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ALKALOIDS FROM DENDROBATID FROGS: STRUCTURES OF TWO ω-HYDROXY CONGENERS OF 3-BUTYL-5-PROPYLINDOLIZIDINE AND OCCURRENCE OF 2,5-DISUBSTITUTED PYRROLIDINES AND A 2,6-DISUBSTITUTED PIPERIDINE

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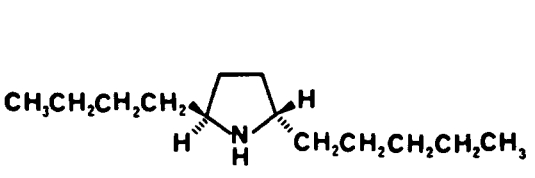
ABSTRACT.—Forty alkaloids were detected and characterized from skin extracts of high- and low-elevation populations of the poison frog *Dendrobates histrionicus* from northwestern Colombia. Combined gc/ms with NH_3 or ND_3 in a chemical ionization mode detected protonated parent ions and determined the number of exchangeable NH and OH hydrogens. Six previously unknown dendrobatid alkaloids were characterized. Two were 2,5-disubstituted pyrrolidines, which included pyrrolidine **197B**, a *trans*-2-butyl-5-pentylpyrrolidine, while a third was a 2,6-dipentylpiperidine. Indolizidines **239AB** and **239CD** had the same relative configuration as the parent alkaloid **223AB** [(5*E*,9*E*)3-butyl-5-propylindolizidine] and contained, respectively, a ω-hydroxy group in the propyl or butyl side chain. The profiles of alkaloids in the new northern populations of *D. histrionicus* are typical of the species in containing a set of about eight histrionicotoxins, in marked contrast to a related species, *Dendrobates lehmanni*, which does not contain histrionicotoxins.

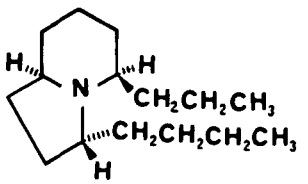
Some 200 alkaloids have been detected from poison frogs of the family Dendrobatidae (1-3 and unpublished data). The dendrobatid alkaloids are now grouped in six major classes: (*a*) batrachotoxins (steroidal); (*b*) histrionicotoxins (spiropiperidines); (*c*) pumiliotoxin-C class (decahydroquinolines); (*d*) pumiliotoxin-A class (6-alkylidene-8-hydroxy-8-methylindolizidines), including an allopumiliotoxin subclass (7-hydroxy derivatives of the pumiliotoxin-A class); (*e*) the tricyclic gephyrotoxins (perhydrobenzoindolizidines); and (*f*) the bicyclic gephyrotoxins (indolizidines). It now appears preferable to refer to the pumiliotoxin-C class simply as decahydroquinolines and to limit the gephyrotoxin classes to only the tricyclic members, with the bicyclic gephyrotoxins being referred to as indolizidines. The trivial class names would be reserved for the complex batrachotoxins, histrionicotoxins, (allo)-pumiliotoxins, and (tricyclic) gephyrotoxins.

Because of the large number of dendrobatrid alkaloids, boldface numerical designations according to nominal mass with identifying letters have been used for identification (1,2). Many of the dendrobatid alkaloids occur as trace constituents, and it has been necessary to develop gs/ms techniques for detailed characterization of minute quantities of many compounds (1). Such techniques include the use of chemical ioniza-

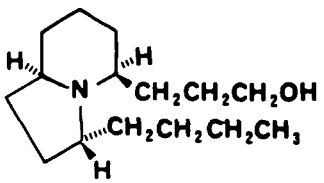
tion ms with NH_3, ND_3, or NO/N_2 as ionizing gases. These techniques led to the identification of two new classes of dendrobatid alkaloids, namely, 2,5-dialkylpyrrolidines and a 2,6-dialkylpiperidine, in extracts of Colombian populations of *Dendrobates histrionicus* Berthold. The structure of pyrrolidine **197B** was defined as that of a *trans*-2-*n*-butyl-5-*n*-pentylpyrrolidine (Figure 1). In addition, three indolizidines, **223AB, 239AB** and **239CD**, have now been isolated in sufficient quantity for structure elucidation by nmr spectrometric analysis. All are levorotatory, and **239AB** and **239CD** proved to be the ω-hydroxy congeners of the parent alkaloid (5*E*,9*E*)3-butyl-5-propylindolizidine (**223AB**) (Figure 1), an alkaloid previously referred to as a gephyrotoxin **223AB** (1). This alkaloid now will be referred to as indolizidine **223AB** and its ω-hydroxy analogs as indolizidines **239AB** and **239CD**.



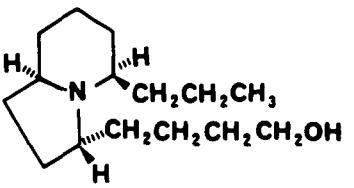
197B



223AB



239AB



239CD

FIGURE 1. Structures of some dendrobatid alkaloids: pyrrolidine **197B**, indolizidine **223AB**, indolizidine **239AB**, and indolizidine **239CD**. The absolute configuration of **223AB, 239AB,** and **239CD** is probably as shown (see text), while that of **197B** is unknown.

EXPERIMENTAL

SOURCE MATERIAL.—One large and three small samples of skin extracts of *D. histrionicus* were analyzed by gc/ms, and the large sample was additionally subjected to chromatographic separation of individual alkaloids, as described below. The large sample of 70 skins was obtained from frogs collected in an elevational range of 800-1070 meters on the summit of Altos del Buey (6°06′N, 77°13′W), which is the high point on the Serranía de Baudó, a small mountain range west of the Andes in the Department of Chocó, northwestern Colombia. Two small samples from Altos del Buey included a high-elevation sample from a campsite at 800-m elevation (5 skins, 1.5 g), and a low-elevation sample from about 260-400 m (5 skins, 1.3 g). A third small sample (10 skins, 2.3 g) was obtained from about 100-m elevation in hills 5 km north of El Valle (6°05′N, 77°25′W), a village on the Pacific Coast about 25 km west of Altos del Buey. Voucher specimens are preserved in the research collection of the American Museum of Natural History (AMNH).

ISOLATION OF ALKALOID FRACTION.—Skins obtained in the field were transported in MeOH with at least 10 ml of MeOH per 5 g of wet skin. Skins were later triturated at least three times with 2-5 volumes of MeOH. The 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 its volume of $CHCl_3$. The combined $CHCl_3$ layers were extracted three or four times, each time with one-fifth their volume of 0.1 N HCl. The combined acid extract was rendered basic (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 were dried over anhydrous Na_2SO_4 and cautiously evaporated under reduced pressure at 30-35°. The alkaloids were then taken up in MeOH so that 100 μl MeOH solution was equivalent to 100 mg of wet skin. This extract was subjected to analysis.

GC/MS ANALYSIS.—Initial chromatographic analyses were with a 1.5% OV-1 column on 80/100

Gas Chrom Q (Applied Science Laboratories) with a flow rate of 30 ml N_2 or He per min and a flame ionization detector. The column was programmed from 150° to 280° at 10° per min. Routinely, methanolic alkaloid fractions equivalent to 2 mg wet-weight skin were injected at a standardized attentuation to facilitate semiquantiative comparisons of alkaloid content from different species or populations of frogs. Tlc analysis, with silica gel GF chromatoplates (Analtech Co.) using $CHCl_3$-MeOH (9:1) for development and iodine vapor for detection, was routinely carried out with methanolic alkaloid fractions equivalent to 10 mg wet-weight of skin to permit quantitative comparisons of alkaloid content. Capillary columns were also used for gc, particularly in conjunction with cims (data not shown).

