Alkaloids in Madagascan Frogs (Mantella): Pumiliotoxins, Indolizidines, Quinolizidines, and Pyrrolizidines

H. M. Garraffo, J. Caceres, J. W. Daly, T. F. Spande, N. R. Andriamaharavo, and M. Andriantsiferana

J. Nat. Prod., **1993**, 56 (7), 1016-1038• DOI: 10.1021/np50097a005 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 7, pp. 1016-1038, July 1993

ALKALOIDS IN MADAGASCAN FROGS (*MANTELLA*): PUMILIOTOXINS, INDOLIZIDINES, QUINOLIZIDINES, AND PYRROLIZIDINES

H.M. GARRAFFO, J. CACERES, J.W. DALY,* T.F. SPANDE,

Laboratory of Bioorganic Chemistry, Building 8, Room 1A17, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

N.R. ANDRIAMAHARAVO, and M. ANDRIANTSIFERANA

Laboratoire de Chimie Organique "Produits Naturels," Université d' Antananarivo, Antananarivo 1001, Madagascar

ABSTRACT.—Brightly colored ranid frogs of the genus *Mantella* are found only in rain forests of Madagascar. Gc-ms and gc-Ft-ir analyses of skin alkaloids of seven different species, including four populations of *Mantella madagascariensis*, are reported. All contain one or more representatives of the pumiliotoxin A (PTX-A) class with the 13,14-dihydro derivatives **309A** and **325A** found in major amounts in the four populations of *M. madagascariensis*, while **307A** (PTX-A) is found in two populations of *M. madagascariensis* and in three additional species, *Mantella aurantiaca, Mantella viridis,* and *Mantella crocea*. The latter three species also contain appreciable quantities of **323A** (PTX-B). The four populations of *M. madagascariensis* show major amounts of two 1,4-disubstituted quinolizidines, **217A** and **231A**, and a 5,8-disubstituted indolizidine, **217B**, in addition to many minor or trace quinolizidines and indolizidines. Such disubstituted quinolizidines and indolizidines are present as trace alkaloids in the six other species of *Mantella*, along with 3,5-disubstituted indolizidines, 3,5-disubstituted pyrrolizidines, the decahydroquinoline *cis*-**195A**, tricyclic alkaloids, and homopumiliotoxins. A new alkaloid class, which appears to contain a quinolizidine moiety, is seen in *M. aurantiaca* and *M. crocea* and is represented by **235C** and several congeners.

Nearly 300 alkaloids have so far been detected in skins of dendrobatid frogs and other amphibians, where their likely purpose is to deter predation (1,2). A code, employing each alkaloid's nominal molecular weight and a letter when necessary to distinguish isomers, is used in boldface to characterize them. There are two major groups of such alkaloids: (A) those that could derive from straight carbon-chain precursors, such as a 2,5-disubstituted pyrrolizidine or a 2,6-disubstituted piperidine (with a second straight-chain component in the 8-alkylindolizidines and the 1-alkylquinolizidines) and (B) alkaloids that contain one or more isoprene units. Among class A (see Figure 1) are cis- or trans-fused 2,5-disubstituted decahydroquinolines (**1**), 3,5-disubstituted pyrrolizidines (**2**), 3,5- or 5,8-disubstituted indolizidines (**3**), 1,4-disubstituted quinolizidines (**4**), 1-azaspiro[5.5]undecan-8-ols (histrionicotoxins) (**5**), and tricyclic alkaloids (**6**), such as the gephyrotoxins and coccinellines. Among class B (see Figure 1) are the pumiliotoxin-A (PTX-A) class (6-alkylidene-8-methyl-8-hydroxyindolizidines) (**7**) with two important subclasses of the 7-hydroxy PTX-A alkaloids (the allopumiliotoxins) and the homopumiliotoxin alkaloids which contain a quinolizidine rather than an indolizidine nucleus; cyclopenta[*b*]quinolizidines (**8**) (3); the pseudophrynamines (3a-prenyl-pyrrolo[2,3-*b*]indoles) (**9**) (4); the pyrrolizidine oximes (hexahydro-2,2-dimethylspiro{cyclopentane-1,1′-[1*H*]pyrrolizine}-7′oximes) (**10**) (5); and the potent steroidal neurotoxins of the batrachotoxin and samandarine groups (**11**).

Skins of non-dendrobatid frogs of ten amphibian families (4,6) have been examined for alkaloids. Only the following three genera contained alkaloids, most of which were of the "dendrobatid types," in particular pumiliotoxins: Myobatrachidae (genus *Pseudophryne*) (4,6), Bufonidae (genus *Melanophryniscus*) (6,7) and Ranidae (genus *Mantella*) (6). Frogs and toads from seventy-five other genera did not contain skin alkaloids (4,6).

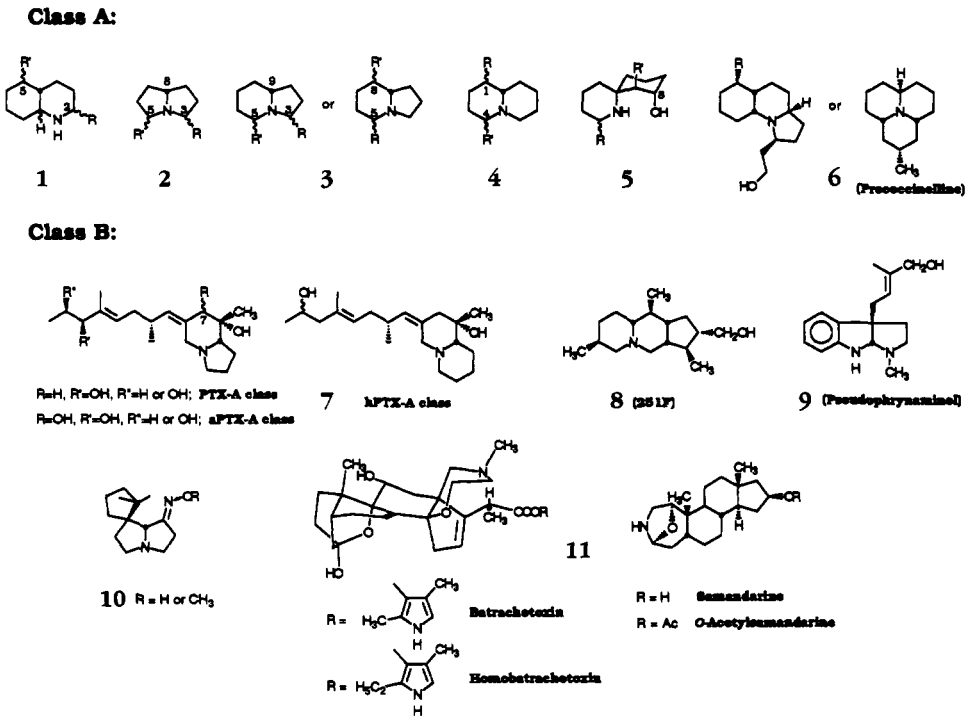


FIGURE 1. Major structural types of amphibian alkaloids (see text for definition of Classes A and B).

Frogs of the family Ranidae in Madagascar are almost entirely species of the endemic subfamily Mantellinae (genera *Mantella* and *Mantidactylus*) (8). This present work extends a preliminary study (6) on alkaloids from two species of Madagascan frogs, *Mantella aurantiaca* (Merguard) and *Mantella madagascariensis* (Grandidier), which was conducted on skin extracts from three frogs obtained through a commercial dealer. Alkaloid profiles from four populations of *M. madagascariensis* are compared, as well as profiles from the following other species: *Mantella* sp. cf. *madagascariensis, Mantella aurantiaca, Mantella betsileo* (Grandidier), *Mantella crocea* (Pintak and Böhme), *Mantella laevigata* (Methuen and Hewitt), and *Mantella viridis* (Pintak and Böhme). Gc in conjunction with ms or Ft-ir spectroscopy provided the basis for structural assignments.

EXPERIMENTAL

SOURCE MATERIAL.—Identification of species was based on literature descriptions (8–11). Voucher specimens are in the collections of the American Museum of Natural History, New York. MeOH extracts were prepared from skins of frogs collected at the following Madagascan sites. Total wet wt of skins in grams is indicated in parentheses following the number of skins.

Mantella madagascariensis (four populations): (A) Andasibe (Nov. 4/89), 10 skins (2.1 g), probably about 18 km north of Andasibe, obtained from local collector; (B) 11 km east by road from Andasibe (Nov. 4/89), 10 skins (2.4 g); (C) 45 km south by road from Moramanga towards Anosibe An' Ala (Nov. 5/89), 10 skins (2.1 g); (D) Ranomafana (Nov. 8/89), 10 skins (2.0 g). *Mantella* sp. cf. *madagascariensis:* (E) Ambavala, a village about 8 km southwest of Sandrakatsky (Dec. 6/90), 6 skins (0.5 g). *M. aurantiaca:* (F) Andasibe (Nov. 4/89), 15 skins (2.3 g), probably about 14 km north of Andasibe, obtained from local collector. *M. betsileo:* (G) Antanambaobe (Dec. 3/90), 11 skins (1.8 g). *M. crocea:* (H) Andasibe (Nov. 4/89), 10 skins (0.9 g), probably about 16 km north of Andasibe, obtained from local collector. *M. laevigata:* (I) Ambodimanga (Dec. 13/90), 6 skins (0.5 g). *M. viridis:* (J) Presumably near Antsiranana (Nov./89), 1 skin (0.24 g), obtained from a commercial dealer.

Extracts of skins of three species of mantellid frogs of the genus *Mantidactylus,* namely *Mantidactylus opiparis* (Peracca), *Mantidactylus femoralis* (Boulenger), and *Mantidactylus lugubris* (Duméril) contained no alkaloids (data not shown).

INSTRUMENTATION.—Initial gc analyses used He carrier gas and a 6 ft column of 1.5% OV-1 on 80–100 mesh Gas Chrom Q (2 mm i.d.) in a Hewlett-Packard Model 5890 flame ionization gas chromatograph equipped with a 3390A recorder-integrator. A Finnigan model 4500 mass spectrometer with an INCOS data system and a 25-m bonded OV-1 fused silica capillary column (Alltech, 0.25 mm i.d.) was used in either the electron impact (eims) or chemical ionization (cims) mode. This instrument was also used with a bleed of ND_3 for completely D-exchanged eims. A Finnigan Model 800 ion trap detector system also was used for gc-ms analysis with either a Hitachi or Varian (model 3400) gas chromatograph fitted with an HP-5 fused silica capillary column (polymer of 5% diphenylsioloxane, 95% dimethylsiloxane, 25 m×0.32 mm) programmed from 100° (initial time, 1 min) to 280° (final time, 10 min) at 10°/min to generate the mass chromatogram. Exact masses were measured using a JEOL SX102 high resolution mass spectrometer fitted with a 15 m×0.20 mm HP-5 column and were in all cases within 5 ppm of calculated masses. A Hewlett-Packard gas chromatograph (model 5890) fitted with an HP-5 column (identical to that of the ion-trap instrument and using the same program) and interfaced with an HP-5965A FTIR instrument having a narrow band (4000–750 cm^{-1}) detector and a 59970 IRD ChemStation data system was used to generate Ft-ir spectra of gc peaks.

ANALYSIS OF ALKALOIDS.—Weighed skins were cut into small pieces and ground with MeOH three times with at least 5 volumes of methanol per 1 volume of skin. The combined MeOH extract was diluted with an equal volume of H_2O, and the aqueous MeOH solution was extracted three times with one-half the volume of $CHCl_3$. The pooled $CHCl_3$ layers were extracted three times with one-third the volume of 0.1 N HCl. The combined acid layers were made basic (pH>9) with 1 N aqueous NH_3 and extracted three times, each time with one-third the volume of $CHCl_3$. The combined $CHCl_3$ layers were dried over anhydrous Na_2SO_4 and cautiously evaporated in vacuo at 30°. The residue was dissolved in a volume of MeOH such that 1 μl of this alkaloid extract was equivalent to 1 mg of the original wet wt of skin.

Gc analyses were carried out with alkaloid extracts equivalent to 2 mg of skin on 1.5% OV-1 packed columns using a program of 150° to 280° at 10° per min (Figures 2 and 3). Chemical ionization gc-ms analyses used NH_3 or ND_3 as the reagent gas. ND_3 reveals the number of exchangeable OH and NH protons (12). Gc-ms (ion trap or electron impact) and gc-Ft-ir analyses with a capillary column permitted the characterization and/or identification of alkaloids.

Acetylation of secondary amines in a small portion of alkaloid extract was carried out after removal of MeOH using a few drops of Ac_2O-pyridine (1:1) at room temperature for 2 h. Solvents were removed with a nitrogen stream and the residue redissolved in MeOH. Hydrogenation of extract used an electrolytic hydrogen generator with H_2 at 2 atm. and a 10% Rh/Al_2O_3 catalyst for 2 h under rapid stirring.

