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ALKALOIDS FROM A PANAMANIAN POISON FROG, *DENDROBATES SPECIOSUS*: IDENTIFICATION OF PUMILIOTOXIN-A AND ALLOPUMILIOTOXIN CLASS ALKALOIDS, 3,5-DISUBSTITUTED INDOLIZIDINES, 5-SUBSTITUTED 8-METHYLINDOLIZIDINES, AND A 2-METHYL-6-NONYL-4-HYDROXYPIPERIDINE

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ABSTRACT.—*Dendrobates speciosus* is a small red or orange frog that occupies a small geographic range in the highlands of western Panama, where it occurs abundantly in some cloud forest habitats. Gc-ms analysis indicated the presence of at least 30 alkaloids in MeOH skin extracts from population samples at the extreme eastern end of the known geographic range. Eleven alkaloids were isolated by cc in quantities sufficient for 2D-nmr spectral analysis, which in some cases confirmed their identity with alkaloids known from other species and in other cases led to assignment of structures. Pumiliotoxin **251D**, pumiliotoxin A [**307A**], pumiliotoxin B [**323A**], and allopumiliotoxin **267A** were identified as major constituents. *N*-Oxides of **323A** and **267A** were also isolated. Indolizidines **195B** and **223AB** with 3-butyl-5-methyl and 3-butyl-5-propyl substituents, respectively, were identified. The 5-substituents of the 8-methylindolizidines **207A** and **235B′** were assigned as $-(CH_2)_3CH{=}CH_2$ and $-(CH_2)_5CH{=}CH_2$, respectively; indolizidine **235B′** from *D. speciosus* is, thus, a positional double-bond isomer of indolizidine **235B** previously isolated from a closely related poison frog, *Dendrobates pumilio*. A piperidine **241D** was isolated and assigned the structure *cis-cis*-2-methyl-6-nonyl-4-hydroxypiperidine.

The Dendrobatidae are a family of small Neotropical frogs, which are currently partitioned among five genera: *Colostethus, Dendrobates, Epipedobates, Minyobates,* and *Phyllobates* (1). The last four genera together are a single evolutionary lineage of "poison frogs" characterized by bright warning colorations and defensive skin alkaloids. MeOH skin extracts from 41 species have been studied, representing about 73% of the named species within this lineage of neotropical poison frogs; these extracts have yielded more than 200 novel alkaloids, representing at least a dozen classes of structurally and pharmacologically diverse compounds (2,3). Because of the large number of dendrobatid alkaloids, boldface numerical designations according to nominal mass with identifying letters have been used (3).

The present report documents the isolation and identification of alkaloids for a Central American species, *Dendrobates speciosus* Schmidt, 1857, a very rare frog until recently. The first three specimens of *D. speciosus* were collected in 1848 by the Polish bontanist Josef Warszewicz, on the Caribbean versant of western Panama in "der Weg zwischen Bocca del toro und dem Vulcan Chiriqui" (4,5). It was not collected again until 1923, when E.R. Dunn and C.B. Duryea obtained specimens at two places apparently near the type locality (6,7). *D. speciosus* again drifted into obscurity until 1976, when C.W. Myers discovered a population in the montane valley of the upper Río Chiriquí, about 25 km southeast of the earlier records. Myers worked on foot from a helicopter base camp, but, in 1981, this highland valley (now flooded due to a hydroelectric dam) was made accessible by a road that crosses a low section of the continental divide between the eastern end of the Cordillera de Talamanca and the western end of the Serranía de Tabasará (8,9). Ease of access into this region has allowed us to demonstrate that *D. speciosus*, although not uniformly distributed, occurs in local, very dense

populations in wet cloud forest at 1140–1410 m above sea level. This area also is the source of specimens studied by German aquarists (10,11). *D. speciosus* is endemic to wet montane forest of western Panama, where it appears confined to a small geographic range on the eastern terminus of the Cordillera de Talamanca, approximately between meridians 82°30′ and 82°12′ W.

