Reviews

Alkaloids from Amphibian Skin: A Tabulation of Over Eight-Hundred Compounds

John W. Daly,*,† Thomas F. Spande, and H. Martin Garraffo

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892-0820

Received May 1, 2005

A diverse array of biologically active, lipid-soluble alkaloids have been discovered in amphibian skin. Such alkaloids include the following: the steroidal samandarines from salamanders, the batrachotoxins, histrionicotoxins, gephyrotoxins, and epibatidine from neotropical poison frogs (Dendrobatidae), the pumiliotoxins, allopumiliotoxins, homopumiliotoxins, and decahydroquinolines from certain genera of anurans from four families (Dendrobatidae, Mantellidae, Bufonidae, and Myobatrachidae), a variety of izidines (pyrrolizidines, indolizidines, quinolizidines, lehmizidines), pyrrolidines, piperidines, various tricyclics (related in structures to the coccinellines), and spiropyrrolizidines from the first three of these four families, the pseudophrynamines from one genus of Australian frogs, and a variety of unclassified alkaloids as yet of undetermined structure. With the exception of the samandarines and the pseudophrynamines, all alkaloids appear to be derived from dietary sources. Although only a few of the over 800 amphibian skin alkaloids have been detected in arthropods, putative arthropod sources for the batrachotoxins and coccinelline-like tricyclics (beetles), the pumiliotoxins (ants, mites), the decahydroquinolines, izidines, pyrrolidines, and piperidines (ants), and the spiropyrrolizidines (millipedes) have been discovered. Ants are likely sources for histrionicotoxins, lehmizidines, and tricyclic gephyrotoxins. Epibatidines represent an important alkaloid class without a putative dietary source. The structures for many of these alkaloids have been rigorously established, while the structures of others represent tentative proposals, based only on mass spectral and FTIR spectral data, along with analogies to structures of well-defined alkaloids.

Introduction

The diverse lipophilic alkaloids that had been detected in amphibian skin, representing over 20 structural classes, were last summarized in 1999.[1] Since that time, over 300 additional alkaloids have been detected and characterized. Structures for many have been established, and some previously proposed structures have been revised. The present overview updates and reviews current structures, spectroscopic properties, and the occurrence of over 800 alkaloids. Structures of those not firmly established by NMR spectroscopic analysis and/or by synthesis should be considered tentative structures, or in some cases merely possible gross structures. The code designations for such alkaloids, first introduced in 1978 for less than 100 alkaloids[2] and then applied to over 200 alkaloids in 1987,[3] to nearly 300 in 1993,[4] and to about 500 in 1999,[1] consist of the nominal molecular weight and an identifying letter(s), both in bold face. A table, listing the over 800 alkaloids with mass spectral data, vapor-phase FTIR spectral data, and other data, is in the Supporting Information. The structures, distribution in nature, synthesis, and the pharmacology of frog skin alkaloids were most recently reviewed in 1999.[1] The present review seeks only to present structures, tentative structures, and spectral properties.

Methods. The extraction of skins with methanol and the preparation of an alkaloid fraction by acid/base partitioning with $HCCl_3$/hexane have been described.[5] Mass spectral analyses have been carried out over the years with both magnetic sector and ion-trap GC-MS spectrometers, using primarily fused-silica-bonded capillary columns for GC separation.[5] Empirical formulas were determined for some alkaloids by high-resolution mass spectral analyses. For other alkaloids, proposed formulas have been based on spectral and chemical properties, including retention times (t_R) on gas chromatography. Molecular weights of alkaloids were confirmed by ammonia chemical ionization (CI) mass spectral analyses. Fragmentation for some alkaloids has been studied by both EIMS/MS and collision-activated dissociation (CAD) NH_3-CI-MS/MS techniques.[6,7] The number of exchangeable hydrogens was determined by GC-MS spectral analysis in the CI mode with ND_3 in place of NH_3 or with D_2O in place of H_2O in HPLC mass spectral analysis in the APCI mode.[8−10] Mass spectra for higher molecular weight alkaloids were obtained in some cases with direct probes or with HPLC mass spectrometry. Vapor-phase FTIR spectral analyses, obtained using fused-silica-bonded capillary GC columns, have provided structural insights into functional groups and stereochemical configurations.[8−19] Perhydrogenation and chemical derivatization (*N*- and *O*-acetylation, butyl- or phenyl-boronation of vicinal hydroxyl groups, *N*-methylation) have been utilized for further characterization of alkaloids, which are often present in complex mixtures.[8,9,13,15] For quantitation of alkaloids, flame-ionization detection after GC separation on a packed column (1.5% OV-1, 5−6 ft) has been used routinely.[3,5] Such traces have been reported for many

* To whom correspondence should be addressed. Tel: (301) 496-4024. Fax: (301) 402-0008. E-mail: jdaly@nih.gov.
† Retired.

10.1021/np0580560 CCC: $30.25
Published on Web 10/06/2005

populations and species of anurans. NMR spectra, vapor-phase FTIR spectra, and spectral analyses have been presented for many of the alkaloids (see references in Table S1 in the Supporting Information).

Results

Structures, tentative structures, and possible gross structures for the majority of alkaloids detected in amphibian skin are depicted in Figures 1−31. Structures that are firmly established by NMR and/or synthesis are indicated by asterisks. One asterisk indicates that the absolute configuration is as shown, while two asterisks usually indicate that the relative configuration is as shown, but the absolute configuration is not known. The remainder of the structures are based on mass spectral analyses, and when available on GC-FTIR spectral analyses, and in some cases partly upon analogies to similar congeneric alkaloids of known structures. Many structures represent only a working postulate, based on present data, and will require further study. In some cases, minor isomers have been detected (see Table S1 in the Supporting Information).

The protocol for preparation of an alkaloid fraction from methanol extracts of amphibian skin has been kept relatively constant, as have the conditions for a flame-ionization GC profile of alkaloids present in the equivalent of 2 mg wet weight of skin. Such semiquantitative flame-ionization GC profiles on 1.5% OV-1 columns have been presented for many species and populations (see ref 1 and citations therein). Detailed tabulations of the distribution of alkaloids from different populations and species of anurans are in preparation.

Code designations, structural class, empirical formulas, retention times for gas chromatography on a capillary column, mass spectral and FTIR spectral data, perhydrogenation and exchange data, and other pertinent comments for the alkaloids are provided in Table S1 of the Supporting Information. This table updates and revises the most recent tabulation and discussion of such alkaloids.[1] Samandarines and batrachotoxins have not been assigned code designations and are not included in this table.

Discussion

Samandarines. The toxic principles of the fire and alpine salamanders (Salamandridae, *Salamandra*) were isolated and structures determined by Schöpf and colleagues.[20a] Nine samandarines have been characterized (Figure 1). There appears to have been little further research on such steroidal alkaloids since our review in 1999.[1] Evidence that the salamanders synthesize the samandarines, presumably from cholesterol, has recently been provided.[20b] Samandarine is the major component in the parotoid glands of the fire (20 mg/gland) and alpine (5 mg/gland) salamanders. The high toxicity (LD_{50} mouse 70 μg) is likely due to potent local anesthetic activity (see ref 1).

Batrachotoxins. The toxic principles of the poison-dart frogs (Dendrobatidae, *Phyllobates*) of the Western Colombian rain forests were shown in 1969 to be steroidal alkaloids, which were named batrachotoxins.[21] The three major alkaloids were batrachotoxin, homobatrachotoxin, and batrachotoxinin-A (Figure 2). A fourth minor alkaloid, pseudobatrachotoxin, was unstable and converted to batrachotoxinin-A on storage. No structure was proposed. Trace amounts of 4β-hydroxybatrachotoxin and 4β-hydroxyhomobatrachotoxin were later described.[22] NMR data have been presented.[21,22] In recent years, batrachotoxins and a number of congeners have been detected in extracts of skin and feathers from two genera of passerine birds (*Pitohui*, *Ifrita*) of Papua New Guinea.[10,23] Such congeners include an acetate, crotonates, and a 4′-hydroxypentanoate. Most recently, batrachotoxin, homobatrachotoxin, batrachotoxinin-A, and congeners, including crotonates were discovered in beetles (Melyridae, *Choresine*).[24] The beetles represent a putative dietary source for the batrachotoxins of poison-dart frogs and the toxic passerine birds. Poison-dart frogs raised in captivity on alkaloid-free diets have no detectable batrachotoxins or other alkaloids in their skin.[25] The poison-dart frog does have batrachotoxin-resistant sodium channels,[25,26] which would allow it to eat batrachotoxin-containing beetles with no ill effects. The sodium channels in the batrachotoxin-containing birds have not yet been studied, nor have birds been raised in captivity on alkaloid-free diets.

Only the three Colombian species of the five neotropical species of dendrobatid frogs of the genus *Phyllobates* have high levels of batrachotoxins, and all three have been used to poison blow-darts.[1] The two Central American species

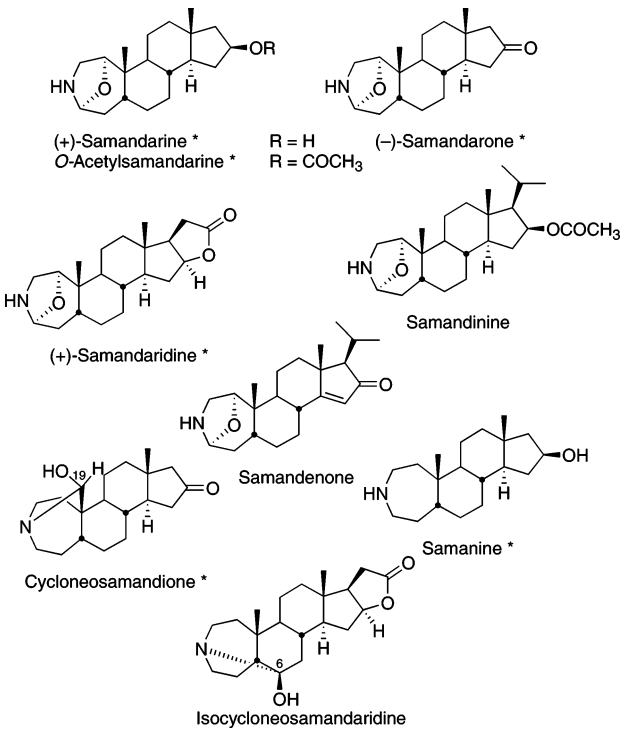


Figure 1. Samandarines. Isocycloneosamandaridine was originally referred to as cycloneosamandaridine in what proved to be an incorrect structural analogy to cycloneosamandione. *Absolute configuration as shown. Apparently synthesized by the salamander.[1,20b]

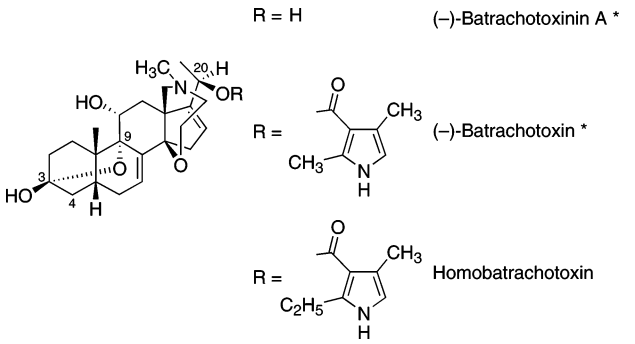
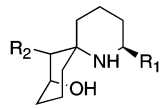


Figure 2. Batrachotoxins (BTX). *Absolute configuration as shown. HomoBTX undoubtedly has the same configuration. Minor amounts of 4β-hydroxyBTX and 4β-hydroxyhomoBTX were detected from one species of poison-dart frog.[22] A large number of other BTXs have been detected in beetles and passerine birds of Papua New Guinea.[10,23,24] None of these were detected in earlier studies on poison-dart frogs. A beetle origin is proposed.[24]



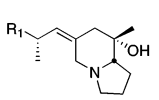
HTX	R_1	R_2
235A *	$CH_2CH{=}CH_2$	$CH{=}CH_2$
237F	*n*-C_3H_7	$CH{=}CH_2$
239H	*n*-C_3H_7	C_2H_5
259A	$CH_2CH{=}CH_2$	$CH{=}CHC{\equiv}CH$
261A	$CH_2CH{=}CH_2$	$CH{=}CHCH{=}CH_2$
263C	$CH_2CH{=}CH_2$	$(CH_2)_2CH{=}CH_2$
265E	*n*-C_3H_7	$(CH_2)_2CH{=}CH_2$
283A *	$CH_2CH{=}CHC{\equiv}CH$	$CH{=}CHC{\equiv}CH$
285A *	$(CH_2)_2CH{=}C{=}CH_2$	$CH{=}CHC{\equiv}CH$
285B *	$CH_2CH{=}CHC{\equiv}CH$	$CH{=}CHCH{=}CH_2$
285C *	$(CH_2)_3C{\equiv}CH$	$CH{=}CHC{\equiv}CH$
285E *	$CH_2CH{=}CHCH{=}CH_2$	$CH{=}CHC{\equiv}CH$
287A *	$(CH_2)_2CH{=}C{=}CH_2$	$CH{=}CHCH{=}CH_2$
287B	$CH_2CH{=}CHCH{=}CH_2$	$CH{=}CHCH{=}CH_2$
287D	$(CH_2)_3C{\equiv}CH$	$CH{=}CHCH{=}CH_2$
291A *	$(CH_2)_3CH{=}CH_2$	$(CH_2)_2CH{=}CH_2$

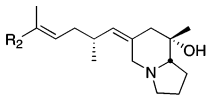
Figure 3. Histrionicotoxins (HTX). *Absolute configuration as shown (negative rotations). The remaining HTXs are assumed to have the same configuration. All double bonds are *cis* except for a minor *trans*-isomer of **283A**, which is probably a photochemical artifact.[17] A neotropical myrmicine ant origin is likely.

have low levels and for some populations of *Phyllobates lugubris* no detectable levels of batrachotoxins. The most toxic of the true Colombian poison-dart frogs (*P. terribilis*) has about 1000 μg of batrachotoxins per frog skin, while the other two true poison-dart frogs, *P. bicolor* and *P. aurotaenia*, have 100 to 200 μg per frog skin. Both of the dialkylpyrrole carboxylates, namely, batrachotoxin and homobatrachotoxin, whose presence is readily detected by a sensitive Ehrlich's reaction, are about 250-fold more toxic than the alcohol batrachotoxinin-A. The LD_{50} for mice of these two extremely toxic alkaloids is about 0.1 μg per mouse. The toxicity is due to the depolarization of nerve and muscle membranes by selective stabilization of sodium channels in an active, open form by the batrachotoxins. Batrachotoxins have been widely used in research on voltage-dependent sodium channels (see ref 1).

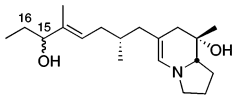
Histrionicotoxins. The major alkaloids in a brightly colored, extremely variable species of South American dendrobatid frogs (*Dendrobates histrionicus*) are the histrionicotoxins. The structure and absolute configuration of histrionicotoxin (**283A**) and isodihydrohistrionicotoxin (**285A**) were determined in 1971.[27] At present 16 histrionicotoxins have been detected (Figure 3). The mass spectra of histrionicotoxins usually are characterized by α-cleavage of the R_1 side-chain and by a major fragment peak at *m/z* 96. Octahydrohistrionicotoxin (**291A**) is an exception, with the mass spectrum showing a loss of a propenyl (C_3H_5) group to yield a major fragment ion at *m/z* 250 and having a base peak at *m/z* 178 somewhat larger than that at *m/z* 96 (Table 1). The vapor-phase FTIR spectra of histrionicotoxins have a diagnostic absorption at 3330 to 3400 cm^{-1} for the hydrogen-bonded OH group.[13] There is no Bohlmann band.

Histrionicotoxins have been detected only in neotropical dendrobatid frogs, with one exception, a mantellid frog obtained through the pet trade.[1] A New World myrmicine ant is suspected to be the dietary source of histrionicotoxins. Dendrobatid frogs (*Dendrobates auratus*) fed on leaf-litter arthropods of Panamá did contain several histrionicotoxins (**283A**, **285A**, **285C**, **287A**).[5] Histrionicotoxins can

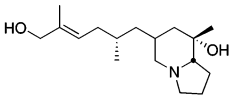




PTX	R_1	PTX	R_2
209F	CH_3	**275H**	$CH{=}CH_2$
223U	C_2H_5	**277B**	CHO
225F	CH_2OH	**277G**	C_2H_5
237A	*n*-C_3H_7	**281F**	see structure below
251D *	*n*-C_4H_9	**289C**	$CH{=}CHCH_3$
253F	$CH_2CHOHCH_3$	**291G**	*n*-C_3H_7
265D	C_4H_7O (OH)	**293E**	$CHOHCH_3$
265G	$(CH_2)_2COCH_3$	**305B**	$COCH_2CH_3$
267C *	$(CH_2)_2CHOHCH_3$	**305D**	$CH{=}CHCH_2OH$
281A	$C_5H_{11}O$ (OH)	**307A** *[b,c]	**C**$HOHCH_2CH_3$ 15*R*
295D	$C_5H_9O_2$	**307G**	$CH_2CHOHCH_3$
295F	$C_5H_{13}O$ (OH)	**307H**	see structure below
297B	$C_5H_{11}O_2$ (2 OH)	**323A** *[b,d]	*threo*-**C**HOH**C**$HOHCH_3$ 15*R*,16*R*
307B	$CH_2CHOHC(CH_3){=}CHCH_2CH_3$		
307F [a]	CH_2CO**C**$H(CH_3)CH_2CH_2CH_3$		
307F'	$CH_2CH_2CH(CH_3)COCH_2CH_3$		
309A [b]	$(CH_2)_2CH(CH_3)CHOHCH_2CH_3$		
325B	$(CH_2)_2CH(CH_3)CHOHCHOHCH_3$		
353A	$C_5H_{19}O_2$ (2 OH)		



307H
Tentative



281F
Tentative

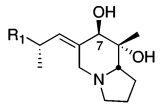
[a] Two isomers **307F"** and **307F"'** have been detected. Probably epimeric at C-14 in bold.
[b] *N*-oxides of **307A**, **309A** and **323A** have been proposed and named, based on apparent MWs, as **307D**, **309C** and **323F**, respectively.[1,34] *N*-Oxides do not show the protonated molecular ion on NH_3-CI-MS, and have significantly longer retention times than the parent alkaloids.
[c] Another isomer **307E** has been proposed. A 15*S*-epimer is considered an artifact.
[d] A rare *erythro*-isomer also has been detected.

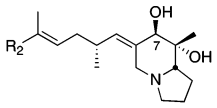
Figure 4. Pumiliotoxins (PTX). *Absolute configuration as shown (positive rotations). Other PTXs, even those (**209F**, **225F**, **307F**) with a negative rotation, are assumed to have the same configuration. Absolute configurations in R_1 and R_2 are unknown except as shown for **307A** and **323A**. Some structures of R_1 and R_2 are tentative. An ant or mite origin is likely.[35a,b]

occur in skin of dendrobatid frogs at levels of up to 200 μg/frog. The name histrionicotoxin is misleading, since these alkaloids have relatively low toxicity. Even 1000 μg in mice was not lethal. But such alkaloids would be noxious to a predator, due to bitterness and a blockade of nicotinic pathways. Histrionicotoxins have been widely used in research as noncompetitive blockers of nicotinic receptor/channels (see ref 1).

