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

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JOHN W. DALY, GEORGE B. BROWN, MONICA MENSAAH-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 (dectahydroquinolines), the hydroxypumiliotoxin-C class, the histriionictoxin class (I-azaspiro [5.5]undecanes), the gephyrotoxin class (perhydropyrophopiperidines and perhydropyrophroquino- lines) 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 Márki 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 histricus, afforded another class of skin alkaloids to which
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>
<thead>
<tr>
<th>Pumiliotoxin-C (PTX-C) class</th>
<th>Hydroxypumiliotoxin-C (HO-PTX-C) class</th>
<th>Pumiliotoxin-A (PTX-A) class</th>
<th>Histrionicotoxin (HTX) class</th>
<th>Gephyrotoxin (GTX) class</th>
</tr>
</thead>
<tbody>
<tr>
<td>167 &quot;C_{11}H_{27}N&quot; (138,H_0)</td>
<td>223E &quot;C_{14}H_{27}NO&quot; (168,H_0)</td>
<td>237A* &quot;C_{15}H_{27}NO&quot; (166,70,H_0)</td>
<td>253A C_{13}H_{27}NO (96,H_0)</td>
<td>223AB C_{15}H_{27}N (180,166,H_0)</td>
</tr>
<tr>
<td>181A &quot;C_{13}H_{27}N&quot; (152,H_0)</td>
<td>225 &quot;C_{14}H_{27}NO&quot; (168,H_0)</td>
<td>237B &quot;C_{15}H_{27}NO&quot; (182,70,H_0)</td>
<td>259* C_{15}H_{27}NO (96,H_0)</td>
<td>239AB* C_{13}H_{27}NO (182,180,H_0)</td>
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<td>181B &quot;C_{13}H_{27}N&quot; (152,H_0)</td>
<td>237E &quot;C_{15}H_{27}NO&quot; (152,H_0)</td>
<td>251D* &quot;C_{14}H_{27}NO&quot; (166,70,H_0)</td>
<td>283A* HTX, C_{14}H_{27}NO (96,H_0)</td>
<td>239CD* C_{13}H_{27}NO (196,166,H_0)</td>
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<tr>
<td>195A* PTX-C, C_{13}H_{27}N (152,H_0)</td>
<td>239A &quot;C_{13}H_{27}NO&quot; (182,H_0)</td>
<td>251E &quot;C_{13}H_{27}NO_2&quot; (168,84,70,H)</td>
<td>285A* Iso-HTX (96,H_0)</td>
<td>257A &quot;C_{13}H_{27}N&quot; (216,H_0)</td>
</tr>
<tr>
<td>195B &quot;C_{13}H_{27}N&quot; (138,H_0)</td>
<td>239B &quot;C_{13}H_{27}NO&quot; (180,H_0)</td>
<td>253 C_{13}H_{27}NO_2 (182,70,H_0)</td>
<td>285B* Neo-HTX (96,H_0)</td>
<td>287C* GTX, C_{13}H_{27}NO (242,H_0)</td>
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<tr>
<td>203* &quot;C_{15}H_{27}N&quot; (138,H_0)</td>
<td>239C &quot;C_{13}H_{27}NO&quot; (196,H_0)</td>
<td>267A* &quot;C_{12}H_{27}NO_2&quot; (182,70,H_0)</td>
<td>285C* Allo-HTX (96,H_0)</td>
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<tr>
<td>205 &quot;C_{13}H_{27}N&quot; (138,H_0)</td>
<td>239D &quot;C_{13}H_{27}NO&quot; (166,H_0)</td>
<td>281A* &quot;C_{13}H_{27}NO&quot; (166,70,H_0)</td>
<td>287A Iso-H_4-HTX (96,H_0)</td>
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<tr>
<td>207 &quot;C_{13}H_{27}N&quot; (138,H_0)</td>
<td>239E &quot;C_{13}H_{27}NO&quot; (166,H_0)</td>