CIMS.—The protonated parent ion (M+1) for each dendrobatid alkaloid was readily detected using gc/ms with NH_3 as the ionizing gas. Next, ND_3 was used as the ionizing gas and afforded a deuterated parent ion (M+2). With ND_3 there was also complete exchange of all OH and NH groups in the alkaloids as they emerged from the gas chromatographic system into the mass spectrometer ion chamber. There is minimal fragmentation with either NH_3 or ND_3 as the ionizing gas. On the other hand, when a mixture of 10 mole percent NO in N_2 is used as the ionizing gas, the spectra obtained for dendrobatid alkaloids are similar to conventional electron impact spectra. Spectra for certain alkaloids of *D. histrionicus* (Altos del Buey) are depicted in Figures 2 and 3.

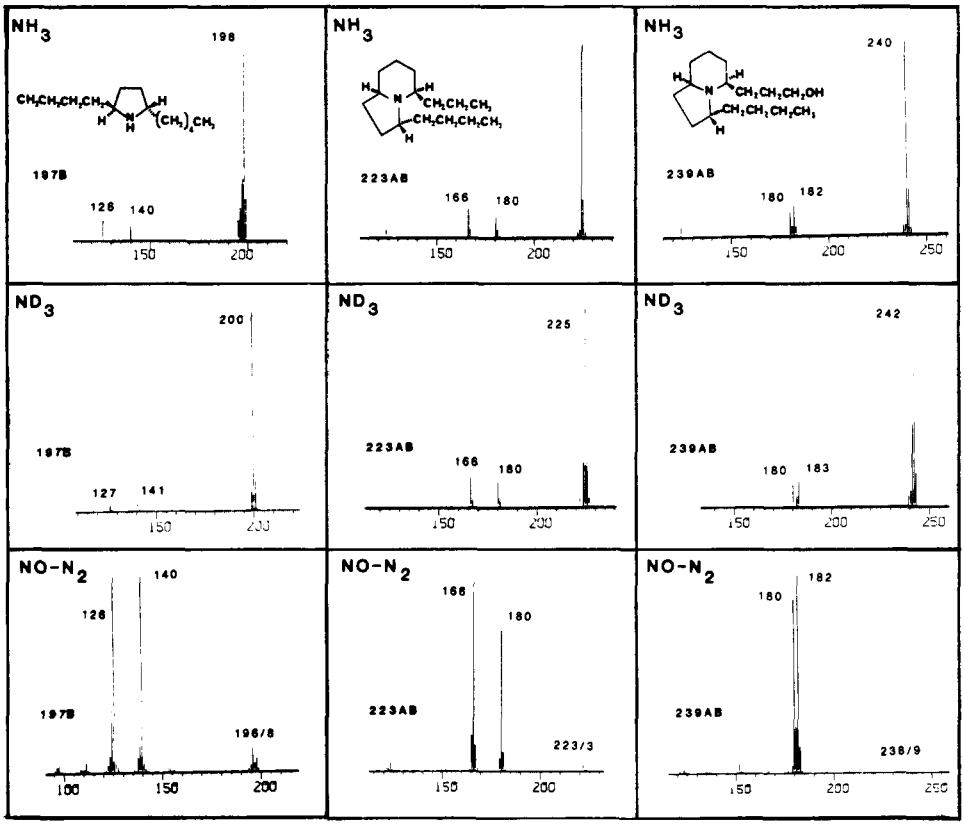


FIGURE 2. Mass spectra for the dendrobatid alkaloids **197B, 223AB,** and **239AB**. *Top*, chemical ionization spectra with NH_3: protonated parent ion and minimal fragmentation. *Middle*, chemical ionization spectra with ND_3: Deuterated parent ion and complete exchange of hydroxyl and secondary amine hydrogen for deuterium. Fragments with exchanged deuterium in hydroxyl or amine functions are readily identified by comparison to chemical ionization spectra with NH_3. *Bottom*, pseudoelectron impact spectra with NO-N_2: Fragmentation patterns are similar to those obtained by eims.

HRMS.—Empirical formulae for alkaloids were obtained by combined gc/hrms using a VG 70/70 mass spectrometer. Sufficient NH_3 was introduced into the source to produce protonated parent ions for mass measurement while retaining enough electron-impact fragmentation to allow both analysis of fragments and production of reference fragment peaks from the tetraiodoethylene standard. This technique was

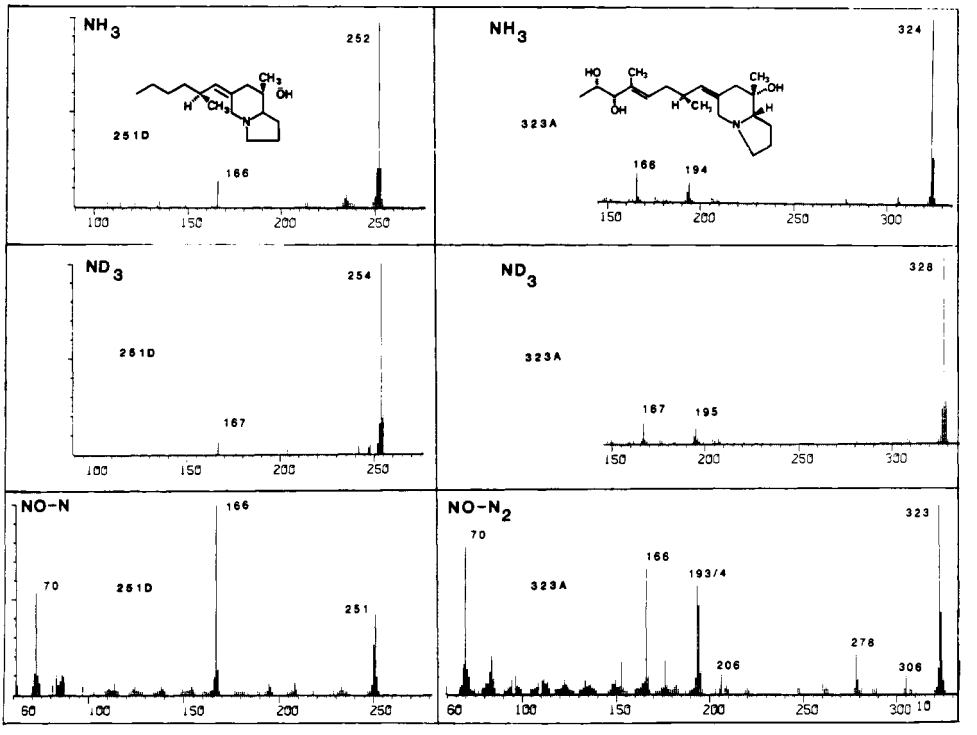


FIGURE 3. Mass spectra for pumiliotoxin **251D** and pumiliotoxin B (**323A**). Chemical ionization spectra as in Figure 1.

necessary since many of the dendrobatid alkaloids exhibit vanishingly small parent ions in the electron impact mode.

PERHYDROGENATION.—In order to assay the degree and, in some instances, the location of unsaturation in the various dendrobatid alkaloids, an aliquot of the methanolic alkaloid fraction equivalent to 50-100 mg wet weight skin was hydrogenated. The alkaloid sample after dilution with MeOH to 500 μl was reduced with 45 psi H_2 for 6 h with shaking in the presence of 50 mg 10% Pd/C. After filtration, the sample was cautiously concentrated to about 25-50 μl and subjected to gc/ms.

PHENYLBORONATION.—A small aliquot (10-50 μl) of the methanolic alkaloid fraction was evaporated to dryness, dissolved in an equivalent volume of 5 mM phenylboronic acid in Me_2CO and subjected after 30 min to gc/ms.