Pumiliotoxins, Allopumiliotoxins, and Congeners. Two pumiliotoxins were reported in 1967 as major alkaloids from one population of a small Panamanian dendrobatid frog, the brightly colored and highly variable *Dendrobates pumilio*.[28] The structures of these two alkaloids, pumiliotoxin A (**307A**) and pumiliotoxin B (**323A**), remained undetermined until 1980, when X-ray crystallography of pumiliotoxin **251D**,[29] isolated from an Ecuadoran dendrobatid frog, *Epipedobates tricolor*, revealed the basic structure of pumiliotoxins and their 7-hydroxy congeners, the allopumiliotoxins.[29,30] At present over 30 alkaloids are considered to be pumiliotoxins (Figure 4) and about 20 alkaloids are considered to be allopumiliotoxins (Figure 5). The proposed structural features of some are tentative, while others have been rigorously established by NMR spectral analysis and/or by synthesis. One alkaloid (**341A**), listed as an allopumiliotoxin, is unusual in having a cyclic ether structure.[31]

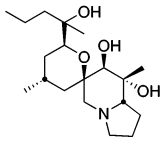
Pumiliotoxins. The mass spectra of pumiliotoxins usually are characterized by major ions at *m/z* 166 and 70. However, in some cases, because of the nature of unsat-





aPTX	R_1	aPTX	R_2
225E	CH_3	**293K**	CHO
237B	$CH=CH_2$	**305C**	$CH=CHCH_3$
241H	CH_2OH	**307C**	*n*-C_3H_7
251I	$CH_2CH=CH_2$	**321C**	$COCH_2CH_3$
253A	*n*-C_3H_7	**323B** *[c]	**C**$HOHCH_2CH_3$ 15*R*
267A *[a,b]	*n*-C_4H_9	**339A** *[d]	$CHOHCHOHCH_3$
293N	C_5H_7O	**341A** *	see structure below
297A	$(CH_2)_2CH(CH_3)CH_2OH$	**341B**	isomer of **341A**
305A	$CH=CHCH=C(CH_3)CH_2CH_3$	**357A**	hydroxy-**341A**
309D	$(CH_2)_2CH(CH_3)CH_2CH_2CH_3$		
325A	$CH_2CH_2CH(CH_3)CHOHCH_2CH_3$		

[a] An *N*-oxide has been isolated[34].
[b] A rare 7-*epi*-isomer has been detected.
[c] AlloPTX **323B** is the 15*R*-epimer. A 15*S*-epimer **323B"** is considered an artifact.
[d] A 7-*epi*-isomer **339B** * also has been detected.



341A

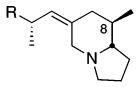
Figure 5. Allopumiliotoxins (aPTX). *Absolute configuration as shown (positive rotations). Other aPTXs are assumed to have the same configuration. Absolute configurations in R_1 and R_2 are unknown except as shown for **323B**. Some structures of R_1 and R_2 are tentative. An ant or mite origin is likely.[35a,b] Certain dendrobatid frogs can produce an allopumiliotoxin from a pumiliotoxin.[36]

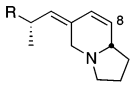
uration in the side-chain, an ion at *m/z* 193 (**277B**, **305B**, **305D**) can be the major fragment. A 6,10-dihydropumiliotoxin structure has been proposed for alkaloid **281F**.[1] The mass spectra and FTIR spectra were similar to those of the 6,10-dihydro derivative of pumiliotoxin **267C**. The vapor-phase FTIR spectra of pumiliotoxins have a diagnostic absorption at 3544 cm^{-1} for the hydrogen-bonded OH at C-8.[12] There are characteristic Bohlmann bands for pumiliotoxins with an absorption at 2798 cm^{-1} and a shoulder at 2750−2600 cm^{-1}.

Allopumiliotoxins. The mass spectra of allopumiliotoxins usually are characterized by an ion at *m/z* 182, a pair of ions at *m/z* 114 and 112, and a major ion at *m/z* 70. The mass spectrum of allopumiliotoxin **293K**, because of the nature of the unsaturation in the side-chain, has a major fragment at *m/z* 209. The vapor-phase FTIR spectra of allopumiliotoxins have a diagnostic absorption at 3521 cm^{-1} for the hydrogen-bonded OH at C-8.[12] The Bohlmann band of allopumiliotoxins near 2800 cm^{-1} is sharper than those of pumiliotoxins and has no shoulder.

8-Deoxypumiliotoxins. The alkaloid **251H**, analyzed by MS, FTIR, and NMR spectroscopic techniques, was reported in 1995 as the first member of a pumiliotoxin subclass of 8-deoxypumiliotoxins.[32] Some dozen alkaloids are now assigned to that subclass (Figure 6). The mass spectra of the 8-deoxypumiliotoxins are characterized by a major ion at *m/z* 150 and a significant ion at *m/z* 70. The vapor-phase FTIR spectra show a Bohlmann band pattern consisting of a major absorption at about 2790 cm^{-1} and a minor absorption at about 2735 cm^{-1}.[32]

8-Dehydrodesmethylpumiliotoxins. In an initial study on alkaloids from mantellid frogs, it was tentatively proposed, based only on mass and FTIR spectral data, that four mantellid alkaloids were dehydrohomopumiliotoxins.[14] The parent member, alkaloid **235C**, has now been isolated and NMR spectroscopic analysis demonstrated that it is not a dehydrohomopumiliotoxin, but instead an 8-dehydrodesmethylpumiliotoxin (Figure 6, unpublished results). A synthetic dehydrohomopumiliotoxin,[33] corresponding to the structure incorrectly proposed for **235C**, has quite different spectral properties from those of **235C**. The mass spectrum of the currently unclassified alkaloid **233K** is very similar to that reported[33] for a synthetic dehydrohomopumiliotoxin of that molecular weight.





8-Deoxypumiliotoxins		8-Dehydrodesmethylpumiliotoxins	
	R		R
193H	CH_3	**221F**	$CH_2CHOHCH_3$
235V	C_4H_9	**233F**	$(CH_2)_2COCH_3$
251H **	$(CH_2)_2CHOHCH_3$	**235C** **	$(CH_2)_2CHOHCH_3$
265X	$(CH_2)_3CHOHCH_3$	**251G**	$CH_2CHOHCHOHCH_3$
281B	$(CH_2)_2CHOHCHOHCH_3$		
281N	$CH_2CHOHCHOHCH_2CH_3$		
289E	$CH_2CH=C(CH_3)COCH_2CH_3$		
291E	$CH_2CH=C(CH_3)CHOHCH_2CH_3$		
293D	$(CH_2)_2CH(CH_3)CHOHCH_2CH_3$	**8-Desmethylpumiliotoxins**	
295C	$(CH_2)_2CH(CH_3)CHOHCH_2OH$		R
309H	$C_7H_{15}O_2$ (1 OH)	**249G**	C_5H_9
309J	$(CH_2)_2CH(CH_3)(CHOH)_2CH_3$	**263N**	C_6H_{11}
		265V	C_6H_{13}

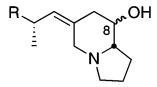
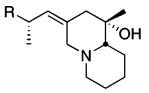


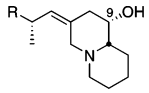
Figure 6. Deoxy-, dehydrodesmethyl-, and desmethylpumiliotoxins. Configurations are based on PTX and aPTX structures. Absolute configurations in R are unknown, and several are tentative, being based on analogies to R_1 and R_2 substituents in PTXs and aPTXs. An ant or mite origin is likely.[35a,b]

The mass spectra of 8-dehydrodesmethylpumiliotoxin **235C** and the three other alkaloids assigned to this subclass (Figure 6) have a characteristic pair of major ions at *m/z* 162 (base peak) and 160. The vapor-phase FTIR spectra of such alkaloids (**233F**, **235C**) have a weak Bohlmann band at 2792 cm^{-1} with a shoulder and an absorption at 3029 or 3020 cm^{-1}, indicating an internal *cis*-double bond.[14]

8-Desmethylpumiliotoxins. The alkaloid **249G** was tentatively proposed to be an 8-desmethylpumiliotoxin,[1] based mainly on the mass spectrum, which is characterized by major fragment ions at *m/z* 152 and 70, as expected from the proposed structure. Two other alkaloids are now proposed to be 8-desmethylpumiliotoxins (Figure 6).

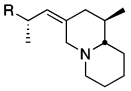
Summary for Pumiliotoxin, Allopumiliotoxins, and Congeners. The pumiliotoxins are very widely distributed in alkaloid-containing anurans from the neotropics (Dendrobatidae, *Dendrobates*, *Epipedobates*, *Minyobates*, *Phyllobates*), semitemperate South America (Bufonidae, *Melanophryniscus*), Madagascar (Mantellidae, *Mantella*), and Australia (Myobatrachidae, *Pseudophryne*). Allopumiliotoxins also occur widely in alkaloid-containing anurans. *N*-Oxides of pumiliotoxin **323A** and allopumiliotoxin **267A** have been isolated.[34] Recently, pumiliotoxins **307A** and **323A** were detected in extracts from formicine ants of two genera (*Brachymyrmex* and *Paratrechina*).[35a] Even more recently, pumiliotoxins **237A** and **251D** and 8-deoxypumiliotoxin **193H** were reported from oribatid mites.[35b] Mites are prey items for certain ants. Thus, it appears possible that some of the pumiliotoxins, allopumiliotoxins, and related congeners found in extracts of various anurans have a mite origin. However, frogs of the dendrobatid genus *Dendrobates* have a pumiliotoxin 7-hydroxylase that can efficiently and enantioselectively convert a dietary pumiliotoxin to a more toxic allopumiliotoxin.[36] This is the only example known for dendrobatid frogs where a dietary alkaloid has been metabolically altered by the frog.





hPTX	
	R
223G *	CH_3
239M *	CH_2OH
251R *	*n*-C_3H_7
265N	*n*-C_4H_9
267P	$CH_2CHOHCH_3$
281K	$(CH_2)_2CHOHCH_3$
317	C_7H_8O
319A	$CH_2CH=C(CH_3)CH_2COCH_3$
319B	$(CH_2)_2C(CH_3)=CHCOCH_3$
319D	$CH_2CH=C(CH_3)COCH_2CH_3$
321B	$CH_2CH=C(CH_3)CH_2CHOHCH_3$
321D	$CH_2CH=C(CH_3)CHOHCH_2CH_3$
321E	$CH_2COCH(CH_3)CH_2CH_2CH_3$
323E	$(CH_2)_2CH(CH_3)CHOHCH_2CH_3$
335	$CH_2CH=C(CH_3)CHOH(CH_2)_2CH_3$
337A	$(CH_2)_2CH(CH_3)CHOH(CH_2)_2CH_3$
337B	$CH_2CH=C(CH_3)(CHOH)_2CH_3$
353C	$(CH_2)_2CH(CH_3)(CHOH)_2CH_2CH_3$

9-Desmethyl-hPTX	
	R
209H	CH_3
267N	$(CH_2)_2CHOHCH_3$
323C	$CH_2CH=C(CH_3)CHOHCHOHCH_3$
339C	$C_7H_{13}O_3$



9-Deoxy-hPTX	
	R
193F	H
207O	CH_3
251W	$CH_2CHOHCH_3$

Figure 7. Homopumiliotoxins (hPTX), desmethylhomopumiliotoxins, and deoxyhomopumiliotoxins. *Absolute configuration as shown (positive rotation). Other hPTXs are assumed to have the same configuration. Absolute configurations in R are unknown, and several are based on analogies to PTXs and aPTXs. An ant origin was tentatively proposed.[35a]

The minor pumiliotoxin congeners have been detected rather rarely in poison frogs. The 8-deoxypumiliotoxins occur in both dendrobatid and mantellid frogs. The 8-dehydrodesmethylpumiliotoxins occur only in certain swamp-dwelling species of mantellid frogs. The 8-desmethylpumiliotoxins have been detected only very rarely in dendrobatid and mantellid frogs.

Major pumiliotoxins, such as **251D**, **267C**, **307A**, **309A**, and **323A**, can be present in skin of alkaloid-containing anurans at levels of up to 200 μg per frog. Major allopumiliotoxins, such as **267A** and **323B**, can be present in skin of alkaloid-containing anurans at levels of up to 100 μg per frog. The pumiliotoxins/allopumiliotoxins are quite toxic, with LD_{50} values for pumiliotoxins **307A** and **323A** and allopumiliotoxin **267A** of about 50 μg per mouse. Pumiliotoxin **251D** is about 5-fold less toxic than its 7-hydroxylated congener, allopumiliotoxin **267A**.[36] The pumiliotoxins, although toxic, at lower doses have marked cardiotonic activity apparently due to prolonging open-time of voltage-dependent sodium channels and, thereby, triggering inositol trisphosphate formation in cardiac and neuronal preparations.[37,38]

Homopumiliotoxins and Congeners. The structure of the parent member (**223G**) of the homopumiliotoxin class was established by NMR spectroscopic analysis in 1987.[39] Later, the absolute configuration was determined by comparison with *O*-acetyl derivatives of the synthetic enantiomers.[40] The structure of **223G** and structures, most of which are tentative, for 17 other homopumiliotoxins are shown in Figure 7.

The mass spectra of homopumiliotoxins are characterized by major ions at *m/z* 180 and 84. The sole exception is **319D**, where the base peak is at *m/z* 207 (Table 1). This appears to be due, as it was in certain pumiliotoxins and one allopumiliotoxin, to the nature of unsaturation in the side-chain. The vapor-phase FTIR spectra of homopumiliotoxins have a moderate broad Bohlmann band at 2753 cm^{-1} and an absorption for a hydrogen-bonded OH at 3555 cm^{-1}.[14]

9-Desmethylhomopumiliotoxins. Three alkaloids (**209H**, **267N**, **323C**) have been proposed to be 9-desmethylhomopumiliotoxins (Figure 7).[1] This subclass, which is analogous to the 8-desmethylpumiliotoxin subclass, was proposed on the basis of characteristic major fragment ions at *m/z* 166 and 84. The vapor-phase FTIR spectra of **267N** and **323C** have a moderate, broad Bohlmann band at about 2753 cm^{-1}, an absorption for a hydrogen-bonded OH at about 3565 cm^{-1}, and a strong absorption at 1111 cm^{-1}.

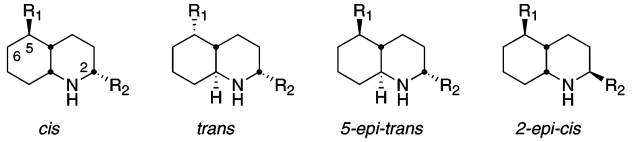
9-Deoxyhomopumiliotoxin. Two alkaloids (**193F**, **2070**) were previously proposed to be 9-deoxyhomopumiliotoxins,[1] a subclass corresponding to the 8-deoxypumiliotoxin subclass, on the basis of a major fragment ion at *m/z* 164 and a significant ion at *m/z* 84. The alkaloid **251W** now has been characterized from a dendrobatid frog where *m/z* 164 and 84 are the dominant ions (unpublished results). It is the third alkaloid tentatively proposed to be a 9-deoxyhomopumiliotoxin. Tentative structures are presented in Figure 7.

Summary for Homopumiliotoxins and Congeners. The homopumiliotoxins occur in dendrobatid, mantellid (*Mantella*), and bufonid (*Melanophryniscus*) anurans, but never at levels of greater than 50 μg per frog. The congeners have a very limited distribution in dendrobatid and/or mantellids. Neither toxicity nor bioactivity data have been reported for homopumiliotoxins. A dietary source for homopumiliotoxins has not been described, but in view of the occurrence of pumiliotoxins in formicine ants[35a] and oribatid mites,[35b] an ant and/or mite dietary source appears likely. The homopumiliotoxins, like the pumiliotoxins, probably will prove to be positive modulators of sodium channels, resulting in cardiotonic activity.

Decahydroquinolines. The parent member *cis*-**195A** of the decahydroquinoline class of alkaloids was isolated along with pumiliotoxins A and B from a Panamanian population of a small dendrobatid frog (*Dendrobates pumilio*), and the structure and absolute configuration were determined by X-ray crystallography in 1969.[41] The original name "pumiliotoxin C" no longer is used because of possible confusion with the true pumiliotoxins. In addition, *cis*-**195A** has very low toxicity. At present about 50 alkaloids, including in some cases four stereoisomers, are considered members of a 2,5-disubstituted decahydroquinoline class (Figure 8). The structures for several are firmly established by NMR spectroscopic analyses and/or synthesis,[1] while others are based on mass spectral and, in some cases, GC-FTIR spectral data.[17]

The mass spectra of decahydroquinolines are dominated by α-cleavage of the side-chain at C-2. In some cases, there is a significant loss of the C-5 substituent by a concerted process, as has been proposed for **269AB**.[17] Many have a methyl group at C-5 and, thus, have a base peak at *m/z* 152. There are a few 5-methyldecahydroquinolines with a ring hydroxyl group and hence a base peak at *m/z* 168. Certain decahydroquinolines have a loss of C_3H_7 that reflects loss from the ring system.[17] The FTIR spectra of decahydroquinolines have either no Bohlmann band, when the hydrogens on carbons α to the nitrogen are *entgegen* (*E*), or a weak Bohlmann band, when the hydrogens on carbons α to the nitrogen are *zusammen* (*Z*).[17] In addition, split absorption peaks in the IR regions 1300 and 1100 cm^{-1} occur for the *cis*-isomers, while single peaks occur in the same regions for the *trans*-isomers, presumably because there is only one ring conformation in the *trans*-isomers rather than two, as for the *cis*-isomers.[15,17]

Decahydroquinolines are common alkaloids in neotropical dendrobatid frogs. But in mantellid (*Mantella*) and



	DHQ	R_1	R_2	
	181D	CH_3	C_2H_5	
cis / 5-epi-trans	**195A** *	CH_3	*n*-C_3H_7	
cis	**195J**	*n*-C_3H_7	CH_3	
	209A	CH_3	$CH_2CH{=}CH_2$	6-OH
	209J	C_2H_5	*n*-C_3H_7	
cis	**211A** **	CH_3	*n*-C_3H_7	6-OH
	211K	CH_3	*n*-C_3H_7	ring-OH
all four	**219A** **	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	
	219C	CH_3	C_5H_7	
	219D	*n*-C_3H_7	$CH_2C{\equiv}CH$	
	221B	C_4H_7	C_2H_5	
	221C	CH_3	C_5H_9	
	221D	*n*-C_3H_7	$CH_2CH{=}CH_2$	
cis / trans / 5-epi-trans	**223F** **	*n*-C_3H_7	*n*-C_3H_7	
	223Q	CH_3	*n*-C_5H_{11}	
	231E	CH_3	$(CH_2)_2CH{=}CHC{\equiv}CH$	
cis	**233C**	$CH_2CH{=}CHCH_3$	$CH_2CH{=}CH_2$	
	235N	CH_3	$CH_2CH{=}CHCH{=}CH_2$	6-OH
cis	**237U**	*n*-C_4H_9	*n*-C_3H_7	
cis / trans / 5-epi-trans	**243A** **	$CH_2CH{=}CHC{\equiv}CH$	$CH_2CH{=}CH_2$	
cis	**245E**	$CH_2CH{=}CHC{\equiv}CH$	*n*-C_3H_7	
cis / trans	**249D**	$(CH_2)_3CH{=}CH_2$	*n*-C_3H_7	
trans	**249E**	*n*-C_3H_7	$(CH_2)_3CH{=}CH_2$	
	251A	CH_3	*n*-C_7H_{15}	
trans	**253D**	$CH_2CHOHCH_2OH$	$CH_2CH{=}CH_2$	
cis	**257A**	$(CH_2)_2CH{=}CHC{\equiv}CH$	$CH_2CH{=}CH_2$	
cis	**263R**	$(CH_2)_4CH{=}CH_2$	*n*-C_3H_7	
cis	**267L**	$CH_2CH{=}CHC{\equiv}CH$	$CH_2CH{=}CHC{\equiv}CH$	
cis / trans / 5-epi-trans	**269AB**	$CH_2CH{=}CHC{\equiv}CH$	$(CH_2)_2CH{=}C{=}CH_2$	
trans	**269A**	$(CH_2)_2CH{=}C{=}CH_2$	$CH_2CH{=}CHC{\equiv}CH$	
trans	**269B**	$CH_2CH{=}CHC{\equiv}CH$	$(CH_2)_3C{\equiv}CH$	
cis / trans	**271D** **	$(CH_2)_2CH{=}C{=}CH_2$	$(CH_2)_3C{\equiv}CH$	
cis / trans / 5-epi-trans	**iso-271D**	$(CH_2)_2CH{=}C{=}CH_2$	$(CH_2)_2CH{=}C{=}CH_2$	
cis / 2-epi-cis	**275B** **	$(CH_2)_3CH{=}CH_2$	$(CH_2)_3CH{=}CH_2$	
	293A	CH_3	*n*-$C_{10}H_{21}$	

Figure 8. Decahydroquinolines. *Absolute configuration as shown for *cis*-**195A** (negative rotation) and *trans*-**219A** (positive rotation). **Absolute configurations for *cis*-**211A**, *trans*-**243A**, and 5-epi-*trans*-**243A** (negative rotations) and for *cis*-**219A** and *cis*-**243A** (positive rotations) are uncertain. The R_1 and R_2 substituents are proposed to be unbranched. A myrmicine ant origin is proposed.[18]

bufonid (*Melanophryniscus*) anurans, decahydroquinolines are extremely rare, with the sole exception of decahydroquinoline **195A**. Like the histrionicotoxins, the 15-, 17-, and 19-carbon decahydroquinolines with highly unsaturated side-chains appear to be restricted to the neotropics. A putative myrmicine ant dietary source has been established for decahydroquinolines.[5,17,18,42] Some of the major decahydroquinolines, such as **195A**, **219A**, **243A**, and **269AB**, can occur in dendrobatid skins at levels as much as 50 μg per frog. There have been limited reports on the toxicity of decahydroquinolines. A minimal lethal dose in mice for *cis*-**195A** and **219A** is over 250 μg per mouse. Several of the decahydroquinolines have activity as noncompetitive blockers of nicotinic receptors (see ref 1).

