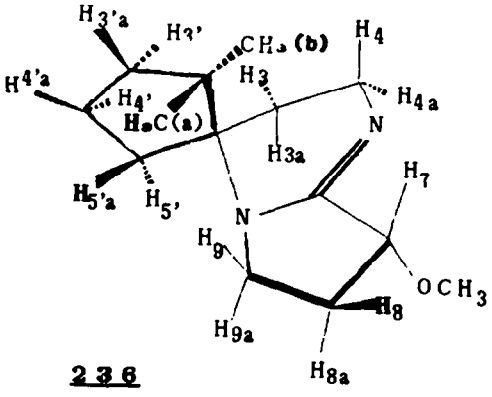
Earlier extracts from a population of Dendrobates pumilio occurring on Isla Bastimentos in Bocas, Panama, contained pumiliotoxins A, B and C as major alkaloids and a number of trace alkaloids (1,5). However, more recent extracts from the same population contained significant amounts of two amidine alkaloids 236 and 252. Structures for these alkaloids can be proposed from ^{13}C NMR (Table 6) and PMR (Table 7, Figure 6) spectral analysis and are consonant with the mass spectral fragmentation (see EXPERIMENTAL). Bands at about 1388 and 1370 cm^{-1} in the infrared spectra of 236 and 252 (spectra not shown) are characteristic of a gem-dimethyl grouping, which is consonant with the formulation of the gross structures of 236 and 252 as shown (Figure 3). One additional amidine alkaloid 222 was isolated in these extracts of Dendrobates pumilio and appears likely, based on ^{13}C NMR (Table 6) and PMR (Table 7) analysis, to be the des-O-methyl analog of 236 (see Figure 3). The Cosy and other NMR studies are compatible with the structural formulation for 236 shown in Table 7. For example, irradiation experiments revealed the following NOE effects: 1) 7H and the geminal dimethyl group; NOE 11% with less shielded 2-CH_3 (a) and 6% with the other 2-CH_3 (b). 2) 3H and 2-CH_3 (b), NOE 8%; 3'-H and 2-CH_3 (b), NOE 5%; 3H and 3'-H, NOE 3%. 3) 5a'H and 2-CH_3 (a), NOE 10%. Proton assignments for 222, 236 and 252 are given in Table 7. Further studies are in progress, since other structures, for example with the $-OCH_3$ at C-9 cannot be rigorously excluded.

Table 6. Carbon 13-magnetic resonance assignments for amidines 222,236 and 252 (solvent $CDCl_3$ except C_6D_6 for 222.

Carbon Number	222	236	252
2	58.6s	58.4s	58.9s
3	35.6t	35.5t	35.3t
4	52.7t	52.7t	53.0t
6	163.9s	164.0s	165.0s
7	67.8d	67.9d	67.9d
8	26.4t	26.8t	70.4d
9	50.4t	50.4t	57.7t
2'	43.5s	43.3s	43.7s
3'	20.0t	19.9t	20.1t
4'	34.3t	34.2t	36.0t
5'	39.3t	39.1t	39.0t
CH_3 (a)	25.9q	25.6q	25.8q
CH_3	23.8q	23.8q	23.8q
CH_3O	----	61.5q	62.2q

Table 7. Proton magnetic resonance assignments for amidines 222, 236 and 252 (solvent C_6D_6).

Hydrogen	222	236	252
3	1.80	1.79	1.73
3a	1.21	1.25	1.39
4	2.88	2.85	2.86
4a	2.42	2.46	2.77
7	3.96	3.88	3.83
8	2.83	2.70	----
8a	2.47	2.43	5.00
9	2.93	2.88	2.80
9a	2.46	2.50	3.23
3'	1.39	1.40	1.40
3'a	1.59	1.63	1.57
4'	1.51	1.53	1.54
4'a	1.67	1.65	1.69
5'	1.07	1.12	1.40
5'a	2.09	2.10	2.33
CH_3 (a)	0.92	0.89	0.81
CH_3 (b)	1.12	1.11	1.04
CH_3O	----	3.81	3.57



