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CLASSIFICATION OF SKIN ALKALOIDS FROM NEOTROPICAL POISON-DART FROGS (*DENDROBATIDAE*)

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JOHN W. DALY, GEORGE B. BROWN, MONICA MENSAH-DWUMAH and CHARLES W. MYERS. Classification of skin alkaloids from Neotropical poison-dart frogs (*Dendrobatidae*). *Toxicon* **16**, 163–188, 1978.—Dendrobatid frogs have evolved an imposing number of unique alkaloids, apparently as a chemical defense against predation. Toxic skin extracts from a majority (18) of the approximately two dozen species of *Dendrobates* (*sensu lato*) were analyzed by thin-layer chromatography, gas chromatography and mass spectrometry. Ninety alkaloids were detected and characterized, with structures being presented for many. The species of *Dendrobates* elaborate at least 5 classes of biosynthetically related alkaloids, namely the *pumiliotoxin-C class* (decahydroquinolines), the *hydroxypumiliotoxin-C class*, the *histrionicotoxin class* (1-azaspiro [5.5]undecanes), the *gephyrotoxin class* (perhydropyrrolopiperidines and perhydropyrroloquinolines) and the *pumiliotoxin-A class* (of as yet unknown structure). A sixth class, the *batrachotoxins*, is a series of highly toxic, steroidal alkaloids that are produced only by species of *Phyllobates* (*sensu stricto*). A wide range of biological activity of the dendrobatid alkaloids is indicated by injection of unresolved alkaloid fractions into white mice: extracts from different species of *Dendrobates* produce reactions as diverse as Straub tail, penile erection, prostration, convulsions and salivation.

INTRODUCTION

THE *Dendrobatidae* are a family of small, Neotropical frogs, some lineages of which have evolved a remarkable array of toxic skin alkaloids. The species of dendrobatids known to possess skin alkaloids are currently sorted among two genera, *Phyllobates* and *Dendrobates*. Three species of South American *Phyllobates* are used by the Emberá and Noanamá Chocó Indians for poisoning blowgun darts. The structures of the dart poisons have been elucidated and the most toxic compounds named batrachotoxin and homobatrachotoxin (Fig. 1; TOKUYAMA *et al.*, 1969; for review of earlier literature see MÄRKI and WITKOP, 1963). The batrachotoxins differ from other dendrobatid alkaloids in their steroidal ring structure and much greater toxicity. The toxicity is due to selective effects on ionic permeability, which leads to an irreversible depolarization of nerve and muscle (ALBUQUERQUE *et al.*, 1971; ALBUQUERQUE and DALY, 1977).

Frogs of the genus *Dendrobates* have elaborated simpler and less toxic alkaloids, of which some have been named after the species from which first isolated. Thus, Panamanian *Dendrobates pumilio* yielded pumiliotoxin A, B, and C (DALY and MYERS, 1967; DALY *et al.*, 1969). The structures of pumiliotoxins A and B are as yet unknown, whereas pumiliotoxin C was shown by X-ray crystallography to be a simple dialkyldecahydroquinoline (Fig. 1). A Colombian frog, *Dendrobates histrionicus*, afforded another class of skin alkaloids to which

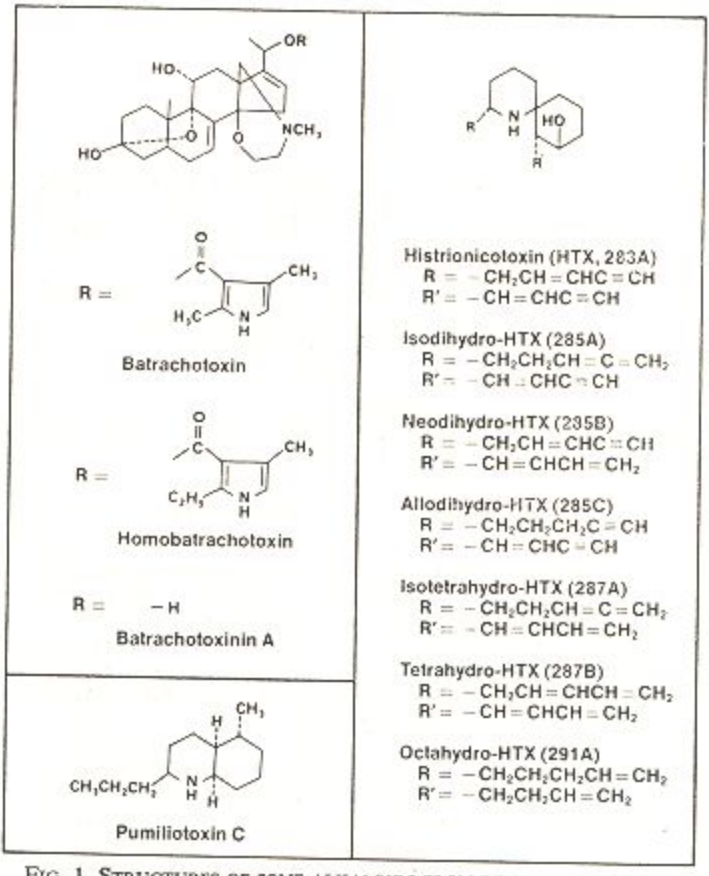


FIG. 1. STRUCTURES OF SOME ALKALOIDS FROM DENDROBATID FROGS.

the name histrionicotoxins was given (DALY *et al.*, 1971; TOKUYAMA *et al.*, 1974). These compounds, which have an unusual spiro-ring structure (Fig. 1), are proving useful for the study of ionic permeability in nerve and muscle (ALBUQUERQUE *et al.*, 1973, 1974; KATO *et al.*, 1975; LAPA *et al.*, 1975). The structure of gephyrotoxin (see Results, **287C**), the first member of yet another class of dendrobatid alkaloids was recently elucidated (DALY *et al.*, 1977).

An ongoing survey of other species of *Dendrobates* has revealed the existence of many additional novel compounds, increasing the known dendrobatid alkaloids to a total approaching 100, the bulk of which occur only in *Dendrobates* (Table 1). The biological significance of this diversity is of considerable interest; also, some of the new compounds merit pharmacological investigation as potentially useful research tools. To facilitate further study, a tentative classification of *Dendrobates* skin alkaloids is presented herein. The taxonomic distribution of batrachotoxins in the species of *Phyllobates* will be discussed elsewhere (MYERS and DALY, in progress), and this structurally well-known class of dendrobatid alkaloids will receive only cursory attention in the present report.

METHODS

Frogs were skinned in the field and the skins stored in 70–100% methanol, when possible at −5°C, otherwise at ambient temperature. Prior to fractionation of extracted substances, the skins were finely minced and macerated in methanol. The methanol was decanted and the skins then extracted and macerated in a fresh portion of methanol. Approximately 20 ml portions of methanol were used for 3–4 g of wet skin. The 2 methanol extracts were combined, diluted with an equal volume of water, and extracted twice, each time with 2 vol of chloroform. Basic chloroform-soluble alkaloids were then extracted thrice from the

TABLE 1. TENTATIVE CLASSIFICATION OF *Dendrobates* SKIN ALKALOIDS

Pumiliotoxin-C (PTX-C) class	Hydroxypumiliotoxin-C (HO-PTX-C) class	Pumiliotoxin-A (PTX-A) class	Histrionicotoxin (HTX) class	Gephyrotoxin (GTX) class
167 "$C_{11}H_{21}N$" (138,H_0)	**223E** "$C_{14}H_{25}NO$" (168,H_2)	**237A*** $C_{15}H_{27}NO$ (166,70,H_2)	**235A** $C_{15}H_{25}NO$ (96,H_4)	**223AB** $C_{15}H_{29}N$ (180,166,H_0)
181A "$C_{12}H_{23}N$" (152,H_0)	**225** "$C_{14}H_{27}NO$" (168,H_0)	**237B** "$C_{15}H_{27}NO$" (182,70,?)	**259*** $C_{17}H_{25}NO$ (96,H_8)	**239AB*** $C_{15}H_{29}NO$ (182,180,H_0)
181B "$C_{12}H_{23}N$" (138,H_6)	**237E** "$C_{15}H_{27}NO$" (152,H_2)	**251D*** "$C_{16}H_{29}NO$" (166,70,H_2)	**283A*** HTX, $C_{19}H_{25}NO$ (96,H_{12})	**239CD*** $C_{15}H_{29}NO$ (196,166,H_0)
195A* PTX-C, $C_{13}H_{25}N$ (152,H_0)	**239A** "$C_{15}H_{29}NO$" (182,H_0)	**251E** "$C_{15}H_{25}NO_2$" (168,84,70,?)	**285A*** Iso-H_2-HTX (96,H_{10})	**257A** "$C_{18}H_{25}N$" (216,H_8)
195B "$C_{13}H_{25}N$" (138,H_0)	**239B** "$C_{15}H_{29}NO$" (180,H_0)	**253** $C_{15}H_{27}NO_2$ (182,70,H_2)	**285B*** Neo-H_2-HTX (96,H_{10})	**287C*** GTX, $C_{19}H_{29}NO$ (242,H_6)
203* "$C_{14}H_{21}N$" (138,H_6)	**239C** "$C_{15}H_{29}NO$" (196,H_0)	**267A*** $C_{16}H_{29}NO_2$ (182,70,H_2)	**285C*** Allo-H_2-HTX (96,H_{10})	
205 "$C_{14}H_{23}N$" (138,H_4)	**239D** "$C_{15}H_{29}NO$" (166,H_0)	**281A*** "$C_{17}H_{31}NO_2$" (166,70,H_2)	**287A** Iso-H_4-HTX (96,H_8)	
207 "$C_{14}H_{25}N$" (138,H_2)	**239E** "$C_{15}H_{29}NO$" (152,H_0)	**281B** "$C_{18}H_{35}NO$" (150,70,H_0)	**287B** H_4-HTX (96,H_8)	
219A* $C_{13}H_{25}N$ (178,H_4)	**239F** "$C_{15}H_{29}NO$" (168,H_0)	**297A** $C_{17}H_{31}NO_3$ (182,70,H_2)	**291A*** H_3-HTX (178,96,H_4)	
219B "$C_{15}H_{25}N$" (152,H_4)	**239G** "$C_{15}H_{29}NO$" (138,H_0)	**297B** $C_{18}H_{35}NO_2$ (166,70,H_4)		
223A "$C_{15}H_{29}N$" (180,H_0)	**251B** "$C_{16}H_{29}NO$" (138,H_2)	**307A*** PTX-A, $C_{19}H_{33}NO_2$ (166,70,H_4)		
223B "$C_{15}H_{29}N$" (166,H_0)	**251C** "$C_{16}H_{29}NO$" (154,H_2)	**307B** Iso-PTX-A, "$C_{19}H_{33}NO_2$" (166,70,H_4)		
223C "$C_{15}H_{29}N$" (152,H_0)	**291B** "$C_{19}H_{33}NO$" (168,H_4)	**307C** Allo-PTX-A, $C_{19}H_{33}NO_2$ (182,70,H_4)		
223D "$C_{15}H_{29}N$" (138,H_0)	**291C** "$C_{19}H_{33}NO$" (152,H_4)	**309A** H_2-PTX-A (166,70,H_2)		
231A "$C_{16}H_{25}N$" (166,H_6)	**295** "$C_{19}H_{37}NO$" (138,?)	**323A*** PTX-B, $C_{19}H_{33}NO_3$ (166,70,H_2)		
231B "$C_{16}H_{25}N$" (152,H_6)	**309B** "$C_{20}H_{39}NO$" (152,?)	**323B** Allo-PTX-B (182,70,H_4)		
233 "$C_{16}H_{27}N$" (166,H_4)		**325*** H_2-Allo-PTX-B (182,70,H_2)		
235B "$C_{16}H_{29}N$" (138,H_2)		**341A*** "$C_{19}H_{35}NO_4$" (84,70,H_2)		
237C "$C_{16}H_{31}N$" (180,H_0)		**341B** "$C_{19}H_{35}NO_4$" (182,70,?)		
237D "$C_{16}H_{31}N$" (138,H_0)		**351** "$C_{21}H_{37}NO_3$" (138,70,?)		
243* $C_{17}H_{25}N$ (202,H_8)		**357** "$C_{20}H_{39}NO_4$" (110,70,H_0)		
251A "$C_{17}H_{33}N$" (152,H_0)		**379** "$C_{23}H_{41}NO_3$" (166,70,H_4)		
269A $C_{19}H_{27}N$ (204,H_{10})		**381** "$C_{23}H_{43}NO_3$" (166,70,?)		
269B $C_{19}H_{27}N$ (202,H_{10})		**395** "$C_{23}H_{41}NO_4$" (182,70,H_4)		
275* $C_{19}H_{33}N$ (152,H_4)				
289 "$C_{20}H_{35}N$" (152,H_4)				

Alkaloids occurring as major compounds in one or more species are designated by an asterisk (*). Empirical formulae in quotations have yet to be confirmed by high resolution spectrometry. One or more major electron-impact mass spectral fragments (in most cases only the base peak) and the number of additional hydrogens in the apparent perhydro-derivative are given in parentheses. Ten additional alkaloids remain unclassified (see Discussion).