D. speciosus is a member of the *histrionicus* group in the genus *Dendrobates* (7). This group of eight species has been rather thoroughly studied with respect to alkaloids; 60 alkaloids have been reported from *Dendrobates histrionicus* and 100 alkaloids from *Dendrobates pumilio* (2,3). Alkaloids have been characterized by gc-ms analysis from the other six members of the *histrionicus* group, namely *Dendrobates arboreus, Dendrobates granuliferus, Dendrobates lehmanni, Dendrobates occultator, D. speciosus,* and a new species from Panama (3). This report documents the isolation and identification of alkaloids present in MeOH skin extracts of *D. speciosus*. Alkaloids of the following classes were detected: histrionicotoxins, indolizidines, pumiliotoxins, decahydroquinolines, and 2,6-disubstituted piperidines. The major alkaloids were pumiliotoxins, indolizidines, and a new piperidine. Structures of the 8-methyl-indolizidines **207A** and **235B′** and the 2-methyl-6-nonyl-4-hydroxypiperidine [**241D**] are established. *N*-Oxides of pumiliotoxin B [**323A**] and of allopumiliotoxin **267A** were isolated. The structures of the alkaloids isolated from *D. speciosus* are shown in Figure 1.

EXPERIMENTAL

SOURCE MATERIAL.—MeOH skin extracts were obtained at the following localitities in western Panama, all in the Province of Chiriquí or on the border (i.e., the continental divide) between the provinces of Chiriquí and Bocas del Toro. Coordinates were determined from a topographic map printed in 1969 (1:50,000, Sheet 3742 II, Quebrada de Yuca). Voucher specimens are preserved in the research collection of the American Museum of Natural History.

Population A.—About 1.5 km N La Sierpe (a place at the now-flooded junction of Río Hornito at Río Chiriquí), Quebrada Los Chorros drainage, 1150 m (8°44′43″N, 82°13′40″W), 29 February 1976 [lit. (3) population 29A].

Population B.—Continental divide above upper Quebrada de Arena, 1250–1410 m (ca. 8°47′N, 82°13′19″–82°14′22″W); (B) January 1983 and (B′) April 1984 [lit. (3) populations 29B and 29B′].

Population C.—Two km airline NE La Sierpe, Quebrada de Frank drainage, 1140–1300 m (8°44′53″N, 82°13′00″–25″W); January 1983, April 1984, and May 1985 [lit. (3) population 29C].

ANALYTICAL INSTRUMENTATION.—Gc analyses were made with a 6-ft 1.5% OV-1 packed column or with a 25-m HP-1 methyl silicone capillary column on a flame ionization instrument programmed from 150° to 280° at 10° per min. A Finnigan 4500 mass spectrometer with an INCOS data system was used for direct probe and gc-ms in both the chemical ionization and electron impact mode. Parent ions of the alkaloids were readily detected as the protonated parent ions $[M + 1]^+$ using NH_3 as the ionizing gas. In the case of the *N*-oxides, protonated parent ions were not detected with NH_3 but could be detected with isobutane as the ionizing gas. ND_3 as the ionizing gas caused complete exchange of all OH and NH protons and afforded deuterated and exchanged parent ions. Empirical formulae for the alkaloids were obtained by combined gc-ms in the high resolution mode using a VG 70/70 spectrometer.

Nmr spectra were obtained in $CDCl_3$ solution on a Nicolet NT-500 or Varian XL-300 MHz spectrometer. All proton shifts (δ) were referenced to internal TMS.

ISOLATION OF ALKALOIDS.—Skins were placed in MeOH in the field with at least 2 volumes of MeOH per 1 volume of wet skin. Subsequent extractions were carried out at NIH by cutting the skin into small pieces and then grinding at least three times with twice the volume of MeOH. Combined MeOH extracts were diluted with an equal volume of H_2O, and the aqueous MeOH was then extracted three times, each time with one-half of the volume of $CHCl_3$. The combined $CHCl_3$ layers were extracted three times with 0.1 N HCl, each time with one-third their volume. The combined acid extract was basified (pH > 9) with 1 N aqueous NH_3 and extracted three times, each time with one-half the volume of $CHCl_3$. The final $CHCl_3$ extracts containing the alkaloids were dried over anhydrous Na_2SO_4 and evaporated in vacuo at 37°. Samples of 5–10 skins from each collection site were fractionated, and the alkaloid fraction isolated as described above was subjected to gc-ms analysis as described by Daly *et al.* (3).