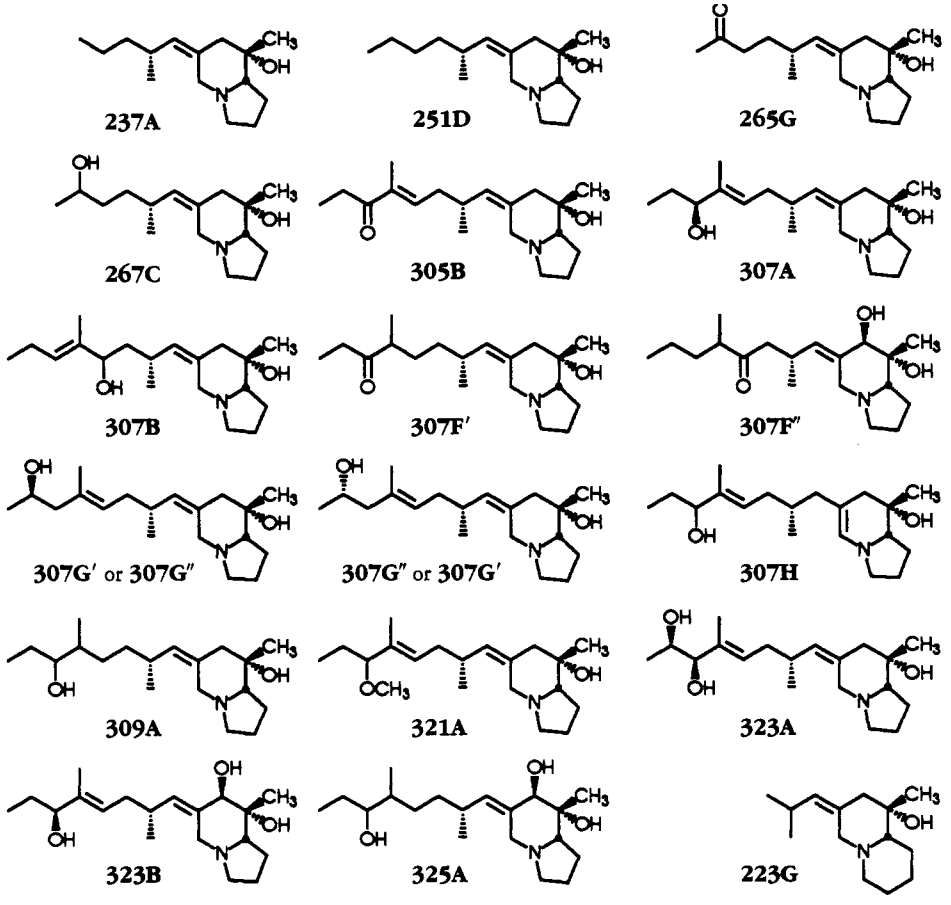
PROPERTIES OF ALKALOIDS.—The alkaloids detected in the extracts of skins of the frogs (*Mantella*) are listed below by class, using the identification code discussed above. The code number and letter is followed by (i) the molecular formula if it was determined in this study by hrms, (ii) the ion trap mass spectrum or, where designated, the eims with intensities relative to the base peak set equal to 100, (iii) exchangeable hydrogens (e.g., 0D, 1D, 2D), (iv) Ft-ir data with selected absorbances in cm^{-1} and intensities in parentheses relative to the maximum absorbance set equal to 100, and (v) derivatives and comments. The ion trap usually yields an $[M+1]^+$ peak for alkaloids, but this is concentration-dependent as are the intensitities of fragment ions. Consult Tables 1–4, Figures 2 and 3, and text for the occurrence of these alkaloids by species.

PTX-A CLASS.—*PTX alkaloids.*—Unless indicated otherwise, PTX alkaloids have ν_{OH} 3544 cm^{-1} (ca. 8) and a characteristic Bohlmann band pattern with a peak at 2798 (intensity ca. 34) and a shoulder at 2750–2700 (ca. 18) cm^{-1} (Figures 4 and 5). Their mass spectra contain significant ions at *m/z* 166 and 70.

237A: *m/z* 238(100), 220(12), 194(18), 166(85), 152(16), 84(30), 70(85); ir 963(15) cm^{-1}; for other properties see Daly and Spande (1). **251D**: *m/z* 252(2), 208(2), 194(6), 166(55), 84(22), 70(100); ir 963(13) cm^{-1}; for other properties see Daly and Spande (1). **265G**: *m/z* 265(2), 222(3), 194(5), 166(100), 148(8), 112(10), 84(15), 70(80); ir 1731(55), 1160(46), 963(21) cm^{-1}. **267C**: *m/z* 268(10), 250(4), 194(3), 176(5), 166(100), 84(20), 70(85); ir 3655(4), 962(18), 835(5) cm^{-1}. **305B**: *m/z* 306 (<1), 290(<1), 260(<1), 246(<1), 206(16), 193(47), 166(50), 150(17), 70(100); ir see Figure 5. **307A**: *m/z* 290(3), 260(2), 206(20), 193(23), 176(12), 166(92), 70(100); ir see Figure 4; for other properties see Daly and Spande (1). **307B**: *m/z* 206(18), 193(27), 176(5), 166(100), 150(8), 70(92); ir see Figure 4; another diastereomer is also seen. **307F**: Diastereomer **307F'** *m/z* 308(2), 264(20), 194(4), 176(6), 166(100), 148(6), 70(90); ir see Tokuyama *et al.* (13); trace amounts of **307F"** detected. **307G**: Diastereomer **307G'** *m/z* 307(8), 262(15), 206(22), 194(10), 193(6), 176(10), 166(100), 70(65); 2D; ir see Figure 4; diastereomer **307G"** *m/z* 308(1), 290(2), 262(3), 206(18), 194(10), 193(22), 176(7), 166(82), 70(100). **307H**: *m/z* 306(13), 288(5), 206(22), 193(100), 176(15), 166(45), 150(25), 84(20), 70(78); ir see Figure 5. **309A**: *m/z* 310(12), 292(5), 262(2), 194(2), 176(2), 166(100), 70(55); 2D; ir See Figure 4. **321A**: 306(22), 290(40), 206(30), 193(25), 166(73), 70(100); ir 1100(54), 968(29) cm^{-1}; for other properties, see Daly and Spande (1). **323A**: *m/z* 278(3), 260(2),

TABLE 1. Pumiliotoxin A (PTX-A) Class Alkaloids Found in Madagascan Frogs (*Mantella*).[a]

Structures



321C 207G, **235J**, **249F**, **251L**- OAc, **317**: Structures unknown

PTX-A Alkaloid	Relative Amount in Species or Population[b]									
	A	B	C	D	E	F	G	H	I	J
PTX **237A**				++						
PTX **251D**	+		+	+			+			
PTX **265G**					+			+		+
PTX **267C**					++	++		+++		
PTX **305B**						+		+		
PTX **307A**	++	++	++			++	+	+++		+++
PTX **307B**						+		+		++
PTX **307F′**	+		+	+						
PTX **307F″**	+			+						
PTX **307G′**							+		++	
PTX **307G″**										+
PTX **307H**										+
PTX **309A**	+++	+++	++	+++						
PTX **321A**										+
PTX **323A**						+++		+++		++
aPTX **321C**							+			
aPTX **323B**						++	+	+++		
aPTX **325A**	++	++	++		+					

TABLE 1. Continued.

PTX-A Alkaloid	Relative Amount in Species or Population[b]									
	A	B	C	D	E	F	G	H	I	J
hPTX **207G**										+
hPTX **223G**							+		+	
hPTX **235J**		+								
hPTX **249F**	+									
hPTX **251L**-*O*Ac			+							
hPTX **317**							+			

[a]A, *Mantella madagascariensis*, Population A; B, *M. madagascariensis*, Population B; C, M. madagascariensis, Population C; D, *M. madagascariensis*, Population D; E, *Mantella* sp. cf. *madagascariensis*; F, *Mantella aurantiaca*; G, *Mantella betsileo*; H, *Mantella crocea*; I, *Mantella laevigata*; J, *Mantella viridis*.

[b]+++=Major alkaloid. ++=Minor alkaloid. +=Trace alkaloid.

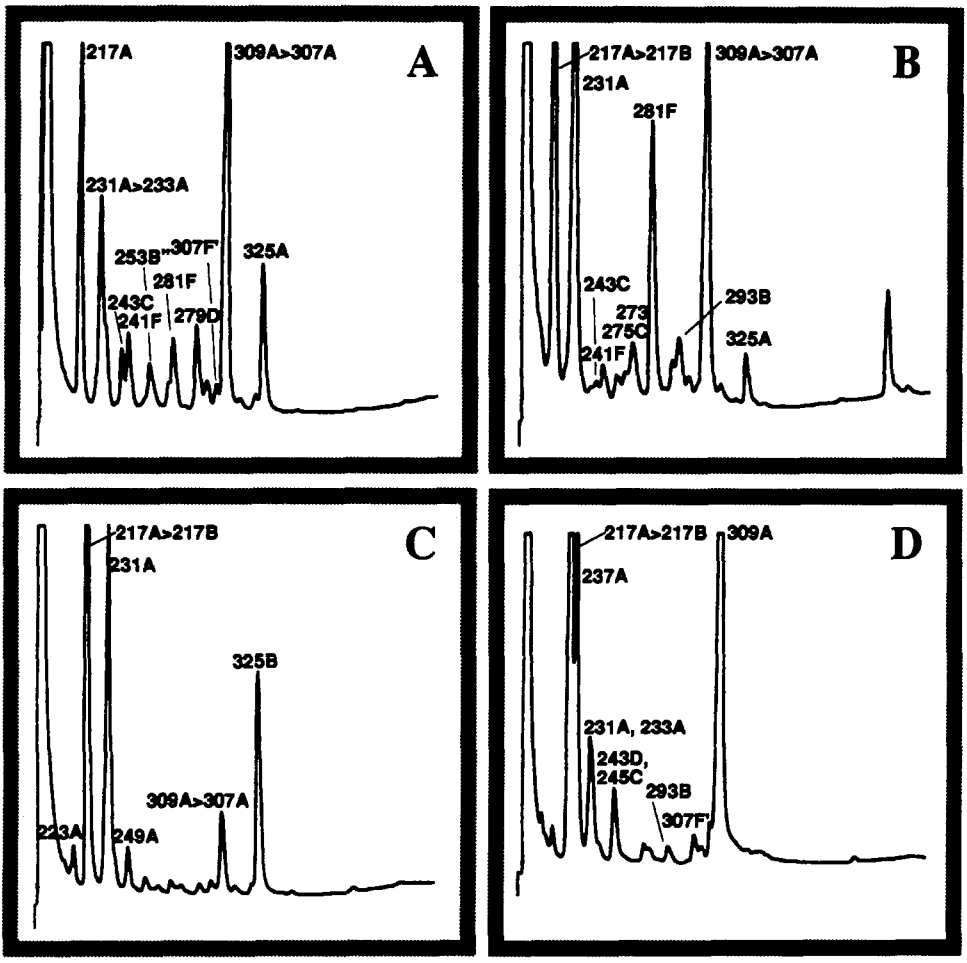
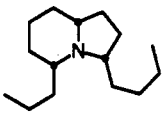


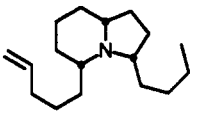
FIGURE 2. Gc profiles from *Mantella madagascariensis*. Location: (A) Andasibe, Madagascar; (B) road east of Andasibe; (C) road south towards Anosibe; (D) Ranomafana. The chromatograms were obtained with a 6-ft (2 mm i.d.) 1.5% OV-1 packed column, with a flame ionization detector and a flow rate of 30 ml/min He. A sample of 2 μl of MeOH alkaloid extract equivalent to 2 mg (wet cut) skin was injected at a column temperature of 150°. After the maximum of the solvent peak (MeOH) was passed (ca. 0.3 min) the column was heated to 280° at 10° per min.

TABLE 2. Indolizidine Alkaloids Found in Madagascan Frogs (*Mantella*).[a]

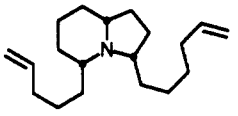
Structures



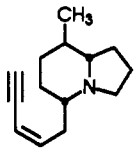
223AB



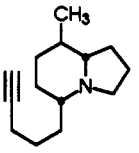
249A



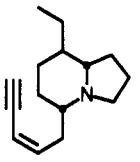
275C



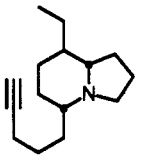
203A



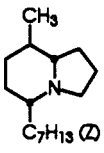
205A



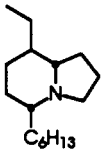
217B



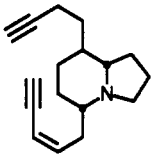
219F



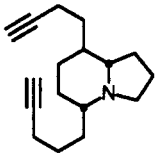
235B



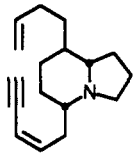
237H



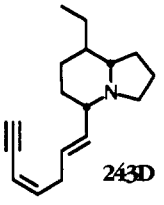
241F



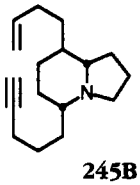
243B



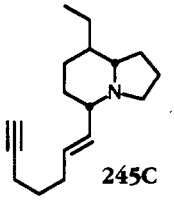
243C



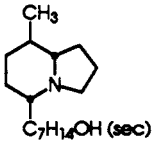
243D



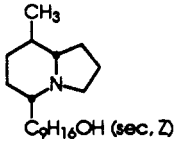
245B



245C



253B



279D

Structure unknown

295B

Indolizidines	Relative Amounts in Species or Populations[b]									
	A	B	C	D	E	F	G	H	I	J
3,5-disubstituted										
223AB										+
249A		++	++							
275C		++	+							
5,8-Disubstituted										
203A				+						
205A			+							
217B	++	++	++	++			+			++
219F							+			
235B	++									
237H										++
241F	++	++								
243B	+									
243C	++	+								
243D			+	++						
245B	+									
245C			+	++						
253B	++									

TABLE 2. Continued.