COLUMN CHROMATOGRAPHY.—The sample of 70 skins of *D. histrionicus*, collected on the summit of Altos del Buey, was purified to yield a number of the major alkaloids in sufficient quantity for detailed analysis. Initial fractionation was as described above, using either $CHCl_3$ (35 skins) or CH_2Cl_2 (35 skins). Similar amounts of alkaloids were isolated with either halocarbon to yield a total of about 100 mg of alkaloids from the 70 skins. A portion of the alkaloids (≲30 mg) was purified by column chromatography on silica gel 60 (63-200 nm, EM Reagents). The alkaloids were introduced in CH_2Cl_2. Elution was with the following: (a) 50 ml CH_2Cl_2-THF (4:1); (b) 50 ml CH_2Cl_2-THF (1:1); (c) 100 ml THF; (d) 50 ml THF-MeOH (1:1). Fractions (5 ml) were analyzed by tlc and gc/ms. The initial fractions (5-8) contained yellow pigments and no alkaloids. Fractions 23-26 contained **251D** (2 mg); Fraction 27: **251D, 267A,** histrionicotoxin, and pumiliotoxin A (1 mg); Fractions 28-29: **267A** (1 mg); Fractions 30-32: **267A** and histrionicotoxins (1 mg); Fractions 33-38: **223AB** and histrionicotoxins (3 mg); Fractions 39-42: pumiliotoxin B (5 mg); Fractions 43-44: **239AB** and pumiliotoxin B (2 mg); Fractions 45-57: **239AB** (7 mg); and Fractions 57-66, **197B, 225B,** and unidentified compounds (3 mg).

Another portion (≲40 mg) was also chromatographed over a silica gel column (2×23 cm). The alkaloids were introduced in $CHCl_3$ and eluted with the following: (a) 105 ml $CHCl_3$-MeOH (20:1); (b) 330 ml $CHCl_3$-MeOH (10:1); (c) 240 ml CHCl-MeOH (5:1); (d) 250 ml $CHCl_3$-MeOH (4:1). All solvents contained about 0.2% 12 N aqueous NH_3. Fractions 70-82 contained **251D** (2 mg); Fractions 83-90: pumiliotoxin A and **251D** (1 mg); Fractions 91-105: **269AB**, pumiliotoxin A, and histrionicotoxins (2 mg); Fractions 106-164: **267A**, histrionicotoxins, and pumiliotoxin A (8 mg); Fractions 165-185; **267A**

and pumiliotoxin A (1 mg); Fractions 186-202: **239AB** (3 mg); Fractions 203-225: **239AB** and pumiliotoxin B (5 mg); and Fractions 226-260: pumiliotoxin B (3 mg).

A final portion (20 mg) was prepurified and then subjected to hplc on a Zorbex CN column (9.4 mm×25 cm; Dupont) with MeOH-0.1% aqueous NH_4HCO_3 (4:1) as solvent at a flow rate of 5 ml/min. The uv detector of an Analtech hplc chromatograph was set at 220 nm. The sample was preparatively chromatographed by multiple 100 μl injections of methanolic alkaloid fraction, each injection corresponding to 400 mg wet-weight skin. Collected fractions (see Figure 4) from the multiple injections were combined and concentrated under reduced pressure, diluted with H_2O and extracted with three 50 ml portions of CH_2Cl_2. After drying over anhydrous Na_2SO_4, the CH_2Cl_2 extract was evaporated to dryness under reduced pressure. Fractions 1 and 2 contained **239AB** (4 mg); Fraction 3: **239AB** and traces of other alkaloids (3 mg); Fraction 4: **267A** and histrionicotoxin (2 mg); Fraction 5: **267A** (1 mg); Fraction 6: **251D** and pumiliotoxin A (1 mg); Fraction 7: **251D, 269AB**, and pumiliotoxin A (2 mg); and Fraction 8: pumiliotoxin B, dihydrohistrionicotoxins, **239B**, and **225B** (4 mg).

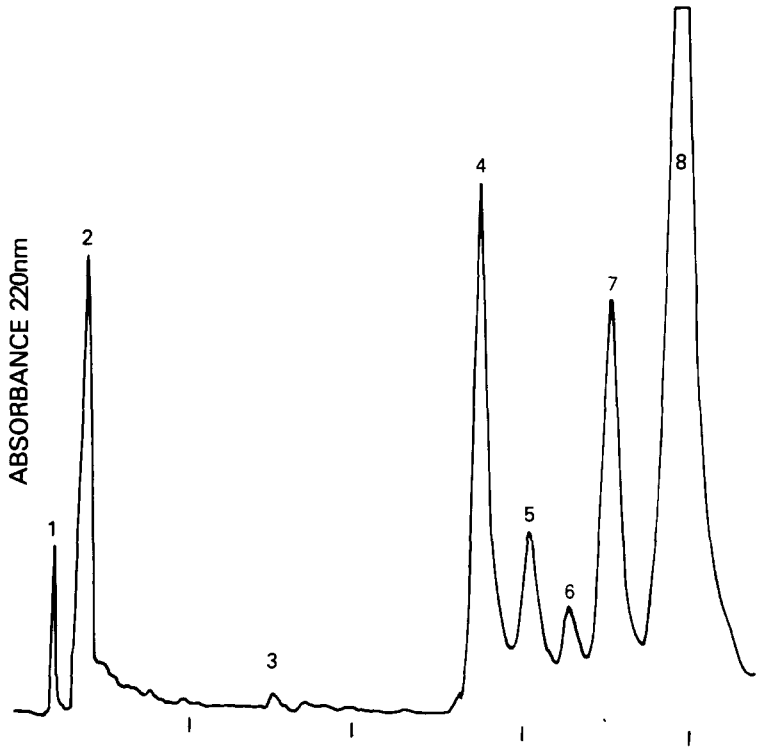


FIGURE 4. High pressure liquid chromatogram of alkaloid fraction from *Dendrobates histrionicus*, high-elevation Altos del Buey population. Zorbax CN column, solvent MeOH-0.1% NH_4HCO_3 (4:1). Detection at 220 nm. Flow rate 5 ml/min. Fractions correspond to designated peaks except for Fraction 3, which included all eluent between peak 2 and 4. The abscissa marks are at 5 min intervals.

Further purification of the fractions that were obtained by silica gel column chromatography and reversed phase hplc was carried out through repetitive centrifugal tlc on silica gel 60 PF 254 containing gypsum (EM Reagents) with a Chromatotron model 7924 (Harrison Research, Palo Alto, CA). Solvents were $CHCl_3$-MeOH ranging from 10:1 mixtures for relatively nonpolar substances such as **251D** to 5:1 mixtures for relatively polar substances such as **239AB** and pumiliotoxin B.

RESULTS AND DISCUSSION

The high- and low-elevation samples of *D. histrionicus* from Altos del Buey, and the low-elevation sample from El Valle, 25 km to the west, were analyzed by gc/ms. The profiles of alkaloids from these several neighboring populations are roughly similar (see Figures 5A, 5B, and 6A for gas chromatographic traces, Figure 7 for thin-layer