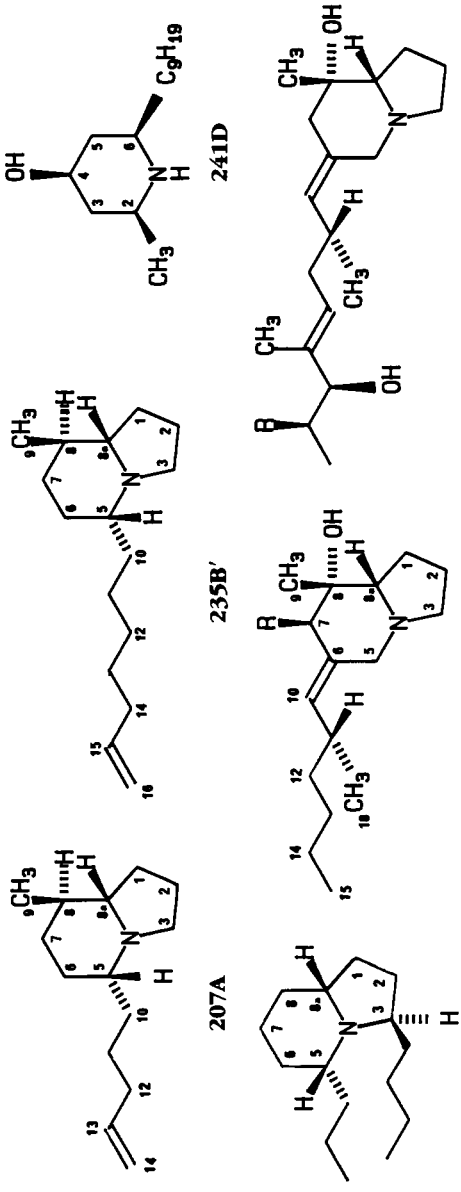

FIGURE 1. Structures of alkaloids isolated from *Dendrobates speciosus* (Population C). The absolute configurations of new structures **207A**, **235B′** and **241D** are unknown.

Based on these analyses, the skins containing a new alkaloid **241D**, which were obtained from population C in April 1984 and March 1985, were combined to give a total of 258 skins, wet wt 25 g. The initial purification of the alkaloids as described above yielded 189 mg of crude alkaloids, which was chromatographed on a Si gel column (Analtech, 10 μm) with CH_2Cl_2-MeOH (10:1). Aliquots of each fraction (3 ml) were analyzed by tlc (Analtech, Si gel uniplates) and by gc-ms. Based on these analyses, seven combined fractions (A–G) were made. Fraction A (1–5) was a 5-mg mixture of low mol wt alkaloids and nonalkaloid substances. The alkaloids were identified by gc-ms analysis as **167A**, **183A**, **195B**, **203A**, and **211B** (3). Fraction B (7–12) contained a single alkaloid **251D** (58 mg). The identity of **251D** was confirmed by nmr comparison with authentic material (12). Fraction C (14–16) contained a single alkaloid (8 mg) with the gc-ms properties of **235B**. However, the alkaloid proved on nmr analysis to be isomeric with **235B** previously isolated from *D. pumilio* (13). Eims *m/z* $[M]^+$ 235 (1), 166 (19), 138 (100), 70 (10); ^1H nmr (300 MHz, $CDCl_3$) δ 5.31 (m, 1H, H-15), 4.95 (m, 2H, H-16), 3.27 (br t, 1H, H-5), 2.49 (br, 1H, H-8a), 1.86 (br, 1H, H-3), 1.45 (br, 1H, H-8), 1.05 (br, 1H, H-3), 1.0–2.15 (br m, 22H), 0.89 (d, $J = 6.2$, 9-*Me*); $[\alpha]^{25}D$ −61° ($c = 0.5$, MeOH).[1] Fraction D (18–23) contained two alkaloids, **223AB** and **307A**. Fraction E (24-28) contained five alkaloids, **195A**, **195C**, **267A**, **241D**, and the *N*-oxide of **267A**. Fraction F (29–40) contained two major alkaloids **207A** and **323A** and an *N*-oxide of **323A**. Fraction G (41–51) (6 mg) contained alkaloids **255**, **307B**, **353** (3), and some nonalkaloidal substances.

Fractions D, E, and F were subjected individually to alumina cc using a step gradient in increments of 20% starting with 100% petroleum ether to 100% Et_2O and then to 100% EtOH, as described below.

Isolation of **223AB** *and* **307A**.—Alumina cc of fraction D separated the two compounds. The first compound to elute was **223AB** (16 mg). The identity of **223AB** was confirmed by nmr comparisons with authentic material (14,15). The second compound to elute was **307A** (1 mg). The identity of **307A** was confirmed by nmr comparisons with authentic pumiliotoxin A (16).