TABLE 2. TOXICITY OF METHANOLIC SKIN EXTRACTS FROM 10 SPECIES OF *Dendrobates*

Species	Gross effects following subcutaneous injection in white mice	Equivalent amount injected (mg skin)
D. auratus (Isla Taboga)	Piloerection, irritation at injection site, locomotor difficulties, gagging, labored breathing, clonic convulsions, prostration, death 6–30 min.*†	60–100
D. granuliferus	Locomotor difficulties, gagging, salivation, prostration, extensor movements of hind limbs, mild clonic convulsions, death 25–35 min.	60
D. histrionicus		
B. Playa de Oro	Locomotor difficulties.	60
E. Quebrada Guangui	Locomotor difficulties, gagging, salivation, labored breathing, clonic convulsions, then prostration, sustained penile erection, thrusting movements, arching of back, convulsions, partial recovery from prostration etc. in 3 hr.	100
G. Guayacana	Minor locomotor difficulties, prostration, recovery after 2 hr.*	100
H. Río Baba	Minor locomotor difficulties, prostration, recovery after 3 hr.	100
I. Río Palenque	Minor locomotor difficulties, prostration, recovery after 2 hr.	100
D. lehmanni	Locomotor difficulties, labored breathing, gagging, salivation, clonic convulsions, prostration, recovered by 3–4 hr.*	100
D. leucomelas	Locomotor difficulties, convulsions, death 11 min.	60
D. minutus (Cerro Campana)	Locomotor difficulties, gagging, salivation, extensor movements of hind limbs, clonic convulsions, death 14–20 min.‡	60
D. pictus	Locomotor difficulties, piloerection, labored breathing, prostration, mild Straub tail, recovery after more than 4 hr.	100
D. pumilio	Initial hyperactivity, irritation at injection site, loss of equilibrium, locomotor difficulties, gagging, labored breathing, salivation, extensor movements of hind limbs, clonic convulsions, death 8–12 min.*§	60
D. tinctorius	Minor locomotor difficulties, gagging, recovered by 1 hr.	60
D. trivittatus (Surinam)	Initial hyperactivity, locomotor difficulties, partial immobilization of limbs, prostration, labored breathing, recovery in 2 hr.	100

Abbreviated localities are given when more than one population of a species is listed in section on Sources and Occurrence of Alkaloids.
*Similar results after injection of alkaloid fraction.
†Similar results with extracts from frogs collected Aug. 1966 (wet season), Feb. 1966 (dry season) and Oct. 1972 and with extracts of brown-variety *auratus* from Chorrera, Panama, Nov. 1967.
‡Similar results with extracts of frogs from ridges above Puerto Pilón, Panama, Jan. 1966 and March 1967, and from Playa de Oro, Río San Juan, Colombia, Feb. 1970.
§Similar results with extracts from frogs collected Nov. 1966 and Nov. 1968.

combined chloroform solutions into one-half volumes of 0·1 N HCl. The combined 0·1 N HCl solutions were adjusted to pH 9 with 1 N aqueous ammonia, followed by re-extraction of the alkaloids into chloroform; 2 extractions, each time with an equal volume of chloroform. The combined chloroform solutions were dried over Na_2SO_4, and evaporated *in vacuo* to dryness. The residue was dissolved in methanol so that 10 µl corresponded to 10 mg of the original wet weight of the skins.

The original methanolic extracts were in many instances assayed for toxicity (Table 2). The final alkaloid fractions were compared and analyzed by thin-layer and gas chromatography. The thin-layer chromatographic system was silica gel GF plates (250 µm, Analtech, Newark, Delaware) with chloroform–methanol (9:1). Detection was with iodine vapor. Conditions for gas chromatography are described in the legend to Fig. 2. Combined gas chromatography–mass spectrometry utilized either methane or helium as the carrier gas. Both the chemical ionization (methane) technique using a Finnigan 1015 mass spectrometer with data system 6000, and the electron impact (70 eV) technique using a LKB 3000 spectrometer and helium as the carrier gas, were employed. Compounds isolated by preparative thin-layer chromatography or by silica-gel column chromatography were further analyzed by chemical ionization and electron impact mass spectrometry. High resolution spectra on isolated alkaloids were provided by Dr. Roger Foltz, Battelle Memorial Institute, Columbus, Ohio. In certain instances, methanolic extracts corresponding to 100–300 µg of skin were reduced overnight with 30 psi hydrogen gas in 0·5 ml of ethanol and 5 mg 10% palladium on charcoal. After filtration and concentration these perhydro-products were analyzed by gas chromatography–mass spectrometry. Identification of alkaloids in various extracts was based primarily on combined gas chromatography–mass spectrometry of the original methanolic alkaloid fraction and the perhydrogenated material.

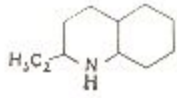
The analysis of pumiliotoxin A and B and their isomers was complicated by incomplete reductions to both H_2- and H_4-derivatives. With most alkaloids the reductions appeared to be complete under the conditions used. Acetylation of alkaloids was under mild conditions described by TOKUYAMA *et al.* (1974).

RESULTS

Alkaloids

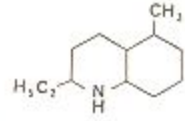
The following compounds have been detected as major, minor or trace constituents of alkaloid fractions from various species of *Dendrobates*. Alkaloids are designated by molecular weight in boldface type (based on mass spectrometry), followed by: (1) trivial name (if any); (2) empirical formula based on high resolution mass spectrometry (postulated formulae in quotations are based on analogy and chromatographic and chemical properties); (3) R_f on thin-layer chromatography (see Methods); (4) approximate emergent temperature on gas chromatography (see legend Fig. 2) and (5) electron impact mass spectrum given in nominal masses (m/e=mass of each ion divided by charge) followed for each ion by the intensity (in parentheses) relative to the base peak set equal to 100. Only the most diagnostic peaks from spectra obtained on combined gas chromatography–electron impact mass spectrometry are given. Peaks that had intensity >25% of the protonated molecular ion on combined gas chromatography–chemical ionization mass spectrometry with methane carrier gas are italicized. The perhydrogenation derivative (H_0=no addition of hydrogen, H_2= addition of 2 hydrogens, etc.) and the m/e for major diagnostic peaks of this derivative are also given. Any other pertinent data and in certain instances tentative structural formulations are included as is the distribution of each alkaloid in various species and populations of frogs.

167. "$C_{11}H_{21}N$", —, 151°, m/e 167(1), 166(1), *138*(100). H_0-derivative. Tentative structure:



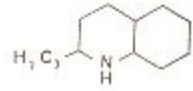
Trace in *D. auratus* (Isla Taboga).

181A. "$C_{12}H_{23}N$", —, 152°, m/e 181(2), 180(1), *152*(100), H_0-derivative. Tentative structure:



Trace in *D. auratus* (Isla Taboga).

181B. "$C_{12}H_{23}N$", —, 153°, m/e 181(2), 180(2), *138*(100). H_0-derivative. Isomer of **181A**. Tentative structure:



Trace in *D. auratus* (Isla Taboga, Río Campana), *D. azureus*, *D. fulguritus*, *D. trivittatus* (Peru).

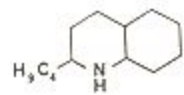
185. ?, 0·2, 153°, m/e 185(1), *170*(100). H_0-derivative.

Trace in *D. auratus* (Isla Taboga).

195A. Pumiliotoxin C (Fig. 1), $C_{13}H_{25}N$, 0·20; 157°, m/e 195(3), *194*(5), 180(1), *152* ($C_{10}H_{18}N$,100), 109(8). H_0-derivative. N-acetyl derivative.

Major in *D. auratus* (Isla Taboga, Río Campana), *D. pumilio;* trace in *D. lehmanni, D. minutus* (Quebrada Guanguí).

195B. "$C_{13}H_{25}N$", 0·23, 156°, m/e 195(2), *194*(1), *138*(100). H_0-derivative. Tentative structure:

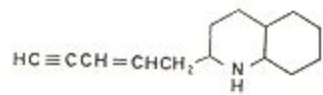


Trace in *D. histrionicus* (Quebrada Guanguí, Río Guapi, Guayacana).

197. ?, —, 160°, m/e 197(1), *180*(100), 126(35). H_0-derivative.

Trace in *D. auratus* (Río Campana).

203. "$C_{14}H_{21}N$", 0·33, 158°, m/e 203(1), *202*(2), *138*(100). H_6-derivative, m/e 209, 138. Tentative structure:



However, **203** did not appear to form an N-acetyl derivative.

Major in *D. fulguritus;* trace in *D. auratus* (Isla Taboga), *D. histrionicus* (Guayacana), *D. minutus* (El Llano-Cartí), *D. pumilio.*

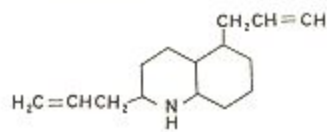
205. "$C_{14}H_{23}N$", —, 158°, m/e 205(1), 204(2), *138*(100). H_4-derivative, m/e 209, 138. Probably dihydroanalog of **203.**

Trace in *D. histrionicus* (Guayacana).

207. "$C_{14}H_{25}N$", —, 158°, m/e 207(1), 206(1), *138*(100). H_2-derivative, m/e 209, 138. Probably tetrahydro-analog of **203.**

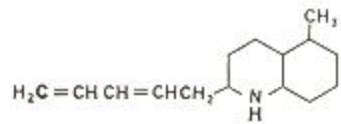
Trace in *D. histrionicus* (Río Guapi, Río Baba).

219A. $C_{15}H_{25}N$, 0·32, 165°, m/e 219(1), *218*(2), *178*($C_{12}H_{20}N$,100). H_4-derivative, m/e 223, 180. N-acetyl derivative. Tentative structure:



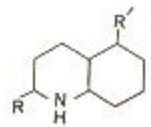
Major in *D. auratus* (Isla Taboga, Río Campana), *D. azureus, D. granuliferus, D. histrionicus* (Playa de Oro, Quebrada Vicordó, Quebrada Docordó), *D. parvulus, D. truncatus;* minor or trace in *D. histrionicus* (Santa Cecilia, Quebrada Guanguí).

219B. "$C_{15}H_{25}N$", —, 162°, m/e 219(1), 218(2), *152*(100). H_4-derivative, m/e 223, 152. Probably analog of **223C** with unsaturated side chain. Tentative structure:



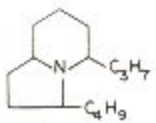
Minor in *D. histrionicus* (Río Guapi, Río Baba), *D.* species (Colombia); trace in *D. azureus.*

223A, B, C, D. "$C_{15}H_{29}N$". Apparently a series of compounds, 0·28–0·32, 158–160°. m/e 223(1), *222*(2) and either *180*(**A**), *166*(**B**), *152*(**C**) or *138*(**D**)(100). H^0-derivative. N-acetyl derivatives of **223A** or **223B** were obtained in certain extracts (*D. auratus, D. histrionicus*), Tentative structures:



	A	B	C	D
R =	C_3H_7,	C_4H_9,	C_5H_{11},	C_6H_{13}
R′ =	C_3H_7,	C_2H_5,	CH_3,	H

In *D. histrionicus* (Guayacana, Quebrada Guanguí) **223AB** represents a single compound [$C_{15}H_{29}NO$, m/e 223(1), 222(2), 180(85), 166(100)] which does not N-acetylate. **223B** is also present (see above). Tentative structure (a perhydropyrrolopiperidine):



223AB

Minor or trace in *D. auratus* (Isla Taboga, **A,B,C**). *D. histrionicus* (various populations, **A,B,C,D,AB**), *D. lehmanni* (**A**), *D. minutus* (El Llano-Cartí, **B,D,** Quebrada Guanguí, **A,B**), *D. occultator* (**A**), *D. pumilio* (**D**), *D. truncatus* (**A,B,C,D**), *D.* species (Columbia, **A,B,C**).