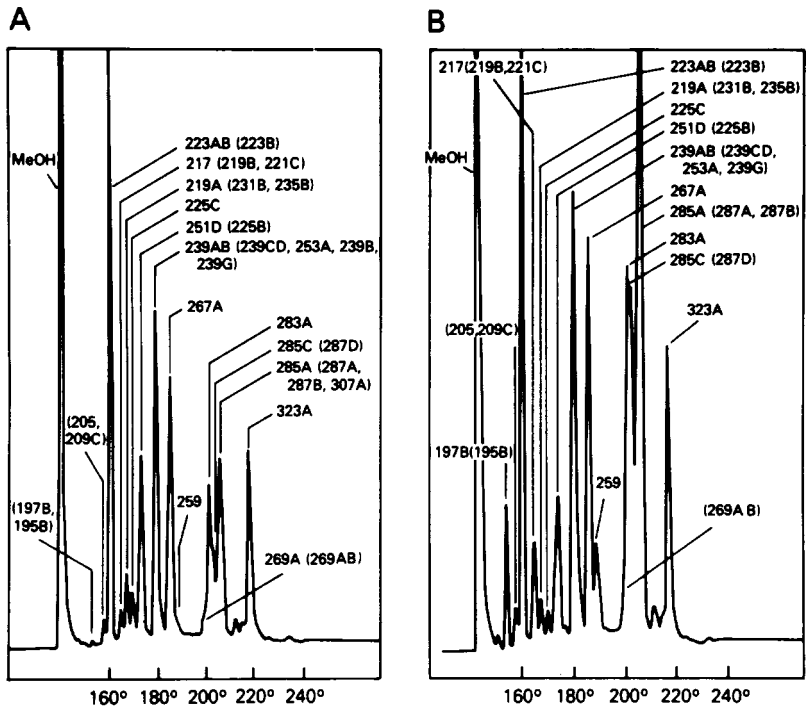


FIGURE 5. Gas chromatograms of alkaloids from two northern Colombian populations of *Dendrobates histrionicus*. *A*. Altos del Buey, high elevation (800 m). *B*. Altos del Buey, low elevation (260-400 m). A sample of 2 μl of methanolic alkaloids equivalent in amount to 2 mg wet skin was injected at 150° onto a 6 foot 1.5% OV-1 on 80/100 Gas Chrom Q column. After the maximum for the solvent peak was passed, the column was programmed at 10° per min from the initial 150° to 280°. Emergent temperatures differ somewhat with different batches of column packing. A flame ionization detector was used. Alkaloids identified and characterized by combined gc-ms are designated by their molecular weights and, where necessary, with an added code letter. Trace constituents are in parentheses.

chromatoplates, and Table 1). Gas chromatographic profiles for two other (non-neighboring) populations of *D. histrionicus* are shown in Figures 6B and 8A, and the profile for a related species, *Dendrobates lehmanni* Myers and Daly, in Figure 8B.

Seven alkaloids from 70 skins of *D. histrionicus* from Altos del Buey (see Table 1) were isolated in sufficient quantities (>2 mg) and purity (>95% by thin layer and gas chromatographic analysis) for nmr spectral analysis. Of these, structures for **251D, 267A**, histrionicotoxin, dihydroisohistrionicotoxin, pumiliotoxin A, and pumiliotoxin B have been previously reported, based on analysis of material isolated from other species or from other populations of *D. histrionicus* (1). The structure of alkaloid **239AB** was defined by analysis of ^{1}H- and ^{13}C-nmr spectra (Table 2). An isomeric alkaloid **239CD** was isolated in sufficient quantities for nmr spectral analysis from another, geographically distant population of *D. histrionicus* (upper Río San Juan, Santa Cecilia, Depto. Risaralda, Columbia; unpublished results). It is only a trace alkaloid in the Altos del Buey population. The nmr spectra defined its structure (Table 2). The parent indolizidine **223AB**, namely, (5*E*,9*E*)3-butyl-5-propyl-indolizidine, was also isolated in sufficient quantities for nmr spectral analysis from the Santa Cecilia population (see Table 2).

Of the various alkaloids, only pumiliotoxin B formed a phenylboronide. Its chemical ionization (NH_3) spectrum [(*m/z* 410 (M+1), 166 (indolizidine ring), 70 (pyr-

TABLE 1. Alkaloids from Northern Populations of *Dendrobates histrionicus*, Altos del Buey Region

Alkaloid[a]	Empirical Formula	Major Mass Spectral Ions[b] *m/z*	Exchangeable Hydrogen[c]	Perhydro-derivative[d]	Occurrence in *D. histrionicus*[e]		
					Altos del Buey		El Valle
					High	Low	
Histrionicotoxins							
259	$C_{17}H_{25}NO$	218,96	2	H_8	+	++	+
283A (HTX)	$C_{19}H_{25}NO$	218,96	2	H_{12}	++	+++	++
285A (Iso-H_2-HTX)	$C_{19}H_{27}NO$	96	2	H_{10}	++	+++	++
285C (Allo-H_2-HTX)	$C_{19}H_{27}NO$	96	2	H_{10}	++	++	+
287A (Iso-H_4-HTX)	$C_{19}H_{29}NO$	96	2	H_8	+	+	+
287B (H_4-HTX)	$C_{19}H_{29}NO$	220,96	2	H_8	+	+	+
287D (Allo-H_4-HTX)	$C_{19}H_{29}NO$	96	2	H_8	+	+	+
Gephyrotoxins (tricyclic)							
287C (GTX)	$C_{19}H_{29}NO$	242	1	H_6	−	−	+
Indolizidines (bicyclic gephyrotoxins)							
223AB	$C_{15}H_{29}N$	180,166	0	H_0	+++	+++	++
239AB	$C_{15}H_{29}NO$	182,180	1	H_0	+++	+++	++
239CD	$C_{15}H_{29}NO$	196,166	1	H_0	+	+	+
Pyrrolidines							
197B	$C_{13}H_{27}N$	140,126	1	H_0	+	++	+++
225C	$C_{15}H_{31}N$	168,126	1	H_0	++	++	+
Piperidines							
225B	$C_{15}H_{31}N$	154	1	H_0	+	+	−
Pumiliotoxin-A Class							
251D	$C_{16}H_{29}NO$	166,70	1	H_2	++	++	−
307A (PTX-A)	$C_{19}H_{33}NO_2$	166,70	2	H_4	+	−	−
323A (PTX-B)	$C_{19}H_{33}NO_3$	166,70	3	H_4	++	++	−
Allopumiliotoxin-A Class							
253A	$C_{15}H_{27}NO_2$	182,70	2	H_2	+	+	−
267A	$C_{16}H_{29}NO_2$	182,70	2	H_2	+++	+++	++
Unclassified							
I[f] **195B**	$C_{13}H_{25}N$	138	0	H_0	+	+	++
I **205**	$C_{14}H_{23}N$	138	0	H_4	+	+	++
209C	$C_{14}H_{27}N$	152	0	H_0	+	+	−
217	$C_{15}H_{23}N$	152	0	H_6	++	++	++
D **219A**	$C_{15}H_{25}N$	178	1	H_4	++	++	+
219B	$C_{15}H_{25}N$	152	0	H_4	+	+	+
D **221C**	$C_{15}H_{27}N$	152	1	H_2	+	+	−
223B	$C_{15}H_{29}N$	166	0	H_0	+	+	−
231B	$C_{16}H_{25}N$	152	0	H_6	+	+	+
I **231C**	$C_{16}H_{25}N$	138	0	H_6	−	−	++
I **235B**	$C_{16}H_{29}N$	138	0	H_2	+	+	++
239B	$C_{15}H_{29}NO$	180	1	H_0	+	−	−
239G	$C_{15}H_{29}NO$	138	1	H_0	+	+	+
269A	$C_{19}H_{27}N$	204	1	H_{10}	++	+	−
D **269AB**	$C_{19}H_{27}N$	204,202	1	H_{10}	+	+	+

[a]Alkaloids are designated by molecular weight in boldface type, followed where necessary by a code letter to distinguish from other alkaloids of the same nominal weight.

[b]Major ions are for electron impact or NO/N_2 cims.

[c]The exchangeable hydrogens were assessed by cims with ND_3.

[d]The degree of unsaturation was determined after catalytic hydrogenation (H_0-no addition of hydrogen).

[e]+++=present as major alkaloid; ++=minor alkaloid; +=trace alkaloid.

[f]The letter I indicates that this alkaloid will be classified as an indolizidine; the letter D, as a decahydroquinoline in a forthcoming summary of dendrobatid alkaloids (J.W. Daly, N. Whittaker, and C.W. Myers, in preparation).

rolidine ring)] was compatible with a boronide at the 15,16-hydroxy groups of the side chain. The allopumiliotoxins **267A** and **253A** did not form phenylboronides, thus indicating a *trans*-diaxial-7,8-dihydroxy moiety in the indolizidine ring as has been the case for nearly all allopumiliotoxins (4).

