Journal of Chemical Ecology, Vol. 26, No. 1, 2000

ARTHROPOD–FROG CONNECTION: DECAHYDROQUINOLINE AND PYRROLIZIDINE ALKALOIDS COMMON TO MICROSYMPATRIC MYRMICINE ANTS AND DENDROBATID FROGS

JOHN W. DALY,[1,*] H. MARTIN GARRAFFO,[1] POONAM JAIN,[1]
THOMAS F. SPANDE,[1] ROY R. SNELLING,[2] CÉSAR JARAMILLO,[3]
and A. STANLEY RAND[3]

[1]*Laboratory of Bioorganic Chemistry*
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Bethesda, Maryland 20892-0820

[2]*Los Angeles County Museum of Natural History*
900 Exposition Boulevard,
Los Angeles, California 90007

[3]*Smithsonian Tropical Research Institute*
P.O. Box 2072,
Panamá, Panama

(Received January 5, 1999; accepted August 18, 1999)

Abstract—Neotropical poison frogs (Dendrobatidae) contain a wide variety of lipophilic alkaloids, apparently accumulated unchanged into skin glands from dietary sources. Panamanian poison frogs (*Dendrobates auratus*) raised in a large, screened, outdoor cage and provided for six months with leaf-litter from the frog's natural habitat, accumulated a variety of alkaloids into the skin. These included two isomers of the ant pyrrolizidine **251K**; two isomers of the 3,5-disubstituted indolizidine **195B**; an alkaloid known to occur in myrmicine ants; another such indolizidine, **211E**; two pyrrolidines, **197B** and **223N**, the former known to occur in myrmicine ants; two tricyclics, **193C** and **219I**, the former known to occur as precoccinelline in coccinellid beetles; and three spiropyrrolizidines, **222**, **236**, and **252A**, representatives of an alkaloid class known to occur in millipedes. The alkaloids **211E**, **197B**, and **223N** appear likely to derive in part from ants that entered the screened cage. In addition, the frog skin extracts contained trace amounts of four alkaloids, **205D**, **207H**, **219H**, and **231H**, of unknown structures and source. Wild-caught frogs from the leaf-litter site contained nearly 40 alkaloids,

---

[*] To whom correspondence should be addressed.

including most of the above alkaloids. Pumiliotoxins and histrionicotoxins were major alkaloids in wild-caught frogs, but were absent in captive-raised frogs. Ants microsympatric with the poison frog at the leaf-litter site and at an island site nearby in the Bay of Panamá were examined for alkaloids. The decahydroquinoline (−)-*cis*-**195A** and two isomers of the pyrrolizidine **251K** were found to be shared by microsympatric myrmicine ants and poison frogs. The proportions of the two isomers of **251K** were the same in ant and frog.

Key Words—Alkaloids, coccinellines, decahydroquinolines, indolizidines, pyrrolidines, pyrrolizidines, dendrobatid frogs, myrmicine ants, coccinellid beetles, millipedes.

INTRODUCTION

The nature of the dietary sources of the diverse lipophilic alkaloids detected in extracts of skin of neotropical dendrobatid (*Dendrobates*, *Epipedobates*, *Minyobates*, and *Phyllobates*) frogs remains a major research challenge for chemical ecologists (Daly, 1998; Daly et al., 1997a). Such lipophilic alkaloids, when detected in skin extracts from mantelline (*Mantella*) frogs of Madagascar, bufonid (*Melanophryniscus*) toads of southeastern South America, and myobatrachid (*Pseudophryne*) frogs of Australia, undoubtedly also have a dietary origin. Dendrobatid (*Dendrobates*, *Epipedobates*, and *Phyllobates*) and mantelline (*Mantella*) frogs raised in captivity on fruit flies and crickets contain no detectable alkaloids in skin extracts, but have the ability to efficiently and selectively accumulate into skin dietary alkaloids provided to them (Daly et al., 1980, 1992, 1994a,b, 1997b). A total of over 500 alkaloids that encompass over 20 structural classes have been detected in frog/toad skin extracts. Because of this large number, the individual alkaloids have been designated with boldface numbers, representing the nominal mass, and, when necessary, with code letters to distinguish those of the same nominal mass (for a current survey, see Daly et al., 1999). Structures of each alkaloid class and certain individual alkaloids of the present study are depicted in Figure 1.

The dendrobatid frogs of the genus *Dendrobates* are known as ant specialists (Toft, 1980, 1995; Donnelly, 1991; Caldwell, 1996) and indeed representatives of six of the more than 20 structural classes of lipophilic alkaloids found in frog skin are now known to occur in extracts of myrmicine ants (Jones et al., 1996, 1999; Spande et al., 1999; Daly et al., 1999 and references therein). These classes are the 2,5-dialkylpyrrolidines, 2,6-dialkylpiperidines, 3,5-dialkylpyrrolizidines, 3,5-dialkylindolizidines, 4,6-dialkylquinolizidines, and 2,5-dialkyldecahydroquinolines. In addition to such "ant alkaloids," there are two other classes of alkaloids found in frog skin that are known to occur in arthropods, namely the tricyclic coccinelline class, known from coccinellid beetles (see Daly et al., 1999 for references) and the spiropyrrolizidine class, known from a