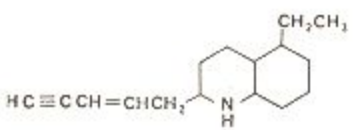
223E. "$C_{14}H_{25}NO$", —, 163°, m/e 223(2), 222(3), *168*(100). H_2-derivative, m/e 223, 168. Possibly an analog of **225** with double bond in side chain.

Trace in *D. lehmanni, D. occultator, D. tinctorius*.

225. "$C_{14}H_{27}NO$", —, 164°, m/e 225(3), 224(6), 208(2), *168*(100), 152(25). H_0-derivative. Possibly a hydroxypumiliotoxin C.

Trace in *D. tinctorius*.

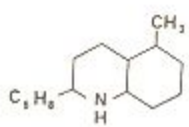
231A. "$C_{16}H_{25}N$", 0·30, 166°, m/e 231(2), *230*(1), *166*(100). H_6-derivative m/e 237, 166. Tentative structure:



However, **231A** did not appear to form an N-acetyl derivative.

Minor in *D. histrionicus* (Río Guapi, Río Palenque), *D. minutus* (El Llano-Cartí).

231B. "$C_{16}H_{25}N$", 0·30, 166°, m/e 231(2), *230*(1), *152*(100), H_6-derivative m/e 237, 152. Tentative structure:

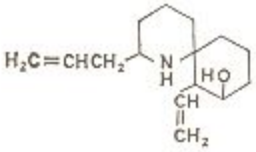


Minor or trace in *D. azureus, D. histrionicus* (Quebrada Docordó, Río Guapi, Río Baba), *D. lehmanni, D. occultator, D. tinctorius, D.* species (Colombia).

233. "$C_{16}H_{27}N$", —, 167°, m/e 233(2), 232(2), *166*(100). H_4-derivative. m/e 237, 166. Probably higher homolog of **223B** with 2 double bonds in the R side chain.

Minor in *D.* species (Columbia)

235A. $C_{15}H_{25}NO$, 0·36, 176°, m/e 235(5), 234(2), 218(15), 294($C_{12}H_{20}NO$, 76), 176($C_{12}H_{18}N$,25), 150(8), 96($C_6H_{10}N$,100). H_4-derivative, m/e 239, 196, 178, 96. O-acetyl derivative Structure:



Minor or trace in *D. auratus* (Isla Taboga, Río Campana), *D. granuliferus, D. histrionicus* (Quebrada Vicordó), *D. parvulus, D. trivittatus, D. truncatus.*

235B. "$C_{16}H_{29}N$", —, 166°, m/e 235(1), 234(1), *138*(100). H_2-derivative, m/e 237, 138. Probably an analog of **237D** with double bond in the side chain.

Trace in *D. histrionicus* (Quebrada Guangui), *D. pumilio.*

237A. $C_{15}H_{27}NO$, 0·58, 167°, m/e 237(4), 236(3), 220(3), *194*(12), *166*(40), 70(100). H_2-derivative, m/e 239(5), 196(5), 168(10), 110(30), 84(100), 70(45). Lowest molecular weight member of the pumiliotoxin-A class of alkaloids (base peak at 70). Major fragment ions at 110, 84 and 70 are typical of the hydrogenated compounds of this series.

Major in *D. abditus;* trace in *D. histrionicus* (Río Baba).

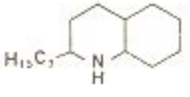
237B. "$C_{15}H_{27}NO$", 0·58, 168°, m/e 237 (1), 236(2), 182(60), 114(30), 112(25), 70(100). An allo-isomer of **237A.** The allo-compounds* of the pumiliotoxin-A class have a major fragment at 182 rather than at 166.

Trace constituent in *D. abditus, D. fulguritus.*

237C. "$C_{16}H_{31}N$", —, 168°, m/e 237(2), 236(1), *180*(100), H_0-derivative. Probably higher homolog of **223B.**

Minor in *D. histrionicus* (Río Baba).

237D. "$C_{16}H_{31}N$", —, 163°, m/e 237(1), 236(2), *138*(100), H_0-derivative. Tentative structure:

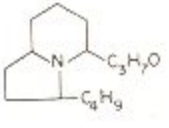


Trace in *D. auratus* (Río Campana).

237E. "$C_{15}H_{27}NO$", 0·25, 180°, m/e 237(1), 236(3), 208(70), *152*(100). H_2-derivative, m/e 239, 152. Possibly a hydroxypumiliotoxin C.

Trace in *D. histrionicus* (Quebrada Guangui).

239AB. $C_{15}H_{29}NO$. Probably one compound, 0·22, 178°, m/e 239(2), 238(3), *182*(**A,** $C_{11}H_{20}NO$,100), *180*(**B,** $C_{12}H_{22}N$,90). H_0-derivative. O-acetyl derivative. Tentative structure:



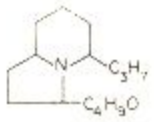
239AB

*The allo- prefix in the pumiliotoxin-A class has no relationship to its usage in the histrionicotoxin class (see **285C**).

In *D. histrionicus* and *D. occultator* **239AB** appears to be one compound of the gephyrotoxin class. In certain frogs **239A** and **239B** may represent trace compounds of the hydroxypumiliotoxin-C class.

Major in *D. histrionicus* (Santa Cecilia, Quebrada Guanguí, **AB**), *D. occultator* (**AB**); minor in *D. histrionicus* (Playa de Oro, Quebrada Docordó, **AB**) *D.* species (Colombia, **B**). A trace compound in *D.* species (Colombia) has an emergent temperature of 168°C and corresponds an isomer of **239B**. In *D. occultator* there was a trace set of isomers of **239A,B** that emerge at 168°C.

239CD. $C_{15}H_{29}NO$. Probably one compound, 0·16, 179°, m/e 239(4), 238(3), *196*(**C**,$C_{12}H_{22}NO$,100), *166*(**D**,$C_{11}H_{25}N$,60). H_0-derivative. O-acetyl derivative. Tentative structure:



239CD

In *D. histrionicus* and *D. occultator* **239CD** appears to be one compound of the gephyrotoxin class (see discussion of **223AB** and **239AB**).

Major in *D. histrionicus* (Quebrada Guanguí) and *D. occultator*. There appears another trace set of isomers which emerge slightly before **239CD.** A trace compound in *D.* species (Colombia) has a retention time of 170° and corresponds to an isomer of **239C** (see **239AB** above).

239E. "$C_{15}H_{29}NO$", —, 176°, m/e 239(2), 238(3), 210(40), *152*(100), H_0-derivative. Possibly a side-chain hydroxy analog of **223C.**

Trace in *D. histrionicus* (Quebrada Guanguí).

239F. "$C_{15}H_{29}NO$", 0·30, 176°, m/e 239(1), *168*(100). H_0-derivative. O-acetyl derivative. Possibly ring hydroxy analog of **223C.**

Trace in *D. histrionicus* (Río Baba).

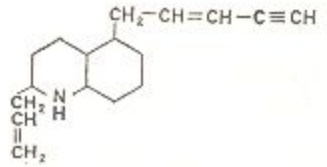
239G. "$C_{15}H_{29}NO$", —, 178°, m/e 239(1), 238(3), *138*(100). H_0-derivative. Possibly side-chain hydroxy analog of **223D.**

Trace in *D. histrionicus* (Quebrada Guanguí).

241. ?, —, 180°, m/e 241(2), 240(3), 166(100), 126(48).

Trace in *D. occultator*. Does not separate from major alkaloids, **239AB,CD.**

243. $C_{17}H_{25}N$, 0·36, 182°, m/e 243(2), 242(1), *202* ($C_{14}H_{20}N$,100). H_8-derivative, m/e 251, 208. N-acetyl derivative. Tentative structure:



Major in *D. auratus* (Isla Taboga, Río Campana), *D. azureus, D. granuliferus, D. histrionicus* (Playa de Oro, Quebrada Vicordó, Quebrada Docordó); minor or trace in *D. pictus, D. tinctorius, D. trivittatus, D. truncatus.* In certain samples a very small amount of an isomer emerges slightly before **243.**

251A. "$C_{17}H_{33}N$", —, 170°, m/e 251(2), 208(6), *152*(100). H_0-derivative. Possibly in pumiliotoxin-C class with $R = C_7H_{15}$ (see **223C**).

Trace in *D. histrionicus* (Río Guapi).

251B. "$C_{16}H_{29}NO$", —, 184°, m/e 251(2), 234(4), *138*(100). H_2-derivative, m/e 253, 138. Probably side-chain hydroxy analog of **235B.**

Trace in *D. lehmanni, D. pumilio.*

251C. "$C_{16}H_{29}NO$", —, 190°, m/e 251(2), 234(4), 154(100). H_2-derivative, m/e 253, 154. Possibly ring hydroxy analog of **235D.**

Trace in *D. minutus* (Quebrada Guanguí, El Llano-Carti).

251D. "$C_{16}H_{29}NO$", 0·52, 172°, m/e 251(5), 250(3), 234(1), 194(3), 166(28), 138(10), 70(100). H_2-derivative, m/e 253, 110, 84, 70. Member of the pumiliotoxin-A class with probably only one double bond.

Major in *D.* species (Colombia); minor in *D. auratus* (Isla Taboga); trace in *D. histrionicus* (Playa de Oro), *D. lehmanni, D. minutus* (Cerro Campana).

251E. "$C_{15}H_{25}NO_2$", —, 175°, m/e 251(3), 250(1), 234(2), 168(30), 84(18), 70(100). Perhaps an unsaturated analog of **253.**

Minor in *D. minutus* (Quebrada Guanguí)

253. $C_{15}H_{27}NO_2$, 0·30, 179°, m/e 253(4), 252(1), 236(22), 210 ($C_{12}H_{20}NO_2$,3), 208(2), 182($C_{10}H_{16}NO_2$,16), 114($C_6H_{12}NO$,27) 112($C_6H_{10}NO$,26), 70(C_4H_8N,100). H_2-derivative, m/e 255, 238, 110(50), 84(100), 70(65). Presently the lowest molecular weight member of the pumiliotoxin-A class to contain two oxygens.

Major in *D. abditus;* minor in *D. lehmanni,* D. auratus (Isla Taboga).

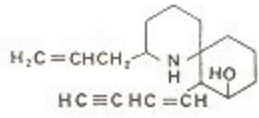
257A. "$C_{18}H_{25}N$", 0·30, 188°, m/e 257(1), 256(2), 216(100). H_8-derivative, m/e 265, 222. **257A** did not form an acetyl derivative and would appear possibly a perhydropyrroloquinoline of the gephyrotoxin class.

Minor in *D. trivittatus.*

257B. ?, 0·35, 192°, m/e 257(60), 256(100), 152(20). Very atypical mass spectrum for a dendrobatid alkaloid. Possibly a degradation artefact, see **265.**

Trace in *D. lehmanni.*

259. $C_{17}H_{25}NO$, 0·36, 190°, m/e 259(4), 242(2), 218($C_{14}H_{20}NO$,18), 200(6), 96($C_6H_{10}N$,100). H_8-derivative, m/e 267(10), 250(13), 224(39), 196(15), 168(19), 152(100), 96(68). O-acetyl derivative. Structure:



Major in *D. tinctorius, D. trivittatus;* minor in *D. auratus* (Isla Taboga), *D. azureus, D. granuliferus, D. histrionicus* (Playa de Oro, Quebrada Vicordó, Quebrada Docordó), *D. occultator, D. truncatus.*

265. ?, 0·35, 198°, m/e 265(50), 264(100), 222(58), 180(72). Very atypical mass spectrum for a dendrobatid alkaloid. There is a possibility that this trace component is actually a degradation artefact from some other compound with R_f values of 0·35–0·49.