The following seven dendrobatid alkaloids are to be added to those previously catalogued for species of *Dendrobates* (1-3). Alkaloids are designated by molecular weight with an added code letter where needed. The empirical formula is based on hrms. The Rf value is for silica gel tlc with $CHCl_3$-MeOH (9:1). The emergent temperature is for gc on a 1.5% OV-1 column programmed from 150° to 280° (1,4). The elec-

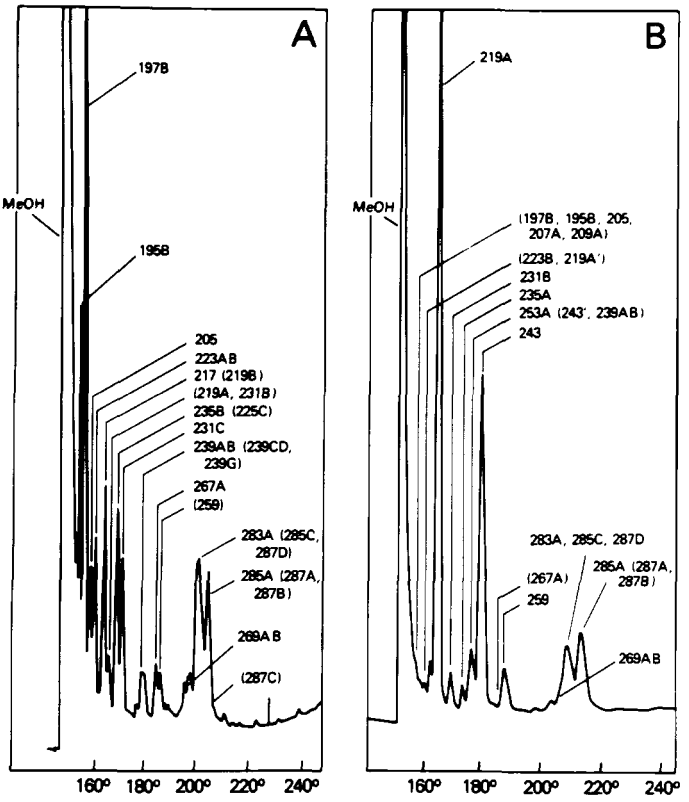


FIGURE 6. Gas chromatograms of alkaloids from two Colombian population samples of *Dendrobates histrionicus*. *A*. El Valle. *B*. Quebrada Docordó, middle Río San Juan. Conditions are as in Figure 5, but different columns and chart speeds were used.

tron impact mass spectrum is given in nominal masses followed for each ion by the intensity (in parentheses) relative to the base peak set equal to 100. The perhydro derivative (H_0=no addition of hydrogen) was obtained by catalytic reduction. The number of exchangeable hydrogens (NH and/or OH) was based on exchange with ND_3 during cims. Alkaloid **197** (2) becomes **197A** because of the following addition:

197B. $C_{13}H_{27}N$, Rf 0.35, 163°, *m/z* 197 (1), 196 (2), 140 (78), 126 (100). H_0-derivative. One exchangeable hydrogen. Major or minor constituent in *D. histrionicus* (Altos del Buey and El Valle) and occurs as trace constituent in one population of *Dendrobates pumilio* O. Schmidt (unpublished results). A member of a pyrrolidine class of dendrobatid alkaloids. Identified as *trans*-2-*n*-butyl-5-*n*-pentyl-pyrrolidine. Absolute configuration unknown.

209C. $C_{14}H_{27}N$, 164°, *m/z* 209 (2), 152 (100). H_0-derivative. No exchangeable hydrogen. Trace constituent in *D. histrionicus* (Altos del Buey) and in one population of *D. pumilio* (unpublished results).

221C. $C_{15}H_{27}N$, 166°, *m/z* 221 (2), 152 (100). H_2-derivative. *m/z* 223, 152. One exchangeable hydrogen. Trace constituent in *D. histrionicus* (Altos del Buey).

225 (1) becomes **225A** because of the following addition:

225B. $C_{15}H_{31}N$, Rf 0.4, 174°, *m/z* 225 (1), 224 (1), 154 (100). H_0-derivative. One exchangeable hydrogen. Trace constituent in *D. histrionicus* (Altos del Buey) and as a minor constituent in an undescribed *Dendrobates* species from Panama (unpublished results). Tentatively proposed to be a 2,6-dipentylpiperidine.

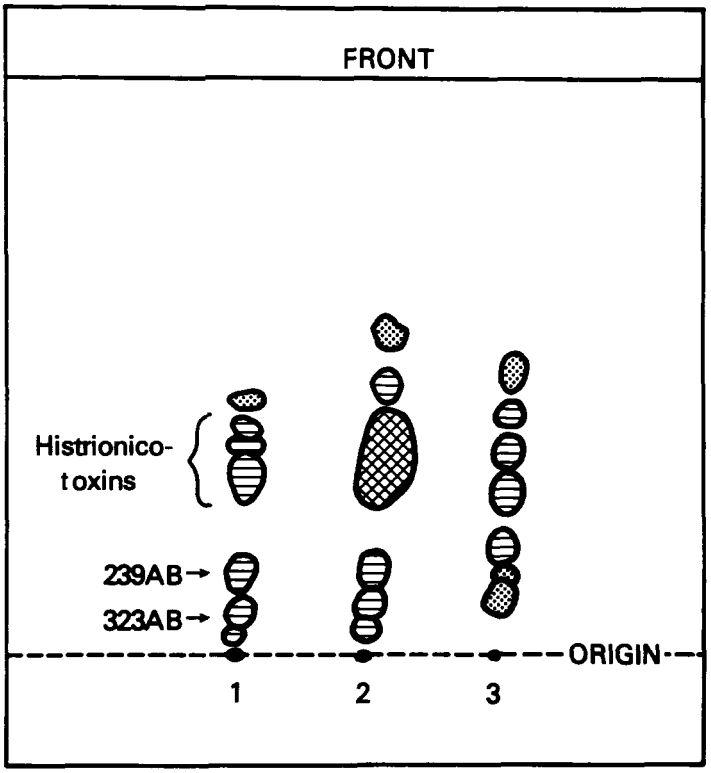


FIGURE 7. Representation of thin-layer chromatoplate of alkaloids from three northern populations of *Dendrobates histrionicus*. 1. Altos del Buey, high elevation (800 m). 2. Altos del Buey, low elevation (260-400 m). 3. El. Valle. A sample of 10 µl of methanolic alkaloids equivalent in amount to 10 mg of wet skin was applied at the origin and the silica gel GF plate developed with $CHCl_3$-MeOH (9:1). Visualization, after chromatography and drying, was by exposure to iodine vapor. Spot intensities depicted as follows: cross-hatched pattern, large amounts; horizontal pattern, moderate amounts; dots, small amounts (see Reference 5). The approximate positions of various alkaloids are indicated.

225C. $C_{15}H_{31}N$, Rf 0.4, 172°, *m/z* 225 (1), 224 (2), 168 (70), 126 (100). H_0-derivative. One exchangeable hydrogen. Trace constituent in *D. histrionicus* (Altos del Buey and El Valle). Tentatively proposed to be a 2-*n*-butyl-5-*n*-heptyl-pyrrolidine.

231C. $C_{16}H_{25}N$, Rf 0.38, 171°, *m/z* 231 (3), 138 (100). H_6-derivative. *m/z* 237, 138. No exchangeable hydrogen. Present as a minor constituent in *D. histrionicus* (El Valle) and in *Dendrobates arboreus* of western Panama (3).