Other Quinolines. In 1993, the decahydroquinoline *cis*-**195A** was reported from a mantellid frog along with a tetrahydroquinoline (**189**) and an octahydroquinoline (**193D**).[14] The proposed structures (Figure 9) were based

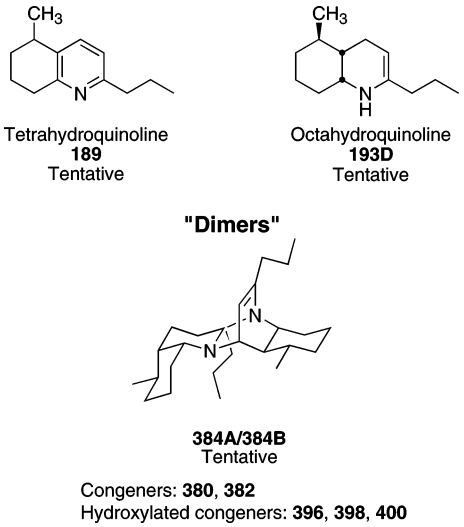
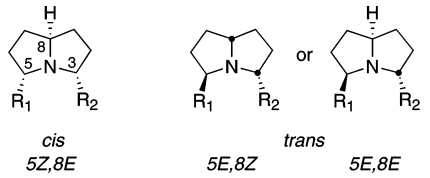


Figure 9. Other quinolines. Structures are tentative. An ant origin is likely.

on mass spectra and FTIR spectra. In addition, two closely related alkaloids, **384A** and **384B**, were reported from mantellid frogs.[14] Tentative structures for the **384A** and **384B** alkaloids were proposed in 1999,[1] on the basis of mass and FTIR spectral and NMR spectroscopic analyses. A proposed structure is shown in Figure 9. Such "dimeric" compounds appear likely to represent Diels–Alder adducts of a hexahydroquinoline with an octahydroquinoline, such as **193D**. They are not isolation artifacts, since they also occur in crude methanol skin extracts (unpublished results). Several congeners have been detected. The mass spectra of both **384A** and **384B** are dominated by loss of a C_3H_7 moiety to yield a base peak at *m/z* 341. With CI mass spectrometry, the protonated species appears to undergo reversal to ions of *m/z* 191 and 193, not an unexpected reversal for such a Diels–Alder adduct. These quinolines occur in certain populations of mantellid frogs, but only in minor or trace amounts. Recently, the **384A/384B** pair has been detected in dendrobatids (unpublished results).

Pyrrolizidines. The occurrence of 3,5-disubstitituted pyrrolizidines in anuran skin was first reported in 1993 for a bufonid (*Melanophryniscus*) toad.[8] Such pyrrolizidines had been known to occur in myrmicine ants since 1980,[43] and one of the anuran pyrrolizidines (*cis*-**223H**) proved to be identical with a pyrrolizidine from a thief ant (*Diplorhoptrum*) (unpublished results). At present about 26 alkaloids, including stereoisomers, are assigned to the 3,5-disubstituted pyrrolizidine class (Figure 10). Both *cis*- and *trans*-isomers occur for several of these pyrrolizidines. The absolute configuration is known only for *cis*-**223H**, where the alkaloids from ant and frog skin have the same absolute configuration (unpublished results).

The mass spectra of such pyrrolizidines are dominated by loss of one or the other of the α-substituents. The loss of a higher alkyl α-substituent is greatly favored over loss of an α-methyl substituent. A weak Bohlmann band or lack thereof in the vapor-phase FTIR spectrum provides evidence for *cis*- versus *trans*-isomers. The *cis*-(5*Z*,8*E*) isomer has a weak Bohlmann band, which is at about 2803 cm^{-1}. Neither of the two *trans*-isomers has a Bohlmann band. It is not certain, however, which of the two *trans*-isomers is present in anuran extracts or if both may occur. The order of emergence on gas chromatography of the *cis*- and *trans*-isomers found in extracts is not consistent. The *cis*-isomer in most cases (**223B**, **223H**, **239K**) emerges first, while the *trans*-isomer in one case (**251K**) emerges first. The *cis*-



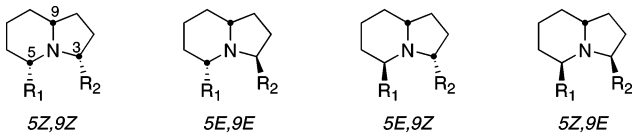
	3,5-P	R_1	R_2
	167F	CH_3	*n*-C_3H_7
cis	**195F**	CH_3	*n*-C_5H_{11}
	209K	CH_3	*n*-C_6H_{13}
	209Q	C_3H_5O	*n*-C_3H_7
cis / trans	**223B**	*n*-C_4H_9	*n*-C_4H_9
cis / trans	**223H***	CH_3	*n*-C_7H_{15}
cis / trans	**223M**	*n*-C_3H_7	*n*-C_5H_{11}
cis	**237G**	CH_3	$(CH_2)_4COCH_2CH_3$
	237R	CH_3	$C_7H_{13}O$ (OH)
cis / trans	**239K**	CH_3	$C_7H_{15}O$ (OH)
	239R	*n*-C_3H_7	$C_5H_{11}O$ (OH)
	239Y	*n*-C_4H_9	C_4H_9O (OH)
	247I	C_4H_7	C_6H_{11}
	249I	*n*-C_4H_9	C_6H_{11}
	249X	*n*-C_3H_7	C_7H_{13}
cis / trans	**251K**	*n*-C_4H_9	*n*-C_6H_{13}
trans	**251O**	*n*-C_3H_7	*n*-C_7H_{15}
cis / trans	**265J**	*n*-C_4H_9	$C_8H_{11}O$ (=O)
	265W	*n*-C_3H_7	$C_7H_{13}O$
cis (2)	**267H**	*n*-C_3H_7	$C_7H_{15}O$ (OH)

Figure 10. 3,5-Disubstituted pyrrolizidines. *Absolute configuration of *cis*-**223H** as shown (positive rotation). Two *trans*-isomers of these pyrrolizidines (5*E*,8*Z* and 5*E*,8*E*) may occur. A myrmicine ant origin is proposed.[18]

(5*Z*,8*Z*) isomers have not been detected in either ants or anurans. A synthetic *cis*-(5*Z*,8*Z*) isomer has a moderate Bohlmann band. The nomenclature refers to the *zusammen* (*Z*) or *entgegen* (*E*) configuration of hydrogens at C-5/C-8 relative to the hydrogen at C-3.

3,5-Disubstituted pyrrolizidine alkaloids occur fairly often in alkaloid-containing dendrobatid, mantellid, and bufonid anurans, but only in minor or trace amounts. The dietary source is undoubtedly myrmicine ants.[18,42] Toxicity apparently has not been investigated.

3,5-Disubstituted Indolizidines. At least three classes of indolizidines occur in alkaloid-containing anurans. The 3,5-disubstituted indolizidine class was first discovered in dendrobatid frogs, and a structure for **223AB** was postulated in 1978[2] and confirmed in 1981.[44] Indolizidine **223AB** was first incorrectly thought to be an unseparable mixture of two alkaloids. All four diastereomers have been found in anuran skin extracts. Remarkably, the isomer isolated from one dendrobatid frog (*Dendrobates histrionicus*) was the 5*E*,9*E* isomer,[45] while the isomer isolated from another frog (*Dendrobates speciosus*) was the 5*Z*,9*Z* isomer.[34] Another 3,5-disubstituted indolizidine, 5*E*,9*E*-**195B**, was reported from a dendrobatid frog in 1986.[46] This alkaloid was an isomer of a 5*Z*,9*Z*-alkaloid, previously described from a myrmicine ant and named in 1973 as monomorine I.[47] All four isomers of **195B** have been detected in anuran skin extracts. Currently, there are nearly 30 alkaloids, including stereoisomers, from anuran skins that are assigned to the 3,5-disubstituted indolizidine class. The structures, some definitive and some tentative, are shown in Figure 11. The absolute configurations of the natural 5*E*,9*E* isomers of **223AB**, **239AB**, and **239CD** are known.



	3,5-I	R_1	R_2
5Z,9Z	**167E**	CH_3	C_2H_5
	181A	C_2H_5	C_2H_5
all four	**195B** **	CH_3	*n*-C_4H_9
5E,9E	**211E**	CH_3	C_4H_9O (OH)
5E,9E	**221H**	*n*-C_3H_7	C_4H_7[a]
all four	**223AB** *	*n*-C_3H_7	*n*-C_4H_9
	223R	CH_3	*n*-C_6H_{13}
	223Z	*n*-C_5H_{11}	C_2H_5
	237E	C_5H_9O (OH)	C_2H_5
5E,9E	**239AB** *	$(CH_2)_3OH$	*n*-C_4H_9
5E,9E	**239CD** *	*n*-C_3H_7	$(CH_2)_4OH$
	239E	$C_5H_{11}O$ (OH)	C_2H_5
5Z,9Z	**239Q**	*n*-C_3H_7	C_4H_9O (OH)
5E,9E	**247C**	$(CH_2)_3CH{=}CH_2$	$(CH_2)_2CH{=}CH_2$
5Z,9Z	**249A**	$(CH_2)_3CH{=}CH_2$	*n*-C_4H_9
	249R	*n*-C_3H_7	C_6H_{11}
	253T	*n*-C_4H_9	C_4H_9O (OH)
	265M	C_5H_9	C_4H_9O (OH)
5E,9E	**271F**	$(CH_2)_3C{\equiv}CH$	$(CH_2)_4C{\equiv}CH$
5Z,9Z	**275C**	$(CH_2)_3CH{=}CH_2$	$(CH_2)_4CH{=}CH_2$

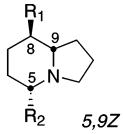
[a]Proposed to have a *trans* double bond.

Figure 11. 3,5-Disubstituted indolizidines. *Absolute configuration as shown for the 5*E*,9*E* isomer of **223AB**, **239AB**, and **239CD** (negative rotations). **Absolute configuration is opposite that shown for 5*E*,9*E*-**195B** (positive rotation). Absolute configurations of other 3,5-disubstituted indolizidines are unknown. All side chains at present are assumed to be unbranched. A myrmicine ant origin is proposed.[18]

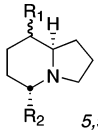
The mass spectra of 3,5-disubstituted indolizidines are dominated by cleavage of one or the other of the two α-substituents. Often there is also a significant fragment ion at *m/z* 124 as a result of loss of both of the α-substituents. Collision-activated CI mass spectra provide evidence as to the ring (five- or six-membered) on which each substituent resides.[6] The vapor-phase FTIR spectra can be diagnostic for the relative configuration of hydrogens at C-3, C-5, and C-9. Indolizidines with all three hydrogens *zusammen* (5*Z*,9*Z*) have a broad complex Bohlmann band pattern with the major absorption at 2791 cm^{-1}. The 5*E*,9*Z* isomer and the 5*E*,9*E* isomer have weak Bohlmann bands at 2803 and 2796 cm^{-1}, respectively, while the 5*Z*,9*E* isomer has virtually no Bohlmann band.

The 3,5-disubstituted indolizidines occur randomly in extracts of dendrobatid (primarily *Dendrobates*), mantellid (*Mantella*), and bufonid (*Melanophrynisus*) anurans, where they are usually minor or trace alkaloids. Myrmicine ants are undoubtedly the dietary source.[18] There is virtually no toxicity data for the 3,5-disubstituted indolizidines. Indolizidine **239CD** has a minimum lethal dose for mice that is greater than 200 μg per mouse. Such indolizidines are noncompetitive blockers of nicotinic receptors (see ref 1).

5,8-Disubstituted Indolizidines. The structures of the first 5,8-disubstituted indolizidines (**205A**, **235B**″) were described in 1987 on the basis of NMR spectroscopic analyses.[39] At present, the 5,8-disubstituted indolizidine class with about 80 examples, including stereoisomers, represents the largest class of alkaloids found in anuran skin (Figure 12). Some structures are rigorously defined, while others, based on mass spectral and in some cases



5,9Z



5,9E

	5,8-I	R_1	R_2
	167A	CH_3	C_2H_5
	181B	CH_3	*n*-C_3H_7
5,9Z	**193E**	C_2H_5	$CH_2CH{=}CH_2$
	195I	CH_3	*n*-C_4H_9
5,9Z	**197C**	CH_2OH	*n*-C_3H_7
5,9Z > 5,9E	**203A** *	CH_3	$CH_2CH{=}CHC{\equiv}CH$
5,9Z	**205A** *	CH_3	$(CH_2)_3C{\equiv}CH$
5,9Z	**207A** *	CH_3	$(CH_2)_3CH{=}CH_2$
	207Q	*n*-C_3H_7	$CH_2CH{=}CH_2$
5,9Z	**209B**	CH_3	*n*-C_5H_{11}
5,9Z	**209I** **	*n*-C_3H_7	*n*-C_3H_7
	209S	CH_3	C_4H_7O
5,9Z	**217B**	C_2H_5	$CH_2CH{=}CHC{\equiv}CH$
5,9Z	**219F**	C_2H_5	$(CH_2)_3C{\equiv}CH$
	219J	C_4H_5	*n*-C_3H_7
5,9Z	**219L**	C_2H_5	$CH_2CH{=}CHCH{=}CH_2$
	221A	CH_3	$(CH_2)_4CH{=}CH_2$
5,9Z	**221I**	C_2H_5	$(CH_2)_2CH{=}CHCH_3$
	221K	*n*-C_4H_9	$CH_2CH{=}CH_2$
5,9Z	**221Y**	C_2H_5	$(CH_2)_3CH{=}CH_2$
	223D	CH_3	*n*-C_6H_{13}
5,9Z	**223J***	*n*-C_3H_7	*n*-C_4H_9
5,9Z	**223V**	*n*-C_4H_9	*n*-C_3H_7
	223AA	C_2H_5	*n*-C_5H_{11}
	225D	CH_3	$C_5H_{11}O$ (OH)
	225M	C_2H_5	C_4H_9O (OH)
5,9Z	**231C**	CH_3	$(CH_2)_3CH{=}CHC{\equiv}CH$
	231G	CH_3	C_7H_9
5,9Z	**233D**	CH_3	$(CH_2)_3CH{=}CHCH{=}CH_2$
5,9Z	**233M**	CH_3	$(CH_2)_5C{\equiv}CH$
5,9Z	**235B'** *	CH_3	$(CH_2)_5CH{=}CH_2$
5,9Z	**235B"** **	CH_3	$(CH_2)_3CH{=}CHCH_2CH_3$
	235Z	C_4H_5	C_3H_7O (OH)
5,9Z	**237D** *	CH_3	*n*-C_7H_{15}
5,9Z	**237H**	C_2H_5	C_5H_9O (OH)
5,9Z	**239C**	C_4H_9O (OH)	*n*-C_3H_7
	239D	*n*-C_3H_7	C_4H_9O (OH)
	239G	CH_3	$C_6H_{13}O$ (OH)
	239U	C_2H_5	$C_5H_{11}O$ (OH)

	5,8-I	R_1	R_2
5,9Z	**241F**	$(CH_2)_2C{\equiv}CH$	$CH_2CH{=}CHC{\equiv}CH$
	243B	C_4H_5	C_5H_7
5,9Z	**243C**	$(CH_2)_2CH{=}CH_2$	$CH_2CH{=}CHC{\equiv}CH$
5,9Z	**243D**	C_2H_5	$CH{=}CHCH_2CH{=}CHC{\equiv}CH$ *E,Z*
5,9Z	**245B**	$(CH_2)_2CH{=}CH_2$	$(CH_2)_3C{\equiv}CH$
5,9Z	**245C**	C_2H_5	$CH{=}CH(CH_2)_3C{\equiv}CH$ *E*
	245D	CH_3	$(CH_2)_4CH{=}CHC{\equiv}CH$
	245I	C_2H_5	$(CH_2)_3CH{=}CHC{\equiv}CH$
	245N	C_4H_5	C_5H_9
5,9Z	**247E**	C_4H_7	$(CH_2)_3CH{=}CH_2$
5,9Z	**247F**	C_2H_5	$(CH_2)_5C{\equiv}CH$
5,9Z	**249J**	C_4H_5	C_4H_9O (OH)
	249L	CH_3	$C_7H_{11}O$
	249O	$(CH_2)_2CH{=}CH_2$	*n*-C_5H_{11}
5,9Z	**251B** **	CH_3	$(CH_2)_3CH{=}CHCHOHCH_3$ *Z*
5,9Z > 5,9E	**251N**	*n*-C_4H_9	*n*-C_5H_{11}
5,9Z	**251U**	CH_3	$C_7H_{13}O$ (=O)
5,9Z	**253B**	CH_3	$C_7H_{15}O$ (OH)
	257C	CH_3	C_9H_{11}
5,9E	**259B**	CH_3	$C_5H_{10}CH{=}CHC{\equiv}CH$
	261D	CH_3	C_9H_{15}
	263F	CH_3	C_9H_{17}
	263K	C_4H_9O (OH)	C_5H_7
	263P	C_2H_5	$C_7H_{11}O$
	265P	*n*-C_4H_9	*n*-C_6H_{13}
	267E	C_4H_9O (OH)	C_4H_7O (=O)
	267S	*n*-C_4H_9	$C_5H_{11}O$ (OH)
	269H	CH_3	$C_7H_{15}O_2$ (2 OH)
	269I	CH_3	$C_7H_{15}O_2$ (CH_2OH, OH)
5,9Z	**271A**	$(CH_2)_2CH{=}CH_2$	$(CH_2)_3CH{=}CHC{\equiv}CH$
	273B	$(CH_2)_2CH{=}CH_2$	$(CH_2)_3CH{=}CHCH{=}CH_2$
	273C	C_2H_5	C_9H_{13}
	275F	$(CH_2)_2CH{=}CH_2$	$(CH_2)_5CH{=}CH_2$
5,9Z	**279D**	CH_3	$C_9H_{17}O$ (OH)
	281I	CH_3	$C_9H_{19}O$ (OH)
	281O	$C_4H_9O_2$ (2 OH)	C_5H_9
	291H	C_4H_7	$C_7H_{13}O$
	295A	CH_3	$C_{10}H_{21}O$ (OH)
	297E	$CH{=}CH_2$	$C_7H_{15}O_3$

Figure 12. 5,8-Disubstituted indolizidines. *Absolute configuration as shown (negative rotations). The configuration at C-8 in the other 5,9*Z*-indolizidines is assumed to be as shown. **Absolute configuration opposite that shown (positive rotations). A 5,9*Z* configuration is indicated by a strong, sharp Bohlmann band. A 5,9*E* configuration is indicated by a weak Bohlman band. An ant origin is likely.

supplemented with FTIR spectral data, must be considered tentative. Several of the 5,8-disubstituted indolizidines (**203A**, **205A**, **207A**, **223J**, **223V**, **235B′**, **235B″**, **237D**, **251B**) have been synthesized (refs 1, 19, 48−50 and citations therein). The absolute configurations of six of these indolizidines have been established[48−50] (see Figure 12), but the presence of both enantiomers is possible.