<td>281B* &quot;C_{13}H_{27}NO&quot; (150,70,H_0)</td>
<td>287B H_4-HTX (96,H_0)</td>
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<tr>
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<td>239F &quot;C_{13}H_{27}NO&quot; (168,H_0)</td>
<td>297A C_{13}H_{27}NO_2 (182,70,H_0)</td>
<td>291A* H_2-HTX (178,96,H_0)</td>
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<tr>
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<td>239G &quot;C_{13}H_{27}NO&quot; (138,H_0)</td>
<td>297B C_{13}H_{27}NO_2 (166,70,H_0)</td>
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<tr>
<td>223A &quot;C_{15}H_{27}N&quot; (180,H_0)</td>
<td>251B &quot;C_{15}H_{27}NO&quot; (138,H_0)</td>
<td>307A* PTX-A, C_{17}H_{27}NO_2 (166,70,H_0)</td>
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<tr>
<td>223B &quot;C_{13}H_{27}N&quot; (166,H_0)</td>
<td>251C &quot;C_{15}H_{27}NO&quot; (154,H_0)</td>
<td>307B Iso-PTX-A, &quot;C_{15}H_{27}NO_2&quot; (166,70,H_0)</td>
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<td></td>
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<td>223C &quot;C_{13}H_{27}N&quot; (152,H_0)</td>
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<td></td>
<td>309A H_2-PTX-A (166,70,H_0)</td>
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<tr>
<td>231A &quot;C_{13}H_{27}N&quot; (166,H_0)</td>
<td>295 &quot;C_{13}H_{27}NO&quot; (138,70,H_0)</td>
<td></td>
<td>323A* PTX-B, C_{17}H_{27}NO_2 (166,70,H_0)</td>
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</tr>
<tr>
<td>231B &quot;C_{13}H_{27}N&quot; (152,H_0)</td>
<td>309B &quot;C_{15}H_{27}NO&quot; (152,H_0)</td>
<td></td>
<td>323B Allo-PTX-B (182,70,H_0)</td>
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<tr>
<td>233 &quot;C_{13}H_{27}N&quot; (166,H_0)</td>
<td>325* H_2-Allo-PTX-B (182,70,H_0)</td>
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<td>341A* &quot;C_{13}H_{27}NO_2&quot; (84,70,H_0)</td>
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</tr>
<tr>
<td>235B &quot;C_{13}H_{27}N&quot; (138,H_0)</td>
<td>341B &quot;C_{13}H_{27}NO_2&quot; (182,70,H_0)</td>
<td></td>
<td>351 &quot;C_{13}H_{27}NO&quot; (138,70,H_0)</td>
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<tr>
<td>237C &quot;C_{13}H_{27}N&quot; (180,H_0)</td>
<td>357 &quot;C_{13}H_{27}NO&quot; (110,70,H_0)</td>
<td></td>
<td>379 &quot;C_{13}H_{27}NO_2&quot; (166,70,H_0)</td>
<td></td>
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<tr>
<td>243 &quot;C_{13}H_{27}N&quot; (202,H_0)</td>
<td>379 &quot;C_{23}H_{41}NO_4&quot; (166,70,H_0)</td>
<td></td>
<td>381 &quot;C_{13}H_{27}NO_2&quot; (166,70,H_0)</td>
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<tr>
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<td>289 &quot;C_{15}H_{27}N&quot; (152,H_0)</td>
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</tr>
</tbody>
</table>