Indolizidines	Relative Amounts in Species or Populations[b]									
	A	B	C	D	E	F	G	H	I	J
279D	++	+								
295B								++		

[a]A, *Mantella madagascariensis*, Population A; B, *M. madagascariensis*, Population B; C, *M. madagascariensis*, Population C; D, *M. madagascariensis, Population D;* E, *Mantella* sp. cf. *madagascariensis*; F, *Mantella aurantiaca*; G, *Mantella betsileo*; H, *Mantella crocea*; I, *Mantella laevigata*; J, *Mantella viridis*.
[b]+++=major alkaloid; ++=minor alkaloid; +=trace alkaloid.

206(10), 193(23), 176(13), 166(22), 153(20), 70(100); ir 3650(5), 3612(8), 1021(30) cm^{-1}; for other properties see Daly and Spande (1).

Allo-PTX alkaloids.—The allo-PTX alkaloids have a Bohlmann band at ca. 2800 cm^{-1} with no shoulder and an absorption at ca. 1010 cm^{-1}. Their mass spectra exhibit major ions at *m/z* 182 and 70.

321C: *m/z* 323(2), 322(3), 304(10), 288(2), 222(2), 209(55), 192(12), 182(32), 114(32), 70(100); a satisfactory ir was not obtained. **323B**: *m/z* 209(8), 192(7), 182(33), 114(22), 70(100); for other properties see Daly and Spande (1). **325A**: *m/z* 326(2), 308(1), 290(3), 206(1), 192(1), 182(38), 166(3), 70(100); ir 3649(8), 3522(8), 2802(32), 1311(27), 1148(27), 1013(33), 990(28) cm^{-1}; for other properties see Daly and Spande (1).

Homo-PTX alkaloids.—The homo-PTX alkaloids have a Bohlmann band pattern with a shoulder at 2800 and peak at 2750 cm^{-1}. The mass spectra typically have ions at *m/z* 180 and 84. Certain of the following alkaloids (**235J**, **249F**, **251L**-OAc) are assigned to the homo-PTX subclass primarily based on their ir spectra. Although exhibiting an *m/z* 84 fragment ion, they lack the *m/z* 180 fragment ion.

207G: *m/z* 207(3), 180(35), 84(100); a satisfactory ir was not obtained. **223G**: *m/z* 208(3), 180(30), 162(12), 84(100); ir see Figure 6; for other properties see Daly and co-workers (1,2). **235J**: *m/z* 235(12), 220(2), 208(2), 192(2), 134(8), 109(18), 84(100); 1D; ir 3551(7), 2755(21), 1121(21), 1056(19), 894(5), ~850(5) cm^{-1}. **249F**: *m/z* 249(12), 220(22), 176(2), 134(2), 123(16), 84(100); ir 3540(8), 2755(23), 1119(28), 1060(15) cm^{-1}. **251L**-*O*Ac: *m/z* 250(18), 222(32), 176(77), 148(40), 134(22), 84(80); ir 2750(22), 1750(64), 1184(54), 1118(32) cm^{-1}. **317**: *m/z* 318(13), 292(3), 262(2), 220(10), 208(15), 207(20), 190(12), 180(100), 164(12), 148(10), 98(18), 84(72); 2D; a satisfactory ir was not obtained.

INDOLIZIDINES.—*3,5-Disubstituted indolizidines.*—The Bohlmann bands are diagnostic for stereochemistry. The ion trap spectra typically show a significant *m/z* 124 fragment.

223AB: *m/z* 180(25), 166(100), 124(33); only the 5*Z*,9*Z* isomer was detected based on ms identification; for properties of all four diastereomers, see Garraffo *et al.* (7). **249A**: *m/z* 248(<1), 206 (<1), 192(100), 180(20), 166(8), 138(12), 124(22), 110(8), 96(10); 0D; ir see Figure 7. **275C**: *m/z* 206(23), 192(100), 135(12), 124(23); 0D; ir 3080(4), 3060(4), 2789(18) (see ir of **249A**, Figure 7), 1640(4), 990(8), 915(12) cm^{-1}.

5,8-Disubstituted indolizidines.—The Bohlmann band pattern (a sharp strong band at ca. 2789 cm^{-1}) is diagnostic for the cis stereochemistry at C-5 and C-9. The ion trap spectra typically show a significant *m/z* 96 fragment.

203A: *m/z* 138(100), 96(55); ir 3328(30), 3039(11), 2789(53) cm^{-1}. **205A**: *m/z* 205(2), 138(100), 96(60); for other properties see Daly and Spande (1). **217B**: *m/z* 218(1), 152(100), 96(53); 0D; ir see Figure 7; coelutes with **219F**. **219F**: *m/z* 152(100), 96(15), 70(10); coelutes with **217B**; a satisfactory ir was not obtained. **235B**: *m/z* 164(10), 151(13), 138(100), 96(40); ir 3010 cm^{-1} (internal cis-double bond); probably identical to **235B"** (14). **237H**: *m/z* 152(100), 96(62); a satisfactory ir was not obtained. **241F**: *m/z* 176(100), 96(50); 0D; ir 3327 (54), 3039(11), 2790(52), 2113(6) cm^{-1}. **243B**: *m/z* 178(100), 96(62); 0D; coelutes with **241F**; a satisfactory ir was not obtained. **243C**: *m/z* 202(1), 178(100), 136(8), 122(8), 108(5), 96(55); 0D; ir 3327(26), 3080(14), 3020(12), 2789(36), 1641(5), 993(8), 913(13) cm^{-1}. **243D**: *m/z* 242(17), 228(3), 214(8), 202(1), 186(8), 176(18), 164(12), 152(32), 122(44), 96(45), 91(50), 70(100); 0D; ir 3328(19), 3035(14), 2789(50), 970(15) cm^{-1}. **245B**: *m/z* 178(100), 96(62); ir 3328(23), 3085(13), 2788(48), 1640(8), 994(8), 914(18) cm^{-1}; coelutes with **243C**. **245C**: *m/z* 244(15), 230(5), 216(20), 206, 204, 202(~10), 188(15), 176(15), 174(17), 164(15), 152(32), 134, 132(15), 122(35), 96(45), 91(48), 79(60), 70(100); 0D; ir 3329(19), 2788(39), 970(17) cm^{-1}. **253B**: *m/z* 138(100), 96(25), 70(10); ir 3650(2), 2786(25), 1131(8) cm^{-1}. **279D**: *m/z* 278(<1), 164(12), 151(20), 138(100), 96(32); ir 3655(3), 3012(18), 2786(35), 1133(20) cm^{-1}. **295B**: *m/z* 250(3), 180(20), 167(22), 154(100), 112(25), 94(12); ir 3656(7),

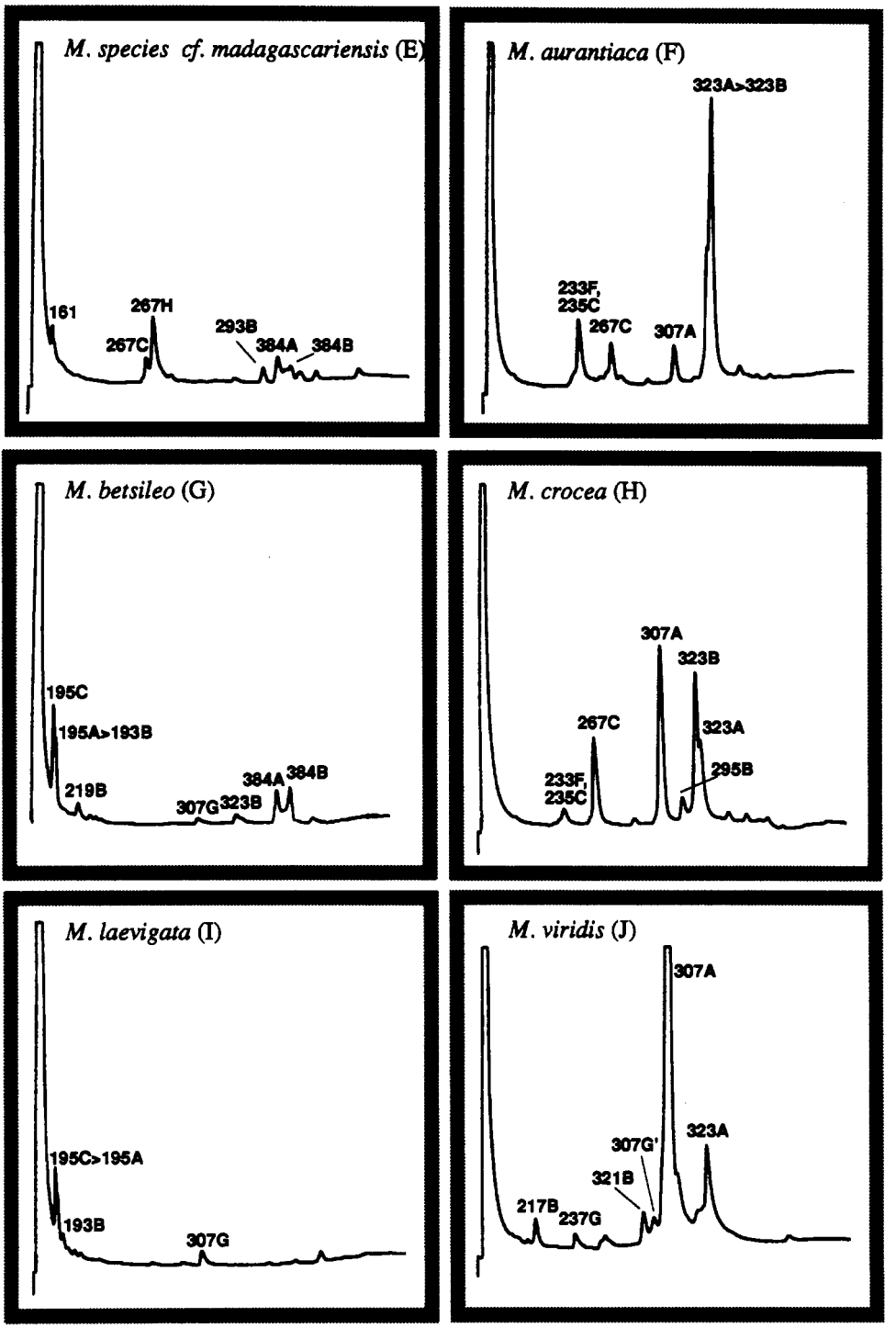
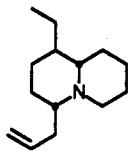


FIGURE 3. Gc profiles of alkaloids from six species of *Mantella*. See legend to Figure 2 for details.

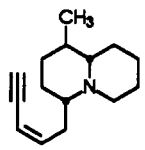
3020(17), 2785(19), 1732(14), 1137(23) cm^{-1}. Tentative classification: an indolizidine with one internal cis-double bond in the side chain and a MeCH(OH)-moiety. *O,O'*-diacetyl derivative *m/z* 380(35), 320(15), 287(2), 222(14), 196(72), 149(20), 136(100), 94(8), 70(10). The ms fragmentation indicates one hydroxyl in the side chain and one in the bicyclic ring system.

TABLE 3. Quinolizidines, Pyrrolizidines, and Decahydroquinolines and Related Alkaloids Found in Madagascan Frogs (*Mantella*).[a]

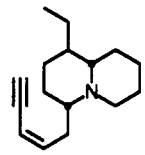
Structures



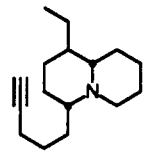
207I



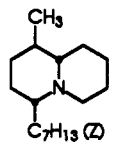
217A



231A

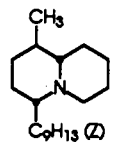


233A



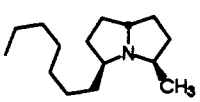
C_7H_{13} (Z)

249C

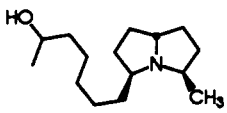


C_9H_{13} (Z) terminal triple bond

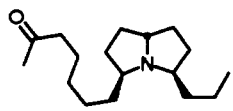
273A



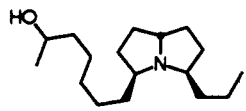
223H′



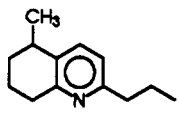
239K′



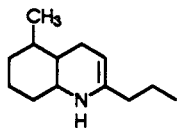
265H′



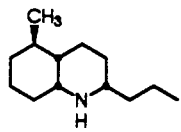
267H′



189



193D



cis-**195A**

Structure unknown

384A/B

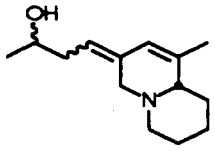
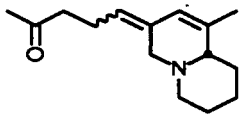
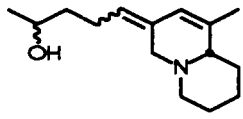
Alkaloid	Relative Amounts in Species or Populations[b]									
	A	B	C	D	E	F	G	H	I	J
Quinolizidines										
207I									+	
217A	+++	+++	+++	+++			+			+
231A	++	+++	+++	++			+	+		+
233A	++			++						+
249C							+			
273A		+								
Pyrrolizidines										
223H′+223H″								+		
239K′+239K″					+					
265H′+265H″					+					
267H′+267H″					+++					
Decahydroquinolines and Related										
189					+		+		+	
193D					+		+		+	
cis-**195A**					+		++[c]	+	++[c]	
384A/B					++		+++		++	

[a] A, *Mantella madagascariensis*, Population A; B, *M. madagascariensis*, Population B; C, *M. madagascariensis*, Population C; D, *M. madagascariensis*, Population D; E, *Mantella* sp. cf. *madagascariensis*; F, *Mantella aurantiaca*; G, *Mantella betsileo*; H, *Mantella crocea*; I, *Mantella laevigata*; J, *Mantella viridis*.