The mass spectra of 5,8-disubstituted indolizidines are dominated by a base peak due to loss of the α-substituent at the 5-position. Many have a methyl group at the 8-position and, thus, have a base peak at *m/z* 138. A subsequent retro-Diels−Alder elimination yields a diagnostic ion at *m/z* 96 for all 5,8-disubstituted indolizidines. The vapor-phase FTIR spectra permit assignment of the relative configuration of the hydrogens at C-5 and C-9.[11] Thus, a strong, sharp Bohlmann band at about 2789 cm^{-1} permits assignment of the 5,9*Z* configuration to most of these indolizidines. Only the alkaloid **259B** of those assigned to the 5,8-disubstituted indolizidine class exhibits a weak Bohlmann band (2810 cm^{-1}) and, therefore, has been proposed to have a 5,9*E* configuration. Alkaloid **223I**, originally proposed to be a 5,8-disubstituted 5,9*E*-indolizidine,[1] is now considered (see Figure 20) to be a pyrrolizidine.[49] The orientation of the 8-substituent for certain 5,9*Z*-indolizidines is equatorial, on the basis of NMR spectroscopic analysis or synthesis for those alkaloids, while the CH_3 substituent at C-8 for 5,9*E*-**259B** could be either equatorial or axial.

5,8-Disubstituted indolizidines occur commonly in dendrobatid and mantellid (*Mantella*) frogs. They are rather uncommon in bufonid (*Melanophryniscus*) toads. In some extracts, amounts of certain 5,8-disubstituted indolizidines, such as **235B″**, can reach levels of 100 μg per frog, but in most cases they are minor or trace alkaloids. A dietary source has not been identified, but **205A** and **235B″** were present in extracts of mixed collections of leaf-litter arthropods, most of which contained ants.[51] In view of the recent detection of a 5,6,8-trisubstituted indolizidine in an ant (unpublished results) and in an oribatid mite,[35b] it seems likely that ants/mites are the dietary sources of both 5,8-disubstituted and 5,6,8-trisubstituted indolizidines. Toxicity data for such indolizidines have not been reported. Several have been reported as potent noncompetitive blockers of nicotinic receptors (see ref 1). Synthetic **235B′** has been reported to be a particularly potent and selective blocker of the $\alpha_4\beta_2$ neuronal nicotinic receptor.[52]

6,7-Dehydro-5,8-Disubstituted Indolizidines. A new major class of indolizidine alkaloids is now proposed, based on mass spectral and vapor-phase FTIR spectral data. In some cases, namely, alkaloid **245F** and **245H** of this class, catalytic hydrogenation yielded a perhydro derivative that had the mass spectra of the expected 5,8-disubstituted indolizidine, i.e., a base peak at *m/z* 152 or 180, respectively, and a diagnostic ion at *m/z* 96. There are currently about 30 alkaloids assigned to the dehydro-5,8-disubstituted indolizidine class (Figure 13).

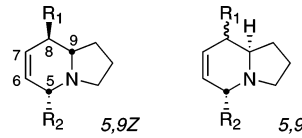
The mass spectra of the dehydro-5,8-indolizidines are characterized by a pair of fragment ions at *m/z* 136 and 134 when R_1 is methyl, at *m/z* 150 and 148 when R_1 is ethyl, at *m/z* 164 and 162 when R_1 is propyl, etc. After loss of the α-substituent at C-5, the resulting ion at *m/z* 136, 150, 164, etc., apparently undergoes aromatization by loss of two hydrogens. A significant fragment ion at *m/z* 120 is always present, presumably due to aromatization by loss of the R_1 substituent and a hydrogen rather than two hydrogens. In some cases it is accompanied by an ion at *m/z* 134. The vapor-phase FTIR spectra of most of the indolizidines of this class exhibit a moderate, sharp Bohlmann band at 2787 cm^{-1}, indicating a 5,9*Z* configuration. There also may be similar 6,7-dehydro-5,6,8-trisubstituted indolizidines and 2,3-dehydro-1,4-disubstituted quinolizidines (see "Other Izidines", below).

The dehydro-5,8-disubstituted indolizidines occur relatively commonly as minor or trace alkaloids in dendrobatid, mantellid, and bufonid anurans. A dietary source is unknown, but ants appear a likely possibility. No toxicity or biological activity data have been reported.

5,6,8-Trisubstituted Indolizidines. The structure of the first member of this class (**223A**) was proposed in 1997,[53] on the basis of NMR spectroscopic analysis of material isolated from a dendrobatid frog (*Dendrobates pumilio*). Later, the proposed configuration at C-6 was corrected, on the basis of comparison with synthetic isomers.[54] At present about 70 alkaloids are assigned to this class (Figure 14). The structures of most are tentative, based only on mass spectral and in some cases FTIR spectral data. The absolute configuration of **223A** is known.

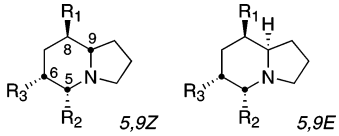
The mass spectra of the 5,6,8-trisubstituted indolizidines are dominated by a fragment resulting from the loss of the α-substituent at C-5. A retro-Diels–Alder reaction then provides a diagnostic ion at *m/z* 110 when R_3 is methyl, at *m/z* 124 when R_3 is ethyl, etc. The presence of a fragment at *m/z* 70, even when weak, then suggests an indolizidine. The 1,4-disubstituted quinolizidines fragment similarly to afford a base peak accompanied by a diagnostic ion at *m/z* 110, but will have a weak fragment at *m/z* 84 instead of one at *m/z* 70. The vapor-phase FTIR spectra of several of the proposed 5,6,8-trisubstituted indolizidines have a sharp, strong Bohlmann band at about 2784 cm^{-1}, indicating a 5,9*Z* configuration, as in **223A**.[53] However, several indolizidines assigned to this class have a weak Bohlmann band at 2811 cm^{-1}, indicating a 5,9*E* configuration. NMR spectroscopic analyses of one of the latter, namely, **249H**, confirmed the relative ring configuration.[55] The conformation was of the less common *cis*-ring fusion; that is, the *N*-lone pair and the H-9 were on the same face. Remarkably, the six-carbon alkenyl substituent of **249H** had a branched chain, the first and, as yet, only documented example of a branched chain in an izidine from anuran skin extracts.

5,6,8-Trisubstituted indolizidines, in particular **223A** and **231B**, are relatively common in dendrobatid frogs, where levels can reach as high as 50 μg per frog. Most of the other alkaloids of this class occur only in minor or trace amounts. Such alkaloids occur commonly in mantellid (*Mantella*) frogs, but rather rarely in bufonid (*Melanophryniscus*) toads. A 5,6,8-trisubstituted indolizidine has been detected in a myrmicine ant (unpublished results) and another such



	De-5,8-I	R_1	R_2
	179	CH_3	*n*-C_3H_7
	191H	CH_3	C_4H_7
	201A	CH_3	$CH_2CH{=}CHC{\equiv}CH$
5,9Z	**207E**	*n*-C_3H_7	*n*-C_3H_7
5,9Z	**219G**	*n*-C_3H_7	$CH_2CH{=}CHCH_3$
	221J	*n*-C_3H_7	*n*-C_4H_9
	221O	CH_3	C_5H_9O (=O)
5,9Z	**221V**	C_5H_9	CH_2OH
	231L	CH_3	C_7H_{11}
	233E	CH_3	$(CH_2)_5CH{=}CH_2$
	233J	*n*-C_4H_9	$(CH_2)_2CH{=}CH_2$
	235Q	CH_3	$C_6H_{11}O$ (=O)
	237P	CH_3	$C_6H_{13}O$ (OH)
	243F	C_2H_5	$(CH_2)_3CH{=}CHC{\equiv}CH$
5,9Z	**245F**	C_2H_5	$(CH_2)_5C{\equiv}CH$
5,9Z	**245H**	$(CH_2)_2C{\equiv}CH$	*n*-C_5H_{11}
	245L	C_4H_7	C_5H_9
5,9Z	**249K**	CH_3	$C_7H_{13}O$ (=O)
	249S	CH_2OH	C_6H_9O
	249T	CH_3	$C_7H_{13}O$ (OH)
	249W	*n*-C_4H_9	*n*-C_5H_{11}
5,9Z	**251P**	CH_3	$C_7H_{15}O$ (OH)
	263L	*n*-C_4H_9	C_5H_9O (=O)
	263O	C_2H_5	$C_7H_{13}O$
	265F	C_4H_9O (OH)	C_4H_7O
	265T	*n*-C_4H_9	$C_5H_{11}O$ (OH)
	265Y	$C_5H_{11}O$ (OH)	*n*-C_4H_9
5,9Z	**269D**	$(CH_2)_2CH{=}CH_2$	$(CH_2)_3CH{=}CHC{\equiv}CH$
	275D	C_2H_5	$(CH_2)_4CH{=}CH(CH_2)_2CH_3$

Figure 13. 6,7-Dehydro-5,8-disubstituted indolizidines. Absolute configurations unknown. Configurations shown are based on the 5,8-disubstituted indolizidines. A 5,9*Z* configuration is based on moderate to strong Bohlmann bands. A 5,9*E* configuration is based on weak Bohlmann bands. An ant origin is likely.



	5,6,8-I	R_1	R_2	R_3
	193G	CH_3	$CH_2CH{=}CH_2$	CH_3
	195D	C_2H_5	C_2H_5	CH_3
	195G	CH_3	*n*-C_3H_7	CH_3
	197G	C_2H_5O (OH)	CH_3	CH_3
	197H	CH_2OH	C_2H_5	CH_3
	207C	CH_3	$(CH_2)_2CH{=}CH_2$	CH_3
	209C	CH_3	*n*-C_4H_9	CH_3
	209E	C_2H_5	*n*-C_3H_7	CH_3
	211L	CH_3	C_3H_7O (OH)	CH_3
	211M	C_3H_7O (OH)	CH_3	CH_3
	217G	CH_3	$CH_2CH{=}CHC{\equiv}CH$	CH_3
	219N	CH_3	C_5H_7	CH_3
	221P	$CH{=}CH_2$	*n*-C_3H_7	C_2H_5
	221Q	CH_3	$(CH_2)_3CH{=}CH_2$	CH_3
	221T	*n*-C_3H_7	$CH_2CH{=}CH_2$	CH_3
	221U	C_2H_5	$CH_2CH{=}CH_2$	C_2H_5
5,9Z	**223A** *	C_2H_5	*n*-C_3H_7	C_2H_5
	223C	CH_3	C_4H_7O	CH_3
	223X	C_2H_5	*n*-C_4H_9	CH_3
	225K	CH_2OH	*n*-C_4H_9	CH_3
	225L	CH_3	C_4H_9O (OH)	CH_3
5,9Z	**231B**	CH_3	$(CH_2)_2CH{=}CHC{\equiv}CH$	CH_3
	231K	C_2H_5	$CH_2CH{=}CHC{\equiv}CH$	CH_3
5,9Z	**233G**	CH_3	$(CH_2)_4C{\equiv}CH$	CH_3
	233L	C_2H_5	C_5H_7	CH_3
5,9Z	**235E**	CH_3	$(CH_2)_2CH{=}CHCH_2CH_3$	CH_3
	237C	*n*-C_3H_7	*n*-C_4H_9	CH_3
	237L	C_2H_5	*n*-C_4H_9	C_2H_5
5,9E	**237M**	CH_2OH	C_5H_9	CH_3
	237N	$CH_2CH{=}CH_2$	CH_2CH_2OH	C_2H_5
	237S	CH_3	*n*-C_6H_{13}	CH_3
	239W	*n*-C_3H_7	CH_2OH	*n*-C_3H_7
	245G	CH_3	$(CH_2)_2CH{=}CHC{\equiv}CH$	C_2H_5
	247B	$CH_2CH{=}CH_2$	C_5H_9	CH_3
5,9E	**249C**	CH_3	C_7H_{13}	CH_3
5,9E	**249H** **	C_2H_5	$C(C_2H_5){=}CHC_2H_5$ *E*	CH_3

	5,6,8-I	R_1	R_2	R_3
	249U	CH_3	C_6H_{11}	C_2H_5
	251M	C_2H_5	*n*-C_5H_{11}	C_2H_5
	251S	CH_3	$C_6H_{11}O$ (OH)	CH_3
	251T	CH_3	C_7H_{15}	CH_3
	251V	*n*-C_3H_7	*n*-C_3H_7	*n*-C_3H_7
	253H	C_2H_5O (OH)	*n*-C_4H_9	C_2H_5
	253K	CH_3	$C_6H_{13}O$ (OH)	CH_3
	253P	C_3H_7O (OH)	*n*-C_3H_7	C_2H_5
	253V	C_2H_5	$C_5H_{11}O$ (OH)	CH_3
	257E	C_4H_7	$CH_2CH{=}CHC{\equiv}CH$	CH_3
5,9E	**259C**	CH_3	$(CH_2)_4CH{=}CHC{\equiv}CH$	CH_3
	261B	CH_3	C_8H_{13}	CH_3
5,9E	**263A**	CH_3	C_8H_{15}	CH_3
	263D	C_2H_5	C_6H_{11}	C_2H_5
	265I	C_2H_5	$C_6H_{11}O$	CH_3
5,9E	**265L**	CH_3	$C_7H_{13}O$ (OH)	CH_3
	265O	CH_2OH	C_7H_{13}	CH_3
5,9E	**265U**	CH_3	$C_7H_{13}O$ (OH)	CH_3
	267J	C_2H_5O (OH)	*n*-C_5H_{11}	C_2H_5
	267R	CH_2OH	*n*-C_7H_{15}	CH_3
	267T	C_2H_5O (OH)	*n*-C_3H_7	*n*-C_4H_9
5,9Z	**267U**	C_3H_7O (OH)	*n*-C_3H_7	*n*-C_3H_7
	267W	CH_3	$C_6H_{11}O_2$	CH_3
5,9Z	**273A**	CH_3	$(CH_2)_5CH{=}CHC{\equiv}CH$	CH_3
5,9E	**275E'**	CH_3	$(CH_2)_5CH{=}CHCH{=}CH_2$	CH_3
5,9E	**275E"**	CH_3	$(CH_2)_7C{\equiv}CH$	CH_3
	277C	*n*-C_4H_9	C_5H_9	C_2H_5
5,9E	**277E'**	CH_3	$(CH_2)_7CH{=}CH_2$	CH_3
5,9E	**277E"**	CH_3	C_9H_{17}	CH_3
	279F	CH_3	$C_8H_{15}O$ (OH)	CH_3
5,9E	**281H**	CH_3	$C_8H_{17}O$ (OH)	CH_3
	281M	C_3H_5O (OH)	$C_5H_{11}O$ (OH)	CH_3
5,9E	**293C**	CH_3	$C_9H_{17}O$ (OH)	CH_3
	341C	$C_7H_{15}O_2$	C_4H_9O (OH)	CH_3
5,9E	**353B**	$C_7H_{15}O$ (OH) or $C_6H_{11}O_2$	$C_4H_7O_2$ or $C_5H_{11}O$ (OH)	C_2H_5

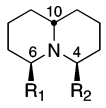
Figure 14. 5,6,8-Trisubstituted indolizidines. *Absolute stereochemistry proposed to be as shown. Alkaloid **249H** is anomalous among izidines in having a branched side-chain. **The relative configuration is as shown. The configuration at C-8 in the other indolizidines is assumed to be as shown. A 5,9*Z* configuration is based on a strong Bohlmann band and analogy to **223A**. A 5,9*E* configuration is based on a weak Bohlmann band with configuration at C-6 and C-8 not certain. An ant or mite origin is likely.

indolizidine in an oribatid mite,[35b] and thus, ants/mites represent likely sources for this class of izidines. No toxicity or biological activity data have been reported for these alkaloids.

4,6-Disubstituted Quinolizidines. A structure for the first of the 4,6-disubstituted quinolizidines, namely, **195C**, was reported in 1999.[18] The relative configuration was established by comparison with the four synthetic diastereomers. At present only six alkaloids are assigned to a 4,6-disubstituted quinolizidine class (Figure 15). The structures of all, except **195C**, are tentative, being based on mass spectral and in two cases FTIR spectral analyses. The absolute configuration of **195C** is unknown.