Isolation of **195A**, **195C**, **267A**, **241D**, *and an N-oxide of* **267A**.—Alumina cc of fraction E gave first compounds **195A** and **195C**, totaling 20 mg, which co-eluted and were identified by gc-ms analysis (3). Compound **267A** (28 mg) eluted next, followed by the *N*-oxide of **267A** (2 mg). The identity of alkaloid **267A** was confirmed by nmr comparisons with authentic material (16). The *N*-oxide of **267A** was first thought to be an isomer of **267A** based on mass spectral analysis, which indicated an apparent molecular ion at *m/z* 267 on ei analysis and an apparent protonated molecular ion at *m/z* 268 on ci analysis with NH_3. The ei mass spectrum of this *N*-oxide was virtually identical to that of **267A**. However, ci analysis with isobutane gave a protonated molecular ion at *m/z* 284. The nmr spectral analysis demonstrated a downfield (lowfield) shift in both the proton and carbon resonances of those nuclei adjacent to nitrogen, suggesting an *N*-oxide structure (see Table 1). The final compound to elute was **241D**, the first piperidine alkaloid to be identified from dendrobatid frogs. Less than one mg of pure **241D** was isolated, mainly because of losses on silica chromatography. Hrms calcd for $C_{15}H_{31}NO$, 241.2406, found 241.2366; eims *m/z* $[M]^+$ 241 (5), 226 (13), 182 (13), 114 (100), 70 (80); ^1H nmr (300 MHz, $CDCl_3$) δ 3.66 (m, 1H, H-4), 2.68 (m, 1H, H-2), 2.54 (m, 1H, H-6), 1.96–1.98 (br t, 2H, H-5e, H-3e), 1.49 (br t, 2H), 1.25 (br m, 14H), 1.13 (d, $J = 6.0$, 3H, CH*Me*), 0.99–1.01 (m, 2H, H-3a, H-5a), 0.88 (t, $J = 7$, 3H, CH_2Me); $[\alpha]^{25}D$ +39° ($c = 0.2$, MeOH).

Isolation of alkaloids **207A**, **323A**, *and an N-oxide of* **323A**.—Alumina cc of fraction F gave first **207A** (20 mg). The structure of **207A** was determined to be that of 5-(pent-4-enyl)-8-methylindolizidine by nmr analysis (17). Eims *m/z* $[M]^+$ 207 (1), 180 (4), 138 (100); ^1H nmr (300 MHz, $CDCl_3$)[2] δ 5.78 (m, 1H, H-13), 5.01 (m, 2H, H-14), 3.90 (br m, 1H, H-5), 2.68 (br m, 1H, H-8a), 2.45 (br m, 1H, H-3), 1.15 (br m, 1H, H-3), 0.99 (d, 3H, CH*Me*). Alkaloid **323A** (17 mg), which eluted second, was identical by nmr comparison with authentic pumiliotoxin B (16). The third compound to elute (1.5 mg) was an *N*-oxide of **323A**. It had a mass spectrum identical to that of **323A** in the ei mode but a different nmr spectrum. The mass spectrum using isobutane as the ionizing gas gave a protonated molecular ion of *m/z* 340 $[M + 1]^+$, as expected of an *N*-oxide. With NH_3 as the ionizing gas the *N*-oxide afforded an apparent protonated molecular ion at *m/z* 324. Further details on pumiliotoxin B *N*-oxide will be published separately.

RESULTS AND DISCUSSION

Analysis of the alkaloid fractions from three different populations of *D. speciosus* by combined gc-ms on 1.5% OV-1 packed columns (3) revealed the presence of 30 alkaloids (see Figure 2 for gas chromatograms for populations B′ and C). The alkaloids

[1]Alkaloid **235B** from *D. pumilio* was dextrorotatory ($[\alpha]^{25}D = +11.3°$, c = 1.0 MeOH) (13).

[2]The spectrum is that of the salt, presumably due to DCl present in the solvent.

TABLE 1. ^{1}H- and ^{13}C-nmr Assignments for **267A**[a] and the *N*-oxide of **267A**.[b]

Assignment	^{13}C nmr		^{1}H nmr	
	267A	*N*-oxide of **267A**	**267A**	*N*-oxide of **267A**
1	22.8	22.6	1.72	1.09, 2.51
2	21.4	20.1	1.72	1.98, 2.39
3	54.5	67.9	2.23 (ax), 3.05 (eq)	3.25 (ax), 3.51 (eq)
5	49.1	60.8	2.61 (ax), 3.60 (eq)	3.80 (ax), 4.36 (eq)
6	133.4	129.0		
7	81.1	80.1	3.72	3.90
8	70.2	71.7		
8a	65.5	69.6	2.49	3.45
9	20.8	19.6	1.22	1.22
10	138.8	143.0	5.34	5.71
11	32.2	32.3	2.39	2.45
12	37.2	36.7		
13	29.8	29.7	1.15–1.35	1.10–1.35
14	22.8	22.7		
15	14.3	14.0	0.89	0.88
18	21.4	20.9	0.99	1.03