[b] +++=Major alkaloid. ++=Minor alkaloid. +=Trace alkaloid.

[c] A trace isomer of slightly shorter Rt also occurs.

TABLE 4. Alkaloids of Unknown and Tentative Structures Found in Madagascan Frogs (*Mantella*).[a]

Structures		
		
221F	**233F** Tentative Structures	**235C** (two diastereomers)

Alkaloid	Relative Amounts in Species or Population[b]									
	A	B	C	D	E	F	G	H	I	J
Tricycles										
191							+			
207J				+						
235K							+			
235C-Class										
221F						+				
233F	+					+		+		
235C	+					++		+		
251G						+[c]				
Others										
161					++				+	
195C					+		+++		+++	
205C				+						
211C									+	
211D					+		+		+	
223A			++						+	
265F						+				
271B							+			
281F	++	+++								
293B		++		+	++					

[a]A, *Mantella madagascariensis*, Population A; B, *M. madagascariensis*, Population B; C, *M. madagascariensis*, Population C; *D, M. madagascariensis*, Population D; E, *Mantella* sp. cf. *madagascariensis*; F, *Mantella aurantiaca*; G, *Mantella betsileo*; H, *Mantella crocea*; I, *Mantella laevigata*; J, *Mantella viridis*.

[b]+++=Major alkaloid. ++=Minor alkaloid. +=Trace alkaloid.

[c]Detected in a prior study (5).

1,4-DISUBSTITUTED QUINOLIZIDINES.—A prominent Bohlmann band (broad) at ca. 2787 cm^{-1} indicates a 1,4-*Z* configuration. All have an *m/z* 110 (20–30%) fragment in ion trap spectra.

207I: *m/z* 206(4), 166(100), 110(27); 0D; ir 3084(6), 2789(17), 1638(4), 991(6), 913(9) cm^{-1}. **217A**: *m/z* 218(4), 152(100), 110(33), 82(5); 0D; ir see Figure 7; a trace of a diastereomer with a longer retention time is also seen. **231A**: *m/z* 232(4), 166(100), 110(34); 0D; ir 3327(13), 3038(6), 2789(20), 2094(1) cm^{-1}. **233A**: *m/z* 166(100), 110(24); 0D; ir 3328(13), 2787(20) cm^{-1}. **249C**: *m/z* 249(5), 152(100), 110(30); a satisfactory ir was not obtained. **273A**: *m/z* 234(5), 190(3), 178(5), 152(100), 110(37); 0D; ir 3327(14), 2787(37), 1251(19), 1148(14) cm^{-1}.

3,5-DISUBSTITUTED PYRROLIZIDINES.—The ms exhibit major fragments corresponding to loss of one or the other of the side chains and a weak *m/z* 110 fragment. The Bohlmann bands, while weak for three of the four diastereomers possible for unsymmetrically substituted structures, are characteristic and different enough for the exo,exo (5*Z*,8*E*) and exo,endo (5*E*,8*Z* or 5*E*,8*E*) cases to be useful in assigning stereochemistry [cf. Jones *et al.* (28) and unpublished work]. The endo,endo (5*Z*,8*Z*) isomer, the only 3,5-disubstituted pyrrolizidine with significant Bohlmann bands, has yet to be detected in nature.

223H: *m/z* 224(2), 124(100), 81(10); two diastereomers (**223H′** and **223H″**) detected [the former is exo,exo (5Z,8*E*)]; ir see Garraffo *et al.* (7). **239K**: *m/z* 224(5), 124(100), 81(10); a satisfactory ir was not

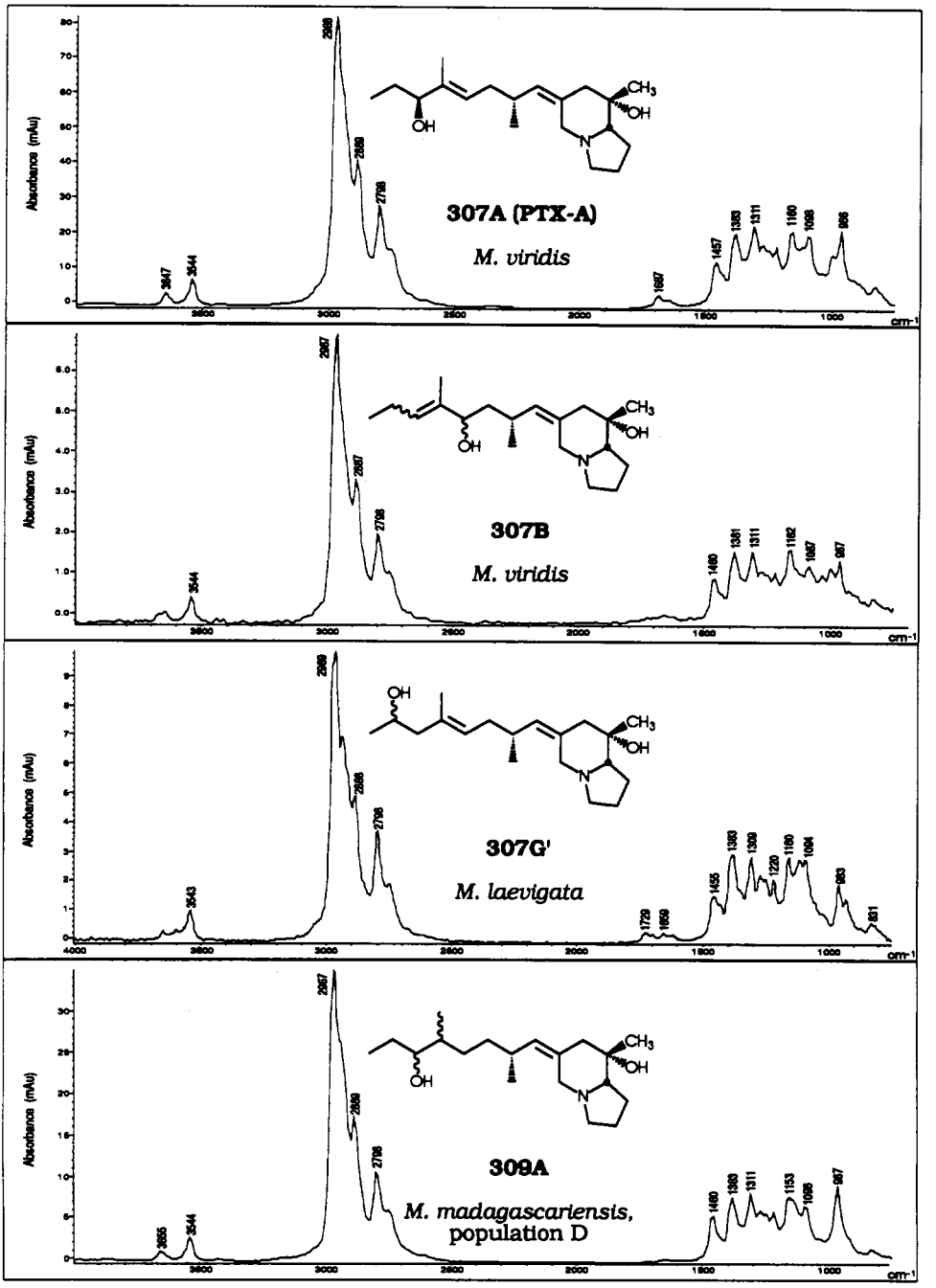


FIGURE 4. Vapor phase Ft-ir spectra of **307A** and analogues from Madagascan frogs.

obtained; two diastereomers (**239K'** and **239K"**) detected [the former is exo,exo (5*Z*,8*E*)]; tentative structures 3-(hydroxyheptyl)-5-methylpyrrolizidines. **265H**: *m/z* 222(30), 152(100), 110(18); ir 2960(100), 2875(50), 1730(11), 1461(11), 1362(16), 1148(13) cm^{-1}; two diastereomers (**265H'** and **265H"**) detected [the former is exo,exo (5*Z*,8*E*)]. **267H**: *m/z* 268(1), 252(3), 224(35) ($C_{14}H_{26}NO$), 152(100, $C_{10}H_{18}N$), 110(15), 70(20); ir 3653(2), 2960(100), 2873(47), 1461(11), 1366(14), 1138(13) cm^{-1}; two diastereomers detected (**267H'** and **267H"**) with nearly identical ir and ion trap spectra [both appear to be exo,exo (5*Z*,8*E*)].

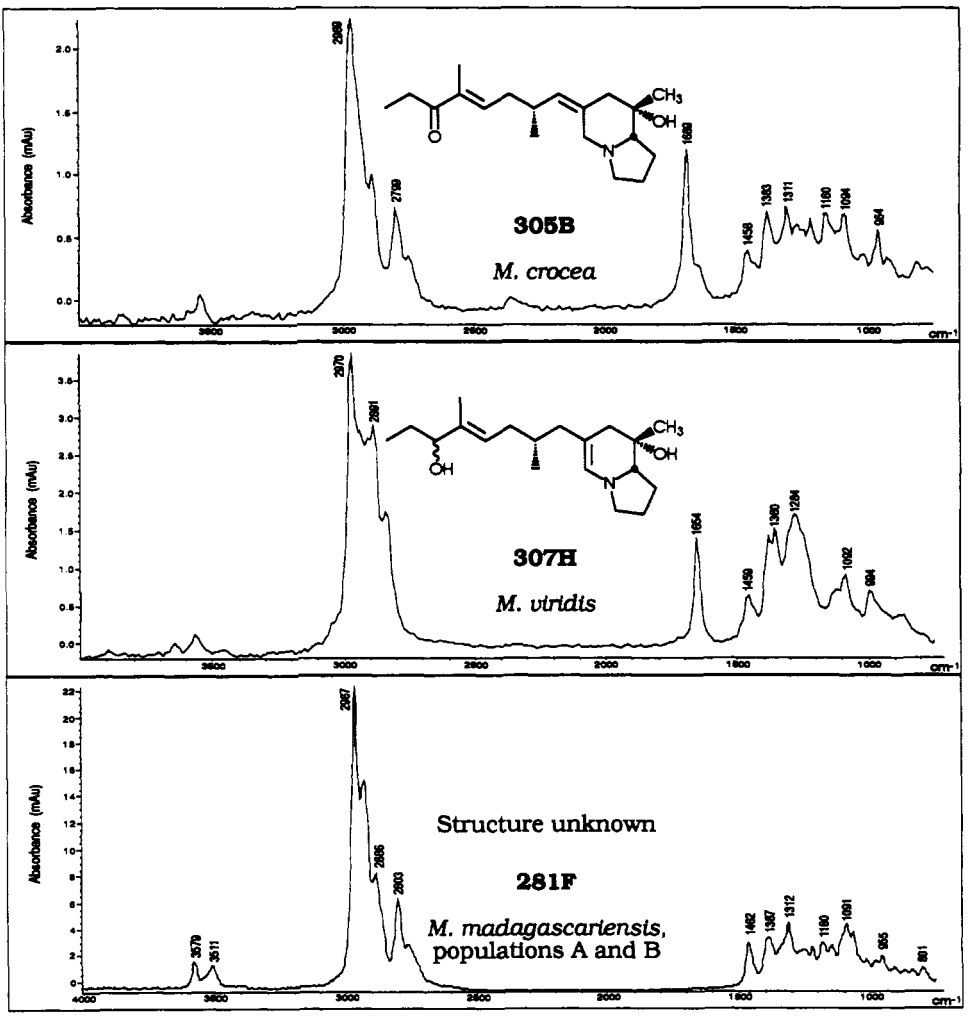


FIGURE 5. Vapor phase Ft-ir spectra of alkaloids from Madagascan frogs.