The mass spectra of 4,6-disubstituted quinolizidines are dominated by fragment ions resulting from loss of one or the other of the substituents at C-4 and C-6. The vapor-phase FTIR spectra of **195C**, **251Y**, and **275I** have weak Bohlmann bands at about 2813 cm^{-1}. Quinolizidine **195C** has hydrogens in a 6*Z*,10*E* configuration relative to the hydrogen at C-4, which is consonant with the weak Bohlmann band.[18]

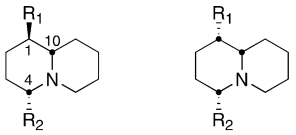
Quinolizidine **195C** is found not uncommonly in dendrobatid frogs and in mantellid frogs as a minor or trace alkaloid. The others of this class occur rarely: **223S** in a mantellid, **237I** in a dendrobatid, and **275I** and **279H** in a bufonid. Quinolizidine **195C** was a major alkaloid from a myrmicine ant (*Diplorhoptrum*),[18] and thus an ant dietary source was proposed. The frog skin and the ant **195C** were the same enantiomer on the basis of separation of racemic **195C** and comparison of retention times on chiral GC



6Z,10E

	4,6-Q	R_1	R_2
6Z,10E	**195C** **	CH_3	*n*-C_3H_7
	223S	C_2H_5	*n*-C_4H_9
	237I	*n*-C_4H_9	*n*-C_3H_7
6Z,10E	**251Y**	*n*-C_4H_9	*n*-C_4H_9
6Z,10E	**275I**	$(CH_2)_3CH{=}CH_2$	$(CH_2)_3CH{=}CH_2$
	279H	C_5H_{11}	C_5H_{11}

Figure 15. 4,6-Disubstituted quinolizidines. Absolute configurations unknown. **Relative configuration as shown. Configurations of the other 4,6-Q's are unknown. A myrmicine ant origin is proposed.[18]



1,4-*trans* 1,4-*cis*

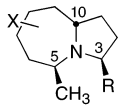
	1,4-Q	R_1	R_2
1,4-*cis*	**207I** *	C_2H_5	$CH_2CH{=}CH_2$
	211Q	C_3H_7O	CH_3
1,4-*trans*	**217A** *	CH_3	$CH_2CH{=}CHC{\equiv}CH$
	219B	CH_3	C_5H_7
	221X	C_2H_5	C_4H_7
1,4-*trans*	**231A**	C_2H_5	$CH_2CH{=}CHC{\equiv}CH$
1,4-*trans*	**233A** *	C_2H_5	$(CH_2)_3C{\equiv}CH$
	233I	C_4H_5	*n*-C_3H_7
	235U	C_2H_5	$(CH_2)_3CH{=}CH_2$
	235W	C_4H_7	*n*-C_3H_7
	237T	CH_3	*n*-C_6H_{13}
1,4-*trans*	**247D**	C_2H_5	$(CH_2)_4C{\equiv}CH$
	251AA	C_2H_5	*n*-C_6H_{13}
	255B	$(CH_2)_2C{\equiv}CH$	$CH_2CH{=}CHC{\equiv}CH$
1,4-*trans*	**257D**	$(CH_2)_2C{\equiv}CH$	$(CH_2)_3C{\equiv}CH$
	259E	C_4H_7	C_5H_7
	263H	CH_3	$(CH_2)_6CH{=}CH_2$
	265C	C_4H_9O (OH)	C_4H_7
	277H	CH_3	$(CH_2)_7CH{=}CH_2$
	279E	C_4H_9O (OH)	C_5H_9
1,4-*trans*	**289F**	CH_3	$C_9H_{13}O$ (OH)
1,4-*trans*	**293L**	CH_3	$C_9H_{17}O$ (OH)
	295E	*n*-C_5H_{11} or C_4H_7O	$C_4H_7O_2$ or $C_5H_{11}O$ (OH)

Figure 16. 1,4-Disubstituted quinolizidines. *Absolute configurations as shown (negative rotations). Relative configuration (1,4-*trans*) is based on FTIR spectral data. An ant or mite origin is likely.

columns (unpublished results). No toxicity or biological activity data have been reported.

1,4-Disubstituted Quinolizidines. The structure of the parent member (**217A**) of the 1,4-disubstituted quinolizidine class was established by NMR spectral analyses of material isolated from a mantellid frog (*Mantella baroni*) and reported in 1996.[56] At present about 20 alkaloids are assigned to this class (Figure 16). Structures of most are tentative, being based only on mass spectral and in some cases FTIR spectral data. The absolute configurations of **207I**, **217A**, and **233A** are known.[19,57a,b]

The mass spectra of these quinolizidine alkaloids are dominated by a fragment resulting from α-cleavage of the



Lehm	R	X
275A **	$(CH_2)_7C{\equiv}CH$	H
275G	$(CH_2)_5CH{=}CHCH{=}CH_2$	H
277A	$(CH_2)_7CH{=}CH_2$	H
289A	$C_9H_{13}O$ (=O, C≡CH)	H
289D	$(CH_2)_5CH{=}CHC{\equiv}CH$	OH
291C	$C_9H_{15}O$	H
291F	$(CH_2)_7C{\equiv}CH$	OH
293F	$C_9H_{17}O$ (=O)	H
293I	$(CH_2)_7CH{=}CH_2$	OH

Figure 17. Lehmizidines. Absolute configuration unknown. **Relative configuration as shown. Other lehmizidines postulated to have the same relative configuration. Positions of ring OH groups are unknown. A myrmicine ant source is likely.

substituent at C-4. A retro-Diels−Alder process then yields a diagnostic ion at *m/z* 110. A small peak at *m/z* 84 confirms that the alkaloid is a quinolizidine, not an indolizidine. The vapor-phase FTIR spectra of these quinolizidines have a Bohlmann band at 2790 cm^{-1} that is broader and less intense than the band of the 5,8-disubstituted or 5,6,8-trisubstituted indolizidines. Quinolizidines **217A** and **233A** have the hydrogens in a 4,10*Z* configuration and the 1- and 4-substituent in a *trans*-configuration.[19] It is assumed that **231A** and **247D** also have the same relative configuration. Upon comparison to synthetic isomers, it was demonstrated that **207I** has the 1- and 4-substituents in a *cis*-configuration.[18,19]

Certain of the 1,4-disubstituted quinolizidines, in particular **217A**, **231A**, and **233A**, are relatively common in certain mantellid frogs and can occur at levels of up to 50 μg per frog. Alkaloids **219B**, **231A**, and **233A** have been detected in both dendrobatid and mantellid frogs. Most of the others occur as trace alkaloids very rarely and only in dendrobatid frogs. None have been reported from bufonid toads. A 1,4-disubstituted quinolizidine recently was tentatively identified from an oribatid mite.[35b] No toxicity data have been reported. The synthetic C-1 epimer of **207I** and synthetic (+)-**207I** were noncompetitive blockers of nicotinic receptors.[52]

Lehmizidines. The structure, including relative configuration of an izidine, **275A**, at that time known only from one population of a Colombian dendrobatid frog (*Dendrobates lehmanni*), was finally established in 2001.[7] Such izidines were designated as lehmizidines after the frog species where they were discovered.[7] The ring system and substitution pattern were established by mass spectral analyses, while the relative configuration was established by comparison to the four synthetic diastereomers of perhydro-**275A**. At present nine alkaloids are assigned to the lehmizidine class (Figure 17). Structures of all, except **275A**, are tentative. The absolute configuration of **275A** is unknown.

The mass spectrum of lehmizidines consists almost entirely of a base peak due to α-cleavage of the substituent at C-3. There is a small fragment due to α-cleavage of the methyl at C-5. The ring systems and substitution pattern were revealed from the collision-activated dissociation (CAD) NH_3-CI mass spectrum of **275A**, which showed the nonynyl side chain on a five-membered ring and the methyl group on a seven-membered ring.[7] The FTIR spectrum of **275A** showed virtually no Bohlmann band.[7] The hydrogens

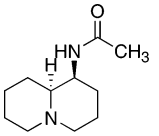


Figure 18. Epiquinamide. The absolute configuration of epiquinamide is not known. A dietary source is unknown.

at C-5 and C-10 were established as 5*Z*,10*E* relative to the hydrogen at C-3.[7]

Lehmizidine **275A** and congeners appear as minor alkaloids only in extracts of the dendrobatid frog *Dendrobates lehmanni*, which is a montane species of Western Colombia. However, trace amounts of lehmizidines now have been detected in some other dendrobatid species (unpublished results). A dietary source has not been discovered, but it appears likely that an ant species, most common in montane regions, will prove to be the source. No toxicity or biological activity data have been reported.

Epiquinamide. An unprecedented quinolizidine was reported in 2003 as a trace alkaloid in extracts from an Ecuadoran dendrobatid frog (*Epipedobates tricolor*).[58] The structure (Figure 18) was elucidated by mass and FTIR spectral and NMR spectroscopic analyses.

The mass spectrum of epiquinamide is dominated by a base peak at *m/z* 137 corresponding to loss of an acetamide moiety. The vapor-phase FTIR spectrum has moderate Bohlmann bands at 2802 and 2765 cm^{-1} and a strong amide carbonyl band at 1706 cm^{-1}.[58]

Epiquinamide is the only member of its class and has been detected in only one extract of a dendrobatid frog. A dietary source is not known. Epiquinamide was isolated by HPLC based on agonist activity at a nicotinic receptor.[58]

Other Izidines. A number of alkaloids detected in extracts of anuran skin have empirical formulas and mass spectra suggesting that they are further bicyclic izidines with either one or two substituents readily lost by α-cleavage. Some appear likely to have ring hydroxyl groups. The fragmentation patterns, however, are not commensurate with those expected of any of the pyrrolizidine, indolizidine, or quinolizidine classes shown in Figures 10−16. Two alkaloids, initially designated **167B** and **209D**, were proposed tentatively to be 5-monosubstituted indolizidines,[4] but during comparison of the natural alkaloids to the synthetic 5-substituted indolizidines corresponding to the proposed structures for **167B** and **209B** (Figure 19), the natural alkaloids were found to be 3,5-disubstituted pyrrolizidines and, therefore, were assigned new code designations of **167F** and **209K**.[1] The structures suggested for the remaining izidines of Figures 19−21 represent only possible gross structures for such alkaloids. Several appear to be dehydroizidines (Figure 19) that, like the dehydro-5,8-disubstituted indolizidines, exhibit a base peak due to α-cleavage followed by ready aromatization of the resultant fragment ion. The 6,7-dehydro-5,6,8-trisubstituted indolizidines are proposed on the basis of a significant fragment ion at *m/z* 134 rather than the *m/z* 120 ion typical of the dehydro-5,8-indolizidines. 2,3-Dehydro-1,4-disubstituted quinolizidines are also proposed. However, other structures than those proposed in Figures 19−21 are possible that also could be rationalized in terms of the mass spectral

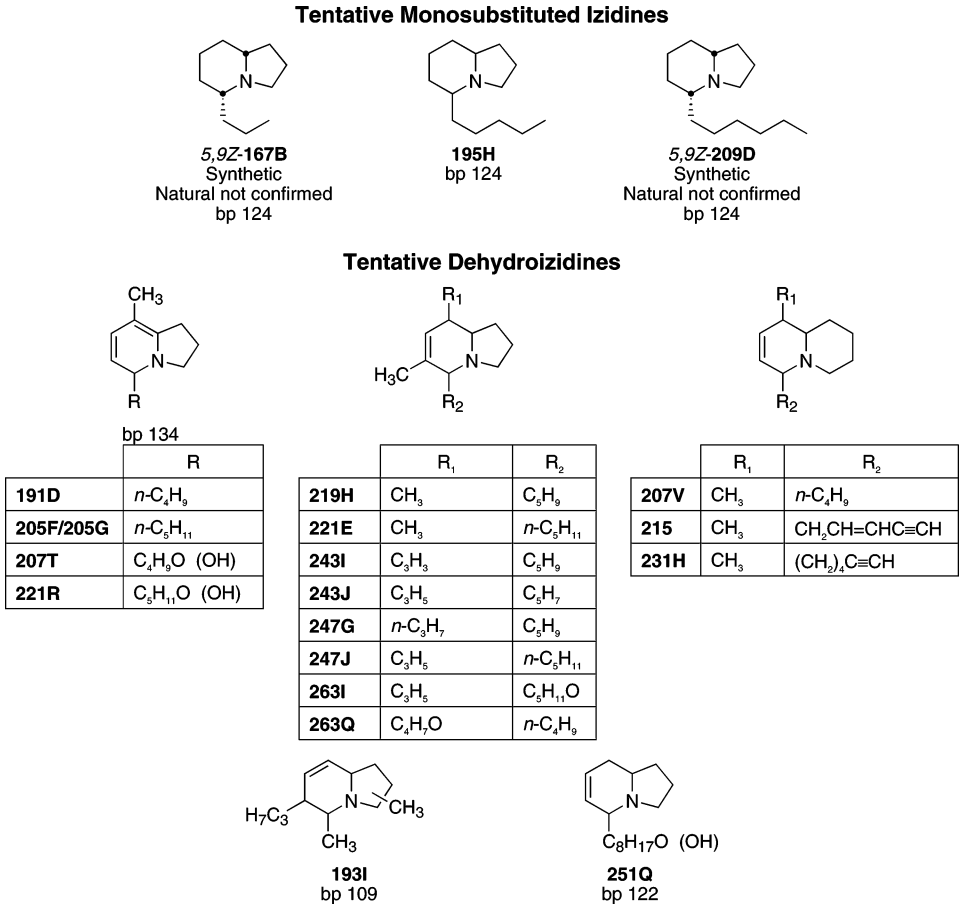
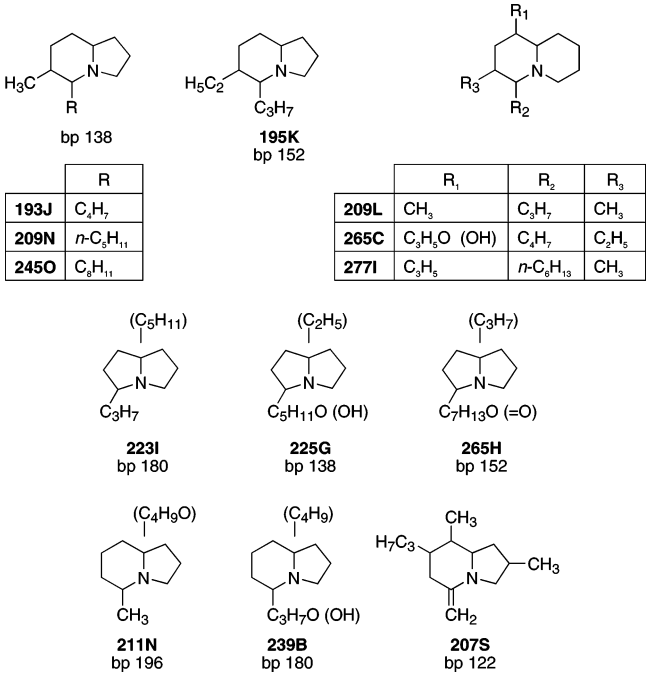


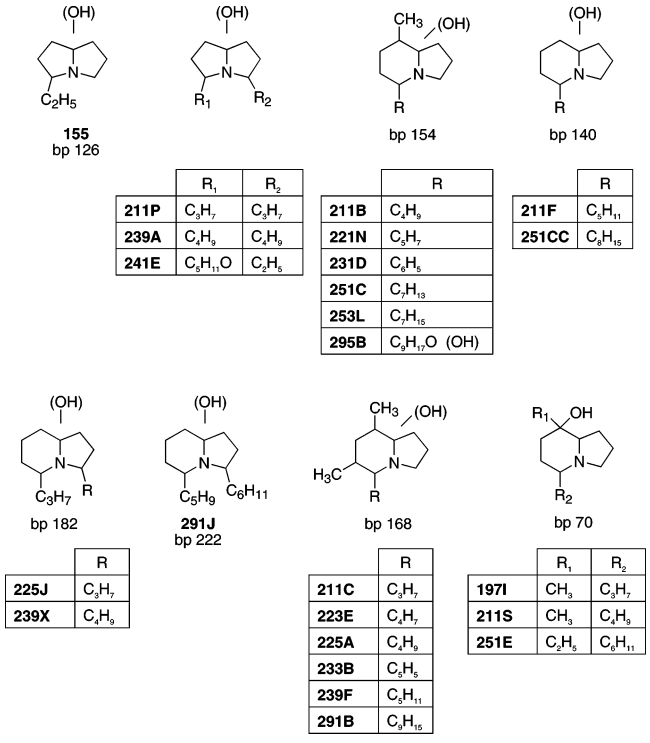
Figure 19. Possible gross structures for monosubstituted and dehydroizidines. The natural occurrence of proposed monosubstituted indolizidines **167B** and **209B** could not be confirmed on comparison to synthetic material. Instead, the proposed structures were incorrect and the alkaloids proved to be 3,5-disubstituted pyrrolizidines and were renamed **167F** and **209K** (see Figure 10). The structures of the dehydroizidines are hypothetical and are based on analogies and mass spectral data and in some cases vapor-phase FTIR spectral data. In some cases, mass spectral base peaks (bp) are indicated. Postulation as a dehydro-5,6,8-I or as a dehydro-1,4-Q for these alkaloids needs to be confirmed by analysis of perhydro derivatives.



	R
193J	C_4H_7
209N	*n*-C_5H_{11}
245O	C_8H_{11}

	R_1	R_2	R_3
209L	CH_3	C_3H_7	CH_3
265C	C_3H_5O (OH)	C_4H_7	C_2H_5
277I	C_3H_5	*n*-C_6H_{13}	CH_3

Figure 20. Possible gross structures for di-, tri-, and tetrasubstituted izidines. The structures are hypothetical and are based on analogies and mass spectral data and in some cases vapor-phase FTIR spectral data. The pyrrolizidine **223I** appears to be trisubstituted, as are perhaps other analogous alkaloids. Mass spectral base peaks (bp) are indicated.



	R_1	R_2
211P	C_3H_7	C_3H_7
239A	C_4H_9	C_4H_9
241E	$C_5H_{11}O$	C_2H_5

	R
211B	C_4H_9
221N	C_5H_7
231D	C_6H_5
251C	C_7H_{13}
253L	C_7H_{15}
295B	$C_9H_{17}O$ (OH)

	R
211F	C_5H_{11}
251CC	C_8H_{15}

	R
225J	C_3H_7
239X	C_4H_9

	R
211C	C_3H_7
223E	C_4H_7
225A	C_4H_9
233B	C_5H_5
239F	C_5H_{11}
291B	C_9H_{15}

	R_1	R_2
197I	CH_3	C_3H_7
211S	CH_3	C_4H_9
251E	C_2H_5	C_6H_{11}

Figure 21. Possible gross structures for ring-hydroxylated izidines. The structures are hypothetical and based on analogies and mass spectral data and in some cases FTIR spectral data. Mass spectral base peaks (bp) are indicated.

fragmentation patterns. Further studies and data are needed. Unfortunately, all of these alkaloids are trace constituents. Dendrobatid frogs (*Dendrobates auratus*) fed on leaf-litter arthropods contained dehydroizidine **219H** as a minor alkaloid.[5]

Pyrrolidines. A major alkaloid in skin extracts of one population of a Colombian dendrobatid frog (*Dendrobates histrionicus*) was identified in 1986 as a 2,5-disubstituted pyrrolidine (**197B**).[45] Such 2,5-disubstituted pyrrolidines had been known since 1976 to be constituents of myrmicine ant venoms.[59] Ten alkaloids from anuran skin currently are assigned to this pyrrolidine class (Figure 22). All, except **197B**, have been detected only as trace alkaloids in anuran skin extracts.

	2,5-Pyr	R_1	R_2
	183B	*n*-C_4H_9	*n*-C_4H_9
trans	**197B** *	*n*-C_4H_9	*n*-C_5H_{11}
	223N	C_6H_{11}	*n*-C_5H_{11}
	225C	*n*-C_4H_9	*n*-C_7H_{15}
cis / trans	**225H**	*n*-C_6H_{13}	*n*-C_5H_{11}
	235F	C_7H_{13}	C_5H_9
trans	**253I**	*n*-C_6H_{13}	*n*-C_7H_{15}
	277D	C_6H_{11}	C_9H_{17}
	279G	*n*-C_6H_{13}	C_9H_{17}

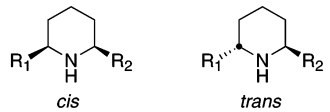
Figure 22. 2,5-Disubstituted pyrrolidines. *Absolute configuration as shown (positive rotation). All side-chains are assumed to be unbranched. A myrmicine ant origin is proposed.[18]

The mass spectra of 2,5-disubstituted pyrrolidines are dominated by fragments resulting from cleavage of one or the other of the α-substituents. Unlike the piperidines, none of the pyrrolidines detected in anuran extracts have an α-methyl substituent. The vapor-phase FTIR spectra distinguish the two isomers: The *cis*-isomer has a weak or very weak Bohlmann band near 2797 cm^{-1}, while the *trans*-isomer has no Bohlmann band.[15] After *N*-methylation these differences are more pronounced.