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*

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

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 from frogs from ridges above Puerto Pilón, Panama, Jan. 1966 and March 1967, and from Playa de Oro, Rio San Juan, Colombia, Feb. 1970.

§Similar results with extracts from frogs collected Nov. 1966 and Nov. 1968.

The 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 Na₂SO₄ 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 H₂- and H₅-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) Rₚ 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 (H₀ = no addition of hydrogen, H₂ = 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. “C₁₁H₂₁N”, —, 151⁰, m/e 167(1), 166(1), 138(100). H₀-derivative. Tentative structure:

[Diagram not provided]

Trace in D. auratus (Isla Taboga).

181A. “C₁₂H₂₃N”, —, 152⁰, m/e 181(2), 180(1), 152(100), H₀-derivative. Tentative structure:

[Diagram not provided]

Trace in D. auratus (Isla Taboga).

181B. “C₁₂H₂₃N”, —, 153⁰, m/e 181(2), 180(2), 138(100). H₀-derivative. Isomer of 181A. Tentative structure:

[Diagram not provided]

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

185. ?, 0-2, 153⁰, m/e 185(1), 170(100). H₀-derivative.

Trace in D. auratus (Isla Taboga).
195A. Pumiliotoxin C (Fig. 1), C₁₃H₂₅N, 0·20; 157°, m/e 195(3), 194(5), 180(1), 152 (C₁₀H₁₈N₁₀), 109(8). H₀-derivative. N-acetyl derivative.

Major in D. auratus (Isla Taboga, Rio Campana), D. pumilio; trace in D. lehmanni, D. minutus (Quebrada Guangui).

195B. “C₁₃H₂₅N”, 0·23, 156°, m/e 195(2), 194(1), 138(100). H₀-derivative. 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 Llanito-Carti), D. pumilio.


Trace in D. histrionicus (Guayacana).


Trace in D. histrionicus (Rio Guapi, Rio Baba).

219A. C₁₅H₂₅N, 0·32, 165°, m/e 219(1), 218(2), 178(C₁₂H₂₀N₁₀). H₄-derivative, m/e 223, 180. N-acetyl derivative. Tentative structure:

Major in D. auratus (Isla Taboga, Rio 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 Guangui).

219B. “C₁₅H₂₅N”, —, 162°, m/e 219(1), 218(2), 152(100). H₄-derivative, m/e 223, 152. Probably analog of 223C with unsaturated side chain. Tentative structure:

Minor in D. histrionicus (Rio Guapi, Rio Baba), D. species (Colombia); trace in D. azureus.
223A, B, C, D. "C₃₅H₉₉N". 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). H₇-derivative. N-acetyl derivatives of 223A or 223B were obtained in certain extracts (D. auratus, D. histrionicus), Tentative structures:

\[
\begin{array}{cccc}
\text{A} & \text{B} & \text{C} & \text{D} \\
R & R' & C_1H_7 & C_1H_2 \\
\end{array}
\]

In D. histrionicus (Guayacana, Quebrada Guanui) 223AB represents a single compound [C₃₅H₉₉NO, m/e 223(1), 222(2), 180(85), 166(100)] which does not N-acetylate. 223B is also present (see above). Tentative structure (a perhydropyrroloperipipedine):

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 Guanui, A,B), D. occultator (A), D. pumilio (D), D. truncatus (A,B,C,D), D. species (Columbia, A,B,C).

223E. "C₃₄H₃₀NO", —, 163°, m/e 223(2), 222(3), 168(100). H₂-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. "C₃₄H₃₀NO", —, 164°, m/e 225(3), 224(6), 208(2), 168(100), 152(25). H₂-derivative.

Possibly a hydroxypumiliotoxin C.

Trace in D. tinctorius.

231A. "C₃₅H₉₉N", 0-30, 166°, m/e 231(2), 230(1), 166(100). H₆-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. "C₃₅H₉₉N", 0-30, 166°, m/e 231(2), 230(1), 152(100), H₆-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). H₄-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. C₁₅H₂₃NO, 0-36, 176°, m/e 235(5), 234(2), 218(15), 294(C₁₂H₂₆NO, 76), 176(C₁₂H₁₈N₂,25), 150(8), 96(C₆H₁₀N,100). H₄-derivative, m/e 239, 196, 178, 96. O-acetyl derivative. Structure:

\[
\begin{align*}
\text{H}_2\text{C} & = \text{CHCH}_2
\end{align*}
\]

Minor or trace in D. auratus (Isla Taboga, Rio Campana), D. granuliferus, D. histronicus (Quebrada Vicordó), D. parvulus, D. trivittatus, D. truncatus.


Trace in D. histronicus (Quebrada Guangui), D. pumilio.