2,5-DISUBSTITUTED DECAHYDROQUINOLINES AND RELATED COMPOUNDS.—Most are characterized by a major cleavage from C-2 and a minor cleavage from C-5 in ion trap ms. Ft-ir can be used to distinguish cis- and trans-fused decahydroquinolines (13) and appears diagnostic in the Bohlmann band region for the relationship (2,8a-*Z* or 2,8a-*E*) of the 2- and 8a-hydrogens of the decahydroquinoline (unpublished work). The Bohlmann band pattern in most amphibian decahydroquinolines is usually similar to that of a cis 2,6-disubstituted piperidine, i.e., a weak peak at 2802 cm^{-1}.

189: *m/z* 190(10), 174(26), 161(100), 146(94),130(8), 117(8), 91(10); 0D; ir 3050(5), 1590(13), 1465(42), 823(10) cm^{-1}; a tetrahydroquinoline. **193D**: *m/z* 193(5), 178(8), 150(100), 122(12), 96(12); 1D; ir 3100(1), 3020(8), 1641(12), 1390(16), 1326(12) cm^{-1}; weak or absent Bohlmann bands; an octahydroquinoline. **195A**: Two $C_{13}H_{25}N$ isomers are detected. One is *cis*-**195A**, *m/z* 196(10), 180(2), 152(100), 135(7), 109(10). For other properties of *cis*-**195A** see Daly and Spande (1). The other isomer, apparently also a cis decahydroquinoline, has a slightly shorter Rt and an enhanced $[M-15]^+$, *m/z* 196(5), 180(32), 152(100), 110(10), 1D; ir identical to *cis*-**195A** except two weak absorptions at ca. 1000 cm^{-1} and slightly different ratio of 1380/1460 cm^{-1}. *N*-acetyl derivative: *m/z* 238(5), 222(10), 194(40), 152(60).

"DIMERIC" ALKALOIDS.—**384A**: $C_{26}H_{44}N_2$, *m/z* 384(1), 341(100) ($C_{23}H_{37}N_2$), 272(4), 230(2), 192(1), 190(8), 162(7), 136(10): eims *m/z* 384(5), 342(28), 341(100), 272(4), 192(2), 190(8), 162(8), 136(10); cims (NH_3) *m/z* 385(73), 341(12), 272(14), 194(52), 192(100); cims (ND_3) *m/z* 388(45), 387(100), 386(95), 343(5), 342(12), 341(17), 274(15), 273(25), 198(37), 195(45), 194(42), 193(48). Thus, exchange indicates 2D, but is incomplete. Eims with a bleed of small amounts of ND_3 indicates no exchange. Ir 3020(4),

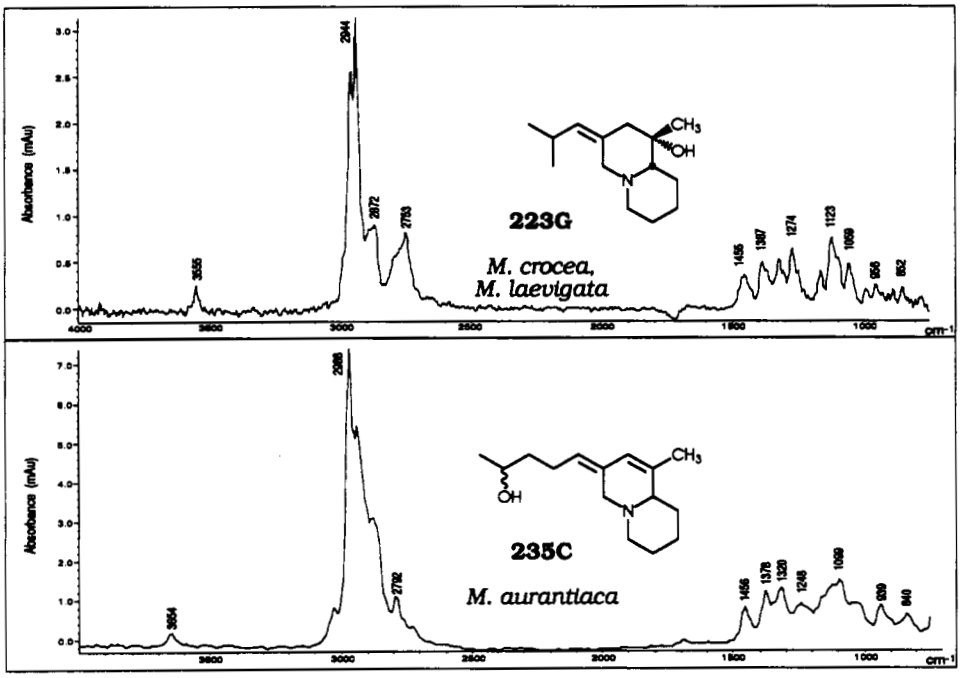


FIGURE 6. Vapor phase Ft-ir spectra of homopumiliotoxin **223G** and alkaloid **235C** from Madagascan frogs. The structure of **235C** is tentative.

1647(11), 1590(4) cm^{-1}; weak or absent Bohlmann bands. H_2-derivative: At least four diastereomers formed on reduction of **384A** and **384B**. Major H_2-diastereomer: *m/z* 386(2), 343(100), 232(5), 195(16), 150(6), 148(5), 122(5). **384B**: *m/z* 384(1), 341(100), 272(1), 228(1), 192(1), 192(8), 150(5), 148(5), 136(5); eims *m/z* 384(2), 342(24), 341(100), 272(2), 192(1), 190(8); cims data similar but not identical to that of **384A**; 2D; ir identical to that of **384A**.

TRICYCLIC ALKALOIDS.—**191**: *m/z* 192(43), 191(28), 176(28), 163(58), 152(20), 148(30), 134(33), 120(100), 106(33), 93(50); a satisfactory ir was not obtained, **207J**: *m/z* 207(18), 192(20), 178(28), 164(30), 152(28), 150(28), 136(100), 122(18), 110(35), 108(53), 96(18); ir 2989(100), ca. 2920(53), 2789(34), 1461(13), 1381(11), 1320(8), 1274(9), 1166(13), 970(13), ca. 920(6) cm^{-1}. **235K**: $C_{16}H_{29}N$; *m/z* 235(21), 234(100), 220(7, $C_{15}H_{26}N$), 206(28), 192(52, $C_{13}H_{22}N$), 178(10), 150(60), 136(62), 122(28); a satisfactory ir was not obtained.

ALKALOIDS OF THE **235C** CLASS.—These are recognized by a pair of ion trap mass spectral peaks at *m/z* 162/160.

221F: *m/z* 220(40), 206(8), 176(10), 162(100), 160(60), 148(18), 134(42), 120(38), 91(35); a satisfactory ir was not obtained. **233F**: $C_{15}H_{23}NO$; *m/z* 232(33), 218(10), 216(3), 190(8), 176(4), 162(100, $C_{11}H_{16}N$), 160(60, $C_{11}H_{14}N$), 146(12), 134(42), 120(40); ir 3020(14), 1731(51), 1163(21) cm^{-1}. **235C**: $C_{15}H_{25}NO$; *m/z* 234(80), 220(10), 218(7), 190(5), 176(15), 162(100, $C_{11}H_{16}N$), 160(58) ($C_{11}H_{14}N$), 148(13), 146(14), 134(52), 124(44), 120(48); 1D; ir see Figure 6. OAc derivative: The following data are for one of two diastereomers of nearly identical ms and ir spectra: *m/z* 276(48), 262(5), 234(18), 218(18), 216(18), 202(15), 176(5), 162(100), 160(58), 134(35), 120(32); ir 3028(8), Bohlmann bands 2790–2700, 1754(66), 1239(100), 1025(18), 949(8), 835(5) cm^{-1}. H_4 derivative: *m/z* 238 (38), 224(8), 210(5), 196(7), 195(8), 194(12), 180(18), 166(16), 152(5), 138(42), 125(40), 124(62), 110(80), 96(80), 84(100), 70(96), 55(55); ir 3654(8), 2932(100), 2790(31), 1456(10), 1381(13), 1360(10), 1173(15), 940(5). **251G**: $C_{15}H_{25}NO_2$; *m/z* 251(26), 250(45), 162(100); 2D; H_2-derivative, see data in Daly *et al.* (6); a congener of **235C** with an additional side-chain hydroxyl group.

UNCLASSIFIED ALKALOIDS.—**161**: $C_9H_{11}N_3$; *m/z* 161(76), 160(100), 146(2), 133(10, $C_8H_9N_2$), 119(8, $C_7H_7N_2$), 107(22), 92(5); 0D; ir 3050(15), 1594(100), 1161(23), 979(19), 842(26), 775(8) cm^{-1}. **195C**: *m/z* 196(8), 180(5), 163(<1), 152(100), 138(1), 124(8), 96(1); ir 2874(31), 2820(8) cm^{-1}. **205C**: *m/z* 205(5), 190(5), 176(23), 140(84), 126(100); a satisfactory ir was not obtained. **211C**: *m/z* 168(100), 124(52); ir (no Bohlmann bands) 3535(4), 1059(20) cm^{-1}. **211D**: *m/z* 212(48), 168(100), 164(25), 149(12),

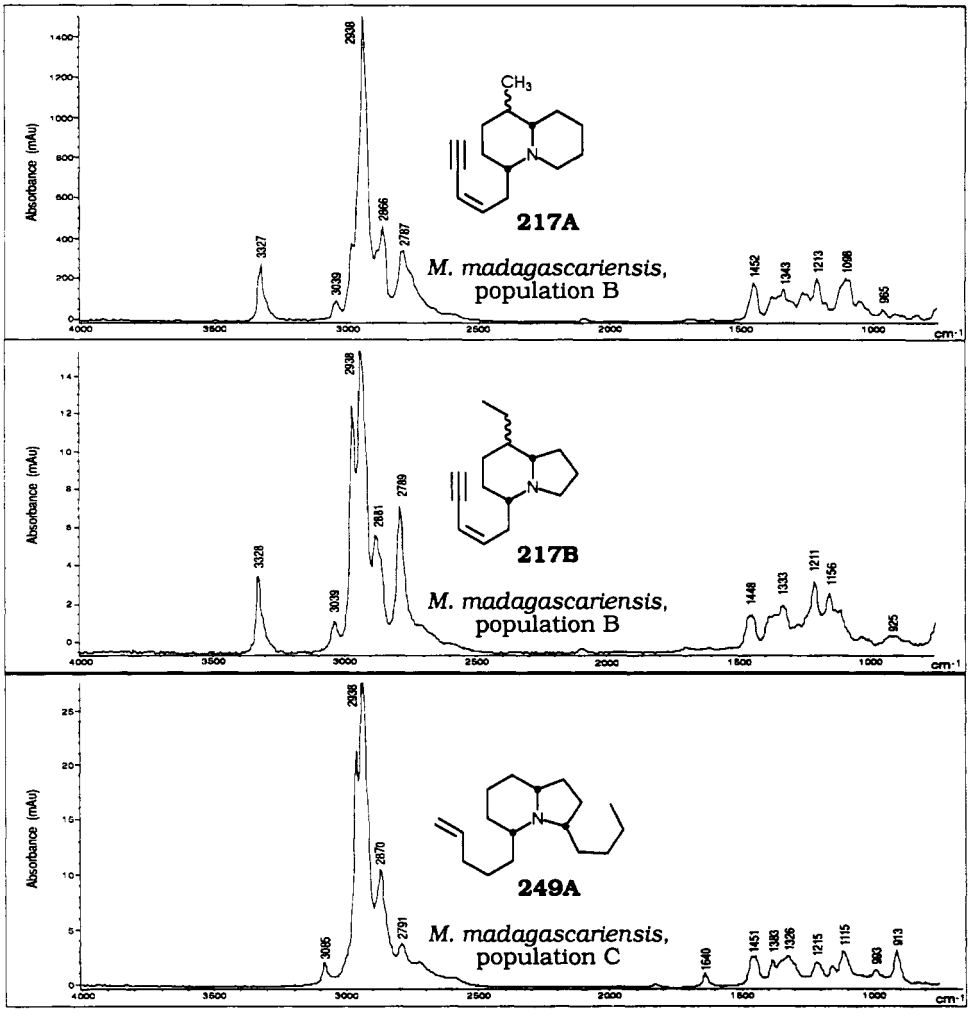


FIGURE 7. Vapor phase Ft-ir spectra of indolizidines and a quinolizidine from Madagascan frogs.

126(100), 110(10). 96(10); 0D; ir 2940(100), 2873(33), 1457(9), 1204(6), 1056(9). **223A**: *m/z* 180(100), 124(27), 96(5), 70(12); 0D: ir 2786(36), 1169(22) cm^{-1}. **265F**: $C_{16}H_{27}NO_2$; *m/z* 265(5), 250(<1), 220(<1), 206(18), 194(100, $C_{12}H_{20}NO$), 192(40, $C_{12}H_{18}NO$). 166(10), 148(15), 136(37), 134(50), 120(37). Mono-OAc derivative: *m/z* 307(8), 264(<1), 248(2), 246(2), 236(100), 206(16), 192(15), 176(45), 174(18), 148(15), 134(100), 120(55); H_2 derivative: *m/z* 266(24), 196(28), 129(12); may be related to the **235C** class. **271B**: *m/z* 271(5), 228(100, $C_{16}H_{22}N$), 200(8), 150(5); ir 2938(100), ca. 2860(39), 1656(17), 1460(17), ca. 1150(17), 1105(17) cm^{-1}. **281F**: *m/z* 280(2), 264(5), 234(5), 222(23), 84(55), 70(100); 2D; ir see Figure 5. **293B**: *m/z* 292(1), 262(1), 150(33), 95(35), 81(70), 67(100); 1D; ir 3655(3), 2790(26), 2750(15), 1162–1100(14), 970(14) cm^{-1}.