The 2,5-disubstituted pyrrolidines occur rarely in dendrobatid frogs and even more rarely in mantellid frogs, almost always as trace alkaloids. Such alkaloids are present in myrmicine ants, which represent the dietary source.[18] The occurrence of **197B** in certain extracts as a major alkaloid is remarkable, since pyrrolidines were accumulated very poorly when fed to a dendrobatid frog.[60] The toxicity of such pyrrolidines has not been reported. Pyrrolidines are noncompetitive blockers of nicotinic receptors (see ref 1).

Piperidines. The presence of a 2,6-disubstituted piperidine (**225B**) in skin extracts from a South American dendrobatid frog was reported in 1986.[45] At that time, the structure of a 4-hydroxy-2-methyl-6-nonylpiperidine (**241D**, see below), a major alkaloid in skin extracts of a montane Panamanian dendrobatid frog (*Dendrobates speciosus*), had been determined, but was not reported until 1988.[34] At present, about 20 alkaloids are assigned to the 2,6-disubstituted piperidine class (Figure 23). Such piperidines were known since 1971 to be present in venom of certain myrmicine ants.[61] One alkaloid (**211J**) is tentatively proposed to be an *N*-methyl-2,6-disubstituted piperidine, solely on the basis of mass spectra and the lack of an exchangeable hydrogen. *N*-Methyl piperidines have been reported from myrmicine ants.[62]

The mass spectra of 2,6-disubstituted piperidines are dominated by fragments resulting from α-cleavage of one or the other substituent. Those with an α-methyl substituent have a base peak at *m/z* 98. The vapor-phase FTIR spectra are diagnostic for the two isomers, with the *cis*-isomer having a small Bohlmann band at about 2780 cm^{-1}



	2,6-Pip	R_1	R_2	
	197E	CH_3	*n*-C_7H_{15}	
	197F	C_2H_5	*n*-C_6H_{13}	
cis / trans	**211D**	*n*-C_3H_7	*n*-C_6H_{13}	
	211I	*n*-C_5H_{11}	*n*-C_4H_9	
	211J	CH_3	*n*-C_7H_{15}	*N*-Methyl
	221L	CH_3	C_9H_{15}	
	223K *	CH_3	C_9H_{17}	
	225B	*n*-C_5H_{11}	*n*-C_5H_{11}	
	225I *	CH_3	*n*-C_8H_{19}	
	237J	CH_3	$C_{10}H_{19}$	
	239I	*n*-C_4H_9	*n*-C_7H_{15}	
cis / trans	**239L**	CH_3	$C_9H_{17}O$ (=O)	
	239O	*n*-C_5H_{11}	*n*-C_6H_{13}	
	241G	CH_3	$C_8H_{15}O_2$ (OH, =O)	
	253J *	CH_3	*n*-$C_{11}H_{23}$	
	253U	*n*-C_5H_{11}	*n*-C_7H_{15}	
	255C	*n*-C_4H_9	$C_6H_{11}O_2$ (OH, =O)	
	255D	*n*-C_5H_{11}	$C_5H_9O_2$ (OH, =O)	
cis / trans	**267K**	CH_3	$C_{11}H_{21}O$ (=O)	
	267X	CH_3	$C_{11}H_{21}O$ (OH)	
trans	**269C**	CH_3	$C_{11}H_{23}O$ (OH)	

Figure 23. 2,6-Disubstituted piperidines. *Absolute configurations shown are based on analogies to the corresponding ant alkaloids. All side-chains are assumed to be unbranched. A myrmicine ant origin is proposed.[18]

and the *trans*-isomer having no Bohlmann band.[15] The *cis*-isomer emerges on gas chromatography before the *trans*-isomer.

The 2,6-disubstituted piperidines occur relatively rarely in dendrobatid frogs and with only a few exceptions not in mantellid frogs and in both groups only in trace amounts. A myrmicine ant source is likely.[18] The ant solenopsins, such as **253J**, are quite toxic to mice and have potent antifungal activity. Such piperidines are noncompetitive blockers of nicotinic receptors (see ref 1).

Other Piperidines. The structure of the unprecedented 4-hydroxy-2,6-disubstituted piperidine **241D** was reported in 1988,[34] on the basis of NMR spectroscopic analysis of material isolated from a population of a Panamanian dendrobatid frog (*Dendrobates speciosus*), where it was a major alkaloid. Four alkaloids are now assigned to this subclass of hydroxylated 2,6-disubstituted piperidines (Figure 24). The absolute configuration of **241D** is known.

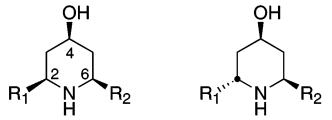
The mass spectra of such piperidines are dominated by fragments resulting from loss of α-substituents. In the case of the three alkaloids having an α-methyl substituent the fragment ion at *m/z* 114 is dominant. The FTIR spectrum of **241D** has a weak Bohlmann band at 2808 cm^{-1}.

Such hydroxypiperidines occur rarely in dendrobatid frogs and have not been reported from ants. Piperidine **241D** is a potent noncompetitive blocker of nicotinic receptors (see ref 1).

Certain alkaloids (**183A**, **185**) have mass spectral properties suggesting that they are disubstituted piperidines with only the smaller substituent in a readily lost α-position. Gross structures are tentatively proposed (Figure 24). Both were detected in skin extracts from dendrobatid frogs.

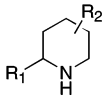
Gephyrotoxins. The structure of the tricyclic gephyrotoxin **287C** was revealed in 1977, on the basis of X-ray

4-Hydroxy-2,6-Disubstituted Piperidines



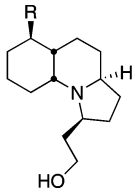
	Pip	R_1	R_2
trans	**213A**	CH_3	*n*-C_7H_{15}
	213B	*n*-C_3H_7	*n*-C_5H_{11}
cis	**241D** *	CH_3	*n*-C_9H_{19}
	255A	CH_3	$C_9H_{17}O$ (=O)

Tentative Disubstituted Piperidines



Pip	R_1	R_2
183A	C_2H_5	*n*-C_5H_{11}
185	CH_3	$C_5H_{11}O$ (OH)

Figure 24. Other piperidines. *Absolute configuration as shown. Gross structures of the two unusual disubstituted piperidines are tentative. An ant origin is likely.



GTX	R
287C *	$CH_2CH{=}CHC{\equiv}CH$
289B	$CH_2CH{=}CHCH{=}CH_2$

Figure 25. Gephyrotoxins. *Absolute configuration as shown based on X-ray crystallography. The other enantiomer may also occur.[1] A neotropical myrmicine ant origin is likely.

analysis of a crystal from material isolated from a Colombian dendrobatid frog (*Dendrobates histrionicus*).[63] The absolute configuration shown in Figure 25 is based on the X-ray analysis, but there remains doubt as to whether that enantiomer is the major one in frog skin (see ref 1).

The mass spectra are dominated by a fragment due to α-cleavage of the CH_2CH_2OH substituent. The FTIR spectrum shows a weak Bohlmann band near 2800 cm^{-1}.

The gephyrotoxins occur relatively rarely as minor alkaloids in dendrobatid frogs and only in extracts in which 19-carbon histrionicotoxins are major alkaloids. It appears likely that the gephryotoxins are of ant origin. Gephyrotoxin **287C** has relatively low toxicity in mice with a minimal toxic dose much greater than 500 μg. It is a noncompetitive blocker of nicotinic receptors (see ref 1).

Coccinelline-like Tricyclics. A coccinelline alkaloid, namely, precoccinelline (**193C**, Figure 26), known since 1971 from coccinellid beetles,[64] was reported as a minor alkaloid in a Panamanian dendrobatid frog (*Dendrobates auratus*) in 1992.[65] Subsequently, another beetle alkaloid, propyleine (**191B**), was identified from a Peruvian dendrobatid frog (*Epipedobates silverstonei*) and from certain populations of *Dendrobates pumilio* (unpublished results).

The structure of a relatively common coccinelline-like tricyclic alkaloid, namely, **205B**, was proposed in 1987,[39] but further analysis of the NMR data led to the correct stereochemical configuration[66] shown in Figure 26. The weak Bohlmann band of **205B** at 2796 cm^{-1} had prompted

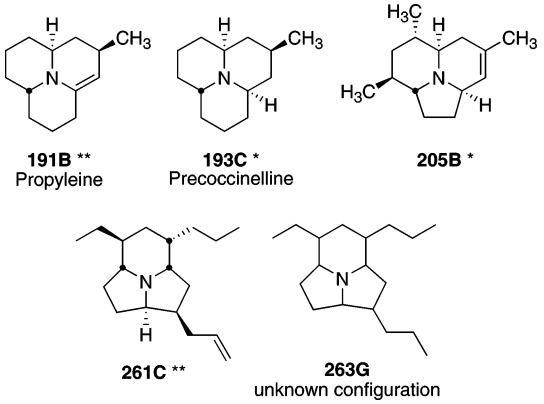


Figure 26. Coccinelline-like tricyclics. *Absolute configuration as shown. **Relative configuration as shown. A beetle origin is likely. A mite origin is also possible.[35b]

re-evaluation of the NMR spectroscopic data. The absolute configuration is known.[67] The structure of another coccinelline-like tricyclic alkaloid (**261C**), isolated from skin extracts of a Madagascan mantellid frog (*Mantella betsileo*), was reported in 2003[68] on the basis of NMR spectroscopic analysis. It has since been found in a Madagascan beetle (Andriamaharavo et al., unpublished results). At present, only five tricyclic alkaloids are assigned coccinelline-like structures (Figure 26). However, among the some 60 alkaloids, which at present are tentatively classified as tricyclics (see Table in Supporting Information), many will probably prove to be coccinelline-like in structure. It should be noted that certain tricyclic alkaloids from myrmicine ants have been given boldface code designations (myrmicines **219A**, **215B**, **217**, **233A**, **237A**, **237B**, etc.),[69] such as those we have used for over four decades. We retain all of our prior code designations for the alkaloids that have been discovered in anuran skin.

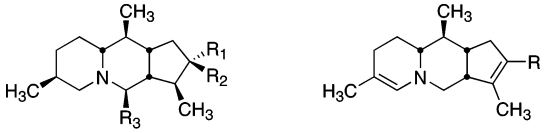
The mass spectra of the coccinelline-like tricyclics exhibit a complex fragmentation pattern with a major $M^+ - 1$ fragment and many other major fragments due to loss of methyl, ethyl, propyl, butyl, etc.[1] Indeed, such complex mass spectra were considered diagnostic in the decision to assign a tricyclic designation to many alkaloids, which otherwise would have been tabulated as "unclassified" (Unclass).

The coccinelline-like tricyclics have been found in dendrobatid, mantellid, and bufonid anurans, but relatively rarely and as only minor or trace alkaloids. Coccinellid beetles are the likely source. However, precoccinelline (**193C**) recently was reported from an oribatid mite.[35b] Toxicity of coccinelline-like tricylics to mice apparently has not been reported. The synthetic unnatural enantiomer of **205B** is a potent and selective blocker for α7 nicotinic receptors.[52]

Cyclopentaquinolizidines. The unique tricyclic alkaloid **251F** was detected in the 1970s in skin extracts of a tiny, Colombian dendrobatid frog (*Minyobates bombetes*).[3] After isolation and detailed NMR spectroscopic analysis of 340 μg of this alkaloid, the structure was determined and reported in 1992.[75] A variety of congeners were present in the extracts, and 10 alkaloids are assigned to this cyclopentaquinolizidine class (Figure 27). The structure of **251F** has been confirmed by synthesis.[76,77]

The mass spectral fragmentation of **251F** with an odd mass fragment at *m/z* 111 as the base peak has been analyzed in detail.[75] The vapor-phase FTIR spectrum of **251F** has a strong Bohlmann band at 2755 cm^{-1}.

Cyclopentaquinolizidine **251F** has been detected only very rarely as a trace alkaloid in dendrobatid frogs. Only



CPQ	R_1	R_2	R_3
235H	CH_3	H	H
249B	CHO	H	H
251F **	CH_2OH	H	H
251F'	H	CH_2OH	H
251J	CH_3	OH	H
265B	CH_2OH	H	CH_3
279B	CH_2OH	H	C_2H_5
279C	CH_3	OH	C_2H_5

CPQ	R
245A	CHO
247A	CH_2OH

Figure 27. Cyclopentaquinolizidines. Absolute configuration unknown. **Relative configuration as shown. Structures of other alkaloids are tentative. A dietary source is unknown.

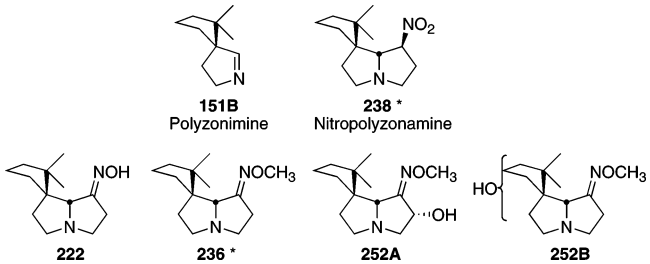


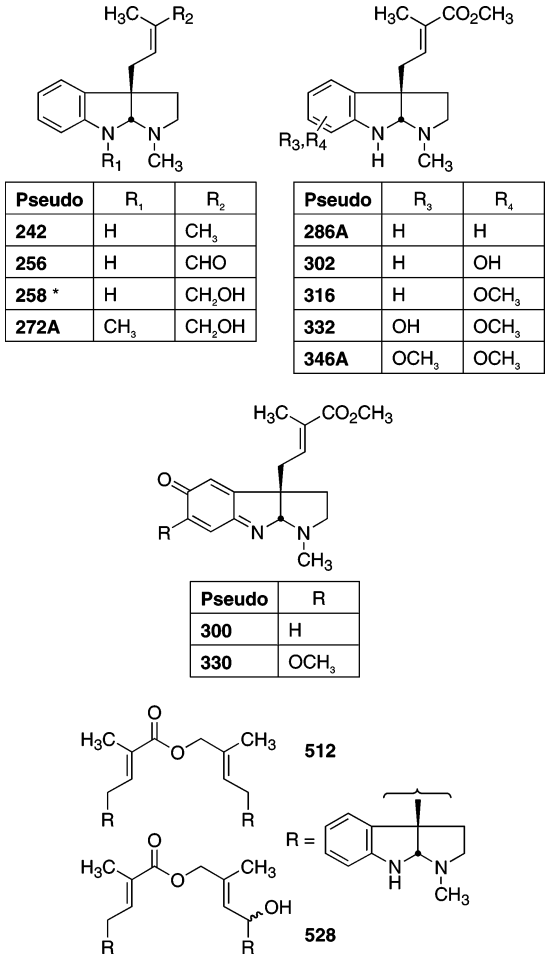
Figure 28. Spiropyrrolizidines. *Absolute configuration as shown. Other alkaloids assumed to have the same configuration. A trace alkaloid, **234**, is proposed to be a dehydro-**236**. Hydroxynitropolyzonamines (**254**) have been detected. A millipede origin is proposed.[74]

in extracts from the small montane frog *Minyobates bombetes* was it a major alkaloid. The biological activity of **251F** has not been investigated.

Spiropyrrolizidines. Three tricyclic alkaloids, isolated from skin extracts of a Panamanian dendrobatid frog (*Dendrobates pumilio*), were first tentatively and incorrectly assigned a tricyclic amidine structure in 1987, on the basis of NMR spectroscopic analysis and other data.[39] However, the apparent amidine infrared absorption at 1660 cm^{-1} was later found to be due to an impurity, and upon further NMR spectroscopic analysis spiropyrrolizidine oxime structures for alkaloids **222**, **236**, and **252A** were established in 1992.[70] At present nine alkaloids are assigned to this class of alkaloids found in anuran skin extracts. Six of these are shown in Figure 28. Polyzonimine (**151B**) and nitropolyzonamine (**238**) had been previously reported in 1975 from a millipede.[71,72]

The mass spectra of the spiropyrrolizidine oximes have dominant base peaks for **222** and **236** at *m/z* 112 and 126, respectively, while **252A** has a base peak at *m/z* 142 and **252B** one at *m/z* 126. The vapor-phase FTIR spectra have characteristic absorptions for the oxime moiety.[70] Bohlmann bands are weak or absent.

The spiropyrrolizidines occur rarely in dendrobatid frogs and only as minor or trace alkaloids. Certain members of the class, in particular the most common member **236**, have been detected in mantellid and bufonid anurans. Alkaloid **252B** has been detected as a trace alkaloid in one extract of a myobatrachid frog[73] and in a few dendrobatid and mantellid extracts (unpublished results). A siphonotid millipede (*Rhinotus*) source for the spiropyrrolizidine oxime **236** has been reported in 2003.[74] No toxicity data are available. Synthetic (±)-*O*-methyloxime **236** and (±)-nitropolyzonamine are potent noncompetitive blockers of nicotinic receptors (see ref 1).



Pseudo	R_1	R_2
242	H	CH_3
256	H	CHO
258 *	H	CH_2OH
272A	CH_3	CH_2OH

Pseudo	R_3	R_4
286A	H	H
302	H	OH
316	H	OCH_3
332	OH	OCH_3
346A	OCH_3	OCH_3

Pseudo	R
300	H
330	OCH_3

512

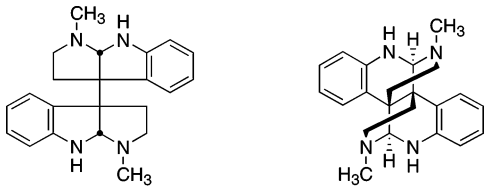
528

Figure 29. Pseudophrynamines. *Absolute configuration as shown (negative rotation). Other pseudophrynamines assumed to have the same configuration. Some structures are tentative. Produced by myobatrachid frogs (genus *Pseudophryne*).[80] Other high molecular weight pseudophrynamines occur.[73]

Pseudophrynamines. Indolic alkaloids were first detected in myobatrachid (*Pseudophryne*) frogs by V. Erspamer in 1976, using the Ehrlich color reaction for indoles.[78] A decade later, the major indolic alkaloids were isolated from skin extracts of an Australian myobatrachid frog (*Pseudophryne coriacea*), and the structures of pseudophrynamines **258** and **286A** were determined by NMR spectroscopic analysis.[79] Several minor congeners were present.[73] The indolic structures of these pseudophrynamines are reminiscent of the physostigmines. Thirteen of the pseudophrynamines are depicted in Figure 29. Some of the structures are tentative. There are other pseudophrynamines (**526**, **540**, **542**) with molecular weights over 500, whose structures are not defined. The absolute configuration of **258** has been proposed to be the same as physostigmine.[79]

The mass spectra of the pseudophrynamines in most cases show two major fragment ions corresponding to the indolic part of the molecule. For example, **258** and **286A** show major fragments at *m/z* 173 and 130. The FTIR spectra of the pseudophrynamines exhibit an absorption band at 3061 cm^{-1} for aromatic H's, a moderate broad Bohlmann band at near 2802 cm^{-1}, and strong, sharp bands in the fingerprint region.[73]

The pseudophrynamines are unique among the skin alkaloids of anurans in being biosynthesized by the frogs rather than coming from a dietary source.[80] They have been detected only in myobatrachid frogs of the genus *Pseudophryne*. Pumiliotoxins/allo-pumiliotoxins are the other class



346B * Chimonanthine

346C * Calycanthine

Figure 30. Other indolic alkaloids. *Absolute configurations of chimonanthine (positive rotation) and calycanthine (negative rotation) as shown. A plant to insect to frog food chain represents a likely origin.

of alkaloids found in skin extracts of these frogs, and they come from a dietary source.[80] Toxicity of pseudophrynamines for mice has not been reported. Synthetic (±)-pseudophrynaminol (**258**) is a potent noncompetitive blocker of nicotinic receptors (see ref 1).