237A. C₁₅H₂₅NO, 0-58, 167°, m/e 237(4), 236(3), 220(3), 194(12), 166(40), 70(100). H₂-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. histronicus (Rio Baba).

237B. “C₁₅H₂₇NO”, 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. “C₁₆H₃₁N^+”, ---, 168°, m/e 237(2), 236(1), 180(100), H₀-derivative. Probably higher homolog of 223B.

Minor in D. histronicus (Rio Baba).

237D. “C₁₆H₃₁N^+”, ---, 163°, m/e 237(1), 236(2), 138(100), H₀-derivative. Tentative structure:

\[
\begin{align*}
\text{H}_2\text{C} & = \text{CCH}_2
\end{align*}
\]

Trace in D. auratus (Rio Campana).

237E. “C₁₅H₂₇NO”, 0-25, 180°, m/e 237(1), 236(3), 208(70), 152(100). H₂-derivative, m/e 239, 152. Possibly a hydroxypumiliotoxin C.

Trace in D. histronicus (Quebrada Guangui).

239AB. C₁₅H₂₉NO. Probably one compound, 0-22, 178°, m/e 239(2), 238(3), 182(A, C₁₁H₂₆NO,100), 180(B, C₁₂H₂₇N,90). H₀-derivative. O-acetyl derivative. Tentative structure:

\[
\begin{align*}
\text{H}_2\text{C} & = \text{CCH}_2
\end{align*}
\]

*The allo-prefix in the pumiliotoxin-A class has no relationship to its usage in the histriionicotoxin 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](image)

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* (Rio 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:

![243](image)

Major in *D. auratus* (Isla Taboga, Rio Campana), *D. azureus*, *D. granuliferus*, *D. histrionicus* (Playa de Oro, Quebrada Vicordó, Quebrada Docordó); minor or trace in *D. pichtus*, *D. tinctorius*, *D. trivittatus*, *D. truncatus*. In certain samples a very small amount of an isomer emerges slightly before 243.
251A. "C₁₇H₃₂N", —, 170°, m/e 251(2), 208(6), 152(100). H₆-derivative. Possibly in pumiliotoxin-C class with R = C₇H₁₅ (see 223C).

Trace in D. histrionicus (Río Guapi).

251B. "C₁₆H₂₉NO", —, 184°, m/e 251(2), 234(4), 138(100). H₂-derivative, m/e 253, 138. Probably side-chain hydroxy analog of 235B.

Trace in D. lehmanni, D. pumilio.

251C. "C₁₆H₂₉NO", —, 190°, m/e 251(2), 234(4), 154(100). H₂-derivative, m/e 253, 154. Possibly ring hydroxy analog of 235D.

Trace in D. minutus (Quebrada Guangú, El Llano-Cartí).

251D. "C₁₆H₂₉NO", 0-52, 172°, m/e 251(5), 250(3), 234(1), 194(3), 166(28), 138(10), 70(100). H₂-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. "C₁₅H₂₃NO₂", —, 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 Guangú)

253. C₁₅H₂₇NO₂, 0-30, 179°, m/e 253(4), 252(1), 236(22), 210 (C₁₂H₂₀NO₂,3), 208(2), 182(C₁₀H₁₅NO₂,16), 114(C₆H₁₂NO,27) 112(C₆H₁₀NO,26), 70(C₄H₈N,100). H₂-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. "C₁₅H₂₅N", 0-30, 188°, m/e 257(1), 256(2), 216(100). H₈-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. C₁₇H₂₅NO, 0-36, 190°, m/e 259(4), 242(2), 218(C₁₄H₂₆NO,18), 200(6), 96(C₆H₁₀N,100). H₈-derivative, m/e 267(10), 250(13), 224(39), 196(15), 168(19), 152(100), 96(68). O-acetyl derivative. Structure:

```
\[ \begin{align*}
H₂C=CHCH₂ \quad \text{NH} \\
H₂C=CHC=CH \\
\end{align*} \]
```

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 R₄ values of 0-35-0-49.