Trace in *D. abditus, D. auratus* (Río Campana), *D. azureus, D. histrionicus* (Río Palenque), *D. lehmanni, D. occultator, D. parvulus, D. tinctorius.*

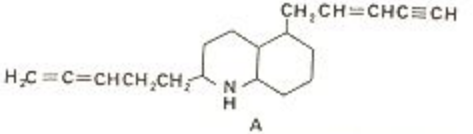
267A. $C_{16}H_{29}NO_2$, 0·31, 186°, m/e 267(15), *250*(33), 182($C_{10}H_{16}NO_2$,18), 114-($C_6H_{12}NO$,52), 112($C_6H_{10}NO$,44), 70(100). H_2-derivative, m/e 269, 252, 110(50), 84(100), 70(60). O-acetyl derivative. A prominent member of pumiliotoxin-A class.

Major in *D. auratus* (Isla Taboga) *D. azureus, D. fulguritus, D. granuliferus, D. lehmanni, D. leucomelas, D. minutus* (all populations), *D. tinctorius;* minor or trace in *D. auratus* (Río Campana), *D. histrionicus* (Santa Cecilia, Playa de Oro, Guayacana), *D. pumilio.*

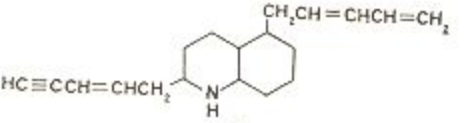
267B. ?, —, 208°, m/e 267(7), 266(4), 250(1), 170(100), 152(4), 112(13). The base peak is atypical for a dendrobatid alkaloid (see **185**).

Trace in *D. auratus* (Isla Taboga) *D. minutus* (El Llano-Carti).

269A,B. $C_{19}H_{27}N$. Probably two isomeric compounds, 0·35, 207°, m/e 269(4), *268*(12), either *204*(**A**) or *202*(**B**)(100). H_{10}-derivative, m/e 279, 208. N-acetyl derivatives. Probably represent members of the pumiliotoxin-C class (see DALY *et al.*, 1977). Tentative structures:



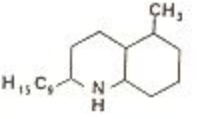
A



B

Minor or trace in *D. granuliferus, D. histrionicus* (Santa Cecilia, Guayacana, Río Baba), *D. occultator, D. trivittatus, D. truncatus.*

275. $C_{19}H_{33}N$, 0·28, 198°, m/e 275(3), *274*(2), 260(5), *152*($C_{10}H_{18}N$,100). H_4-derivative, m/e 279, 278, 152. Tentative structure:



However, **275** did not appear to form an N-acetyl derivative.

Major in *D. lehmanni;* trace in *D. auratus* (Isla Taboga).

281A. "$C_{17}H_{31}NO_2$", —, 205°, m/e 281(4), 280(2), 264(2), 194(12), 166(72), 70(100). H_2-derivative, m/e 283(1), 282(2), 266(4), 208(40), 138(10), 110(10), 84(100), 70(85). Loss of OH is less than in other compounds of the pumiliotoxin-A class (see **253** and **267A**).

Major in *D. minutus* (Cerro Campana); trace in *D. abditus.*

281B. "$C_{18}H_{35}NO$", —, 200°, m/e 281(4), 264(12), 208(25), 206(20), 150(65), 98(5), 96(20), 70(100). H_0-derivative. Atypical spectrum for dendrobatid alkaloid. Probably in pumiliotoxin-A class.

Trace in *D. granuliferus.*

283A. Histrionicotoxin (Fig. 1), $C_{19}H_{25}NO$, 0·50, 210°, m/e 283(9), 282(2), 266(5), 250(2), 218(48), 200(27), 160(22), 96(100). H_{12}-derivative, 0·36, 214°, m/e 295(12), 294(2), 278(13), 252(18), 224(73), 196(27), 180(100), 168(39), 96(68). O-acetyl derivative.

Major in *D. azureus, D. granuliferus, D. histrionicus* (all populations), *D. occultator, D. parvulus, D. pictus, D. tinctorius, D. trivittatus, D. truncatus.*

283B,C. $C_{17}H_{33}NO_2$, **B:** 0·36, 197°, m/e 283 (<1), 282(1), 254(2), 212($C_{12}H_{22}NO_2$,40), 152($C_{10}H_{18}N$,23), 140($C_9H_{18}N$,100). **C:** 0·40, 195°, m/e 283 (<1), 282(1), 240(5), 226($C_{13}H_{24}NO_2$,28), 224($C_{15}H_{30}N$,10), 166($C_{11}H_{20}N$,60), 126($C_8H_{16}N$,100). H_0-derivatives. N-acetyl derivatives. Do not completely separate on gas chromatography. Perhaps in

pumiliotoxin-A class, but without a double bond and because of this show no major fragment at m/e 70. Loss of C_5H_{11} is seen with **283B** and loss of C_4H_9 with **283C.**

Major in *D. histrionicus* (Quebrada Guanguí) and *D. occultator*.

285A. Isodihydrohistrionicotoxin (Fig. 1), $C_{19}H_{27}NO$, 0·39, 215°, m/e 285(7), 284(2), 268(8), 252(12), 238(3), 218(6), 200(9), 190(4), 176(24), 162(18), 96(100). H_{10}-derivative, m/e see H_{12}-derivative of **283A.** O-acetyl derivative.

Major in *D. azureus, D. granuliferus, D. histrionicus* (all populations), *D. occultator, D. parvulus, D. pictus, D. tinctorius.*

285B. Neodihydrohistrionicotoxin (Fig. 1), $C_{19}H_{27}NO$, 0·46, 211°, m/e 285(4), 284(1), 268(3), 250(2), 220(37), 202(9), 160(20), 96(100). H_{10}-derivative, m/e see H_{12}-derivative of **283A.**

Minor in *D. histrionicus* (Playa de Oro, Quebrada Guanguí, Guayacana, Río Baba), *D. pictus, D. trivittatus, D. truncatus.*

285C. Allodihydrohistrionicotoxin, $C_{19}H_{27}NO$, 0·40, 211°, m/e 285(4), 284(1), 268(2), 252(2), 218(5), 200(3), 190(4), 176(15), 162(17), 96(100). H_{10}-derivative, m/e see H_{12}-derivative of **283A.** See Fig. 1 for structure (from DALY *et al.*, 1977).

Major in *D. histrionicus* (all populations)*, *D. pictus, D. trivittatus, D. truncatus;* minor in *D. granuliferus, D. tinctorius.*

285D. ?, —, 190°, m/e 285(3), 270(2), 256(2), 180(35), 140(100). Atypical spectrum for a dendrobatid alkaloid.

Trace in *D.* species (Colombia).

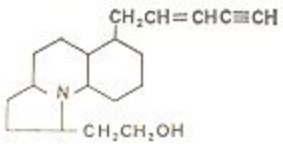
287A. Isotetrahydrohistrionicotoxin (Fig. 1), $C_{19}H_{29}NO$, 0·42, 216°, m/e 287(12), 286(4), 270(3), 220(30), 202(34), 176(45), 162(60), 148(24), 96(100). H_8-derivative, m/e see H_{12}-derivative of **283A.**

Minor in *D. azureus, D. histrionicus* (all Colombian populations), *D. pictus.*

287B. Tetrahydrohistrionicotoxin (Fig. 1), $C_{19}H_{29}NO$, 0·43, 213°, m/e 287(13), 286(2), 270(2), 220(43), 202(18), 176(6), 162(4), 148(4), 96(100). H_8-derivative, m/e see H_{12}-derivative of **283A.**

Minor in *D. histrionicus* (Santa Cecilia, Quebrada Docordó, Quebrada Vicordó, Guayacana, Río Baba, Río Palenque), *D. parvulus.*

287C. Gephyrotoxin (Formerly referred to as HTX-D, TOKUYAMA *et al.*, 1974) $C_{19}H_{29}NO$, 0·20, 218°, m/e 287(5), 286(3), 242($C_{17}H_{24}N$,100), 222($C_{14}H_{24}NO$,45), 122(14). H_6-derivative, m/e 293(5), 292(3), 248(100), 222(32). O-acetyl derivative. A perhydropyrroloquinoline, the parent member of the gephyrotoxin class of dendrobatid alkaloids (see DALY *et al.*, 1977).



Minor in *D. histrionicus* (Santa Cecilia, Playa de Oro, Río Guapi, Guayacana).‡

*An allotetrahydrohistrionicotoxin, with a terminal double bond in the 4-carbon side chain, was not detected in the present survey. This trace compound has only been isolated from large samples of the Guayacana population of *D. histrionicus* (see DALY *et al.*, 1977).

‡A dihydrogephyrotoxin, with a terminal double bond in the 5-carbon side chain, was not detected in the present survey, but traces have been isolated from large samples of the Guayacana population of *D. histrionicus* (see DALY *et al.*, 1977).

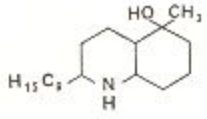
289. "$C_{20}H_{35}N$", —, 216°, m/e 289(2), 287(2), 274(3), *152*(100). H_4-derivative, m/e 293, 152. Possibly a member of pumiliotoxin-C class of alkaloids.

Trace in *D. lehmanni*.

291A. Octahydrohistrionicotoxin (Fig. 1), $C_{19}H_{33}NO$, 0·35, 212°, m/e 291(12), 290(2), 274(14), 250(54), 222(24), 194(18), 192(12), 178(100), 96(52). H_4-derivative, m/e see H_{12}-derivative of **283A.** O-acetyl derivative.

Major in *D. histrionicus* (Río Baba), minor in *D. granuliferus, D. histrionicus* (Río Guapi, Guayacana, Río Palenque), *D. parvulus, D. tinctorius, D. truncatus*.

291B. "$C_{19}H_{33}NO$", 0·12, 221°, m/e 291(2), 290(3), 276(6), *168*(100). H_4-derivative, m/e 295, 168. Possibly ring hydroxylated analog of **275.** Tentative structure:



Trace in *D. lehmanni*.

291C. "$C_{19}H_{33}NO$", 0·20, 220°, m/e 291(1), 290(2), 276(4), 210(10), *152*(100). H_4-derivative, m/e 295, 152. Possibly a side-chain hydroxylated analog of **275.**

Trace in *D. lehmanni*.

295. "$C_{19}H_{37}NO$", 0·09, 224°, m/e 295(3), 278(4), *138*(100). Possibly a side-chain

Trace in *D. auratus* (Isla Taboga, Río Campana).

297A. $C_{17}H_{31}NO_3$, 0·13, 225°, m/e 297(3), 296(4), 280(9), 236(2), 210(3), 194(4), 193(3), 182(21), 114(27), 112(16), 70(100). H_2-derivative, m/e 299(4), 282(12), 256(6), 224(4), 110(50), 84(100), 70(75). Lowest molecular-weight member of the pumiliotoxin-A class to contain 3 oxygens.

Major in *D. minutus* (Cerro Campana).

297B. $C_{18}H_{35}NO_2$, 0·35, 222°, m/e 297(10), 166(92), 70(100), H_4-derivative. Pumiliotoxin-A class.

Trace in *D. auratus* (Isla Taboga).

301. "$C_{21}H_{35}N$", —, 213°, m/e 301(< 1), 260(100). Possibly a member of pumiliotoxin-C class with an allyl R-substituent and a C_9H_{15} R′-substituent (see **223A,B,C,D**).

Trace in *D. azureus*.

307A. Pumiliotoxin A, $C_{19}H_{33}NO_2$, 0·36, 216°, m/e 307(5), *290*(3), 278($C_{17}H_{28}NO_2$4), 206($C_{13}H_{20}NO$,10), 194($C_{12}H_{20}NO$,16), 176($C_{12}H_{18}N$,10), 166($C_{10}H_{16}NO$,85), 70(C_4H_8N,100). H_2-derivative, m/e 309(3), 308(2), 210(8), 166(30), 110(45), 84(100), 70(40). H_4-derivative, m/e 311(3), 110(32), 84(100), 70(55). Partial conversion to O-acetyl derivative. A $CHOHCH_2CH_3$ moiety is present. One double bond is readily reduced, the other slowly.