Other Indolic Alkaloids. Two indolic plant alkaloids, chimonanthine and calycanthine (Figure 30), were found as minor alkaloids in extracts of a Colombian poison-dart frog (*Phyllobates terribilis*).[22] The mass spectrum of chimonanthine is dominated by an ion at *m/z* 173, while the mass spectrum of calycanthine consists almost exclusively of the M^+ ion. Another plant alkaloid, morphine, has been reported in extracts from a bufonid toad (*Bufo marinus*).[81] The dietary source of chimonanthine/calycanthine/morphine presumably involves a food chain from plant to arthropod to frog.

Pyridinic Alkaloids. The discovery of a potent analgetic alkaloid in skin extracts of an Ecuadoran dendrobatid frog (*Epipedobates tricolor*), obtained in the late 1970s, was triggered by the observation that on injection of the extract in mice there occurred a pronounced Straub tail (an arching of the tail over the back),[2] a response known to be elicited by morphine alkaloids. The responsible alkaloid was isolated and shown to be 200 times more potent than morphine as an analgetic. The analgesia was not blocked by an opioid antagonist, and hence the alkaloid was not morphine-like. The alkaloid, termed **208/210**, had an empirical formula of $C_{11}H_{13}N_2Cl$.[3] Although further data suggested the presence of a chloropyridine moiety, structure elucidation was not accomplished until NMR spectroscopic techniques advanced in sensitivity to the point where the structure could be defined with an irreplaceable sample of about 700 μg that had been isolated in the early 1980s. The structure of epibatidine (**208/210**, Figure 31) was reported in 1992, on the basis of NMR spectroscopic analysis of the *N*-acetyl derivative.[82] A *N*-methylepibatidine (**222/224A**) now has been detected. In addition, a structurally related alkaloid, phantasmidine (**222/224B**), recently isolated from skin extracts of a population of an Ecuadoran dendrobatid frog (*Epipedobates* tricolor),[58] is under investigation (Fitch et al., unpublished results). The frog has been called the "phantasmal poison-arrow frog". The empirical formula for phantasmidine is $C_{11}H_{11}N_2OCl$. A partial structure has been defined by NMR spectroscopic analysis (unpublished data) and is shown in Figure 31. It is a potent nicotinic agonist.

The mass spectrum of epibatidine has a base peak at *m/z* 69. The vapor-phase FTIR spectrum has no Bohlmann band and has absorptions characteristic of the pyridine moiety. Phantasmidine has a base peak at *m/z* 167 ($C_8H_6NOCl^+$) and major fragment ions at *m/z* 82 and 56.

Epibatidine has been detected only in certain South American frogs of the genus *Epipedobates*. Presumably, the dietary source of this nicotine-like alkaloid involves a food chain of plant to arthropod to frog. Epibatidine owes both

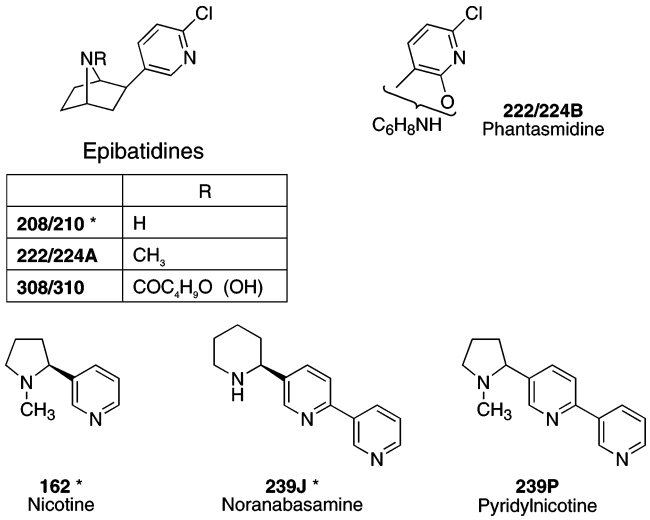


Figure 31. Epibatidines and other pyridinic alkaloids. *Absolute configuration of epibatidine (**208/210**) as shown (free base, negative rotation). *Absolute configuration of noranabasamine (negative rotation), based on an analogous plant alkaloid, anabasamine. A plant to insect to frog food chain for all these pyridinic alkaloids represents a likely origin.

the high toxicity with an LD_{50} about 0.4 μg per mouse and the potent analgetic activity (200-fold greater than morphine) to activation of nicotinic receptors.[83] Epibatidine has become a major research tool (see ref 1) and has stimulated extensive synthetic efforts to develop less toxic nicotinic agonists for potential use as analgetics.

Three other pyridine alkaloids have been detected in skin extracts of dendrobatid frogs. These are nicotine (**162**), noranabasamine (**239J**), and a pyridylnicotine (**239P**) (Figure 31). Nicotine has been detected in both dendrobatid and mantellid frogs, but very rarely and only in trace amounts. Noranabasamine was isolated from a Colombian poison-dart frog,[22] while the pyridylnicotine was detected as a trace alkaloid in extracts of another Colombian dendrobatid frog, *Dendrobates lehmanni* (unpublished results). The mass spectra of these three pyridyl alkaloids exhibit a base peak at *m/z* 84, corresponding to the piperidine or the *N*-methylpyrrolidine ring. The vapor-phase FTIR spectrum of **239P** has a strong, sharp Bohlmann band at 2791 cm^{-1}. In view of identity with or structural similarities to plant nicotinoid alkaloids, these three pyridine alkaloids probably originate through a food chain from a plant to arthropod to frog. However, another such pyridyl alkaloid, anabaseine, has been reported from a myrmicine ant.[84]

Unclassified Alkaloids. About 150 alkaloids detected in frog skin extracts cannot be placed in any of the 24 structural classes mentioned above, on the basis of the mass spectral data and in some cases vapor-phase FTIR spectra (see Table S1 in the Supporting Information). Isolation of such unclassified (Unclass) alkaloids and then NMR spectroscopic analysis will be required. However, most of these alkaloids occur only in trace amounts, and thus, probably less than 10 μg will be available for isolation. Most are present in dendrobatid and/or mantellid extracts, while only a few are from bufonids.

On the basis of mass spectral data some of the unclassified alkaloids can be grouped together. A few appear quite unique based on mass spectral properties.

Mass Spectra: Dominant $M^+/(M-1)^+$ Ions. Ten alkaloids exhibit such spectra with virtually no other fragment ions. All have been detected rarely as trace alkaloids from dendrobatid frogs. Six (**151A**, **153A**, **167D**, **181E**, **191C**, **257B**) have an exchangeable NH. The other four (**153B**, **167C**, **193A**, **195E**) have no exchangeable hydrogens. Possible structures of these alkaloids are not apparent. Alkaloid **191C** has fragment ions at *m/z* 119 and 91, indicating an aromatic ring. It may not be an alkaloid.

Mass Spectra: Major *m/z* 58 Ion. Sixteen alkaloids (**181C**, **183C**, **195L**, **197D**, **199**, **209M**, **211G**, **211R**, **223T**, **239S**, **241B**, **253E**, **267I**, **267O**, **267Q**, **269E**) exhibit a significant ion at *m/z* 58 ($C_3H_8N^+$). For many it is the base peak. However, seven alkaloids (**181C**, **183C**, **195L**, **197D**, **209M**, **211R**, **223T**) have moderate to large peaks due to loss of CH_3, and one (**239S**) has a base peak due to loss of C_2H_5. One alkaloid (**209M**) has a base peak due to loss of OH. One (**267Q**) has the M^+ ion as the base peak, and one (**199**) has a base peak at *m/z* 86 ($C_4H_8NO^+$). Alkaloid **181C** has a strong, sharp Bohlmann band, while alkaloid **269E** has a broader, strong Bohlmann band and a C=O absorption. The empirical formulas suggest that most have the equivalent of one or two rings or double bonds. Alkaloid **181C** with two ring/double bond equivalents cannot be hydrogenated and, thus, must have two rings. Two alkaloids (**183C**, **223T**) have no exchangeable hydrogens, but such data are lacking for the other members of this class. Seven alkaloids apparently have no oxygen, while eight have one and one (**211R**) has two. One alkaloid (**241B**) has no rings or double bonds. Thus, a tentative structure for **241B**, first described in 1993,[85] might be $C_{13}H_{27}CH_2N(CH_3)_2$. Indeed, the most likely but not only possible source of a *m/z* 58 ion would be a $-CH_2N(CH_3)_2$ moiety. However, the data do not allow postulation of even a tentative common structural class for these alkaloids. Most of these alkaloids have been detected from dendrobatid frogs, although a few have been found in mantellids and bufonids.

Mass Spectra: Significant *m/z* 67 Fragment Ion. Four alkaloids (**167G**, **191G**, **293B**, **305F**) exhibit a significant odd mass fragment ion at *m/z* 67 ($C_4H_5N^+$). The base peaks for these alkaloids are at *m/z* 152, 94, 150, and 124, respectively. No structures can be proposed, nor have FTIR data been obtained.

Mass Spectra: Major *m/z* 70 Fragment Ion. A major fragment ion at *m/z* 70 is typical of pumiliotoxins, 8-desmethylpumiliotoxins, and allopumiliotoxins. It is possible that a similar hydroxyindolizidine moiety is present in some of the seven alkaloids (**253N**, **279D**, **309G**, **323D**, **337C**, **339F**, **351**) that have an *m/z* 70 fragment ion with an intensity of 60% or greater compared to the base peak and an oxygen in their empirical formulas. However, none appears to be of the pumiliotoxin class, based on the other fragment ions. Alkaloid **253N** has a major odd mass fragment ion at *m/z* 109. An alkaloid **207B** has fragment ions at *m/z* 166 and 70 and, thus, appears likely to be an unusual pumiliotoxin. Without further data it is listed as unclassified. Alkaloids **207L** and **217D** have a base or major peak, respectively, at *m/z* 70 but no oxygen in the empirical formula.

Mass Spectra: Major *m/z* 82 Fragment Ion. Seven unclassified alkaloids (**205D**, **209G**, **223L**, **233H**, **281E**, **305F**, **434**) show a major fragment ion (>40% of base peak) at *m/z* 82 ($C_5H_8N^+$). It is the base peak only for **205D** and **223L**. Three other unclassified alkaloids (**153C**, **390**, **392**) have significant *m/z* 82 fragments. For **209G** and **233H**, loss of C_5H_{11} or CH_2OH affords a base peak at *m/z* 138 or 202, respectively, while in **153C** the *m/z* 95 fragment ion is the base peak. Another unclassified alkaloid, **207F**, with a base peak corresponding to loss of CH_3 has a small *m/z* 82 fragment ion. FTIR data have not been obtained. No structures are proposed.

Mass Spectra: Major *m/z* 84 Fragment Ion. A major fragment ion at *m/z* 84 is typical of homopumiliotoxins. Nine unclassified alkaloids (**235J**, **249F**, **251L**, **265K**, **267M**, **279A**, **279J**, **293G**, **325C**) exhibit a mass spectrum with base peak or major fragment ion at *m/z* 84 ($C_5H_{10}N^+$), and several have an FTIR spectrum commensurate with those of homopumiliotoxins. It is possible that a similar hydroxyquinolizidine ring system is present in at least some of these alkaloids. Certain of those alkaloids had been tentatively considered to be homopumiliotoxins despite the lack of a diagnostic fragment at *m/z* 180.[1] All except **267M** and **325C** have the equivalent of four rings or double bonds in their structures. The major loss of $C_3H_7O_2$ and of $C_5H_9O_3$ for **251L** and its *O*-acetate **293G**, respectively, cannot be rationalized in terms of the homopumiliotoxin ring system.

Mass Spectra: Major *m/z* 86 Fragment Ion. Six unclassified alkaloids (**161B**, **223BB**, **227**, **249Y**, **251BB**, **255E**) show a major *m/z* 86 ($C_4H_8NO^+$) fragment ion. FTIR spectra were not obtained for any of these trace alkaloids. Structures are not proposed.

Mass Spectra: Major *m/z* 110 Fragment Ion. Three alkaloids (**241I**, **265R**, **281D**) exhibit a base peak at *m/z* 110 ($C_7H_{12}N^+$). Six other unclassified alkaloids (**191G**, **207F**, **209G**, **253E**, **275J**, **325C**) have a major *m/z* 110 ion (>40% of base peak). Such an ion often accompanies the base peak in many 5,6,8-trisubstituted indolizidines and 1,4-disubstituted quinolizidines, but is always a minor fragment. Instead, these may be monosubstituted pyrrolizidines. Another alkaloid (**253N**) exhibits an odd-mass base peak at *m/z* 109 in addition to a major *m/z* 110 ion. Structures are not proposed.

Mass Spectra: Significant Fragment Ion at *m/z* 116. Four alkaloids (**253O**, **269G**, **283D**, **283E**) exhibit a significant fragment ion (>35% of base peak) at *m/z* 116 ($C_5H_{10}NO_2^+$). Infrared spectra were not obtained for any of these trace alkaloids. Structures are not proposed.

Mass Spectra: Significant Fragment Ion at *m/z* 118. One alkaloid (**287F**) exhibits a base peak at *m/z* 118 ($C_5H_{12}NO_2^+$) and a major fragment ion at *m/z* 88 ($C_4H_{10}NO^+$). A structure is not proposed.

Mass Spectra: Major Fragment Ion at *m/z* 120. Three alkaloids (**243H**, **245K**, **247K**) have a base peak at *m/z* 120 ($C_8H_{10}N^+$) and a significant fragment ion at *m/z* 80 ($C_5H_6N^+$). Structures are not proposed.

Mass Spectra: Significant Fragment Ion at *m/z* 122. Six alkaloids (**209R**, **223CC**, **235P**, **235S**, **265R**, **293J**) have a significant fragment ion (>20% of base peak) at *m/z* 122 ($C_8H_{12}N^+$). It is the base peak in **209R**. Structures are not proposed.

Mass Spectra: One Major Fragment Ion. A large number of alkaloids exhibit a single major fragment ion, suggestive of a structure with only one α-substituent adjacent to nitrogen in a bicyclic or tricyclic ring system. For these alkaloids, minor fragment ions were not diagnostic for any of the proposed izidine classes. The presence of a significant fragment ion at *m/z* 70 is typical for the indolizidine ring system, while a significant fragment ion at *m/z* 84 is typical for the quinolizidine ring system. A fragment ion at *m/z* 70 also could arise from other classes of alkaloids containing a five-membered pyrrolidine moiety. The presence of a significant fragment ion at *m/z* 70 or at *m/z* 84 is noted in parentheses for the following alkaloids: **193K**, **207N** (70), **211H**, **217C** (70), **219M**, **231I**, **231J**, **235D**, **235K**, **235R** (70), **235X** (70), **239N** (70), **239T**, **249N**, **249Q**, **251Z**, **263J**, **267B**, **271E**, **279K** (70), **305E** (84), **307J**, **309E**, **319C** (70), **322G** (84), **323H** (70), **339D** (70), **365**, **369** (70). Three of these alkaloids (**235X**, **305E**, **369**) exhibit a significant fragment ion due to loss of OH. Alkaloid **235X** has a base peak due to loss of CH_3 and is unusual in having a major fragment ion at *m/z* 188. Alkaloid **235S** appears related in structure to **235X**, losing methyl to yield a major fragment ion and having a minor fragment ion at *m/z* 188. Two alkaloids (**319C**, **369**) have odd-mass base peaks at *m/z* 193 and 185, respectively. Alkaloid **231J** shows a base peak at *m/z* 146 due to loss of C_6H_{13} and is possibly a highly unsaturated 5,8-disubstituted indolizidine.

Fifteen unclassified alkaloids (**207D**, **231F**, **231M**, **235O**, **239B**, **241C**, **253C**, **263B**, **267G**, **271B**, **281C**, **301**, **309B**, **309F**, **357B**) show a major fragment ion, presumably due to loss of an α-substituent, but no other significant fragment ions. Some may be tricyclics. The apparent major loss of 27 mass units from **207D** and of 51 from **231F** are difficult to rationalize.

Mass Spectra: Two Major Fragment Ions. A number of alkaloids exhibit two major fragment ions. In some cases, these two ions appear likely to be due to loss of one or the other of two substituents from a bicyclic or tricyclic ring system. In others, alternative cleavages of a single substituent or further cleavage following the initial loss of a substituent must be involved.

For 13 alkaloids, the nature of the two apparent losses of substituents and for three the presence of a significant fragment ion at *m/z* 70 or 84 is as follows: **211N** (CH_3 > C_4H_9O), **223W** (C_7H_{15} > C_4H_9), **223Y** (C_2H_5 > $C_5H_{11}O$), **223DD** (C_3H_7 > C_5H_{11}), **235G** (C_2H_5 > C_3H_5), **235BB** (C_3H_5>C_3H_7O), **253M** (C_3H_7 > $C_5H_{11}O_2$, 70) **253Q** (CH_3 > $C_3H_7O_2$), **269F** (CH_2OH > C_4H_9O), **271C** (C_5H_5 > CH_3), **293J** ($C_3H_7O \approx C_5H_{11}O$, 84), **323I** ($C_4H_9$ > $C_8H_{17}O_2$, 70), **357B** (CH_3 > OH).

For 22 alkaloids, the two major fragment ions must result from either alternate cleavages of a single substituent or further cleavage following the initial loss of a substituent. These are as follows with the presence of a significant fragment ion at *m/z* 70 or 84 indicated in parentheses: **205C**, **235L**, **237K** (84), **239I** (70), **239V** (70), **241A**, **249V**, **273D**, **275J** (70), **279J** (84), **281G** (70), **281J**, **283B**, **283C**, **285D**, **291I** (70), **297C**, **305G** (84), **307I**, **339E** (70), **341D** (70), **371** (70). Alkaloid **249V** probably is a dehydroizidine. Alkaloids **283B** and **283C** appear closely related, as do alkaloids **291I** and **307I**. Alkaloid **339E** shows a significant loss of OH. Two alkaloids (**235L**, **239V**) show a base peak at *m/z* 170 due, respectively, to loss of C_5H_5 or a C_5H_9 side-chain; both also have a major ion at *m/z* 152.