267A. C_{16}H_{29}NO_{2}, 0·31, 186°, m/e 267(15), 250(33), 182(C_{10}H_{18}NO_{2},18), 114-(C_{6}H_{12}NO,52), 112(C_{6}H_{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. leucomes, D. minutus (all populations), D. tinctorius; minor or trace in D. auratus (Rio 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-Cartti).

269A,B. C_{15}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:

![Chemical Structure Image]

Minor or trace in D. granuliferus, D. histrionicus (Santa Cecilia, Guayacana, Rio 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:

![Chemical Structure Image]

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_{33}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. abdittus.

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_{6}-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(66). O-acetyl derivative.


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_{6}H_{18}N,23), 140(C_{6}H_{19}N,100). C: 0·40, 195°, m/e 283 (<1), 282(1), 240(5), 226(C_{13}H_{24}NO_{2},28), 224(C_{13}H_{20}N,10), 166(C_{13}H_{20}N,60), 126(C_{6}H_{18}N,100). H_{6}-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 \( \text{C}_5\text{H}_{11} \) is seen with 283B and loss of \( \text{C}_4\text{H}_9 \) with 283C.

Major in *D. histrionicus* (Quebrada Guanui) and *D. occulator*.

**285A.** Isodihydrohistrionicotoxin (Fig. 1), \( \text{C}_{19}\text{H}_{27}\text{NO} \), 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). \( \text{H}_{10}\text{-derivative, m/e see } \text{H}_{12}\text{-derivative of 283A. O-acetyl derivative.} \)


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

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

**285C.** Allodihydrohistrionicotoxin, \( \text{C}_{19}\text{H}_{27}\text{NO} \), 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). \( \text{H}_{10}\text{-derivative, m/e see } \text{H}_{12}\text{-derivative of 283A. See Fig. 1 for structure (from Daly et al., 1977).} \)

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), \( \text{C}_{19}\text{H}_{29}\text{NO} \), 0-42, 216°, m/e 287(12), 286(4), 270(3), 220(30), 202(34), 176(45), 162(60), 148(24), 96(100). \( \text{H}_8\text{-derivative, m/e see } \text{H}_{12}\text{-derivative of 283A.} \)

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

**287B.** Tetrahydrohistrionicotoxin (Fig. 1), \( \text{C}_{19}\text{H}_{29}\text{NO} \), 0-43, 213°, m/e 287(13), 286(2), 270(2), 220(43), 202(18), 176(6), 162(4), 148(4), 96(100). \( \text{H}_8\text{-derivative, m/e see } \text{H}_{12}\text{-derivative of 283A.} \)

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

**287C.** Gephyrotoxin (Formerly referred to as HTX-D, Tokuyama et al., 1974) \( \text{C}_{19}\text{H}_{29}\text{NO} \), 0-20, 218°, m/e 287(5), 286(3), 242(\( \text{C}_{17}\text{H}_{24}\text{N} \)), 222(\( \text{C}_{14}\text{H}_{24}\text{NO} \)), 122(14). \( \text{H}_8\text{-derivative, m/e see 293(5), 292(3), 248(100), 222(32). O-acetyl derivative. A perhydro-pyrroloquinoline, the parent member of the gephyrotoxin class of dendrobatid alkaloids (see Daly et al., 1977).} \)

\[
\text{CH}_2\text{CH}=\text{CHC}≡\text{CH}\\
\text{CH}_2\text{CH}_2\text{OH}
\]

Minor in *D. histrionicus* (Santa Cecilia, Playa de Oro, Rio 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. \( \text{C}_{29}\text{H}_{35}\text{N}^+ \), —, 216\(^\circ\), 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 \text{D. lehmanni}.

291A. Octahydrohistrionicotoxin (Fig. 1), C\(_{19}\text{H}_{33}\text{NO}\), 0-35, 212\(^\circ\), m/e 291(12), 290(2), 274(14), 250(54), 222(24), 194(18), 192(12), 178(100), 96(32). H\(_4\)-derivative, m/e see H\(_2\)-derivative of 283A. O-acetyl derivative.