DISCUSSION

PTX-A-CLASS ALKALOIDS.—All of the Madagascan mantellid frogs examined contained at least one and some as many as six alkaloids of the PTX-A class (Figures 2, 3, Table 1). The four sampled populations of *M. madagascariensis* show a wide variability in their profiles of PTX and allo-PTX alkaloids, a phenomenon seen previously in populations of the Australian myobatrachid frog *Pseudophryne coriacea* (4) and in neotropical dendrobatid frogs (16,17). In addition to rather common representatives of this class, namely the PTX alkaloids **237A**, **251D**, **267C**, **307A** (PTX-A), **323A**

(PTX-B), and the allo-PTX alkaloid **323B,** extracts of *M. madagascariensis* contain substantial amounts of the 13,14-dihydro derivatives, PTX **309A** and allo-PTX **325A,** previously encountered as minor or trace alkaloids in dendrobatid frogs (13,14). The ir spectra of the dihydro PTX-A class alkaloids are characterized by the presence of the 965 cm^{-1} absorption of the 6,10-double bond ($\delta_{=CH}$ trisubstituted), but absence of the analogous absorption (990 cm^{-1}) for the 13,14-double bond (see Figure 4). Significant ms fragment ions at *m/z* 166 or 182 also indicate an intact 6,10 double bond in **309A** or **325A**, respectively.

A simple PTX-A class alkaloid not reported before, **265G**, occurs at trace levels in three species, namely *Mantella* sp. cf. *madagascariensis, M. crocea,* and *M. viridis*. The proposed structure, a 14-keto analogue of **267C**, is supported by the ketone absorption at 1731 cm^{-1} and absorptions consistent with the PTX-A class (3541 cm^{-1}, Bohlmann band pattern at 2799–2750, and the $\delta_{=CH}$ at 963 cm^{-1} for the 6,10-double bond) and an ms showing the usual strong fragment ions at *m/z* 166 and 70 of the PTX-A class, but also a weak ion (3%) at *m/z* 222 corresponding to a cleavage of 43 amu from the side chain.

Other previously unreported PTX-A class alkaloids that occur as traces in certain Madagascan extracts are the α,β-unsaturated ketone **305B** and two 16-hydroxy isomers of PTX-A, **307G′** and **307G″**, the last two with slightly longer and shorter retention times, respectively, than **307A**. Pumiliotoxin **307G′** lacks the usual *m/z* 290 fragment of PTX-A $[M-OH]^+$, which represents cleavage of the allylic alcohol at C-15. It has a significant $[M-45]^+$, $[M-MeCH(OH)]^+$, fragment and exhibits an ir spectrum (see Figure 4) slightly different from PTX-A in the trisubstituted double bond $\delta_{=CH}$ region (990–995 cm^{-1}). More important, the usual side-chain hydroxyl ν_{OH} at 3650 cm^{-1} appears now as a split absorption band, typical of most homoallylic alcohols in the vapor phase [7; see also Pouchert (18), spectra #206D, 207D, 208B, 208C], presumably due to hydrogen bonding of this hydroxyl group with the π electrons of the 13,14 double bond. The other 16-hydroxy diastereomer **307G″** has ms and Ft-ir spectra very similar to those of **307G′**, including the same split absorption band at 3650 cm^{-1}; however, slight differences were seen in the fingerprint region. The ν_{OH} absorption for the 8-hydroxyl group in **305B** and the two diastereomers **307G′** and **307G″** is seen at 3544 cm^{-1}, the usual position in PTX-A alkaloids for this tertiary alcohol, hydrogen-bonded to nitrogen.

The ketonic alkaloid **307F′** occurs in trace amounts in certain populations of *M. madagascariensis*, while the isomeric **307F″**, originally referred to as **307F** (13), was either barely or not detectable. The designation **307F** is now used as a general code designation to refer to material reported in early extracts (1) where the exact location (C-13 or C-15) of the carbonyl remains unknown. The isomer **307F′** was previously detected accompanying **307F″** in the dendrobatid frog *Dendrobates pumilio* (13,14); it occurred there in amounts much less than those of **307F″**. We have considered **307F″** and **307F′** as possible isolation artifacts; however, the detection of a different ratio of these ketones in the Madasgascan frog extracts, compared to those in the *D. pumilio* extracts, suggests that they are not artifactual.

Alkaloid **321A**, another presumed isolation artifact (13), is detected in *M. viridis* as a trace alkaloid. It should be noted that *M. crocea* has a large amount of the likely precursor **307A**, and **321A** was not detected there. In dendrobatid frogs, extracts from the same population can give remarkably different ratios of **307A** and **321A** under different isolation conditions (19), where variables such as alkaloid concentration and time are of likely significance.

Small amounts of two diastereomers of **307B**, possible allylic rearrangement products of **307A**, are seen in *M. auriantica, M. crocea,* and *M. viridis*. These alkaloids are

recognized by an enhanced *m/z* 193 ms fragment ion and *m/z* 70> *m/z*166 in intensity as well as subtle changes in the 990–995 cm^{-1} ir region (Figure 4).

Extracts of *M. auriantica, M. crocea,,* and *M. viridis* contained approximately 1% of the amount of **323A** as the 15*R*,16*S*-erythro diastereomer (*erythro*-**323A**), detected by the slightly shorter retention time of its diacetate relative to that of *threo*-**323A** diacetate (19). The erythro isomer had been detected before in the non-dendrobatid frog *Pseudophryne coriacea* of Australia, where its proportion relative to *threo*-**323A** was much higher (19). Rearrangement of *threo*-**323A** to *erythro*-**323A** does not occur under a variety of conditions (19).

M. viridis contains a trace alkaloid, **307H**, exhibiting an enamine absorption ($\nu_{C=C}$ 1654 cm^{-1}) in the ir spectrum (see Figure 5), a typical side chain OH absorption at 3650 cm^{-1}, and intact 13,14-double bond ($\delta_{=CH}$ 993 cm^{-1}) but a modified 8-hydroxyl ν_{OH} at 3589 cm^{-1} and no Bohlmann bands. It would appear likely to represent a 5,6-double bond isomer of pumiliotoxin **307A**.

Only three allo-PTX alkaloids were detected in the Madagascan frogs. Allo-PTX **323B** and its dihydro derivative **325A** have been previously isolated and characterized from dendrobatid frogs (13,14). Allo-PTX alkaloids are recognized by Ft-ir and by characteristic *m/z* 182 and 70 fragments in their mass spectra. A structure for **321C** is not proposed. It is present as a trace alkaloid in *M. betsileo*, and a satisfactory Ft-ir could not be obtained.

Several trace homo-PTX alkaloids are seen in the Madagascan frogs. Homo-PTX **223G** could be identified with certainty in *M. laevigata* and *M. betsileo*. It had previously been isolated from a dendrobatid frog (14). Insufficient data exist for the other apparent homo-PTX alkaloids to permit the assignment of definitive structures. Indeed, classification as homo-PTX alkaloids is tentative for certain of these trace alkaloids (see below). Remarkably, alkaloid **251L** occurs mainly as an acetate; it contains an acetylated hydroxyl group (ir 1750, 1184 cm^{-1}). The only other example of an amphibian alkaloid with an *O*-acetate is *O*-acetyl samandarine, which is a major alkaloid in extracts from the European fire salamander *Salamandra salamandra* (20).

Homo-PTX alkaloids are recognized by a characteristic Bohlmann band pattern in their Ft-ir spectra, which has a shoulder on the higher wavenumber side of the major absorption at 2754 cm^{-1} (see **223G**, Figure 6) rather than on the lower wavenumber side as in PTX and allo-PTX alkaloids. There is also a shift in the absorption frequency of the tertiary 9-hydroxyl group to 3555 cm^{-1} when compared with the equivalent 8-hydroxyl group of PTX alkaloids (3544 cm^{-1}). The mass spectra of homo-PTX alkaloids usually show characteristic *m/z* 180 and 84 fragment ions. Although the mantellid trace alkaloids **207G**, **235J**, **249F**, and **251L**-*O*-Ac all showed the characteristic Bohlmann band pattern of a homo-PTX alkaloid, the characteristic *m/z* 180 peak was missing in **235J** and **249F**. A satisfactory mass spectrum for **251L** could not be obtained because of an overlap with another alkaloid. Alkaloid **251L** occurs mainly as **251L**-*O*-Ac, in which the characteristic *m/z* 180 peak is missing. It appears likely that the new alkaloid class represented by **235C**, discussed below, is related to the homo-PTX alkaloids.

One population of the toad *Melanophryniscus stelzneri* has minor amount of homo-PTX alkaloids with a C_{10}-side chain, relatively common for alkaloids of the PTX-A class (7). A trace alkaloid, **317**, in *M. betsileo*, has ms data indicating a C_{10}-side chain.

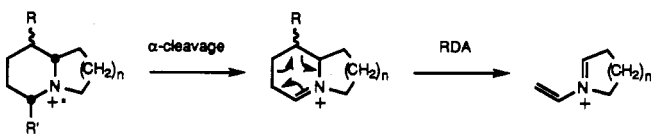
Alkaloids **309A** and **325A**, found in mantellid frogs, are expected to share the potent myotonic and cardiotonic activities of **307A** (PTX-A) and **323A** (PTX-B), which are due to effects on sodium channels (21) and perhaps to the release of calcium from intracellular stores (22,23). Activity is side-chain dependent with maximum activity so far residing in *threo*-**323A** (PTX-B).

INDOLIZIDINES AND QUINOLIZIDINES.—Two 3,5-disubstituted indolizidines, **249A** and **275C**, are seen in two populations of *Ma. madagascariensis*. These indolizidines are characterized by pairs of major α-cleavage fragment ions in their ms and a significant *m/z* 124 ion arising from a McLafferty-type rearrangement in the ion trap detector (*m/z* 124 is minor in eims [cf. Garraffo *et al.* (7)]. Jones *et al.*, (25) have reported the occurrence of the 5*E*,9*Z* diastereomer of **249A** in venom glands of the New Zealand ant *Monomorium smithii*. The Bohlmann band pattern of **249A** from *M. madagascariensis* indicates a 5Z,9Z stereochemistry, and as seen in Figure 7, the Bohlmann band pattern is significantly different from the 5,8-disubstituted type, being broader with some fine structure. Alkaloid **249A** from *M. madagascariensis* has an ir spectrum identical to the first eluting isomer in a synthetic mixture of the four diastereomers of 3-butyl-5-(pent-4-enyl)indolizidine, which had been identified as the 5*Z*,9*Z* isomer (25). The stereochemistry of **275C**, having an ir spectrum similar to **249A**, is assigned by analogy. A trace of 5*Z*,9*Z*-**223AB**, previously seen in dendrobatid frogs, is seen in *M. viridis*.

Skins of the populations of *Ma. madagascariensis* contain substantial amounts of unsaturated 5,8-disubstituted indolizidines and 1,4-disubstituted quinolizidines; in one population these exceed the amounts of alkaloids of the PTX-A class (Tables 2, 3).

The 5,8-disubstituted indolizidines found in these extracts are recognized in ion trap ms by base peaks of *m/z* 138 or 152 arising from α-cleavage of the side chain at C-5 and a significant (usually >50%) *m/z* 96 ion produced by a retro-Diels-Alder fragmentation (Scheme 1).

The 1,4-disubstituted quinolizidines found here are recognized in ion trap ms by analogous base peaks at *m/z* 152 and 166, accompanying a retro-Diels-Alder fragment ion at *m/z* 110, which is usually of lesser intensity than the *m/z* 96 ion of the 5,8-disubstituted indolizidines (Scheme 1).



$[M]^+$, n=1,2	n=1: *m/z* 138 (R=Me)	n=1: *m/z* 96 (~50%)
R=Me, Et	or 152 (R=Et)	
R′=*n*-alkyl, *n*-alkenyl, etc.	n=2: *m/z* 152 (R=Me)	n=2: *m/z* 110 (~30–50%)
	or 166 (R=Et)	

SCHEME 1

Characteristic and intense Bohlmann band patterns in their Ft-ir spectra (Figure 7) permit the assignment of 5,9-*Z* (indolizidine) or 4,10-*Z* (quinolizidine) stereochemistry in every case so far encountered in the Madagascan frogs. Bohlmann bands [Garraffo *et al.* (7)] arise when two or more C-H bonds adjacent to nitrogen are oriented trans antiparallel to the lone pair of electrons on nitrogen (Figure 8). The indolizidine Bohlmann bands are much sharper, while those of the quinolizidines are broader and extend to lower frequencies.