[a]Values for **267A** are essentially identical to those previously reported (16).
[b]δ, $CDCl_3$, 5-mm sample tube, Varian Assoc. XL-300 nmr spectrometer, 75.4 MHz ^{13}C frequency.

from each of the three populations (A, B, and C) are tabulated, and relative amounts are shown in Table 2. Capillary gc analysis revealed additional isomeric alkaloids. Ten alkaloids were isolated by hplc and Si gel or alumina cc in quantities and purity sufficient for detailed nmr spectral analysis using a variation of homonuclear 2D and heteronuclear 2D nmr spectroscopy (17). Six of the ten isolated alkaloids, namely pumiliotoxin **251D**, allopumiliotoxin **267A**, pumiliotoxin A [**307A**], pumiliotoxin B [**323A**], and indolizidine **223AB**, were shown to be identical to known dendrobatid alkaloids by ms and nmr spectroscopy (see Experimental). Two of the isolated alkaloids proved to be previously unreported *N*-oxides of allopumiliotoxin **267A** and of pumiliotoxin B [**323A**]. Two of the other new isolated alkaloids, **207A** and **235B′**, belonged to the indolizidine class of alkaloids: Both were shown to be 8-methyl-5-substituted indolizidines. The substituents at the 5 position were shown by nmr spectral analysis to be pent-4-enyl and hept-6-enyl, respectively. Thus, the indolizidine **235B′** isolated from *D. speciosus* proved to be an isomer of the indolizidine **235B** isolated from *D. pumilio*, which has the double bond at a different position in the 5-heptenyl side chain. A heretofore uncharacterized alkaloid [**241D**] was isolated and shown to be a *cis-cis*-2-methyl-6-nonyl-4-hydroxypiperidine.

Pumiliotoxin-A class.—The pumiliotoxin-A class of dendrobatoid alkaloids comprises mainly 6-alkylidene-8-methyl-8-hydroxy indolizidines. Three subclasses have been proposed (2,3): A parent pumiliotoxin subclass, an allopumiliotoxin subclass, which differs by the presence of an additional 7-hydroxy group, and a new homopumiliotoxin subclass (13), whose one member differs by being a quinolizidine rather than an indolizidine.

Alkaloids of the pumiliotoxin subclass occur in all species of the *histrionicus* group. Indeed, pumiliotoxin A and B were first isolated as major alkaloids of a population of *D. pumilio* (18) from the *histrionicus* group. *D. speciosus* also contains alkaloids of the pumiliotoxin subclass as major alkaloids, namely pumiliotoxin **251D**, pumiliotoxin A, and pumiliotoxin B (Table 2). A mixture of 15*R* and 15*S*-epimers, **307A** and **307A′**, of pumiliotoxin A was isolated by Si gel chromatography from *D. speciosus* as

TABLE 2. Alkaloids from *Dendrobates speciosus*.