269AB. $C_{19}H_{27}N$, 0.35, 207°, *m/z* 269 (4), 268 (12), 204 ($C_{14}H_{22}N$, 100), 202 ($C_{14}H_{20}N$, 50). H_{10}-derivative, *m/z* 279, 208. One exchangeable hydrogen. *N*-acetyl derivative. This compound has been previously considered to be a mixture of two alkaloids, one affording a base peak at *m/z* 204 (**269A**) and one affording a base peak at *m/z* 202 (**269B**) (1,2). However, in view of the consistency in nearly all extracts of a 2 to 1 ratio for the 204 and 202 peaks and the lack of separation even on capillary columns, it seems likely that this is one compound, probably of the decahydroquinoline class (4). In a few extracts, there do appear to be isomers affording a larger ion at *m/z* 204 to be designated **269A** or *m/z* 202 to be designated **269B.** Occurs in eight species of *Dendrobates* and in six populations of *D. histrionicus*.

Dendrobatid frogs produce an amazing array of alkaloids, with over 200 having

TABLE 2. Alkaloids from Two Other Colombian Populations of *Dendrobates histrionicus* (I,II) and from Animal-Trade Specimens of *D. histrionicus* (III) and *Dendrobates lehmanni* (IV) from Unknown Localities in Colombia. (Alkaloids Shared with Altos del Buey Populations are Indicated by Asterisks [see Table 1 and legend]. Gas Chromatograms for I and II are in Figure 7 and II and IV are in Figure 8.)

I. *D. histrionicus*. Quebrada Docordó, Río San Juan, Chocó (June 1983, 5 skins, 1.0 g). Major alkaloids: **243, 219***. Minor alkaloids: **287D***, **285A***, **285C***, **283A***, **259***, **253A***, **235A**, **231B***. Trace alkaloids: **287A***, **287B***, **269AB***, **267A***, an isomer of **243** (designated **243'**), **239AB**, **223B***, an isomer of **219A** (designated **219A'**), **209B**, **207A**, **205**, **197B***, **195B***.

II. *D. histrionicus*. Guayacana, Nariño (various samples, 1972-1978). Major alkaloids: **285A***, **283A***. Minor alkaloids: **291A**, **287A***, **287B***, **287C***, **285B**, **285C***, **223AB***. Trace alkaloids: **289B**, **287D***, **285E**, **269AB***, **267A***, **223B***, **223D**, **205***, **203**, **195B***.

III. *D. histrionicus*. Colombia (April, 1979; 1 skin, 0.2 g). Major alkaloids: **283A***, **285A***, **285C***. Minor alkaloids: **219A***, **243***, **259***, **269AB***, **285B**, **287A***. Trace alkaloids: **223AB***, **231B***, **235A**, **287B***.

IV. *D. lehmanni*. Colombia (April, 1979; 1 skin, 0.2 g). Major alkaloids: **275**. Minor alkaloids: **231A**, **251D***, **267A***, **275'**, **307A***, **307B**, **323A***, **341A**. Trace alkaloids: **181C**, **195C**, **217***, **221A**, **223A**, **231B***, **235E**, **253A***, **251B**, **257B**, **307C**.

been so far detected and characterized from 40 species of *Dendrobates* and *Phyllobates*. An account of the occurrence and properties of these diverse alkaloids is in preparation (J.W. Daly, N. Whittaker, and C.W. Myers) and will bring up to date the two previous tabulations (1,2). Some of the presently unclassified alkaloids (1-3 and Table 1) will at that time be placed in indolizidine or decahydroquinoline classes of dendrobatid alkaloids. For example, alkaloid **219A** has now been shown by x-ray crystallographic analysis to be [2*S*,4a*S*,5*S*,8a*S*]2,5-diallyl-*trans*-decahydroquinoline (T. Tokuyama, N. Nishimori, I. Karle, M.W. Edwards and J.W. Daly, *Tetrahedron*, in press).

A new class of dendrobatid alkaloids, comprising two alkaloids that appear to be 2,5-disubstituted pyrrolidines, was detected in the new populations of *D. histrionicus*. One of the pyrrolidines, namely **197B** has now been detected also in one population of *D. pumilio*. Gc of alkaloids from *D. histrionicus* (El Valle) with a mixture of authentic *cis*- and *trans*-2-*n*-butyl-5-*n*-pentylpyrrolidine on a capillary OV-17 column (50 meters) from 170-220° at 5°/min permitted the identification of alkaloid **197B** as a *trans*-2-*n*-butyl-5-*n*-pentylpyrrolidine (retention time 12.8 min, *cis*-isomer 12.6 min). The absolute configuration of **197B** is unknown. The *trans*-isomer has also been detected in a South African fire ant, *Solenopsis punctaticeps*, and in the Old World Pharaoh's ant *Monomorium pharaonis* (6).

A second new class of dendrobatid alkaloid was detected in the samples of *D. histrionicus* from Altos del Buey and El Valle, namely, a 2,6-disubstituted piperidine. Such 2,6-disubstituted piperidines have been proposed as potential precursors to the histrionicotoxins, gephyrotoxins, indolizidines, and decahydroquinolines (1). Alkaloid **225B** appears, based on its mass spectral properties, to be a 2,6-dipentylpiperidine. Previously, a similar alkaloid was detected in extracts from the skin of a single specimen of *Dendrobates trivittatus* Spix obtained in 1978 at Pebas, Peru, and shown by mass spectral characterization to be a 2-heptyl-6-butylpiperidine (unpublished results).

Classification of the many trace alkaloids from dendrobatid frogs has now been facilitated by the use of ND_3 as an ionizing gas. With ND_3 in the mass spectrometer, a quantitative exchange of NH and OH groups occurs as alkaloids enter the ionizing chamber of the mass spectrometer from the gc inlet (Figures 2,3). Quantitative exchange also occurs with compounds introduced on direct inlet probes (unpublished results). The ND_3 results can, in conjunction with other data, including nature of fragments, permit conclusions as to sites of exchange. There has been one prior report on

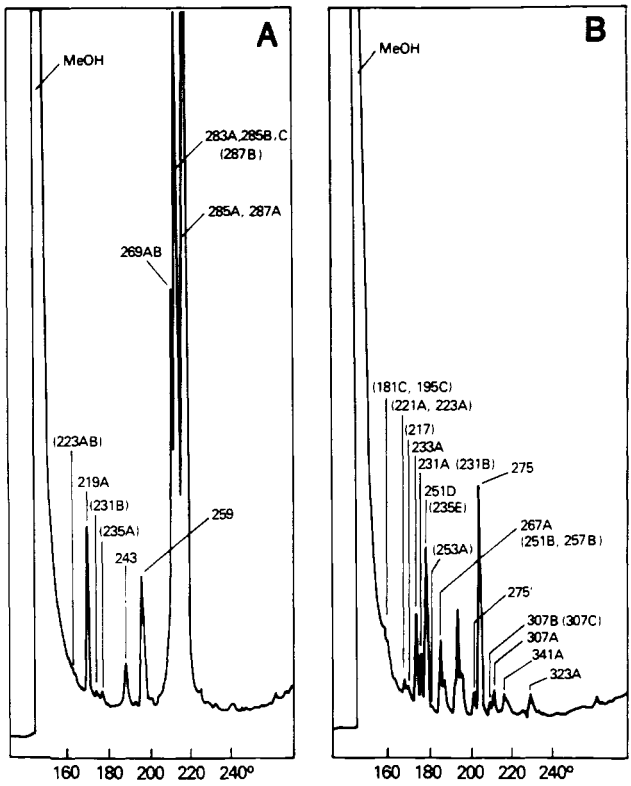


FIGURE 8. Gas chromatograms of alkaloids from two specimens of *Dendrobates* imported illegally from Colombia and seized by U.S. Customs (received through Dr. F.E. Russell). Conditions are as in Figure 5; see Table 2 for listing of alkaloids. *A. Dendrobates histrionicus*, representing a distinctive "unknown" population (see text), probably from western Colombia. Identification to species is confirmed by the alkaloid profile, especially including the set of histrionicotoxins emerging as a pair of distinctive peaks at about 210-218°. *B. Dendrobates lehmanni*, a species closely related to *D. histrionicus* but having a distinctive color pattern (Figure 10) and a different set of alkaloids. Histrionicotoxins are lacking and the rare alkaloid **275** is present as a major compound.

the use of ND_3 in ms for determination of exchangeable hydrogens (7), but the technique has not been applied to natural products. The results with the indolizidine **239AB** are illustrative. One exchangeable hydrogen is present and is lost with the $-C_3H_7O$ side chain (Figure 2). Thus, a hydroxyl moiety is present in this side chain. The nitrogen has no exchangeable hydrogen. The gross structure of the indolizidine **239AB** was inferred from mass spectral data and biosynthetic considerations to be that of a 3-butyl-5-hydroxypropylindolizidine (1,2). The position of the hydroxy moiety in the three-carbon side chain was undefined.