Major in \text{D. histrionicus} (Rio Baba), minor in \text{D. granuliferus}, \text{D. histrionicus} (Rio Guapi, Guayacana, Rio Palenque), \text{D. parvulus}, \text{D. tinctorius}, \text{D. truncatus}.

291B. \( \text{C}_{19}\text{H}_{33}\text{NO}^+ \), 0-12, 221\(^\circ\), 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:

\[
\text{HOCH}_2
\]

Trace in \text{D. lehmanni}.

291C. \( \text{C}_{19}\text{H}_{33}\text{NO}^+ \), 0-20, 220\(^\circ\), 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 \text{D. lehmanni}.

295. \( \text{C}_{19}\text{H}_{35}\text{NO}^+ \), 0-09, 224\(^\circ\), m/e 295(3), 278(4), 138(100). Possibly a side-chain Trace in \text{D. auratus} (Isla Taboga, Rio Campana).

297A. C\(_{17}\text{H}_{33}\text{NO}_3^+ \), 0-13, 225\(^\circ\), 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 \text{D. minutus} (Cerro Campana).

297B. C\(_{18}\text{H}_{35}\text{NO}_2^+ \), 0-35, 222\(^\circ\), m/e 297(10), 166(92), 70(100), H\(_4\)-derivative. Pumiliotoxin-A class.

Trace in \text{D. auratus} (Isla Taboga).

301. \( \text{C}_{21}\text{H}_{35}\text{N}^+ \), —, 213\(^\circ\), m/e 301(< 1), 260(100). Possibly a member of pumiliotoxin-C class with an allyl R-substituent and a C\(_9\text{H}_{15}\) R' substituent (see 223A,B,C,D).

Trace in \text{D. azureus}.

307A. Pumiliotoxin A, C\(_{19}\text{H}_{33}\text{NO}_2^+ \), 0-36, 216\(^\circ\), m/e 307(5), 290(3), 278(C\(_{17}\text{H}_{28}\text{NO}_2^+ \), 206(C\(_{12}\text{H}_{26}\text{NO}_1^+ \), 194(C\(_{12}\text{H}_{20}\text{NO}^+ \), 176(C\(_{12}\text{H}_{16}\text{N}^+ \), 166(C\(_{10}\text{H}_{16}\text{NO}_5^+ \), 70(C\(_4\text{H}_{8}\text{N}^+ \), 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\(_2\)CH\(_3\) moiety is present. One double bond is readily reduced, the other slowly.

Major in \text{D. pumilio}; minor trace in \text{D. auratus} (Isla Taboga), \text{D. granuliferus}, \text{D. lehmanni}, \text{D. minutus} (Quebrada Guangui, \text{D. occulator}, \text{D. viridis}, \text{D. species} (Colombia).

307B. Isopumiliotoxin A, \( \text{C}_{19}\text{H}_{33}\text{NO}_2^+ \), 0-36, 211\(^\circ\), 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 \text{D. auratus} (Isla Taboga), \text{D. granuliferus}, \text{D. lehmanni}, \text{D. minutus} (Cerro Campana, Quebrada Guangui), \text{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}), 70(100). H_{2}-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_{33}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_{2}-derivative of 307A.

Minor in D. minutus (Quebrada Guaniguí), D. viridis.

309B. “C_{26}H_{39}NO”, 0.09, 220°, m/e 309(1), 152(100). Possibly member of hydroxypumiliotoxin-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 CHOCHOHCH_{2} moiety is present (cf., CHOCHOHCH_{2} 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 Guaniguí), D. minutus (Quebrada Guaniguí), 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_{2}-derivative of 323A. Separates poorly from coexisting 323A.

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

325. Alloidihydropumiliotoxin B, “C_{19}H_{33}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 Guaniguí, El Llanito-Cartí).

341A. “C_{19}H_{33}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_{t} value.