Gc-Ft-ir spectra also permit in most cases an assignment of the unsaturation pattern of R and R′ in such alkaloids. Easily distinguished are the terminal acetylene ($\nu_{\equiv CH}$, 3320; $\nu_{C\equiv C}$, 2100 cm^{-1}), terminal ethylene ($\nu_{=CH_2}$, 3080, $\delta_{=CH}$, 990<910 cm^{-1}), and internal *cis*- ethylene ($\nu_{=CH-}$, 3020 cm^{-1}) linkages. So far no internal acetylenes have been encountered in any frog skin alkaloids, and *trans*-ethylene linkages ($\delta_{=CH}$ 965 cm^{-1}) are rare.

Of the major indolizidines and quinolizidines detected in the Madagascan frogs,

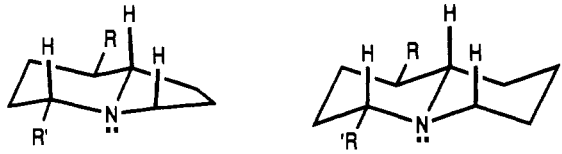


FIGURE 8. Depiction of GH bonds responsible for Bohlmann bands in indolizidine and quinolizidines. Bold hydrogens are trans-antiparallel to the nitrogen lone-pair.

indolizidine **217B** and quinolizidine **231A** appear to occur commonly in dendrobatid frogs (16). The stereochemistry of the indolizidine 8-substituent or the quinolizidine 1-substituent is unknown; however, we have provisionally assigned to them an equatorial configuration analogous to the configuration of the 8-Me group in 5,8-disubstituted indolizidines of known structure (14,24).

Two populations of *M. madagascariensis* contain indolizidines (**241F**, **243B**, **243C**, **245B**) with an unsaturated C_4 unit at C-8 and variously unsaturated C_5 side chains at C-5. Related alkaloids with base peaks of *m/z* 176 or 178 have been seen previously in the dendrobatid frog *Minyobates bombetes* (unpublished data).

Two populations of *M. madagascariensis* contain two related trace alkaloids, **243D** and **245C**, with complex ion trap spectra. Losses of 15, 29, 43, 57, 71 amu, etc., are detected, with a slightly prominent *m/z* 152 peak for both alkaloids. Both Ft-ir spectra show one terminal acetylene, a Bohlmann band pattern resembling an indolizidine, and a sharp 973 cm^{-1} absorption (possibly a $\delta_{=CH}$ for a trans olefin). An additional internal cis double bond (ca. 3020 cm^{-1}) appears to be present in **243D**. It is proposed that these indolizidines are 5,8-disubstituted and that the unsaturation adjacent to C-5 retards the usual facile α-cleavage. The proposed structures in Table 2 are provisional until further quantities of alkaloids become available for study.

Extracts of *Ma. crocea* contain a minor **295B** alkaloid having ν_{OH} at 3656 cm^{-1}, cis double bond with $\nu_{=CH}$ at 3020 cm^{-1}, and Bohlmann bands typical of an indolizidine. The ms indicates a MeCH(OH)- moiety to be present. Acetylation yields a di-*O*-acetyl derivative showing consecutive losses of 59 (OAc) and 60 (HOAc) amu in the ms. The major fragment (*m/z* 154) of **295B** is now seen at *m/z* 196, indicating that the second hydroxyl group is in the bicyclic ring, most probably in the pyrrolidine ring of an indolizidine. The *m/z* 112 ion of **295B** may be a hydroxy analogue of the usual *m/z* 96 ion arising from 5,8-disubstituted indolizidines, in this case generating an *m/z* 94 by dehydration. The *m/z* 94 ion is also seen in the diacetyl derivative. Additional data are required before a structure can be proposed for **295B**.

The indolizidines and quinolizidines are expected to be potent noncompetitive blockers of nicotinic (26,27) and perhaps other receptor-modulated channels.

3,5-DISUBSTITUTED PYRROLIZIDINES.—Skins of two species of *Mantella* contain 3,5-disubstituted pyrrolizidines. Such pyrrolizidines have characteristic ms and ir spectra. In addition to major ions resulting from loss of one or the other substituent, the 3,5-disubstituted pyrrolizidines frequently show a weak (10–20%) peak in ion trap ms at *m/z* 110 from a McLafferty rearrangement analogous to that (*m/z* 124) seen in 3,5-disubstituted indolizidines. The Bohlmann band pattern is diagnostic for the configuration of three of the four possible diastereomers of such pyrrolizidines. The two diastereomers of **223H** from *M. crocea* have ms identical to that reported (28) for the thief ant alkaloid, (5*Z*,8*E*)-3-heptyl-5-methylpyrrolizidine. Mass spectra do not distinguish the four diastereomers of 3-heptyl-5-methyl-pyrrolizidine (28), but the retention times

indicate that the relative configuration of one diastereomer in *M. crocea*, to be termed **223H′**, is identical to the 5*Z*,8*E* thief ant alkaloid, while the configuration of the other is unkown.

A major hydroxypyrrolizidine, **267H** (ν_{OH} 3650 cm^{-1}), and a trace ketonic pyrrolizidine, **265H** ($\nu_{C=O}$ 1730 cm^{-1}), are seen in *Mantella* sp. cf. *madagascariensis*. In each case, two diastereomers were detected. The major diastereomer **267H′** has a Bohlmann band pattern very similar to synthetic (5*Z*,8*E*)-3-heptyl-5-methylpyrrolizidine (28) and thus appears to have an "exo,exo" configuration (see Table 3). Also found in *Mantella* sp. cf. *madagascariensis* is a hydroxylated analogue (**239K**) of **223H**. Two diastereomers were present. Both appear to have the exo,exo configuration.

DECAHYDROQUINOLINES AND RELATED COMPOUNDS.—In general, the wide variety of decahydroquinolines usually seen in dendrobatid extracts or, as encountered recently, in extracts of toads of the genus *Melanophryniscus* (7) are not seen in frogs of the genus *Mantella*. *M. betsileo* and *M. laevigata* extracts contain the dendrobatid alkaloid *cis*-**195A**, formerly known as pumiliotoxin C (see Table 3), as a major component, accompanied by an isomer of slightly shorter retention time. The minor isomer appears to be a cis decahydroquinoline. It is not 5-*epi*-*cis*-**195A** (the methyl epimer of *cis*-**195A**), based on comparison with synthetic material obtained from a derivative provided by P. Grieco (15). Extracts of *Mantella* sp. cf. *madagascariensis* contain only *cis*-**195A**. All three extracts also contain a dehydro derivative, **193D**, of slightly longer retention time than *cis*-**195A**. Alkaloid **193D** has one exchangeable hydrogen, an ir absorption typical of an enamine [$\nu_{C=C}$(s), 1641 cm^{-1}, $\nu_{=CH}$ at 3020 cm^{-1}, cis], and an ms showing an *m/z* 150 base peak. A tentative structure (see Table 3), with a 2,3 double bond, would require a rearrangement to account for the *m/z* 150 base peak in the ms. All three extracts also contain an aromatic alkaloid **189** (see Table 3) having no exchangeable hydrogens. It is assigned a tetrahydroquinoline structure on the basis of many similarities between the ir spectrum and that of 2,3-cyclohexenopyridine [Pouchert (18), spectrum #1521A].

"DIMERIC" ALKALOIDS.—Possibly related to **193D** are the major dimeric alkaloids **384A** and **384B** of *M. betsileo*. These dimeric alkaloids also accompany **193D** in extracts from *Mantella* sp. cf. *madagascariensis* and *M. laevigata*. Alkaloids **384A** and **384B** occur in a roughly 1:1 ratio. They have identical ir spectra, indicating an internal double bond (3020 cm^{-1}) and an enamine or imine (1647 cm^{-1}) absorption overlapping a weak absorption at 1590 cm^{-1}. Cims with ND_3 indicates two exchangeable hydrogens each for **384A** and **384B**, although the exchange is incomplete. Surprisingly, eims with a ND_3 bleed, usually a very reliable method for obtaining spectra on deuterium-exchanged materials, shows little exchange in the molecular ion or any of the fragments. It may be that exchange under eims conditions is possible but only much slower in the presence of lower amounts of ND_3. The hrms established for **384A** a $C_{26}H_{44}N_2$ molecular ion and indicates that the major fragmentation, $[M]^+ \mapsto 341$ (100%), is due to loss of a propyl group. Other significant ms fragments are *m/z* 272 and a weak pair at *m/z* 192/190, whose ratio was appreciably different for **384A** and **384B**. Curiously, cims with ND_3 yielded substantial fragmentation accompanying the deuteronated molecular ion, leading to a greatly enhanced pair of ions at *m/z* 194 and 192 (ca. 45%). It would appear that the action of the NH_3 or ND_3 reagent gases leads to some reversion to monomers in the mass spectrometer. Acetylation (Ac_2O, overnight) fails to give an *N*-acetyl derivative, and hydrogenation halts at the uptake of two hydrogens. The major dihydro-384 diastereomer (one of four produced by hydrogenation of the mixture) gave an *m/z* 343 base peak on ms. A structure is not proposed for these apparent dimeric alkaloids.

TRICYCLIC ALKALOIDS.—A trace tricyclic alkaloid **191** occurs in *M. betsileo* and was

considered likely to be related to precoccinelline (MW 193), an insect alkaloid now detected in certain dendrobatid frogs (17) and bufonid toads (7); however, the mass spectrum is different from that reported for propyleine, which is a mixture of dehydro precoccinellines [cf. Tursch *et al.* (29), Mueller and Thompson (30)]. Traces of a tricyclic alkaloid, **207J**, possibly a homologue of the insect alkaloid precoccinelline, are seen in one population of *M. madagascariensis*. Another tricyclic alkaloid, **235K**, is detected in *M. betsileo* and is clearly different in ms from the tricyclic **235I** recently detected in the toad *M. stelzneri* of Argentina (7). All of these tricyclic alkaloids have complex ms, showing no predominant fragments.

ALKALOIDS OF THE **235C** CLASS.—Extracts of *Ma. aurantiaca* and *Ma. crocea* (Figure 3) contain an unknown alkaloid, **235C**, with a characteristic pair of ms fragment ions at *m/z* 162 (100) and 160 (40) and a weak fragment at *m/z* 190 $[M-45]^+$ [loss of MeCH(OH)]. Capillary gc reveals, at a slightly shorter retention time, lesser amounts (roughly one part to six parts of **235C**) of a related **233F** alkaloid showing a weak $[M-43]^+$ fragment and a ketone absorption (1731 cm^{-1}) in the ir spectrum. Alkaloid **235C** has one exchangeable hydrogen. The mixture of **235C** and **233F** in the crude extract is converted by hydrogenation (Pt, 2 h) to a mixture of three diastereomers (20:1:1) of mol wt 239, indicating structures with two rings and two unsaturated linkages for **235C** and two rings and three unsaturated linkages for **233F**. Alkaloid **235C** was originally reported (6), because of incomplete reduction, to form a dihydro derivative. The hrms indicate formulae of $C_{15}H_{25}NO$ and $C_{15}H_{23}NO$, respectively, for **235C** and **233F**. The ir spectrum of **235C** (see Figure 6) indicates a non-hydrogen-bonded hydroxyl group (ν_{OH}, 3650 cm^{-1}), a cis vinyl hydrogen ($\nu_{=CH}$ 3020), weak Bohlmann bands, and absorptions at 1099 (ν_{C-O} non-terminal) and 939 cm^{-1} ($\delta_{=CH}$ trisubstituted). The major tetrahydro-diastereomer formed on reduction of **235C** shows little change in the ν_{OH} absorption and a Bohlmann band pattern similar to that of a quinolizidine. The hrms indicates a $C_{11}H_{16}N$ formula for the *m/z* 162 ion of **235C** and shows the oxygen atom of **235C** and **233F** to reside in C_4H_9O and C_4H_7O moieties, respectively. The observation of no hydrogen bonding for the hydroxyl group of **235C** probably rules out a homoallylic position (see above discussion for **307G**). Ms fragments indicating losses of 15, 17, 45, 59, and 73 amu from the molecular ion support a side chain structure of MeCH(OH) $(CH_2)_2$- for **235C**.

Acetylation (Ac_2O/pyridine, 2 h) of **235C** gives a barely resolved 2:1 mixture of diastereomeric monoacetates ($[M]^+$ 277; ir 1754, 1239 cm^{-1}), accompanied by four other minor monoacetates. All show nearly identical ms, having a major $[M-1]^+$ peak and losses of 43, 59, 61, and 75 amu from $[M]^+$. The *m/z* 162/160 fragment ions remain unchanged. The ease of acetylation rules out a tertiary alcohol, and the separation of acetate diastereomers on gc supports **235C** being a mixture of secondary alcohol diastereomers. The minor monoacetates probably arise from the other minor regioisomers of **235C** (see below). The ketonic alkaloid **233F** is not acetylated, as expected.

Tentative structures are proposed for **235C** and **233F** in Table 4. Alkaloid **235C** is accompanied in *M. aurantiaca* by at least two isomers (e.g., **235C'**, **235C''**) at the trace level. Alternative C-4 and C-6 alkylidene structures are ruled out, since tetrahydro-**235C** shows no single major cleavage ion, such as would result from an α-cleavage of a side chain.