The use of NO/N_2 mixtures to obtain "pseudo" electron impact mass spectra has been previously reported (8). In the case of dendrobatid alkaloids, the NO/N_2 spectra (Figures 2,3) are similar to electron impact spectra (2).

Isolation of indolizidine **239AB** in quantities sufficient for nmr spectral analysis revealed that a ω-hydroxy group was present and left only the question of relative

stereochemistry at carbons 3, 5, and 9 unresolved. It was felt that comparison of carbon-13 resonances at carbons 3, 5, and 9 to those of the four isomeric 3-butyl-5-propylindolizidines would reveal the stereochemistry. Chemical shifts for the four isomeric 3-propyl-5-butylindolizidines and tentative chemical shifts for indolizidine **239AB** are presented in Table 3. The 5*E*,9*E*-configuration appears most compatible with the

TABLE 3. Carbon-13 Magnetic Resonance Peaks of 3,5-Disubstituted Indolizidines

	3-Butyl-5-propylindolizidine[a]				3-Butyl-5(3′-hydroxypropyl) indolizidine[b] (**239AB**)		3(4′-Hydroxybutyl)-5-propyl-indolizidine[c] (**239CD**)
	Free base Isomer				Free base	Hydro-chloride	Free base
	5*Z*,9*Z*	5*E*,9*Z*	5*Z*,9*E*	5*E*,9*E*			
	($CDCl_3$)	(C_6D_6)	(C_6D_6)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)
C-3	65.3	56.4	54.8	58.5	58.7	59.7	58.5
C-5	62.3	52.7	52.3	56.6	55.9	58.9	56.7
C-9	67.6	58.4	58.5	59.0	59.8	61.8	59.1
$-CH_2OH$		—	—	—	62.4	62.5	62.7

[a]Data from reference 9 and the following ^{13}C-resonances for natural (5*E*,9*E*) **223AB** (δ, $CDCl_3$): 59.0 (d), 58.5 (d), 56.6 (d), 35.9 (t), 32.4 (t), 30.9 (t), 30.0 (t), 29.1 (t), 26.3 (t), 24.9 (t), 24.6 (t), 22.9 (t), 18.9 (t), 14.5 (q), 14.1 (q). These resonances are identical to those of synthetic material from three different laboratories (ref. 10 and O.E. Edwards and M. Natsume, personal communications). The previously reported resonances for synthetic material (11) appear slightly in error due perhaps to calibration.

[b]The ^{13}C resonances for **239AB** were as follows (δ, $CDCl_3$): Free base 62.5 (t), 59.8 (d), 58.7 (d), 55.9 (d), 30.7 (t), 29.9 (t), 29.2 (t), 28.8 (t), 28.6 (t), 27.9 (t), 25.8 (t), 25.3 (t), 24.0 (t), 22.7 (t), 14.0 (q). Hydrochloride* 62.4, 61.8, 59.7, 58.9, 29.5, 29.0, 28.6, 28.2, 28.0, 27.8, 26.4, 25.5, 23.4, 22.5, 13.8. *Apparently sufficient HCl was present in $CDCl_3$ to convert this sample of **239AB** to the salt. The proton resonances of **239AB** were as follows (δ, $CDCl_3$): 3.8-4.0 (3H, CH_2OH, multiplet), 3.3 (3H, multiplet), 2.5 (3H, multiplet), 1.8 (4H, multiplet), 1.3 (13H, broad), 0.97 (3H, CH_3, triplet).

[c]The ^{13}C resonances for **239CD** were as follows (δ, $CDCl_3$): 62.7 (t), 59.1 (d), 58.5 (d), 56.7 (d), 35.8 (t), 33.0 (t), 32.1 (t), 30.8 (t), 29.9 (t), 26.3 (t), 25.3 (t), 24.6 (t), 23.1 (t), 18.9 (t), 14.5 (q). The proton resonances of **239CD** were as follows (δ, $CDCl_3$): 3.8-4.0 (3H, CH_2OH, multiplet), 3.2 (1H, multiplet), 3.0 (1H, multiplet), 2.4 (4H, multiplet), 1.8 (4H, multiplet), 1.3 (13H, broad), 0.98 (3H, CH_3, triplet).

resonances. The congeneric indolizidine **223AB**, formerly referred to as gephyrotoxin **223AB**, has the 5*E*,9*E* configuration (9) and is a likely precursor of indolizidines **239AB** and **239CD**. The carbon-13 magnetic resonance spectra of **223AB, 239AB,** and **239CD** are quite similar with respect to the peaks of the indolizidine carbons (Table 2). In addition, these three indolizidines are all levorotatory: Indolizidine **223AB** $[\alpha]^{16}D$ −35° (c 0.49, MeOH); indolizidine **239AB** $[\alpha]^{16}D$ −38° (c 1.0, MeOH); and indolizidine **239CD** $[\alpha]^{16}D$ −52° (c 0.19, MeOH). Recently, (3*R*,5*R*,9*R*)3-butyl-5-propylindolizidine was synthesized and found to be levorotatory (10), suggesting that the natural indolizidines also have the 3*R*,5*R*,9*R* configuration.

D. histrionicus occupies a long narrow range on the Pacific side of the Andes, in western Colombia and northwestern Ecuador. The species displays extreme interpopulational variation in size, habits, color and color pattern, and in the nature of its alkaloids (5). This extraordinary frog has now been selectively canvassed for alkaloids from virtually one end of its range to the other, from latitude 6°N to nearly 1°S. The alkaloids of nine populations of *D. histrionicus* from along the Pacific side of northwestern South America were compared in an earlier study (5). Histrionicotoxins were present in all populations. Tricyclic gephyrotoxins occurred in four of the nine populations. Indolizidines occurred in five of the nine populations. It is now apparent (2) that three alkaloids of either the pumiliotoxin-A class or its allopumiliotoxin subclass occurred in three of these nine populations of *D. histrionicus*. The new northern populations reported here from Altos del Buey and El Valle contain 34 alkaloids, which include histrionicotoxins, indolizidines, pumiliotoxins, allopumiliotoxins, a piperidine, and a variety of unclassified alkaloids. The northern populations of *D. histrionicus* from the Altos

del Buey region are unusual in their high content of (allo)pumiliotoxins, containing five representatives.