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

341B. “C_{19}H_{33}NO_{4}”, —, 225°, m/e 341(3), 324(4), 182(60), 114(20), 112(30), 70(100). Probably a hydroxy analog of dihydroallopumiliotoxin B (325).

Trace in D. lehmanni.

351. “C_{20}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_{36}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_{6}-derivative. Higher dihydro-homolog of 341A.

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

379. “C_{25}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_{2}-derivative. m/e 383, 368, 110, 84, 70.

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

381. “C_{25}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 Llanito-Cartí).
395. \( \text{C}_{23}\text{H}_{41}\text{NO}_4 \), 0·10, 243\(^\circ\), 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 \( D. \) \textit{pumilio}, \( D. \) \textit{auratus} (Isla Taboga).

Sources and occurrence of alkaloids

The following 18 species of \textit{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 \textit{Dendrobates}, including even \( D. \) \textit{trivittatus}—the nomenclatural type species of \textit{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).

\textit{D. abditus}:

\textit{D. auratus}:


\textit{D. azureus}:

\textit{D. fulguritus}:

\textit{D. granuliferus}:
Palmar Norte, Puntarenas, Costa Rica, July 1967, 9 skins, 0·7 g. Major alkaloids: pumiliotoxin B (323A), 219A, histrionicotxin (283A), isodihydrohistrionicotixin (285A), 243, 267A. Minor alkaloids: 259, alloidihydrohistrionicotxin (287C), pumiliotoxin

*More trace compounds are listed for several species (\textit{D. histrionicus}, \textit{D. lehmanni}, \textit{D. occulator}) than were given by Myers and Daly (1976a) 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. 2. Gas Chromatograms of Alkaloid Fraction from Population Samples of *Dendrobates histrioicus*.


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 x 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 cm³/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°C 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% dimethylidichlorosilane in toluene. Alkaloids corresponding to chromatogram peaks are designated (for further details see Results).

**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.
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:


(B) Playa de Oro, upper Rio San Juan, Colombia, Feb. 1971, 8 skins, 2-4 g. Major alkaloids: 219A, 243, histrionicotoxin (283A), isodihydrohistrionicotoxin (285A). Minor
alkaloids: allodihydrohistrionicotoxin (285C), neodihydrohistrionicotoxin (285B), isotetrahydrohistrionicotoxin (287A), gephyrotoxin (287C), 239AB, 259. Trace alkaloids: 251D, 267A.


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


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


(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. 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: histronicotoxin (283A), isodihydrohistronicotoxin (285A), 219A. Minor alkaloids: tetrahydrohistronicotoxin (287B) octahydrohistronicotoxin (291A). Trace alkaloids: 235A, 265 (results not shown).


**D. trivittatus**:


(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. 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


**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, 1976a) 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 methyldecalhydroquinoline 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

*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.

\[
\text{Pumiliotoxin—C class}
\]

*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 group of the hydroxydecahydroquinolines has been tentatively assigned to the carbon bearing the R'-substituent. Hydroxypumiliotoxin-C class alkaloids should form N,O-diacyl 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 C₁₉-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, allottetra-

\[
\text{Histrionicotoxin class}
\]

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 perhydropropyrolo-bridge (gephos = bridge) as in gephyrotoxin (287C) (a perhydropropyroloquinoline) and 239AB and 239CD, (perhydropropyrolopiiperidines). One of six alkaloids of this class, a dihydropygephyrotoxin, 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 283BC 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 283BC 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 (Meyer 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 Guanui 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, 1976a), 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 batarachotoxins of Phyllobates aurataenia 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 substituted, unsaturated piperidine. Cyclization of the enamine 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.

\[
\begin{align*}
&\text{R} \quad \text{N} \\
&\text{O} \quad \text{R'} \\
&\text{HO} \quad \text{CH}_2 \text{R'} \\
&\text{R} \quad \text{N} \\
&\text{R} \quad \text{K}
\end{align*}
\]

**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 (Rhadincaea spp.) and a mouse opossum (Marmosa sp.) usually released proferred 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, 1976a: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, 1976a)—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 (1976a) 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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