Other Unclassified Alkaloids. While most of the alkaloids sequestered from diet into frog skin contain only one nitrogen, there are some exceptions. Alkaloid **135** ($C_5H_5N_5$) proved to be adenine (unpublished results). Oleamide (**281L**) was detected in a mantellid extract. Whether it is a natural amide or an artifact from its use in plastics is uncertain (see ref 86). It, along with linoleamide and stearamide, was present in glands of one of two sympatric myrmicine ants.[87] Alkaloid **161A** ($C_9H_{11}N_3$) is an aromatic heterocycle. Alkaloid **392** ($C_{22}H_{36}N_2O_4$) and an apparent *O*-acetyl derivative, alkaloid **434**, were first reported from a mantellid frog in 1996.[88a] The former has been studied in detail, including NMR spectroscopic analysis (unpublished results), but as yet a definitive structure cannot be proposed. Alkaloid **392** forms a mono-*O*-acetate isomeric with alkaloid **434**. Alkaloid **390** appears to be a dehydro congener of alkaloid **392**. Alkaloid **395** ($C_{23}H_{41}NO_4$) with a base peak at *m/z* 326 has not been isolated for

NMR spectroscopic analysis. A recent report documents further occurrence of alkaloids, including unclassified, in mantellid frogs.[88b]

Summary

Four decades of research on alkaloids in anuran skin extracts have revealed over 800 compounds, most of which, as yet, are unknown elsewhere in nature. This is particularly remarkable, since the vast majority of these alkaloids appear to be sequestered into anuran skin glands unchanged from dietary sources. Evidence that the dietary origins for most of the primary structural classes are ants, mites, beetles, and millipedes has been obtained. Nonetheless, the structures and dietary origins of the many alkaloids that cannot, on the basis of mass spectral properties, be put into one of over 20 major classes of anuran skin alkaloids remains a challenge for the future. The biosynthetic pathways leading to such alkaloids and the functional significance for the arthropods of the alkaloids found sequestered in anuran skin remain virtually uninvestigated. For the majority, the pharmacological activity has not been investigated.

Acknowledgment. The past four decades of research on alkaloids from amphibian skins would not have prospered without the input over those decades from chemist Dr. T. Tokuyama (now retired), biologist Dr. C. W. Myers (now retired), and X-ray crystallographer Dr. I. L. Karle. During the last decade of discovering the dietary origin in arthropods of those frog skin alkaloids, Dr. J. P. Dumbacher, Dr. T. H. Jones, and R. A. Saporito have been invaluable colleagues. Many others have contributed to the discovery of further alkaloids, including the following postdoctorals: Drs. N. R. Andriamaharavo, M. W. Edwards, R. W. Fitch, P. Jain, and T. Kaneko. In addition, many students have been involved in GC analysis of extracts, leading to the discovery of a number of the alkaloids listed in this report. They include J. Caceres, V. C. Clark, M. Dennig, L. Giddings, C. Johnson, S. Lewis, R. A. Saporito, S. I. Secunda, S. S. Strange, and J. M. Wilham. Invaluable synthetic compounds have come from many chemists, including Drs. L. E. Overman, E. J. Corey, and, in recent years Dr. N. Toyooka. The dedication and skill of M. Grothe in the preparation of this review is gratefully acknowledged.

Supporting Information Available: Tabulation of alkaloids (code, class, empirical formula, t_R value, mass spectrum, FTIR spectral features, and other information). This is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Daly, J. W.; Garraffo, H. M.; Spande, T. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: New York, 1999; Vol. 13, pp 1−161.
(2) Daly, J. W.; Brown, G. B.; Mensah-Dwumah, M.; Myers, C. W. *Toxicon* **1978**, *16*, 163−188.
(3) Daly, J. W.; Myers, C. W.; Whittaker, N. *Toxicon* **1987**, *25*, 1023−1095.
(4) Daly, J. W.; Garraffo, H. M.; Spande, T. F. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 1993; Vol. 43, pp 185−288.
(5) Daly, J. W.; Garraffo, H. M.; Spande, T. F.; Jaramillo, C.; Rand. A. S. *J. Chem. Ecol*. **1994**, *20*, 943−955.
(6) Garraffo, H. M.; Spande, T. F.; Jones, T. H.; Daly, J. W. *Rapid Commun. Mass Spectrom*. **1999**, *13*, 1553−1563.
(7) Garraffo, H. M.; Jain, P.; Spande, T. F.; Daly, J. W.; Jones, T. H.; Smith, L. J.; Zottig, V. E. *J. Nat. Prod.* **2001**, *64*, 421−427.
(8) Garraffo, H. M.; Spande, T. F.; Daly, J. W.; Baldessari, A.; Gros, E. G. *J. Nat. Prod*. **1993**, *56*, 357−373.
(9) Tokuyama, T.; Daly, J. W.; Garraffo, H. M.; Spande, T. F. *Tetrahedron* **1992**, *48*, 4247−4258.
(10) Dumbacher, J. P.; Spande, T. F.; Daly, J. W. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 12970−12975.
(11) Tokuyama, T.; Tsujita, T.; Shimada, A.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Tetrahedron* **1991**, *47*, 5401−5414.
(12) Tokuyama, T.; Tsujita, T.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Tetrahedron* **1991**, *47*, 5415−5424.
(13) Spande, T. F.; Garraffo, H. M.; Daly, J. W.; Tokuyama, T.; Shimada, A. *Tetrahedron* **1992**, *48*, 1823−1836.
(14) Garraffo, H. M.; Caceres, J.; Daly, J. W.; Spande, T. F.; Andriamaharavo, N. R.; Andriantsiferana, M. *J. Nat. Prod*. **1993**, *56*, 1016−1038.
(15) Garraffo, H. M.; Simon, L. D.; Daly, J. W.; Spande, T. F.; Jones, T. H. *Tetrahedron* **1994**, *50*, 11329−11338.
(16) Toyooka, N.; Tanaka, K.; Momose, T.; Daly, J. W.; Garraffo, H. M. *Tetrahedron* **1997**, *53*, 9553−9574.
(17) Spande, T. F.; Jain, P.; Garraffo, H. M.; Pannell, L. K.; Yeh, H. J. C.; Daly, J. W.; Fukumoto, S.; Imamura, K.; Tokuyama, T.; Torres, J. A.; Snelling, R. R.; Jones, T. H. *J. Nat. Prod*. **1999**, *62*, 5−21.
(18) Jones, T. H.; Gorman, J. S. T.; Snelling, R. R.; Delabie, J. H. C.; Blum, M. S.; Garraffo, H. M.; Jain, P.; Daly, J. W.; Spande, T. F. *J. Chem. Ecol*. **1999**, *25*, 1179−1193.
(19) Michel, P.; Rassat, A.; Daly, J. W.; Spande, T. F. *J. Org. Chem*. **2000**, *65*, 8908−8918.
(20) (a) Schöpf, Cl. *Experientia* **1961**, *17*, 285−328. (b) Mebs, D.; Pogoda, W. *Toxicon* **2005**, *45*, 603−606.
(21) Tokuyama, T.; Daly, J. W.; Witkop, B. *J. Am. Chem. Soc*. **1969**, *91*, 3931−3938.
(22) Tokuyama, T.; Daly, J. W. *Tetrahedron* **1983**, *39*, 41−47.
(23) Dumbacher, J. P.; Beehler, B. M.; Spande, T. F.; Garraffo, H. M.; Daly, J. W. *Science* **1992**, *258*, 799−801.
(24) Dumbacher, J. P.; Wako, A.; Derrickson, S. R.; Samuelson, A.; Spande, T. F.; Daly, J. W. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 15857−15860.
(25) Daly, J. W.; Myers, C. W.; Warnick, J. E.; Albuquerque, E. X. *Science* **1980**, *208*, 1383−1385.
(26) Albuquerque, E. X.; Warnick, J. E.; Sansone, F. M.; Daly, J. W. *J. Pharmacol. Exp. Therap*. **1973**, *184*, 315−329.
(27) Daly, J. W.; Karle, I.; Myers, C. W.; Tokuyama, T.; Waters, J. A.; Witkop, B. *Proc. Natl. Acad. Sci. U. S. A.* **1971**, *68*, 1870−1875.
(28) Daly, J. W.; Myers, C. W. *Science* **1967**, *156*, 970−973.
(29) Daly, J. W.; Tokuyama, T.; Fujiwara, T.; Highet, R. J.; Karle, I. L. *J. Am. Chem. Soc*. **1980**, *102*, 830−836.
(30) Tokuyama, T.; Daly, J. W.; Highet, R. J. *Tetrahedron* **1984**, *40*, 1183−1190.
(31) Jain, P.; Spande, T. F.; Garraffo, H. M.; Daly, J. W. *Heterocycles* **1999**, *50*, 903−912.
(32) Jain, P.; Garraffo, H. M.; Spande, T. F.; Yeh, H. J. C.; Daly, J. W. *J. Nat. Prod*. **1995**, *58*, 100−104.
(33) Armstrong, P.; Feutren, S.; McAlonan, H.; O'Mahony, G.; Stevenson, P. J. *Tetrahedron Lett*. **2004**, *45*, 1627−1630.
(34) Edwards, M. W.; Daly, J. W.; Myers, C. W. *J. Nat. Prod*. **1988**, *51*, 1188−1197.
(35) (a) Saporito, R. A.; Garraffo, H. M.; Donnelly, M. A.; Edwards, A. L.; Longino, J. T.; Daly, J. W. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 8045−8050. (b) Takeda, W.; Sakata, T.; Shimano, S.; Enami, Y.; Mori, N.; Nishida, R.; Kuwahara, Y. *J. Chem. Ecol*. **2005**, in press.
(36) Daly, J. W.; Garraffo, H. M.; Spande, T. F.; Clark, V. C.; Ma, J.; Ziffer, H.; Cover, Jr., J. F. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11092−11097.
(37) Daly, J. W.; McNeal, E.; Gusovksy, F.; Ito, F.; Overman, L. E. *J. Med. Chem*. **1988**, *31*, 477−480.
(38) Daly, J. W.; Gusovsky, F.; McNeal, E. T.; Secunda, S.; Bell, M.; Creveling, C. R.; Nishizawa, Y.; Overman, L. E.; Sharp, M. J.; Rossignol, D. P. *Biochem. Pharmacol*. **1990**, *40*, 315−326.
(39) Tokuyama, T.; Nishimori, N.; Shimada, A.; Edwards, M. W.; Daly, J. W. *Tetrahedron* **1987**, *43*, 643−652.
(40) Kibayashi, C.; Aoyagi, S.; Wang, T.-C.; Saito, K.; Daly, J. W.; Spande, T. F. *J. Nat. Prod*. **2000**, *63*, 1157−1159.
(41) Daly, J. W.; Tokuyama, T.; Habermehl, G.; Karle, I. L.; Witkop, B. *Liebig's Ann. Chem.* **1969**, *729*, 198−204.
(42) Daly, J. W.; Garraffo, H. M.; Jain, P.; Spande, T. F.; Snelling, R. R.; Jaramillo, C.; Rand, A. S. *J. Chem. Ecol.* **2000**, *26*, 73−85.
(43) Jones, T. H.; Blum, M. S.; Fales, H. M.; Thompson, C. R. *J. Org. Chem*. **1980**, *45*, 4778−4780.
(44) Spande, T. F.; Daly, J. W.; Hart, D. J.; Tsai, Y.-M.; Macdonald, T. L. *Experientia* **1981**, *37*, 1242−1245.
(45) Daly, J. W.; Whittaker, N.; Spande, T. F.; Highet, R. J.; Feigl, D.; Nishimori, N.; Tokuyama, T.; Myers, C. W. *J. Nat. Prod*. **1986**, *49*, 265−280.
(46) Tokuyama, T.; Nishimori, N.; Karle, I. L.; Edwards, M. W.; Daly, J. W. *Tetrahedron* **1986**, *42*, 3453−3460.
(47) Ritter, F. J.; Rotgans, I. E. M.; Talman, E.; Vermiel, P. E. J.; Stein, F. *Experientia* **1973**, *29*, 530−531.
(48) Enders, D.; Thiebes, C. *Synlett* **2000**, *12*, 1745−1748.
(49) Toyooka, N.; Kawasaki, M.; Nemoto, H.; Daly, J. W.; Spande, T. F.; Garraffo, H. M. *Heterocycles* **2005**, *65*, 5−8.
(50) Toyooka, N.; Nemoto, H.; Kawasaki, M.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Tetrahedron* **2005**, *61*, 1187−1198.
(51) Daly, J. W.; Kaneko, T.; Wilham, J.; Garraffo, H. M.; Spande, T. F.; Espinosa, A.; Donnelly, M. A. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 13996−14001.
(52) Tsuneki, H.; You, Y.; Toyooka, N.; Kagawa, S.; Kobayashi, S.; Sasaoka, T.; Nemoto, H.; Kimura, I.; Dani, J. A. *Mol. Pharmacol*. **2004**, *66*, 1061−1069.
(53) Garraffo, H. M.; Jain, P.; Spande, T. F.; Daly J. W. *J. Nat. Prod*. **1997**, *60*, 2−5.
(54) Toyooka, N.; Fukutome, A.; Nemoto, H.; Daly, J. W.; Spande, T. F.; Garraffo, H. M.; Kaneko, T. *Org. Lett*. **2002**, *4*, 1715−1717.
(55) Tokuyama, T.; Shimada, A.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Heterocycles* **1998**, *49*, 427−436.

(56) Jain, P.; Garraffo, M. H.; Yeh, H. J. C.; Spande, T. F.; Daly, J. W.; Andriamaharavo, N. R.; Andriantsiferana, M. *J. Nat. Prod*. **1996**, *59*, 1174−1178.
(57) (a) Toyooka, N.; Nemoto, H. *Tetrahedron Lett*. **2003**, *44*, 569−570. (b) Huang, H.; Spande, T. F.; Panek, J. S. *J. Am. Chem. Soc.* **2003**, *125*, 626−627.
(58) Fitch, R. W.; Garraffo, H. M.; Spande, T. F.; Yeh, H. J. C.; Daly, J. W. *J. Nat. Prod*. **2003**, *66*, 1345−1350.
(59) MacConnell, J. G.; Blum, M. S.; Buren, W. F.; Williams, R. N.; Fales, H. M. *Toxicon* **1976**, *14*, 69−78.
(60) Daly, J. W.; Secunda, S. I.; Garraffo, H. M.; Spande, T. F.; Wisnieski, A.; Cover, J. F. *Toxicon* **1994**, *32*, 657−663.
(61) MacConnell, J. G.; Blum, M. S.; Fales, H. M. *Tetrahedron* **1971**, *26*, 1129−1139.
(62) Jones, T. H.; Blum, M. S.; Fales, H. M. *Tetrahedron* **1982**, *38*, 1949−1958.
(63) Daly, J. W.; Witkop, B.; Tokuyama, T.; Nishikawa, T.; Karle, I. L. *Helv. Chim. Acta* **1977**, *60*, 1128−1140.
(64) Tursch, B.; Daloze, D.; Dupont, M.; Pasteels, J. M.; Tricot, M. C. *Experientia* **1971**, *27*, 1380−1381.
(65) Daly, J. W.; Secunda, S. I.; Garraffo, H. M.; Spande, T. F.; Wisnieski, A.; Nishihira, C.; Cover, J. F., Jr. *Toxicon* **1992**, *30*, 887−898.
(66) Tokuyama, T.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Anal. Asoc. Quim. Argentina* **1998**, *86*, 291−298.
(67) Toyooka, N.; Fukutome, A.; Shinoda, H.; Nemoto, H. *Angew. Chem., Int. Ed*. **2003**, *42*, 3808−3810.
(68) Kaneko, T.; Spande, T. F.; Garraffo, H. M.; Yeh, H. J. C.; Daly, J. W.; Andriamaharavo, N. R.; Andriantsiferana, M. *Heterocycles* **2003**, *59*, 245−258.
(69) Schröder, F.; Franke, S.; Francke, W. *Tetrahedron* **1996**, *52*, 13539−13546.
(70) Tokuyama, T.; Daly, J. W.; Garraffo, H. M.; Spande, T. F. *Tetrahedron* **1992**, *48*, 4247−4258.
(71) Smolanoff, J.; Kluge, A. F.; Meinwald, J.; McPhail, A.; Miller, R. W.; Hicks, K.; Eisner, T. *Science* **1975**, *188*, 734−736.
(72) Meinwald, J.; Smolanoff, J.; McPhail, A. T.; Miller, R. W.; Eisner, T.; Hicks, K. *Tetrahedron Lett*. **1975**, 2387−2370.
(73) Daly, J. W.; Garraffo, H. M.; Pannell, L. K.; Spande, T. F. *J. Nat. Prod*. **1990**, *53*, 407−421.
(74) Saporito, R. A.; Donnelly, M. A.; Hoffman, R. L.; Garraffo, H. M.; Daly, J. W. *J. Chem. Ecol*. **2003**, *29*, 2781−2786.
(75) Spande, T. F.; Garraffo, H. M.; Yeh, H. J. C.; Pu, Q.-L.; Pannell, L. K.; Daly, J. W. *J. Nat. Prod*. **1992**, *55*, 707−722.
(76) Tabor, D. F.; You, K. K. *J. Am. Chem. Soc*. **1995**, *117*, 5757−5762.
(77) Wrobleski, A.; Sahasrabudhe, K.; Aube, J. *J. Am. Chem. Soc*. **2004**, *126*, 5475−5481.
(78) Roseghini, M.; Erspamer, V.; Endean, R. *Comp. Biochem. Physiol*. **1976**, *54C*, 31−43.
(79) Spande, T. F.; Edwards, M. W.; Pannell, L. K.; Daly, J. W. *J. Org. Chem*. **1988**, *53*, 1222−1226.
(80) Smith, B. P.; Tyler, M. J.; Kaneko, T.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *J. Nat. Prod*. **2002**, *65*, 439−447.
(81) Oka, K.; Kantrowitz, J. D.; Spector, S. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 1852−1854.
(82) Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. *J. Am. Chem. Soc*. **1992**, *114*, 3475−3478.
(83) Badio, B.; Daly, J. W. *Mol. Pharmacol*. **1994**, *45*, 563−569.
(84) Wheeler, J. W.; Olubajo, O.; Storm, C. B.; Duffield, R. M. *Science* **1981**, *211*, 1051−1052.
(85) Daly, J. W.; Highet, R. J.; Myers, C. W. *Toxicon* **1984**, *22*, 905−919.
(86) Hanuš, L. O.; Fales, H. M.; Spande, T. F.; Basile, A. S. *Anal. Biochem*. **1999**, *270*, 159−166.
(87) Do Nascimento, R. R.; Jackson, B. D.; Morgan, E. D.; Clark, W. H.; Blom, P. E. *J. Chem. Ecol*. **1993**, *19*, 1993−2005.
(88) (a) Daly, J. W.; Andriamaharavo, N. R.; Andriantsiferana, M.; Myers, C. W. *Am. Mus. Novitates* **1996** (3177), 1−34. (b) Clark, V. C.; Raxworthy, C. J.; Rakotomalala, V.; Sierwald, P.; Fisher, B. L. *Proc. Natl. Acad. Sci.* **2005**, *102*, 11617−11622.
(89) Garraffo, H. M.; Edwards, M. W.; Spande, T. F.; Daly, J. W.; Overman, L. E.; Severini, C.; Erspamer, V. *Tetrahedron* **1988**, *44*, 6795−6800.
(90) Edwards, M. W.; Garraffo, H. M.; Daly, J. W. *Synthesis* **1994** (11), 1167−1170.
(91) Daly, J. W.; Witkop, B.; Tokuyama, T.; Nishikawa, T.; Karle, I. L. *Helv. Chim. Acta* **1977**, *60*, 1128−1140.
(92) Tokuyama, T.; Yamamoto, J.; Daly, J. W.; Highet, R. J. *Tetrahedron* **1983**, *39*, 49−53.
(93) Tokuyama, T.; Uenoyama, K.; Brown, G.; Daly, J. W.; Witkop, B. *Helv. Chim. Acta* **1974**, *57*, 2597−2604.
(94) Daly, J. W.; Noimai, N.; Kongkathip, B.; Kongkathip, N.; Wilham, J. M.; Garraffo, H. M.; Kaneko, T.; Spande, T. F.; Nimit, Y.; Nabhitabhata, J.; Chan-Ard, T. *Toxicon* **2004**, *44*, 805−815.

NP0580560