Major in *D. pumilio;* minor or trace in *D. auratus* (Isla Taboga), *D. granuliferus, D. lehmanni, D. minutus* (Quebrada Guanguí, *D. occultator, D. viridis, D.* species (Colombia).

307B. Isopumiliotoxin A, "$C_{19}H_{33}NO_2$", 0·36, 211°, m/e 307(12), 306(4), 290(2), 194(24), 193(45), 166(100), 70(56). H_2-derivative, m/e 309(1), 280(3), 208(10), 138(25), 110(60), 84(100), 70(45). H_4-derivative, m/e 311(1), 110(25), 84(100), 70(30). Isomer of pumiliotoxin A without a large fragment ion for loss of OH (see **281A**).

Minor or trace in *D. auratus* (Isla Taboga), *D. granuliferus, D. lehmanni, D. minutus* (Cerro Campana, Quebrada Guanguí), *D. pumilio*.

307C. Allopumiliotoxin A, $C_{19}H_{33}NO_2$, 0·39, 214°, m/e 307(9), 290(11), 182($C_{10}H_{16}NO_2$,62), 70(100). H_4-derivative, m/e 311(3), 110(30), 84(100), 70(35). Isomer of pumiliotoxin A with a large fragment ion at 182 rather than 166.

Minor in *D. lehmanni, D. pumilio.*

309A. Dihydropumiliotoxin A, "$C_{19}H_{35}NO_2$", 0·38, 218°, m/e 309(9), 306(3), 292(2), 280(4), 206(4), 194(15), 176(5), 166(100), 110(10), 84(20), 70(51). H_2-derivative, m/e see H_4-derivative of **307A.**

Minor in *D. minutus* (Quebrada Guanguí), *D. viridis.*

309B. "$C_{20}H_{39}NO$", 0·09, 220°, m/e 309(1), *152*(100). Possibly member of hydroxy-pumiliotoxin-C class with hydroxy moiety in the side chain.

Trace in *D. auratus* (Isla Taboga), *D. granuliferus.*

323A. Pumiliotoxin B, $C_{19}H_{33}NO_3$, 0·17, 230°, m/e 323(10), *306*(5), 290(2), 278(12), 260(2), 206(15), 194(26), 193(22), 176(15), 166(75), 70(100) (see **307A** for empirical formulae). H_2-derivative, m/e 325(5), 166(25), 110(24), 84(100), 70(38). H_4-derivative, m/e 327(3), 326(2), 312(1), 310(2), 282(14), 264(12), 110(30), 84(100), 70(43). A $CHOHCHOHCH_3$ moiety is present (cf., $CHOHCH_2CH_3$ in **307A**), O-acetyl derivative.

Major in *D. auratus* (Isla Taboga), *D. granuliferus, D. lehmanni, D. leucomelas, D. occultator, D. pumilio, D. viridis;* minor or trace in *D. abditus, D. fulguritus, D. histrionicus* (Quebrada Guanguí), *D. minutus* (Quebrada Guanguí), *D.* species (Colombia).

323B. Allopumiliotoxin B, "$C_{19}H_{33}NO_3$", 0·20, 228°, m/e 323(5), 306(10), 210(4), 209(3), 182(50), 70(100). H_4-derivative similar to H_4-derivative of **323A.** Separates poorly from coexisting **323A.**

Minor or trace in *D. adbitus, D. leucomelas, D. pumilio, D.* species (Colombia).

325. Allodihydropumiliotoxin B, "$C_{19}H_{35}NO_3$", 0·20, 232°, m/e 325(3), 308(12), 280(3), 210(2), 182(56), 114(16), 112(9), 70(100). H_2-derivative, m/e similar to H_4-derivative of **323A.**

Major in *D. fulguritus, D. minutus* (Quebrada Guanguí, El Llano-Cartí).

341A. "$C_{19}H_{35}NO_4$", 0·48, 222°, m/e 341(2), 324(3), 323(1), 306(1), 298(3), 266(4), 254(7), 112(60), 84(42), 70(100). H_2-derivative, m/e 343(1), 342(2), 328(2), 266(10), 138(100), 110(5), 84(15), 70(15). Probably a hydroxy analog of a dihydropumiliotoxin B. High R_f value.

Major in *D. auratus* (Río Campana), minor or trace in *D. auratus* (Isla Taboga), *D. granuliferus, D. viridis.*

341B. "$C_{19}H_{35}NO_4$", —, 223°, m/e 341(1), 324(4), 182(60), 114(20), 112(20), 70(100). Probably a hydroxy analog of dihydroallopumiliotoxin B (**325**).

Trace in *D. lehmanni.*

351. "$C_{21}H_{37}NO_3$", 0·15, 230°, m/e 351(6), 350(2), 336(4), 152(38), 138(65), 70(100). Probably a member of the pumiliotoxin-A class, but the large peak at 138 is atypical.

Trace in *D. auratus* (Río Campana).

357. "$C_{20}H_{39}NO_4$", 0·30, 240°, m/e 357(1), 340(2), 339(1), 324(8), 272(4), 138(10), 110(60), 84(30), 70(100). H_0-derivative. Higher dihydro-homolog of **341A.**

Minor in *D. auratus* (Río Campana); trace in *D. auratus* (Isla Taboga).

379. "$C_{23}H_{41}NO_3$", 0·26, 229°, m/e 379(5), 364(2), 362(1), 360(2), 206(28), 194(26), 193(33), 166(100), 70(72). H_4-derivative, m/e 383, 368, 110, 84, 70.

Minor in *D. auratus* (Isla Taboga), *D. granuliferus, D. lehmanni;* trace in *D. histrionicus* (Quebrada Guanguí), *D. pumilio, D.* species (Colombia).

381. "$C_{23}H_{43}NO_3$", —, 230°, m/e 381, 366, 364, 362, 194, 166, 70. Probably a dihydro-derivative of **379.** Assignment of peak intensities difficult since it emerges with **325.**

Trace in *D. minutus* (El Llano-Cartí).

395. "$C_{23}H_{41}NO_4$", 0·10, 243°, m/e 339(3), 322(3), 294(4), 276(5), 222(4), 209(15), 208(11), 192(14), 182(45), 114(25), 70(100). The parent ion of this alkaloid in electron impact spectra appears to be at m/e 339, but chemical ionization (methane) affords a protonated parent ion at 396(100) and a loss of water fragment at 378(20). H_4-derivative, m/e 399(4), 382(6), 356(5), 324(3), 138(10), 110(25), 84(100), 70(65). Pumiliotoxin-A class.

Trace in *D. pumilio, D. auratus* (Isla Taboga).

Sources and occurrence of alkaloids

The following 18 species of *Dendrobates* have been examined for alkaloids. Data given under each name includes: (1) abbreviated locality; (2) date of collection; (3) numbers of skins in pooled sample; (4) wet weight of skins and (5) a listing of the major, minor and trace alkaloids that were detected in the sample.* Unless noted otherwise, a gas chromatogram is shown in Figs. 2, 3, or 4. Voucher specimens for each geographic sample of frogs are preserved in the amphibian collection of the American Museum of Natural History.

We are aware that SILVERSTONE (1975, 1976) has excluded several of the following species from the genus *Dendrobates*, including even *D. trivittatus*—the nomenclatural type species of *Dendrobates* (MYERS and DALY, 1971). The definition of monophyletic groups within the Dendrobatidae has been the source of considerable confusion, as will be discussed elsewhere (MYERS and DALY, in progress).

D. abditus: Volcán Reventador, Napo, Ecuador, Feb. 1974, 3 skins, 0·18 g. Major alkaloids: **237A** and **253**. Minor alkaloids: pumiliotoxin B (**323A**), allopumiliotoxin B (**323B**). Trace alkaloids: **237B, 265, 281A** (results not shown, see MYERS and DALY, 1976*b*).

D. auratus:

(A) Isla Taboga, Panama, March 1972, 10 skins, 3·3 g. Major alkaloids: pumiliotoxin C (**195A**), **219A, 243**, **267A**, pumiliotoxin B (**323A**). Minor alkaloids: **259, 251D, 235A, 307A,B, 379**. Trace alkaloids: **167, 181A, B, 185, 203, 223A,B,C, 253, 267B, 275, 295, 297B, 309B, 341A, 357, 395.**

(B) Río Campana, Panama Prov., Panama, March 1972, 2 skins, 0·8 g. Major alkaloids: pumiliotoxin C (**195A**), **219A, 243, 341A**. Minor alkaloids: **235A, 267A, 357**. Trace alkaloids: **181B, 197, 237D, 265, 295, 351** (results not shown).

D. azureus: Sipaliwini Savanna, Surinam, July 1972, 5 skins, 2·0 g. Major alkaloids: **243**, histrionicotoxin (**283A**), **219A**, isodihydrohistrionicotoxin (**285A**), **267A**. Minor alkaloids: **259**, isotetrahydrohistrionicotoxin (**287A**). Trace alkaloids: **181B, 219B, 231B, 265, 301**.

D. fulguritus: El Llano-Cartí road, Panama Prov., Panama, March 1974, 6 skins, 0·2 g. Major alkaloids: **267A**, allodihydropumiliotoxin B (**325**), **203**. Minor alkaloids: pumiliotoxin B (**323A**). Trace alkaloids **181B, 237B**.

D. granuliferus: Palmar Norte, Puntarenas, Costa Rica, July 1967, 9 skins, 0·7 g. Major alkaloids: pumiliotoxin B (**323A**), **219A**, histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**), **243, 267A**. Minor alkaloids: **259**, allodihydrohistrionicotoxin (**285C**), pumiliotoxin

*More trace compounds are listed for several species (*D. histrionicus, D. lehmanni, D. occultator*) than were given by MYERS and DALY (1976*a*) for the same samples. These additions are the result of advances and refinements in the computer-assisted detection of trace compounds by combined gas chromatography–mass spectrometry.

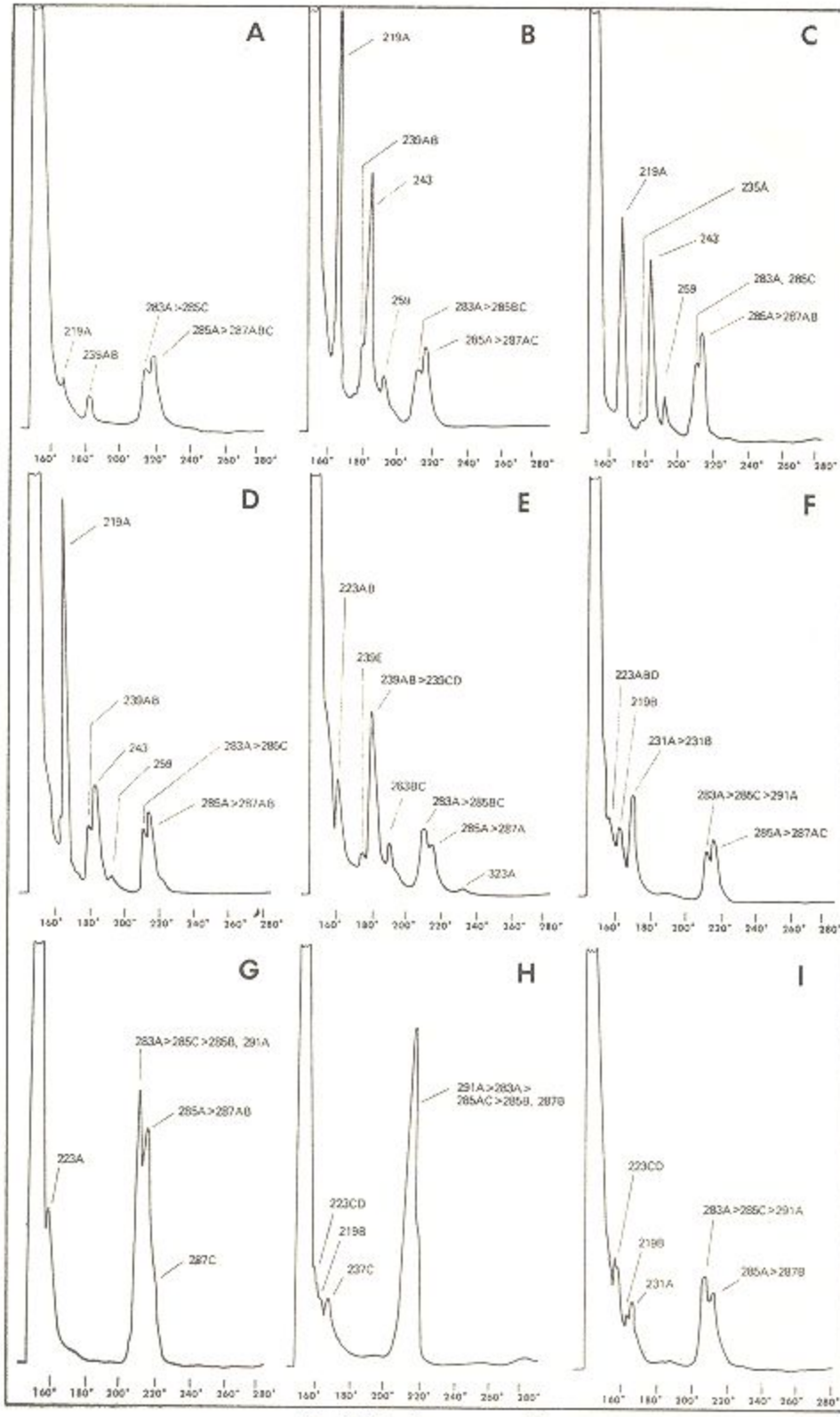


FIG. 2. Caption on page 179.