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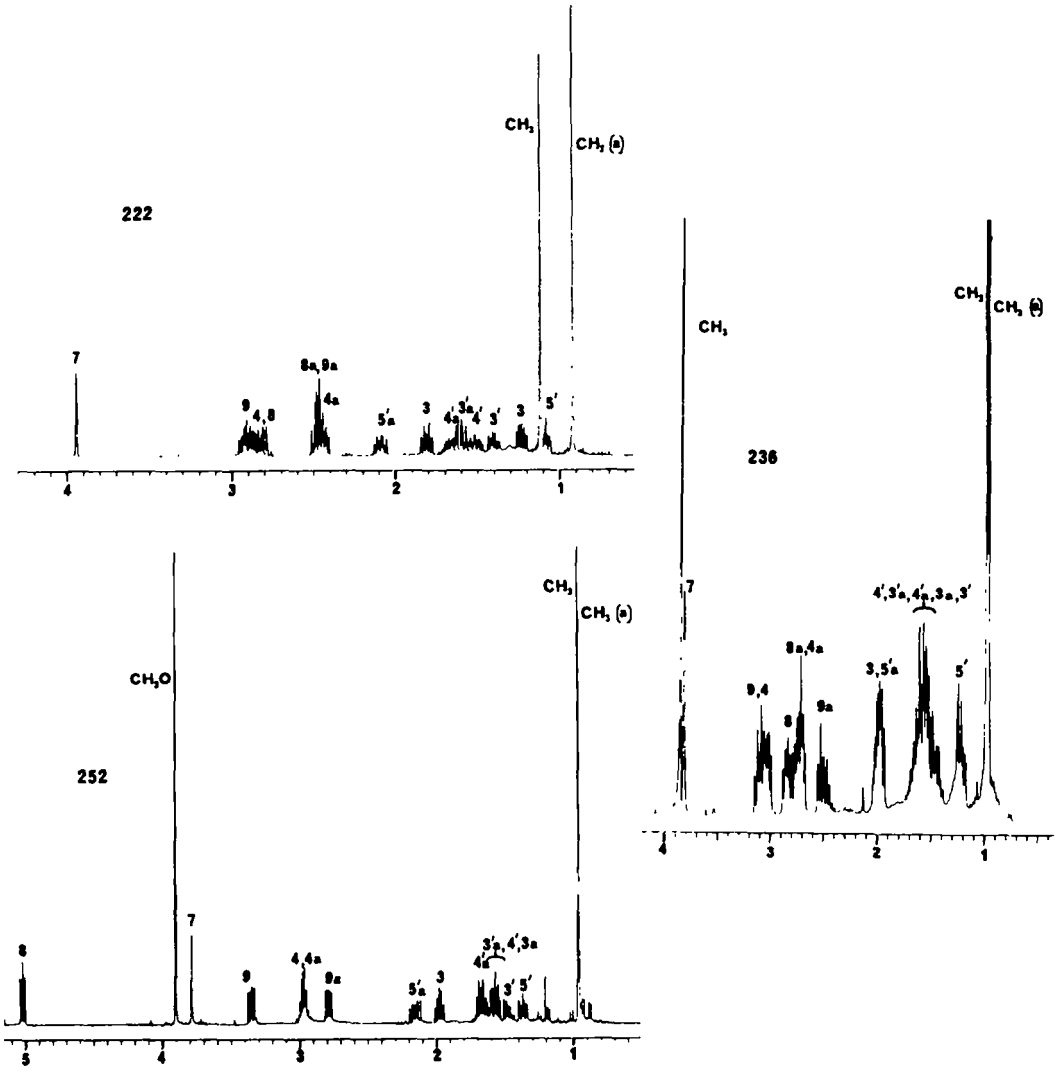

Figure 6. Proton magnetic resonance spectra (400 MH_z) of amidines 222, 236 and 252. Chemical shifts in δ. Solvent: $CDCl_3$ except for C_6D_6 for 222. Note marked changes in chemical shifts in $CDCl_3$ compared to C_6D_6 (Table 7).

These amidine alkaloids have not been detected in other dendrobatid frogs and in only one of nineteen of the populations of Dendrobates pumilio (3). The apparent emergence of amidines 236 and 252 as significant alkaloid components in this one population of Dendrobates pumilio over a period of less than twenty years is remarkable.

EXPERIMENTAL

High-resolution mass spectral data were obtained on JEOL D-300 mass spectrometer (electron impact, 70 eV). Combined gas chromatography-mass spectrometry was on a 1.5% OV-1 Chromasorb G AW-DMCS column programmed from 150-280° at 10°/min with a Finnegan 1015 mass spectrometer. NMR were obtained on JEOL FX 100 or GX 400 spectrometer or a Varian XL-300 MHz spectrometer. For a structure analysis 2D-NMR, homo- and heteronuclear shift correlation spectroscopies were routinely used. NOE effects were observed through 1D-differential spectra.

Isolation of Alkaloids

Methanolic extracts from 1080 skins (Jan. 1983) and from 2540 skins (Oct. 1983) of Dendrobates pumilio from Isla Bastimentos, were partitioned between aqueous methanol-chloroform. Alkaloids were then extracted from the chloroform layer with 0.05 N HCl. After adjusting the pH to >10 with aqueous ammonia, the aqueous layer was extracted with hexane and then with

chloroform. The hexane and chloroform layers were evaporated in vacuo to dryness to afford 0.75 g and 0.73 g, respectively, of crude alkaloids from the 2540 skin sample.