Alkaloids[a]	Empirical Formula[b]	Major Mass Spectral Spectral Ions[c] (*m/z*)	Occurrence in Various Populations[d,e]		
			A	B (B′)	C
Histrionicotoxins					
235A (3)	$C_{15}H_{25}NO$	194,96	+	−(−)	−
Pumiliotoxin-A Class					
251D	$C_{16}H_{29}NO$	166,70	++	++(+++)	+++
307A	$C_{19}H_{33}NO_2$	166,70	−	+++(+++)	+[f]
307B (3)	$C_{19}H_{33}NO_2$	166,70	−	+(+)	+[f]
323A	$C_{19}H_{33}NO_3$	166,70	+	+++(+++)	+++
353 (3)	"$C_{19}H_{31}NO_5$"	166,70	−	+(+)	+[f]
Allopumiliotoxins					
267A	$C_{16}H_{29}NO_2$	182,70	++	+++(+++)	+++
Homopumiliotoxins					
223G (13)	$C_{14}H_{27}NO$	180,84	+	−(−)	−
Indolizidines					
167A (3)	$C_{11}H_{21}N$	138	+	+(−)	++
167B (3)	"$C_{11}H_{21}N$"	124	−	−(+)	−
181B (3)	"$C_{12}H_{23}N$"	138	−	+(+)	++
195B(3)	$C_{13}H_{25}N$	138	+	+(+)	+
203A (3)	$C_{14}H_{21}N$	138	+++	+(++)	+[f]
207A	$C_{14}H_{25}N$	138	++	++(++)	++
223AB	$C_{15}H_{29}N$	180,166	+++	+++(+++)	++
235B′	$C_{16}H_{29}N$	138	+	+++(+++)	++
237D (3)	"$C_{16}H_{31}N$"	138	−	++(++)	−
Decahydroquinolines					
195A (3)	$C_{13}H_{25}N$	152	−	++(++)	+
Piperidines					
241D	$C_{15}H_{31}NO$	114	++	++(+)	+++
255	$C_{15}H_{29}NO_2$	114	++	++(+)	+[f]
Unclassified					
183A	$C_{12}H_{25}N$	154	−	++(++)	+[f]
195C (3)	$C_{13}H_{25}N$	152	−	++(++)	+++
207E (3)	"$C_{13}H_{25}NO$"	164,84	+	−(−)	−
211B (3)	"$C_{13}H_{25}NO$"	160	−	+(+)	+[f]
223A (3)	"$C_{15}H_{29}N$"	180	−	−(++)	−
225A (3)	"$C_{14}H_{29}NO$"	168	−	++(−)	−
231B (3)	$C_{16}H_{25}N$	152	++	−(−)	++
251C (3)	"$C_{19}H_{33}N$"	154	−	−(+)	−
275A (3)	$C_{19}H_{33}N$	152	−	−(+)	−
291B (3)	"$C_{19}H_{33}NO$"	168	−	−(+)	−

[a]Alkaloids are designated by mol wt in boldface type, followed where necessary by a code letter to distinguish from other alkaloids of the same nominal weight [see Daly and Spande (2) and Daly *et al.* (3)].

[b]Empirical formulae by hrms. Tentative empirical formulae not confirmed by hrms are in quotations.

[c]Major ions by eims.

[d]+++, present as a major alkaloid; ++, present as a minor alkaloid; +, present as a trace alkaloid; −, not present. Analysis based on gc analysis of alkaloid fractions from 5 or 10 frog skins on a 6-ft 1.5% OV-1 column programmed from 150° to 280° with a flame ionization detector, followed by combined gc-cims (NH_3 or ND_3) on a Finnigan 1015 mass spectrometer. Additional data were obtained by eims with a VG 70/70 mass spectrometer and with capillary columns on a Finnigan 4500 mass spectrometer.

[e]Populations: A, 1.5 km N. La Sierpe, Chiriqui, Panama, Feb. 1976; B, divide above Quebrada de Arena, Chiriqui, Panama, Jan. 1983; B′, (in parentheses), April 1984; C, Quebrada de Frank, Chiriqui, Panama, Jan. 1983.

[f]Not detected in original 10-skin sample, but detected as trace alkaloids in pooled sample from this population (see Experimental).