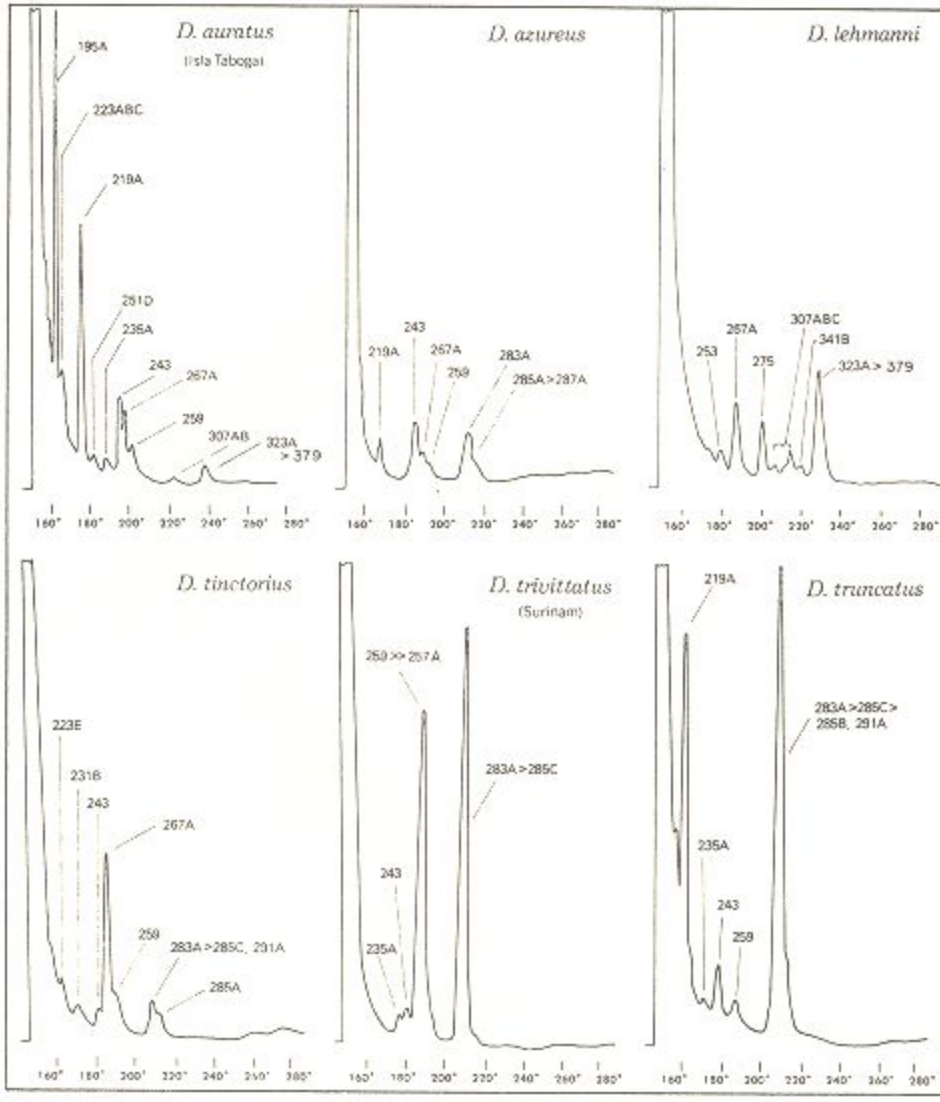


FIG. 3. GAS CHROMATOGRAMS OF ALKALOID FRACTION FROM VARIOUS SPECIES OF MEDIUM-SIZED TO LARGE *Dendrobates*.
Methodology as in Fig. 2. See text for sources.

FIG. 2. GAS CHROMATOGRAMS OF ALKALOID FRACTION FROM POPULATION SAMPLES OF *Dendrobates histrionicus*.
A. Sta. Cecilia, upper Río San Juan. B. Playa de Oro, upper Río San Juan. C. Vicordó, middle Río San Juan. D. Docordó, middle Río San Juan. E. Guanguí, upper Río Saija drainage. F. Río Guapi. G. Guayacana. H. Río Baba. I. Río Palenque. Localities, which are arranged from north (Colombia) to south (Ecuador), are shown in MYERS and DALY (1976*a*, map 1).

Methanolic alkaloid fraction (2 µl) equivalent to 2 mg of skin was injected directly into a 1·5% OV-1-Chromasorb G AW-DMCS (80–100 mesh, Applied Science Laboratories, State College, Pa.) glass column (U-shaped, 5 × 2 mm i.d.). The injection port was 280°C, the column 150°C and the flame ionization detector 300°C, with a 20–25 cm^3/min flow rate of nitrogen (Finnigan 9500 gas chromatograph). Immediately after the maximum for the solvent was passed the column temperature was programmed at 10°C per min from initial 150° to 280°C. Emergent temperatures for alkaloids differ somewhat with different batches of column packing. The columns after initial packing had been silanized by an injection of 40 µl of 5% dimethyldichlorosilane in toluene. Alkaloids corresponding to chromatogram peaks are designated (for further details see Results).

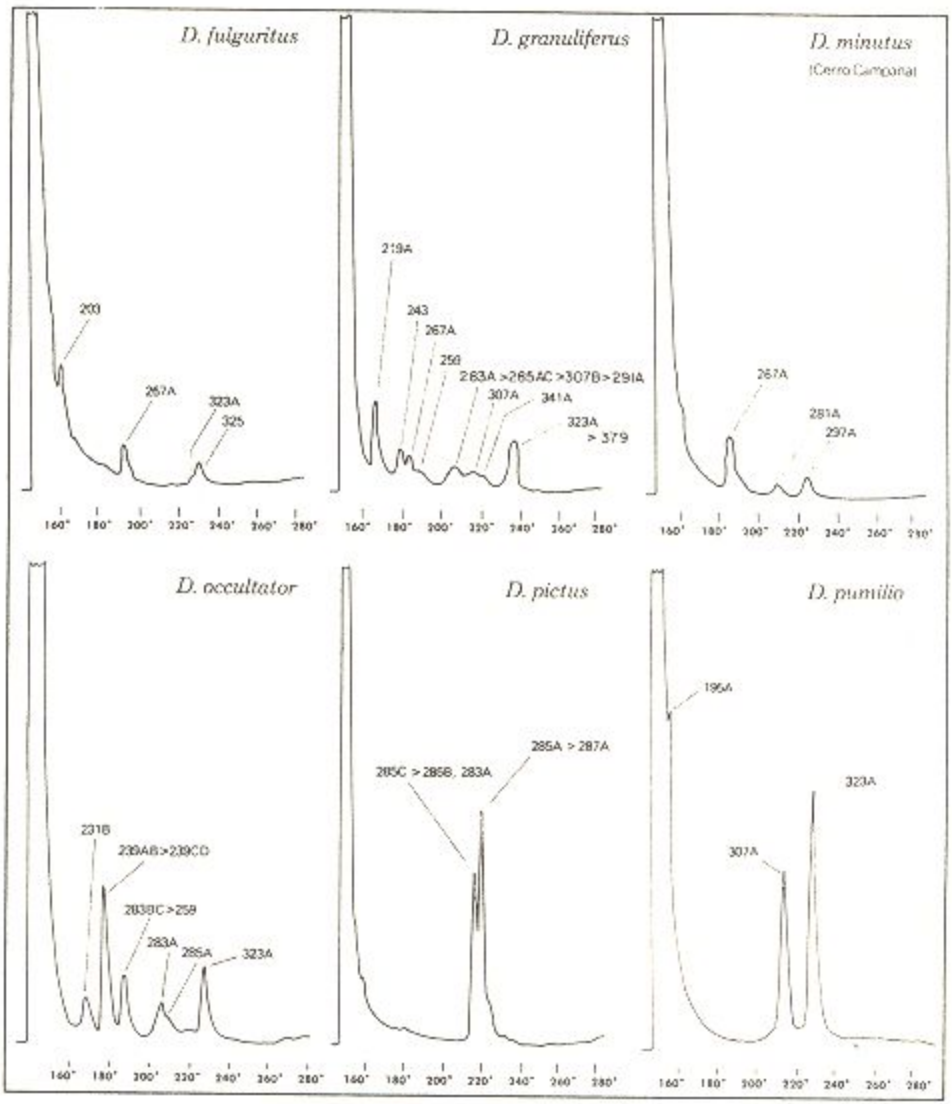


FIG. 4. GAS CHROMATOGRAMS OF ALKALOID FRACTION FROM VARIOUS SPECIES OF SMALL TO MEDIUM-SIZED *Dendrobates*.
Methodology as in Fig. 2. See text for sources.

A (**307A**), **341A**, isopumiliotoxin A (**307B**), octahydrohistrionicotoxin (**291A**), **379**. Trace alkaloids: **235A**, **269A,B**, **281B**, **309B**. This sample was 7 yr old at the time of gas chromatographic analysis.

D. histrionicus:

(A) Santa Cecilia, upper Río San Juan, Colombia, Feb. 1970, 12 skins, 3·0 g. Major alkaloids: histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**), **239AB**. Minor alkaloids: allodihydrohistrionicotoxin (**285C**), tetrahydrohistrionicotoxins (**287A,B**), gephyrotoxin (**287C**), **219A**. Trace alkaloids: **223A,B**, **267A**, **269A,B**.

(B) Playa de Oro, upper Río San Juan, Colombia, Feb. 1971, 8 skins, 2·4 g. Major alkaloids: **219A**, **243**, histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor

alkaloids: allodihydrohistrionicotoxin (**285C**), neodihydrohistrionicotoxin (**285B**), isotetrahydrohistrionionicotoxin (**287A**), gephyrotoxin (**287C**), **239AB, 259**. Trace alkaloids: **251D, 267A**.

(C) Quebrada Vicordó, Río San Juan, Colombia, Feb. 1971, 3 skins, 1·1 g. Major alkaloids: **219A, 243**, histrionicotoxin (**283A**), iso- and allodihydrohistrionicotoxins (**285A,C**). Minor alkaloids: tetrahydrohistrionicotoxins (**287A,B**), **235A, 259**.

(D) Quebrada Docordó, Río San Juan, Colombia, Feb. 1971, 8 skins, 2·2 g. Major alkaloids: **219A, 243**, histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor alkaloids: allodihydrohistrionicotoxin (**285C**), tetrahydrohistrionicotoxins (**287A,B**), **239AB, 259**. Trace alkaloid: **231B**.