Another alkaloid, **221F**, detected in trace amounts in *M. aurantiaca*, is closely related to **235C** in having the *m/z* 162/160 pair and the same fragment pattern as **235C** below these masses. The fragmentations $[M-15]^+$ and $[M-45]^+$ are also seen. A tentative structure is proposed in Table 4.

No trace of the **251G** alkaloid seen in *M. aurantiaca* in the 1984 study (5) and

assumed to be a side-chain hydroxy congener of **235C** was detected in extracts of *M. aurantiaca* obtained in 1989.

Thus, Madagascan frogs of the genus *Mantella* contain an alkaloid class that appears to be analogous to the homopumiliotoxins. Such alkaloids have not been seen in dendrobatid frogs.

UNCLASSIFIED ALKALOIDS.—The trace alkaloid **161** from *Mantella* sp. cf. *madagascariensis* and *M. laevigata* has a molecular formula of $C_9H_{11}N_3$, thus highly unsaturated, representing an unusual new class of amphibian alkaloids. A structure is not proposed for **161**.

A bicyclic alkaloid **195C** is detected as a major alkaloid in *M. betsileo* and *M. laevigata* and as a trace in *Mantella* sp. cf. *madagascariensis*. The ms had a base peak of *m/z* 152 with a weak fragment at *m/z* 124 (8%) and no exchangeable hydrogens. The Ft-ir spectrum is similar in the Bohlmann band region to *exo,exo*-3,5-dibutylpyrrolizidine, but differs significantly in the fingerprint region, probably ruling out, at least, a 3,5-dipropylpyrrolizidine as a possible structure, although other pyrrolizidine structures might be compatible with the ir and the *m/z* 124 ion observed. An alkaloid **195C** has been reported in many dendrobatid frogs (16) and was suggested to have either a pyrrolizidine or indolizidine structure (17). Further studies are needed to ascertain whether **195C** represents one or several alkaloids and the nature and substitution pattern of the bicyclic ring system.

There are several trace alkaloids detected in the Madagascan frogs for which only very limited data could be obtained. These are **205C**, which has major fragments at *m/z* 140 and 126, **211C**, which has major fragments at *m/z* 168 and 124, and **211D**, which has major fragments at *m/z* 168 and 126. All appear likely to be relatively simple alkaloids with two readily cleaved side chains adjacent to nitrogen.

An alkaloid **223A** is present in one population of *M. madagascariensis* and in *M. viridis*. It is a bicyclic amine, probably the same as alkaloid **223A** reported to be present in many dendrobatid frogs (1,16). The structure is uncertain. A propyl substituent is readily lost. Alkaloid **223A** from the dendrobatid frog *Dendrobates auratus* was recently proposed to be a 1,4-dipropylquinolizidine (17), but additional data suggests that it may prove to be an indolizidine (17, note added in proof). Alkaloid **223A** may or may not prove to represent a single compound in amphibian extracts.

Another unknown alkaloid, **265F**, observed in *M. auriantica* has, instead of the *m/z* 162/160 pair of **235C**, a pair of fragment ions at *m/z* 194(100) and 192(40), for which hrms indicates formulae of $C_{12}H_{20}NO$ and $C_{12}H_{18}NO$. Acetylation results in a single base peak at *m/z* 236 (see Experimental) indicating a hydroxyl group in that fragment. The other oxygen resides in a C_4H_7O moiety (one double bond equivalent) easily cleaved from acetylated **265F**. The ir spectrum of **265F** indicates no carbonyl absorption. A structure is not proposed for **265F**. It may prove to be related to the alkaloids of the **235C** class.

A trace alkaloid **271B** detected in *M. betsileo*, with an enamine-like ir spectrum, yields a highly unsaturated major fragment $C_{16}H_{22}N$ at *m/z* 228, apparently through loss of a propyl substituent.

Two populations of *M. madagascariensis* contain significant amounts of a new alkaloid **281F** with two exchangeable hydrogens. The Bohlmann band pattern in the Ft-ir is identical to that of dihydro-**267C** (see Table 1 for **267C**). There are two hydroxyl ν_{OH} absorptions at 3580 and 3513 cm^{-1} and no $\delta_{=CH}$ for a trisubstituted double bond in the region 990–950 cm^{-1} (see Figure 6). Two $\nu_{C\text{-}O}$ are detected at 1093 and 1050 cm^{-1}, the latter suggesting a primary alcohol. The ms exhibits a major fragment at *m/z* 222 $[M-59]^+$ but no peaks (e.g., *m/z* 166, 194) typical of the PTX-A class with an intact 6,10 alkylidene side chain. It appears likely to be of the PTX-A class but having the 6,10-

double bond reduced and one double bond in the side chain. A structure is not proposed for **281F**.

Another alkaloid, **293B**, detected in minor amounts in two populations of *M. madagascariensis*, also may be related to the PTX-A class. Alkaloid **293B** showed significant ms peaks at *m/z* 150 and 67 rather than the usual *m/z* 166 and 70 of the PTX-A class. The Ft-ir indicates a side chain hydroxyl ν_{OH} at 3655 cm^{-1} but not the ring hydroxyl of the PTX-A class. An intact 6,10 double bond ($\delta_{=CH}$ at 970 cm^{-1}) could be present.

SUMMARY.—Skin extracts of *M. madagascariensis* contain by far the largest amounts of alkaloids and the greatest variety of indolizidines and quinolizidines of the five species of *Mantella* (Figures 2 and 3, Tables 2 and 3). Only *M. aurantiaca* and *M. crocea* contain substantial amounts of the new **235C** class of amphibian alkaloids, although a sympatric population of *M. madagascariensis* does contain traces of three such alkaloids. Extracts of *M. betsileo, M. laevigata,* and *Mantella* sp. cf. *madagascariensis* all have much lower amounts of alkaloids. All contain several of the same alkaloids, including **195A**, **195C**, **193D**, and the "dimers" **384A** and **384B** (Tables 3, 4).

The current survey of Madagascan frogs finds the presence of **235C**, **267C**, **323A**, and **323B** in *M. aurantiaca* as originally reported for extracts from two skins of *M. aurantiaca* obtained through a commercial dealer [Daly *et al.* (6), in which **267C** was erroneously proposed to be a new alkaloid, **267D**]. However, in the present extract, pumiliotoxin **307A** is found while pumiliotoxin **339A** is absent, and **233F** is found while **251G** is absent. The species *M. aurantiaca* occurs in isolated populations in swamp forests to the North of Andasibe, and each population may have its own profile of alkaloids.

In 1984 one skin of *M. madagascariensis*, obtained through a commercial dealer, yielded an extract containing three histrionicotoxins, **283A**, **285A**, and **285C**, as major alkaloids along with allo-PTX **323B** and an unusual, ringless, saturated tertiary amine, $C_{16}H_{35}N$ (**241B**), with a base peak at *m/z* 58 (5). There is no way of knowing where in Madagascar this single specimen was collected, nor its history after collection. The histrionicotoxins, the saturated amine, and allo-PTX **323B** were not detected in any of the four populations of *M. madagascariensis* of the present survey, nor were any of the other alkaloids (**195A**, **207A**, **235C**, **269A**) reported as minor or trace constituents in that one-skin sample. We do find significant amounts of **195A** [mistakenly called **195C** in Daly *et al.* (6)] in *Mantella* sp. cf. *madagascariensis, M. betsileo,* and *M. laevigata* and alkaloid **235C** in *M. aurantiaca* and *M. crocea*, and the 5,8-disubstituted indolizidines **203A** and **205A**, analogous to **207A**, in *M. madagascariensis.* However, the histrionicotoxins originally reported in *M. madagascariensis* have not been detected in any wild-caught Madagascan frogs of the present study. We are baffled by this discrepancy and can only assume that the origin or character of that one frog obtained through a dealer has a significant role.

Several classes of alkaloids at one time termed "dendrobatid," viz. the pumiliotoxins, allopumiliotoxins, homopumiliotoxins, the decahydroquinolines, quinolizidines and indolizidines, have now been found to occur in several species of ranid frogs of the genus *Mantella.* The occurrence of such dendrobatid alkaloids with their unique structures in certain genera of anurans from four different families poses interesting questions of origin (6).

ACKNOWLEDGMENTS

The authors (M.A. and J.W.D.) thank the University of Antananarivo and Emile Tsizaraina, General Secretary of MESUP (Madagascar), for a travel grant, and Astra Research and Development Laboratories, Sweden, for support of field work. We thank Dr. Lewis Pannell (NIDDK) for all cims and hrms data.

LITERATURE CITED

1. J.W. Daly and T.F. Spande, in: "Alkaloids: Chemical and Biological Perspectives." Ed. by S.W. Pelletier, John Wiley and Sons, New York, 1986, Vol. 4, Chapter 1, pp. 1–274.
2. J.W. Daly, H.M. Garraffo, and T.F. Spande, in: "The Alkaloids." Ed. by G.A. Cordell, Academic Press, San Diego, 1993, Vol. 43, Chapter 3, pp. 185–288.
3. T.F. Spande, H.M. Garraffo, H.J.C. Yeh, Q-L. Pu, L.K. Pannell, and J.W. Daly, *J. Nat. Prod.*, **55**, 707 (1992).
4. J.W. Daly, H.M. Garraffo, L.K. Pannell, T.F. Spande, C. Severini, and V. Erspamer, *J. Nat. Prod.*, **53**, 407 (1990).
5. T. Tokuyama, J.W. Daly, H.M. Garraffo, and T.F. Spande, *Tetrahedron*, **48**, 4247 (1992).
6. J.W. Daly, R.J. Highet, and C.W. Myers, *Toxicon*, **22**, 905 (1984).
7. H.M. Garraffo, T.F. Spande, J.W. Daly, V. Erspamer, A. Baldessari, and E.G. Gros, *J. Nat. Prod.*, **56**, 357 (1993).
8. K. Busse, *Amphibia-reptilia*, **2**, 23 (1981).
9. T. Pintak and W. Böhme, *Salamandra*, **24**, 119 (1988).
10. T. Pintak and W. Böhme, *Salamandra*, **26**, 58 (1990).
11. R.M.A. Blommers-Schlosser, *Beaufortia*, **29**, 1 (1979).
12. J.W. Daly, N. Whittaker, T.F. Spande, R.J. Highet, D. Feigl, N. Nishimori, T. Tokuyama, and C.W. Myers, *J. Nat. Prod.*, **49**, 265 (1986).
13. T. Tokuyama, T. Tsujita, A. Shimada, H.M. Garraffo, T.F. Spande, and J.W. Daly, *Tetrahedron*, **47**, 5401 (1991).
14. T. Tokuyama, N. Nishimori, A. Shimada, M.W. Edwards, and J.W. Daly, *Tetrahedron*, **43**, 643 (1987).
15. P.A. Grieco and D.T. Parker, *J. Org. Chem.*, **53**, 3658 (1988).
16. J.W. Daly, N. Whittaker, and C.W. Myers, *Toxicon*, **25**, 1023 (1987).
17. J.W. Daly, S.I. Secunda, H.M. Garraffo, T.F. Spande, A. Wisniewski, C. Nishihira, and J.F. Cover Jr., *Toxicon*, **30**, 887 (1992).
18. C.J. Pouchert, Ed., "Aldrich Library of FTIR Spectra, Vapor Phase Spectra," Aldrich Chemical Co., Milwaukee, WI, 1989, Vol. 3.
19. H.M. Garraffo, M.W. Edwards, T.F. Spande, J.W. Daly, L.E. Overman, C. Severini and V. Erspamer, *Tetrahedron*, **44**, 6795 (1988).
20. G. Habermehl, *Justus Liebigs Ann. Chem.*, **679**, 164 (1964).
21. F. Gusovsky, D.P. Rossignol, E.T. McNeal, and J.W. Daly, *Proc. Natl. Sci. USA*, **85**, 1272 (1988).
22. E.X. Albuquerque, J.E. Warnick, M.A. Maleque, F.C. Kauffman, R. Tamburini, Y. Nimit, and J.W. Daly, *Mol. Pharmacol.*, **19**, 411 (1981).
23. K.S. Rao, J.E. Warnick, J.W. Daly, and E.X. Albuquerque, *J. Pharmacol. Exp. Therap.*, **243**, 775 (1987).
24. M.W. Edwards, J.W. Daly, and C.W. Myers, *J. Nat. Prod.*, **51**, 1188 (1988).
25. T.H. Jones, A. Laddago, A.W. Don, and M.S. Blum, *J. Nat. Prod.*, **53**, 375 (1990).
26. R.S. Aronstam, J.W. Daly, T.F. Spande, T.K. Narayanan, and E.X. Albuquerque, *Neurochem. Res.*, **11**, 1227 (1986).
27. J.W. Daly, Y. Nishizawa, M.W. Edwards, J.A. Waters, and R.S. Aronstam, *Neurochem. Res.*, **16**, 489 (1991).
28. T.H. Jones, M.S. Blum, H.M. Fales, and C.R. Thompson, *J. Org. Chem.*, **45**, 4778 (1980).
29. B. Tursch, D. Daloze, and C. Hootele, *Chimia*, **26**, 74 (1972).
30. R.H. Mueller and M.E. Thompson, *Tetrahedron Lett.*, 1097 (1980).

Received 7 October 1992