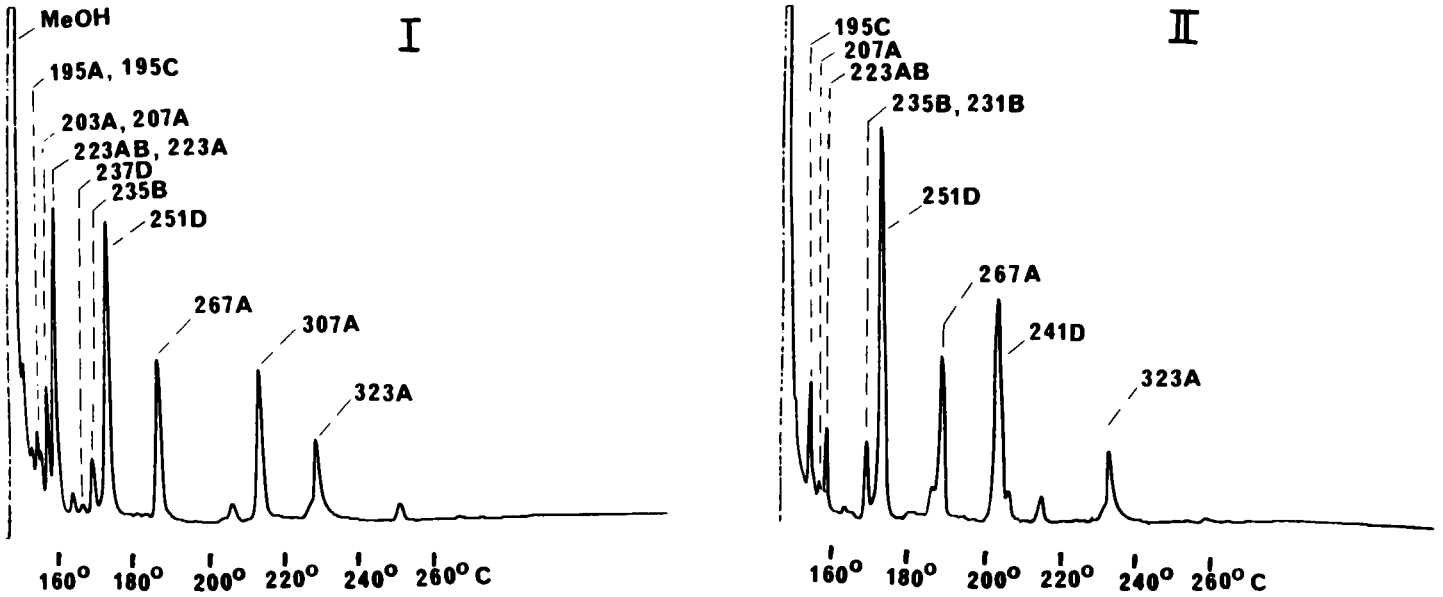

FIGURE 2. Gas chromatographic profile of alkaloids from *Dendrobates speciosus*. I. Population B′, 10 skins, April 1984. II. Population C, 10 skins January 1983. The chromatogram was obtained on a Hewlett-Packard 5890 with a 6-ft × 2-mm i.d. 1.5% OV-1 on 80/100 mesh Gas Chrom Q column and a flame ionization detector. A sample of 2 μl of MeOH alkaloid fraction equivalent to 2 mg wet wt skin was injected at a column temperature of 150°. After the maximum of the solvent peak (MeOH) was passed, the column was programmed from 150 to 280° at 10°/min. Alkaloids identified by combined gc-ms analysis. Trace alkaloids are not designated.

was also found in the case of *D. pumilio* (16). It has now been shown that pumiliotoxin A readily epimerizes at the 15 position under acid conditions and that only the 15*S*-isomer occurs naturally in *D. pumilio* and presumably other dendrobatid frogs (H.M. Garraffo and T.F. Spande, unpublished data). Pumiliotoxin B was accompanied by a small amount of the *N*-oxide.

Allopumiliotoxin **267A**, the 7-axial hydroxy derivative of pumiliotoxin **251D**, is widespread as a major alkaloid in dendrobatid poison frogs and occurs in certain populations of all species of the *histrionicus* group of *Dendrobates*. *D. speciosus* is no exception, and all three populations contain allopumiliotoxin **267A** as a major alkaloid (Table 2). Allopumiliotoxin **267A** was accompanied by a small amount of the *N*-oxide.

Indolizidines.—Two major subclasses of simple disubstituted indolizidines have been identified from dendrobatid frogs. The first class consists of 3,5-disubstituted indolizidines, as exemplified by indolizidine **223AB**, formerly known as gephyrotoxin **223AB** (14) and its side chain hydroxy derivatives, indolizidines **239AB** and **239CD** (15). Indolizidine **223AB** occurs in most species of the *histrionicus* group. In *D. speciosus* it is a major alkaloid (Table 2).

The second class of simple disubstituted indolizidines from dendrobatid frogs consists of 5-substituted 8-methylindolizidines, as exemplified by indolizidine **205A** with a 5-$CH_2CH_2CH_2C \equiv CH$ substituent, which had been isolated from a population of *D. pumilio* and the structure determined by nmr analysis (13). Indolizidine **205A** and proposed analogs **203A** and **207A** (13) occur in many dendrobatid poison frogs and in all species of the *histrionicus* subgroup of *Dendrobates*. In *D. speciosus*, indolizidine **207A** occurs as a minor constituent. The ^{1}H-nmr assignments (see Experimental) are consistent with the formulation shown in Figure 1 with a 5-$(CH_2)_3CH{=}CH_2$ substituent. The relative configuration of 5*E*,9*E* with 5-equatorial and 8-equatorial substituents is assigned based on homonuclear 2D-nmr analysis (19) and is the same as that proposed for indolizidine **205A** (13). Indolizidine **207A** has recently been recorded based on gc-ms from an non-dendrobatid frog, namely a Madagascan ranid of the subfamily Mantellinae, *Mantella madagascarensis* (20). Another 5-substituted 8-methylindolizidine **235B** with a 5-*Z*-$(CH_2)_3CH{=}CHCH_3$ side chain has been isolated from one population of *D. pumilio* and the structure determined by nmr analysis (13). The indolizidine of mol wt 235 isolated from *D. speciosus* proved on ^{1}H-nmr analysis (see Experimental for assignments) to be a double bond positional isomer of the indolizidine **235B** isolated from *D. pumilio*. Indolizidine **235B′** from *D. speciosus* shows the ABX pattern of a terminal vinyl group, unlike indolizidine **235B** from *D. pumilio*, which has a *Z*-4,5-double bond in the 8-heptenyl side chain. The isomer with the terminal double bond will be referred to as **235B′**. The positional isomer with the *Z*-4,5-double bond will be referred to as **235″**. Because of lack of separation of the two **235B** isomers on packed columns and because of identical mass spectral fragmentation, the designation **235B** will be used to include both isomers. The occurrence of **235B′** and **235B″** as components of indolizidine **235B** detected by gc-ms in alkaloid fractions from other dendrobatid frogs (3) requires further analysis on capillary columns.