(E) Quebrada Guanguí (Río Saija), Cauca, Colombia, Feb. 1973, 10 skins, 3·2 g. Major alkaloids: **239AB, 239CD, 283B,C**, histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor alkaloids: **223AB**, allo- and neodihydrohistrionicotoxins (**285B,C**), isotetrahydrohistrionicotoxin (**287A**), **239E**, pumiliotoxin B (**323A**). Trace alkaloids: **195B, 219A, 235B, 237E, 239E,G, 323A, 379** and isomers of **239C,D**.

(F) Río Guapi, Cauca, Colombia, Feb. 1973, 5 skins, 1·4 g. Major alkaloids: histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor alkaloids: **231A**, allodihydrohistrionicotoxin (**285C**), isotetrahydrohistrionicotoxin (**287A**), gephyrotoxin (**287C**), octahydrohistrionicotoxin (**291A**), **219B, 231B**. Trace alkaloids: **195B, 207, 223A,B,D, 251A**.

(G) Guayacana, Nariño, Colombia, Oct. 1972, 10 skins, 2·9 g. Major alkaloids: histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor alkaloids: neo- and allodihydrohistrionicotoxins (**285B,C**), tetrahydrohistrionicotoxins (**287A,B**), gephyrotoxin (**287C**), octahydrohistrionicotoxin (**291A**), **223AB**. Trace alkaloids: **195B, 203, 205, 223B,D, 267A, 269A,B** [also allotetrahydrohistrionicotoxin and dihydrogephyrotoxin, see notes under **285C** and **287C**].

(H) Río Baba, Pichincha, Ecuador, Feb. 1974, 5 skins, 0·9 g. Major alkaloids: octahydrohistrionicotoxin (**291A**), histrionicotoxin (**283A**), iso- and allodihydrohistrionicotoxin (**285A,C**). Minor alkaloids: neodihydrohistrionicotoxin (**285B**), tetrahydrohistrionicotoxin (**287B**), **237C, 223C,D, 219B**. Trace alkaloids: **207, 231B, 237A, 239F, 269A,B**.

(I) Río Palenque Biol. Station, Pichincha, Ecuador, Feb. 1974, 5 skins, 0·9 g. Major alkaloids: Histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor alkaloids: allodihydrohistrionicotoxin (**285C**), tetrahydrohistrionicotoxin (**287B**), octahydrohistrionicotoxin (**291A**), **223C,D, 219B, 231A**. Trace alkaloids: **265**.

D. lehmanni: Anchicayá Valley, Valle, Colombia, Jan. 1973, 8 skins, 2·8 g. Major alkaloids: pumiliotoxin B (**323A**), **267A, 275**. Minor alkaloids: **253**, pumiliotoxin A's (**307A,B,C**), **341B, 379**. Trace alkaloids: **195A, 223A,E, 231B, 251B,D,E, 257B, 265, 289, 291B,C, 341B**.

D. leucomelas: Guri Dam, Bolívar, Venezuela, Nov. 1968, 6 skins, 1·5 g. This extract was analyzed only by preparative thin-layer chromatography and mass spectrometry. Major alkaloids: pumiliotoxin B (**323A**), **267A**. Minor alkaloids: allopumiliotoxin B (**323B**), (results not shown).

D. minutus:

(A) Cerro Campana, Panama Prov., Panama, March 1972, 36 skins, 1·2 g. Major alkaloids: **267A, 297A, 281A**. Trace alkaloids: **251D, 307B**.

(B) El Llano-Cartí road, Panama Prov., Panama, March 1974, 10 skins 0·25 g. Major

alkaloids: **267A**, allodihydropumiliotoxin B (**325**). Minor alkaloids: **231A**. Trace alkaloids: **203, 223B,D, 251C, 267B, 381** (results not shown).

(C) Quebrada Guanguí (Río Saija), Cauca, Colombia, Feb. 1973, 9 skins, 0·25 g. Major alkaloids: allodihydropumiliotoxin B (**325**), **267A**, dihydropumiliotoxin A (**309A**). Minor alkaloids: **251E**, pumiliotoxin A (**307A**), pumiliotoxin B (**323A**). Trace alkaloids: **195A, 223A,B, 251C, 307B** (results not shown).

(D) Playa de Oro, upper Río San Juan, Colombia, Feb. 1970, 6 skins, 0·15 g. This extract was analyzed only by preparative thin-layer chromatography and mass spectrometry. Major alkaloid: **267A** (results not shown).

D. occultator: Quebrada Guanguí and Le Brea (Río Saija), Cauca, Colombia, Feb. 1973, 4 skins, 0·6 g. Major alkaloids: **239AB, 239CD**, histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**), pumiliotoxin B (**323A**), **283B,C**. Minor alkaloids: **231B, 259**. Trace alkaloids: **223A,E, 241, 265, 269A,B, 307A** and isomers of **239A,B,C,D**.

D. parvulus: Mocoa, Putumayo, Colombia, Feb. 1970, 25 skins, 2·4 g. This extract was analyzed only by preparative thin-layer chromatography and mass spectrometry. Major alkaloids: histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**), **219A**. Minor alkaloids: tetrahydrohistrionicotoxin (**287B**) octahydrohistrionicotoxin (**291A**). Trace alkaloids: **235A, 265** (results not shown).

D. pictus: Boquerón de Padre Abad, upper Río Aguaytía, Loreto, Peru, Nov. 1974, 10 skins, 1·7 g. Major alkaloids: dihydrohistrionicotoxins (**285A,B,C**), histrionicotoxin (**283A**). Minor alkaloid: isotetrahydrohistrionicotoxin (**287A**). Trace alkaloid: **243**.

D. pumilio: Isla Bastimentos, Panama, Oct. 1972, 10 skins, 0·9 g. Major alkaloids: pumiliotoxins A,B and C (**307A, 323A, 195A**), Trace alkaloids: **203, 223D, 235B, 251B, 267A, 307B,C, 323B, 379, 395**.

D. tinctorius: Raleigh Cataracts, Coppename River, Surinam, Feb. 1973, 1974, 3 skins, 0·8 g. Major alkaloids: **267A, 259**, histrionicotoxin (**283A**), isodihydrohistrionicotoxin (**285A**). Minor alkaloids: **231B, 243**, allodihydrohistrionicotoxin (**285C**), octahydrohistrionicotoxin (**291A**). Trace alkaloids: **223E, 225, 265**.

D. trivittatus:

(A) Raleigh Cataracts, Coppename River, Surinam, Feb. 1972, 10 skins, 3·4 g. Major alkaloids: histrionicotoxin (**283A**), **259**, allodihydrohistrionicotoxin (**285C**). Minor alkaloids: neodihydrohistrionicotoxin (**285B**), **257A, 243** and **235A**. Trace alkaloids: **269A,B**.

(B) Tingo Maria, Huánuco, Peru, Nov. 1974, 2 skins, 0·9 g. The profile and quantities of alkaloids were virtually identical to those of above population from Surinam (results not shown). Trace alkaloids: **181B, 269A,B**.

D. truncatus: Mariquita, Tolima, Colombia, Jan. 1970, 24 skins, 7·1 g. Major alkaloids: histrionicotixon (**283A**), allodihydrohistrionicotoxin (**285C**), **219A**. Minor alkaloids: neodihydrohistrionicotoxin (**285B**), octahydrohistrionicotoxin (**291A**), **243, 235A, 259**. Trace alkaloids: **223A,B,C,D, 269A,B**.

D. viridis: Anchicayá Valley, Valle, Colombia, Jan. 1973, 2 skins, 0·06 g. This very small sample was analyzed only by quantitative chemical ionization mass spectrometry and thin-layer chromatography. The following compounds were present in small amounts. Major

alkaloids: pumiliotoxin B (**323A**), dihydropumiliotoxin A (**309A**). Minor alkaloids: pumiliotoxin A (**307A**), **341A** (results not shown, see MYERS and DALY, 1976*a*).

Dendrobates sp: An undescribed species from Quebrada de la Chapa, Valle, Colombia, Feb. 1974, 7 skins, 0·4 g. Major alkaloid: **251D**. Minor alkaloids: **219B**, **223C**, **233**, **239B**, pumiliotoxin B's (**323A,B**), **379**. Trace alkaloids: **223A,B**, **231B**, **285D**, **307A**, and isomers of **239A,B,C** (results not shown, MYERS and DALY, in progress).

DISCUSSION

Chemistry

Extracts from 18 species of *Dendrobates* contained significant amounts of alkaloids as shown by thin-layer chromatography and gas chromatography (Figs. 2, 3 and 4). Through the use of thin-layer, gas and column chromatographic separation, and mass spectrometry, 90* different alkaloids (Table 1) have been detected and characterized. These data were analyzed in relationship to known structures of pumiliotoxin C, the histrionicotoxins and gephyrotoxin and to the probability that common biosynthetic pathways will have led to the formation of series of closely related alkaloids in related frogs. Except for a few compounds with incomplete data, the *Dendrobates* alkaloids are grouped into 5 classes. Tentative structures have been formulated and discussed for many (see Results). Isolation of much larger quantities of the partially characterized alkaloids will be necessary to establish definitive structures by NMR analysis, or X-ray crystallographic analysis and to permit pharmacological evaluation of their potential usefulness. Such efforts are in progress.

Pumiliotoxin-A class. Twenty-four compounds have been assigned to this class. Their mass spectra are in all cases typified by a major peak at m/e 70. On reduction, peaks at m/e 84 and 110 are prominent. Major peaks at m/e 166 and 182 are present in the spectra of many of these compounds.

The basic ring system of the pumiliotoxin-A class of alkaloids remains uncertain in spite of extensive unpublished studies on pumiliotoxin A and pumiliotoxin B. At present it would appear that a piperidine ring system is present, but any further speculation as to the nature of other rings and the position of double bonds is not yet warranted. In a previous paper (MYERS and DALY, 1976*a*) pumiliotoxins A, B and C were treated as a group, as were the histrionicotoxins. Pumiliotoxin A and B are now classified as members of the pumiliotoxin-A class, all of which contain one or more hydroxyl substituents. Pumiliotoxin C, which has no hydroxyl substituent, is now classified as the parent member of the pumiliotoxin-C class.

Pumiliotoxin-C class. Most of the 26 alkaloids tentatively assigned to this class have base peaks at m/e 138, 152, 166 or 180. Hydrogenation does not affect these base peaks but does, in some instances, reveal unsaturation in the R-substituent adjacent to nitrogen. In pumiliotoxin C the base peak at 152 is due to cleavage of the propyl side chain adjacent to nitrogen, to yield a positively charged methyldecahydroquinoline fragment. Compounds with base peak at m/e 138 are proposed to lack the methyl group, while compounds with base peaks at m/e 166 and 180 would have, respectively, ethyl or propyl substituents in place of the methyl group. Four compounds, **219A**, **243**, and **269A**, **B**, have unsaturation in both the R- and R′-substituent. Pumiliotoxin-C class alkaloids should readily form N-acetyl derivatives as is the

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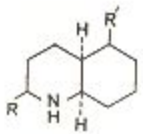
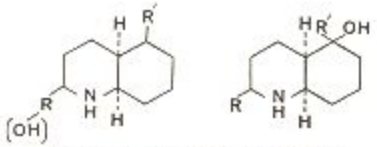
*Not counting two additional alkaloids, dihydrogephyrotoxin and allotetrahydrohistrionicotoxin, which were not detected in our small sample analyses. These new trace compounds (see notes under **285C** and **287C**) were recently characterized (DALY *et al.*, 1977) from large samples of the Guayacana population of *Dendrobates histrionicus*.

case for **195A, 219A**, **223B**, **243**, and **269A,B**, (see however **203, 231A**, **275**) This distinguishes them from the gephyrotoxin class alkaloids which do not N-acetylate and from a possible deoxyhistrionicotoxin class which would not readily N-acetylate based on unpublished studies with synthetic perhydrodeoxyhistrionicotoxin. As yet no compelling evidence for deoxyhistrionicotoxins has been obtained. Clearly the assignments of many of the pumiliotoxin-C and hydroxypumiliotoxin-C class alkaloids must, at present, be considered quite tentative.