The earlier study of geographic variation (5) in *D. histrionicus* revealed that populations vary somewhat in the total quantity of alkaloids produced. This is also true at Altos del Buey, with the low-elevation population containing a notably larger amount of alkaloids than the high-elevation population (Figure 5). The earlier study also demonstrated a nearest-neighbor effect in alkaloid similarity comparisons, with individual populations tending to share more compounds with their nearest neighbors than with more distant populations. Comparison of current results with the earlier data would seem difficult, since advances in techniques now permit the detection and characterization of many trace alkaloids that would previously have gone undetected. Nonetheless, populations recently studied show results similar to the pattern previously detected. An alkaloid similarity value for a pair of populations is calculated by $100\ C/N_1$, where C is the number of shared alkaloids and N_1 is the number of alkaloids in the population sample having the smaller number of compounds. In the following, samples B-E each represent N_1 as compared with sample A from the summit of Altos del Buey.

A) Altos del Buey, high (32 alkaloids)
B) Altos del Buey, low=100% (30, all shared)
C) El Valle=92% (22 shared of 24)
D) Quebrada Docordó=74% (17 shared of 23)
E) Guayacana, Nariño=68% (13 shared of 19)

Population C above is only about 25 km west of Altos del Buey, whereas D is 185 km to the south-southeast, and E is 530 km to the south-southwest, close to the Ecuadorian border. Data for population D (Quebrada Docordó) above are based on five recently analyzed skins (June 1983), whereas population E (Guayacana) has been extensively studied, and its alkaloid profile is well known (2). These two populations also had been studied by earlier techniques that detected fewer alkaloids, and the following comparisons with Altos del Buey (population A above) are made from the 1976 publication (5): Quebrada Docordó with 7 of 10 alkaloids shared=70% (vs. 74% above); Guayacana with 6 of 9 alkaloids shared=67% (vs. 68% above). Thus, in these few comparisons, increasing the number of detectable alkaloids by resampling with improved technique more than doubled the number of alkaloids detected but did not materially change the similarity comparisons. The original value of 56% for shared alkaloids between the Quebrada Docordó and Guayacana populations is also virtually unchanged (57%) with present data (Table 2).

One purpose of collecting *D. histrionicus* on Altos del Buey was to compare alkaloids from that population with those of the closely related *D. lehmanni*, which occurs on the western flank of the Andes about 270 km to the south-southeast. Altos del Buey is one of the few places where the lowland *D. histrionicus* occurs as high (1070 m) as the geographically restricted *D. lehmanni* (850-1200 m), and it furthermore is one of the very few populations in which occasional individuals exhibit a tendency towards cross-banding (Figure 9E), in marked contrast to the spotted, blotched, marbled, or nearly unicolor patterns of other populations of *D. histrionicus*. *D. lehmanni* is thought to have speciated from a disjunct highland *D. histrionicus* population that embarked on a markedly different evolutionary course, as suggested by the vivid orange and black cross-banded pattern (broken bands in about 10-15%, Reference 5) and normally white digit tips (Figure 10) and different kinds of skin alkaloids. Alkaloid similarity comparisons between *D. lehmanni* and various populations of *D. histrionicus* originally ranged from 0-13%, whereas the range is 38-100% for paired comparisons within *D. histronicus* (5). *D. lehmanni* shares six (29%) of its 21 alkaloids (2) with the Altos del Buey populations, with all but one of the shared compounds being of the pumiliotoxin-A or allo-

FIGURE 9. *Dendrobates histrionicus* representing two populations from northwestern Colombia (6°N) latitude), near the northern end of its geographic range; about 1.5 times life size. *A-B*. Color pattern variation in specimens (AMNH 102115, 102119) from near El Valle, showing "normal" (A) and extreme (B) patterns. *C-E*. Color pattern variation in specimens (AMNH 102156, 102157, 102155) from 800-1000 m elevation near the summit of Altos del Buey. Specimen E is a rare crossbanded variant reminiscent of *Dendrobates lehmanni* (Figure 10). *From color transparencies by C.W. Myers, American Museum of Natural History (AMNH).*

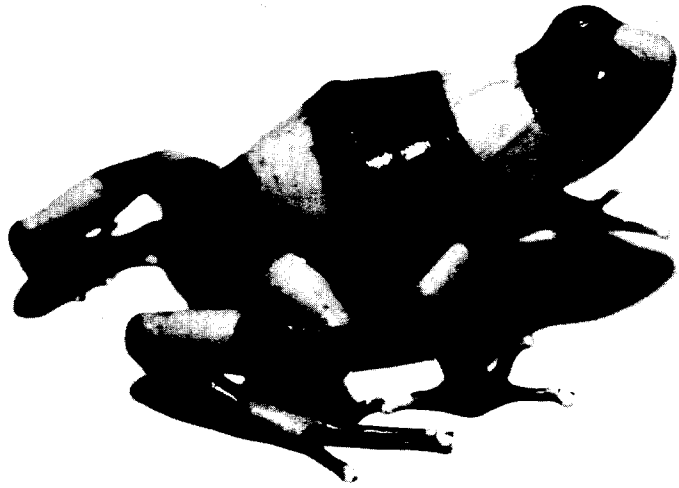


FIGURE 10. *Dendrobates lehmanni* (AMNH 88153), a western Colombian species related to *Dendrobates histrionicus*; about two times life size. The distinctive orange and black cross-banded pattern is approached in the variational repertory of some distant populations of *D. histrionicus* (Figure 9E). The alkaloid profile of *D. lehmanni* differs markedly from all the color-pattern diverse populations of *histrionicus*. *From a transparency by C.W. Myers.*

pumiliotoxin class. *D. lehmanni* remains biochemically distinctive in completely lacking histrionicotoxins. All populations of *D. histrionicus*, including those of Altos del Buey, contain a variety of histrionicotoxins that emerge together as a distinctive pair of peaks at about 210-218° on gc (Figures 5, 6, 8A).

A new alkaloid profile for *D. lehmanni* is given in Figure 8B and Table 2, based on a specimen of unknown locality seized as contraband by U.S. Customs. The extract contained 21 alkaloids, the same number as shown by reanalysis of *D. lehmanni* extract from the type locality (2), but only 14 compounds (67%) are shared. Another *D. lehmanni* specimen imported in the animal trade in a different year gave a profile similar to Figure 8B (data not shown), suggesting that dealers were not obtaining frogs from their original locality from which the species was described scientifically (5). In addition to the absence of histrionicotoxins, alkaloid **275** appears characteristic of *D. lehmanni*, having been detected as a major alkaloid in the initial study of *D. lehmanni* (5) and in the subsequent specimens from the animal trade. Of unknown structure, alkaloid **275** has been detected only as a trace compound in three other species, and even in these (*Dendrobates auratus, D. pumilio, Dendrobates speciosus*), the compound was found only in one of several to many populations of each species studied.

Another distinctive *Dendrobates* of unknown locality has appeared in the Colombian animal trade within the last decade. It is a brown frog densely vermiculated and spotted with yellow and with a reddish suffusion over the head. Skin extracts of two specimens imported at different times yielded similar profiles, one of which is shown in Figure 8A. This specimen contained 13 alkaloids of which eight were histrionicotoxins, one an indolizidine (**223AB**), and four were alkaloids that are presently unclassified. These results confirm the identity of this frog as a population of *D. histrionicus*, presumably deriving from a single locality in Pacific western Colombia.

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of *D. histrionicus* from Quebrada Docordó, Colombia, to Dr. F.E. Russell (University of Arizona) for the specimens of *D, histrionicus* and *D. lehmanni* confiscated by U.S. Customs, and to Prof. H.-P. Husson for information on synthetic (−)indolizidine **223AB** prior to publication. Fieldwork in Colombia was initiated and facilitated under the auspices of the late F. Carlos Lehmann Valencia, former director of the Museo Departamento de Historia Natural, in Cali. We also are grateful to courtesies from the Sección de Recursos Naturales, CVC, Colombia, in support of our fieldwork. Mr. Michael G.A. Hill, of Medellín, is gratefully acknowledged for assistance on the 1978 expedition to Altos del Buey, which was made possible by a grant to the American Museum of Natural History from the Camille and Henry Dreyfus Foundation.

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