Piperidines.—2,6-Disubstituted piperidines have been suggested (21) as possible precursors in dendrobatid frogs for histrionicotoxins, gephyrotoxins, 3,5-disubstituted indolizidines, and 2,5-disubstituted decahydroquinolines. However, such piperidines have not been detected in extracts of dendrobatid frogs until recently when the occurence was proposed (15) of a 2,6-dipentyl piperidine [**225B**] from a Colombian population of *D. histrionicus* and of a 2-butyl-6-heptyl-piperidine [**239I**] from an Amazonian specimen (Peru) of *Epipedobates trivittatus* [formerly *Dendrobates trivittatus*, see Myers (1)]. Such simple 2,6-disubstituted piperidines were not detected in extracts of *D.*

speciosus. However, a 2,6-disubstituted hydroxy piperidine **241D** was isolated and represents the first such piperidine alkaloid isolated from a dendrobatid frog. This alkaloid was a major constituent in population C from lower elevations but was present in only small or trace amounts in nearby population A and in population B from higher elevations. In other dendrobatid frogs piperidine **241D** has now been detected as a trace constituent in extracts from certain populations of *D. pumilio* (3). It appears likely that alkaloid **255** from *D. speciosus* is a side chain keto derivative of **241D**.

Hrms of **241D** afforded a molecular formula of $C_{15}H_{31}NO$ with a base peak at *m/z* 114 ($C_6H_{12}NO^+$) consistent with the loss of a nine-carbon side chain from a 2-methyl-6-nonyl-4-hydroxypiperidine (see below). Chemical ionization gc-ms with ND_3 confirmed the presence of two exchangeable protons, in this case on OH and NH, both of which were retained in *m/z* 114 fragment. Analysis of the double quantum filtered COSY of **241D** led directly to assignment of the structure as a *cis,cis*-2-methyl-6-nonyl-4-hydroxypiperidine. The structure has been confirmed by synthesis.[3] The ^{1}H-nmr assignments are given in the Experimental section. The downfield C-4 proton at 3.66 ppm showed coupling to a pair of protons at 1.96 ppm and a pair at 1.00 ppm. Each of the protons at 1.96 ppm showed coupling to the proton at 3.66 ppm. However, the downfield proton of the pair at 1.96 ppm was coupled to individual protons at 2.54 ppm and 0.99 ppm, while the upfield proton (1.98 ppm) showed coupling to protons at 2.68 and 1.01 ppm. The assignment of protons at 0.99 and 1.96 ppm as a geminal pair was confirmed by Heteronuclear Multiple Quantum Coherence spectroscopy: A carbon resonance at 42.2 ppm (C-5) correlates with a geminal pair at 1.99 and 0.99 ppm, while a carbon resonance at 45.0 ppm (C-3) correlates with a geminal pair at 1.01 and 1.98 ppm. The two remaining piperidine ring protons may be assigned by following the coupling patterns in the double quantum filtered COSY. Piperidine rings prefer, when possible, the more stable chair conformation (22). This, in combination with an analysis of the coupling constants for protons of the piperidine ring, indicates that the hydroxyl and alkyl substituents of **241D** are all equatorial. The coupling constants for the *trans*-diaxial protons were determined in each case to be approximately 11 Hz, while the adjacent axial-equatorial couplings were approximately 2.4 Hz. These values are consistent with previous studies of 2,6-disubstituted-4-hydroxy piperidines (23).

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