Pumiliotoxin—C class

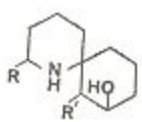
Hydroxypumiliotoxin-C class. The 16 alkaloids, tentatively assigned to this class, show base peaks either at m/e 138, 152, 166, or 180 (as in the pumiliotoxin-C class) or at m/e 154, 168, 182, or 196. It is proposed that the hydroxyl group typical of this class is present in either; (1) the R side chain, wherein mass spectral fragmentation leads to decahydroquinoline base peaks at 138, 152, 166, or 180, or (2) in the decahydroquinoline portion of the molecule, wherein mass spectral fragmentation leads to hydroxydecahydroquinoline base peaks at 154, 168, 182, or 196. In view of biosynthetic considerations (see below), the hydroxy



Hydroxypumiliotoxin—C class

group of the hydroxydecahydroquinolines has been tentatively assigned to the carbon bearing the R′-substituent. Hydroxypumiliotoxin-C class alkaloids should form N,O-diacetyl derivatives in contrast to hydroxy-members of the gephyrotoxin class, which form only O-acetyl derivatives. Acetylation data are as yet unavailable since all the alkaloids in question are trace constituents.

Histrionicotoxin class. This series of 10 alkaloids (see below) is better characterized than the other *Dendrobates* alkaloids. All the histrionicotoxin series of alkaloids have mass spectra with a major peak at m/e 96 (see TOKUYAMA *et al.*, 1974, for fragmentation patterns). In earlier papers, the term "histrionicotoxins" has been applied only to the C_{19}-alkaloids, but, as defined here, the histrionicotoxin class of alkaloids includes also the lower homologs **235A** and **259**. Nine histrionicotoxins are listed in Table 1; a tenth compound, allotetra-



Histrionicotoxin class

hydrohistrionicotoxin (see note under **285C**), was not detected by methods of the present study. Histrionicotoxins are characterized by the unusual spiropiperidine ring structure; the

heretofore unique allenic and acetylenic moieties (DALY *et al.*, 1971) have now been found to occur also in the pumiliotoxin-C and gephyrotoxin classes.

Gephyrotoxin class. Six alkaloids are assigned to this class of compounds. All contain the perhydropyrrolo-bridge (*gephyros*=bridge) as in gephyrotoxin (**287C**) (a perhydropyrroloquinoline) and **239AB** and **239CD**, (perhydropyrrolopiperidines). One of six alkaloids of this class, a dihydrogephyrotoxin, is not listed in Table 1 (see comment under **287C**). These tricyclic and bicyclic compounds presumably form via cyclization of precursor alkaloids of the pumiliotoxin-C class or of a 2,6-disubstituted piperidine, respectively. [A 2,6-disubstituted piperidine has been detected in a new extract from Peruvian *D. trivittatus*.]

Other alkaloids from Dendrobates. Ten alkaloids have not been assigned to any foregoing class. Very little data are available on the trace alkaloids (**185**, **197**, **241**, **257B**, **265**, **267B**, **285D**) and some may actually represent degradation artefacts.

The two major unclassified alkaloids **283B** and **C**, possibly belong in the pumiliotoxin-A class despite not having the major peak at m/e 70 nor in giving evidence for the presence of the double bonds typical of this class. It is thought that the mass spectra of **283B,C** are more typical of spectra of saturated analogs in the pumiliotoxin-A class rather than of the simpler spectra of the gephyrotoxin and hydroxypumiliotoxin-C classes; in addition, alkaloids **283B,C** contain two oxygens as do many other members of the pumiliotoxin-A class. The last unclassified alkaloid, namely **301**, possibly belongs in the pumiliotoxin-C class, based on the simplicity of its mass spectrum.

Batrachotoxin class. This class of 4 steroidal alkaloids has been detected only in frogs of the dendrobatid genus *Phyllobates* (MYERS and DALY, in progress). These are the true poison-dart frogs, whose toxins bear no biosynthetic relationship to the *Dendrobates* alkaloids. The batrachotoxins have structural features that appear to be unique in nature (Fig. 1), namely the hemiketal and homomorpholine bridges fused to the steroidal skeletons of all batrachotoxins, and the dialkyl carboxylate R-substituents of batrachotoxin and homobatrachotoxin. The last two compounds are among the most toxic of nonprotein poisons.

Toxicity and pharmacology

Batrachotoxins are strong cardiotoxins, which, on subcutaneous administration to mice, cause locomotor and equilibrium difficulties, followed by labored breathing, violent convulsions, and death even at doses of less than 0·1 μg (MÄRKI and WITKOP, 1963). At higher doses, for example 1 μg death occurs in mice within 1 min. Batrachotoxins selectively increase cell-membrane permeability to sodium ions, thereby eliciting depolarization in a variety of nerve and muscle preparations. Selective activation of sodium channels by batrachotoxin has provided an important tool for the study of the function of sodium channels in nerve and muscle (ALBUQUERQUE *et al.*, 1971; ALBUQUERQUE and DALY, 1977).

Histrionicotoxins are relatively nontoxic and cause only slight locomotor difficulties and prostration after subcutaneous administration of 100 μg to mice (DALY *et al.*, 1971). At a 40 μg dose, isodihydrohistrionicotoxin had virtually no effect in mice, in contrast to the marked toxicity of pumiliotoxin-A class alkaloids at this dose level (see below). The histrionicotoxins have proven useful pharmacological tools because of their ability to antagonize the conductance changes that normally follow activation of nicotinic receptors by acetylcholine in muscle (ALBUQUERQUE *et al.*, 1973; KATO *et al.*, 1975; LAPA *et al.*, 1975). Histrionicotoxins, in addition, antagonize potassium conductance changes associated with action potentials in nerve and muscle.

Pumiliotoxins A and B are much more toxic than the histrionicotoxins or pumiliotoxin C. Subcutaneous injection of 100 μg of either pumiliotoxin A or B in mice caused locomotor difficulties, partial paralysis of hind limbs, salivation, extensor movements, and finally, clonic convulsions and death in less than 10 min (DALY and MYERS, 1967). Even at a 20 μg dose, pumiliotoxin B caused death in less than 20 min. Another member of the pumiliotoxin-A class, **267A**, was less toxic and at 40 μg caused in mice initial hyperactivity and vocalization, with accompanying hypersensitivity to stimuli and locomotor difficulties, but no deaths. Pumiliotoxin B potentiates muscle contractures in both diaphragm and atrial preparations (MENSAH-DWUMAH and DALY, 1977).

The pumiliotoxin-C and hydroxypumiliotoxin-C classes of alkaloids have not been studied in detail and little is known of their pharmacology. Pumiliotoxin C, at a subcutaneous dose of 100 μg in mice, caused locomotor difficulties followed by prostration, with recovery within 1 hr. Another member of the pumiliotoxin-C class, **219A**, at 80 μg caused in mice salivation, piloerection, and marked locomotor difficulties due to paralysis of hind limbs; recovery was complete after 4 hr. Pumiliotoxin C has little effect on neuromuscular transmission, a marked contrast to the potent effects of histrionicotoxins (MENSAH-DWUMAH and DALY, 1977).

Gephyrotoxin (**287C**) had little effect at an 80 μg dose in mice, although spontaneous activity was noticeably reduced. Gephyrotoxin is a relatively potent muscarinic antagonist in ileum and atrial preparations (MENSAH-DWUMAH and DALY, 1977). The compound **239CD**, of the gephyrotoxin class, at 80 μg caused in mice locomotor difficulties, piloerection and prostration with complete recovery only after 4 hr.

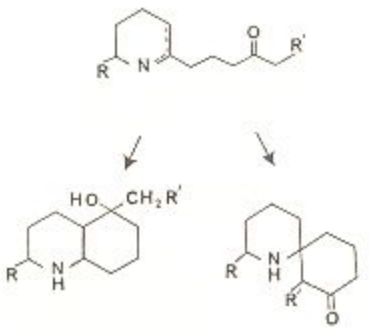
The pronounced toxicity of extracts of *D. auratus, D. granuliferus, D. lehmanni, D. leucomelas, D. minutus,* and *D. pumilio* would appear, based on the above results, to be due primarily to pumiliotoxin B and/or other members of the pumiliotoxin-A class of alkaloids, with lesser toxic contributions from the pumiliotoxin-C class and gephyrotoxin class and little or no contribution from histrionicotoxins.

Preliminary screening of methanolic extracts from ten species of *Dendrobates* has revealed the presence of at least 2 other alkaloids with very interesting pharmacological properties (Table 2). Extracts of *D. histrionicus* from Quebrada Guangui elicited clonic convulsions, sustained penile erections and thrusting movements in mice. Such effects were not observed with extracts from other populations of *D. histrionicus,* and the responsible alkaloid has yet to be identified. Extracts from *Dendrobates pictus* (and also from "*Phyllobates*" *anthonyi* and "*P.*" *espinosai,* unpublished results) contained an extremely potent analgesic agent that causes Straub tail in mice; this alkaloid has been isolated by preparative thin-layer chromatography and efforts to characterize it are in progress.

Biosynthesis

Biosynthetic pathways for formation of the dendrobatid alkaloids have not been established, but the relative constancy of alkaloid profiles, even after prolonged maintenance of frogs in captivity (MYERS and DALY, 1976*a*), indicates that diet has little influence on the pathways. No significant incorporation of radioactive acetate, mevalonate, or cholesterol into the piperidine-based alkaloids of *D. pumilio* and *D. auratus* or into the batrachotoxins of *Phyllobates aurotaenia* was detected (JOHNSON and DALY, 1971). It seems probable that pathways to the histrionicotoxin series of alkaloids and the (hydroxy)pumiliotoxin-C series share a common precursor, a disubstituted, unsaturated piperidine. Cyclization of the enеamine form could lead into the (hydroxy)pumiliotoxin-C class, while cyclization of the imine tautomer could lead into the histrionicotoxin class (see following diagram). Cyclization

of the side chain onto the nitrogen moiety would yield the gephyrotoxin class alkaloids.



Evolutionary and taxonomic implications

The dendrobatid alkaloids have proved to be not only unexpectedly numerous, but also remarkably diverse in structure and pharmacology. Like many plant alkaloids (McKey, 1974; Levin, 1976), the dendrobatid toxins clearly have a defensive function, as supported by the following observations: (1) The toxins are released in secretions following the slightest damage to the frogs' skin. (2) Captive snakes (*Rhadinaea* spp.) and a mouse opossum (*Marmosa* sp.) usually released proffered frogs immediately and with evident distaste. The skin secretions are also distasteful to human beings and some cause an unpleasant feeling of tightening in the throat. (3) Most of the toxic dendrobatids are brightly colored, and the bold patterns of some species might make these diurnal frogs recognizable even to predators that lack color vision (Myers and Daly, 1976*a*:214). It follows from considerations above that the toxins probably evolve under strong selective pressure. This has not, however, resulted in narrow limits of intraspecific variability, but rather the converse. Some species exhibit extraordinary interpopulation variability in skin toxins, coloration and escape behaviour (Daly and Myers, 1967; Myers and Daly, 1976*a*)—which suggests an unusual capability for rapidly adjusting to different suites of predators in complex tropical ecosystems, although variational patterns probably are also complicated by chance restriction of heterozygosity in small founder or newly fragmented populations. In any case, some predators probably are keeping pace with the evolution of dendrobatid toxins, although the snake *Leimadophis epinephelus* is the only predator so far known to be able to feed on a variety of poison frogs (Myers, unpublished data).

The capability for elaborating skin toxins has been a major evolutionary development that can be deduced to have opened new avenues of adaptation within the family Dendrobatidae. The dendrobatid toxins, therefore, would seem to provide a systematic character of potentially high information content. Maximum utility of this character will be dependent on the acquisition of sufficient *comparative* data and, ultimately, on an ability to detect primitive and derived states on the basis of the molecular data. Although a biochemical survey of dendrobatid frogs is far from complete, the accumulating data are nonetheless useful in testing proposed relationships and in helping define the limits of certain species that are highly variable in coloration, morphology, and even behaviour. Myers and Daly (1976*a*) assessed biochemical and other variation in *Dendrobates histrionicus* and differentiated two previously undescribed "sibling" species (*D. lehmanni* and *D. occultator*). The occurrence of batrachotoxins is being utilized in conjunction with other characters in redefining *Phyllobates* as a strictly monophyletic genus (Myers and Daly, in progress).

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