The hexane extract was chromatographed on a DIOL column (Merck, prepacked Lobar column, size B) first with n-hexane and triethylamine (200:1) and then with n-hexane, chloroform and triet ylamine (80:20:1) to afford nine main fractions (H-1 to H-9) as monitored by refractive index. GLC-mass spectral analysis on OV-1 and OV-225 columns revealed the main alkaloids of each fraction as follows: Fraction H-1, 138 mg, 195A and 205B; Fraction H-2, 141 mg 235B; Fraction H-3, 22 mg 205A; Fraction H-4, 8 mg 223G; Fraction H-5, 80 mg 321; Fraction H-6, 16 mg 307F; Fraction H-7, 44 mg 236; Fraction H-8, 200 mg 307A, 252; Fraction H-9, 47 mg 323A and 323B.

The chloroform extract was chromatographed on a reversed phase silica gel column (Merck, prepacked Lobar column, RP-8, size B) with acetonitrile, water and triethylamine (40:60:1) to afford five main fractions (C-1 to C-5). GLC-mass spectral analysis revealed the main alkaloids of each fraction as follows: Fraction C-1, 35 mg 339A; Fraction C-2, 12 mg mixture of alkaloids; Fraction C-3, 322 mg 323A; Fraction C-4, 30 mg 323A and 323B; Fraction C-5, 66 mg 252, 222 and 211A.

Each fraction was further separated and purified on the DIOL column. Fraction H-1 was repeatedly chromatographed with n-hexane containing triethylamine (0.3%) to separate 205B (13 mg) from 195A. Fraction H-8 was re-chromatographed with n-hexane, chloroform and triethylamine (80:20:1) to yield almost pure samples of 15-OCH_3-323B (20 mg), 307A (70 mg), 252 (27 mg), 251B (22 mg) and 211A (6 mg). Chromatography of the fraction C-5 with n-hexane, chloroform and triethylamine (50:50:1) afforded almost pure samples of 252 (10 mg), 211A (10 mg), and 222 (18 mg). Alkaloid 251B is under investigation.

Mass Spectra and Other Properties

The empirical formulae as determined by high resolution mass spectrometry, the electron impact mass spectra with intensities relative to a base peak set equal to 100 and other data on the new dendrobatid alkaloids are as follows:

Pumiliotoxins

307F, $C_{19}H_{33}NO_2$, m/z 307 (12), 194 (24), 193 (46), 166 (100), 70 (68). Gas chromatographic (GLC) elution temperature on a 1.5% OV-1 column programmed from 150-280°C at 10° per min and with a flow rate of 30 cc/min was 211°C. This is nearly identical to that of pumiliotoxin 307B, which also occurs in extracts of D. pumilio. It is possible that 307F is an artefact derived during isolation from 307A or 307B. $[\alpha]_D$, -8.5° (c 1.0, CH_3OH).

321, $C_{20}H_{35}NO_2$, m/z 321 (3), 304 (8), 166 (65), 70 (100). GLC elution temperature 223°C. It is probable that 321 is an artefact derived during isolation by reaction of methanol with 307A to yield two major isomers epimeric at C-15. $[\alpha]_D$, +21.3° (c 1.0, CH_3OH).

Homopumiliotoxins

223G, $C_{14}H_{27}NO$, m/z 223 (18), 190 (22), 180 (39), 98 (27), 84 (100). GLC elution temperature 163°C.

Decahydroquinolines

211A, $C_{13}H_{25}NO$, m/z 211 (3), 168 (100), 152 (32), 150 (13). GLC elution temperature 166°C. $[\alpha]_D$ -11.7° (c 1.0, $CHCl_3$).

Indolizidines

205A, $C_{14}H_{23}N$, m/z 205 (1), 204 (2), 138 (100). GLC elution temperature 158°C. $[\alpha]_D$ -35° (c 0.24, CH_3OH).

235B, $C_{16}H_{29}N$, m/z 235 (1), 234 (1), 138 (100). GLC elution temperature 166°C. $[\alpha]_D$, +11.3° (c 1.0, CH_3OH).

Azatricyclododecenes

205B, $C_{14}H_{23}N$, m/z 205 (38), 204 (54), 190 (100). GLC elution temperature 158°C, $[\alpha]_D$, -8.5° (c 0.59, $CHCl_3$).

Amidines

222, $C_{13}H_{22}N_2O$, m/z 222 (1), 221 (2), 112 ($C_5H_8N_2O$, 100). GLC elution temperature 180°C.

236, $C_{14}H_{24}N_2O$, m/z 236 (12), 126 ($C_6H_{10}N_2O$, 100). GLC elution temperature 172°C. $[\alpha]_D$, +55.6° (c 1.0, CH_3OH).

252, $C_{14}H_{24}N_2O_2$, m/z 252 (4), 251 (3), 142 ($C_6H_{10}N_2O$, 100). GLC elution temperature 179°C. $[\alpha]_D$, +18.4° (c 0.47, CH_3OH), -4.3° (c 0.47, $CHCl_3$).

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