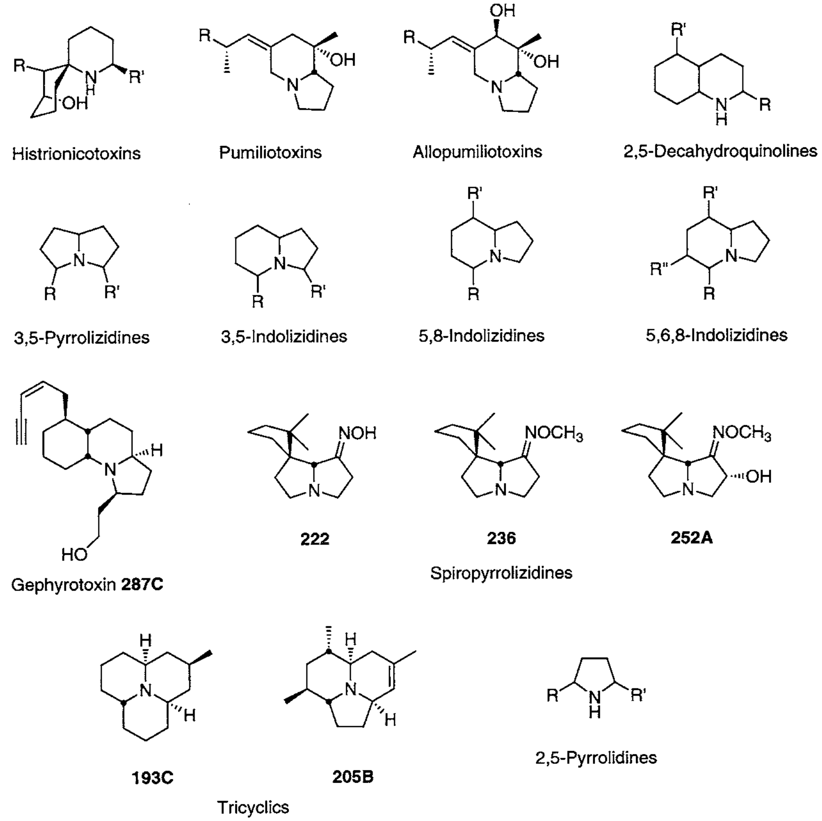

FIG. 1. Structures of various classes of alkaloids and certain individual alkaloids detected in Panamanian poison frogs (*Dendrobates auratus*) as documented in Table 1. For further structural details, including relative or absolute configuration of certain alkaloids and the nature of the *R* substituents, see Daly et al. (1999).

millipede (Meinwald et al., 1975). Of the eight structural classes (a–h) of alkaloids now known from frogs and arthropods, only a few individual alkaloids are known from both frogs and arthropods as in the following tabulation (see Daly et al., 1999):

Class a. Of the nearly twenty 2,5-disubstituted pyrrolidines reported from myrmicine ants only five have been detected in frog skin extracts, while the remaining 11 from frog skin extracts have not been reported from ants.

Class b. Of the 20 or more 2,6-disubstituted piperidines found in myrmicine ants, only three have been detected in frog skin extracts, while the remaining 6 from frog skin extracts have not been reported from ants.

Class c. Of the five 3,5-disubstituted pyrrolizidines previously reported from ants, three have been detected in frog skin extracts, while the remaining 18 from frog skin extracts have not been reported from ants.

Class d. Of the 10 or more 3,5-disubstituted indolizidines found in myrmicine ants, only four also have been detected in frog skin extracts (see Jones et al., 1996; Daly et al., 1999), while the remaining 16 from frog skin extracts have not been reported in ants.

Class e. The one 4,6-disubstituted quinolizidine found in a myrmicine ant (Jones et al., 1999) also has been detected in frog skin extracts; one other such quinolizidine has been detected in frog skin.

Class f. Of the four 2,5-disubstituted decahydroquinolines recently reported from myrmicine ants (Jones et al., 1999; Spande et al., 1999), two have been detected in frog skin extracts, but nearly 40 other decahydroquinolines from frog skin extracts have not been reported from myrmicine ants.

Class g. Of the five spiropyrrolizidines detected in frog skin extracts, only nitropolyzonamine has been reported from millipedes. A related spiropyrrolidine, polyzonimine, has been reported from both frog skin extracts and a millipede.

Class h. Only two of the tricyclic coccinelline class alkaloids reported in beetles have been identified in frog skin extracts, but there are 15 or more other tricyclic alkaloids in frog skin alkaloids that may prove to be of the coccinelline class (see Daly et al., 1999).

The occurrence of the above eight structural classes in frogs and arthropods includes data from the present paper and is summarized in Table 1. The present report of *trans*-**251K** in ants brings to 22 the number of alkaloids known to occur in both frogs and arthropods. Thus, the vast majority of over 500 alkaloids detected in frog skin extracts have not yet been identified from a possible dietary source. Furthermore, until the present report, none of the 22 alkaloids had been identified in arthropods from a region where the frogs occur.

Arthropods obtained with Berlese funnels from leaf-litter have been reported to provide a variety of alkaloids to captive-raised poison frogs (*Dendrobates auratus*) in Panamá (Daly et al., 1994b). No known myrmicine ant alkaloids were detected, but one beetle alkaloid was detected. In the present study, arthropods, contained in the fresh leaf-litter that was placed in large, screened outdoor cages during raising of the poison frog *Dendrobates auratus*, provided 14 of the 40 alkaloids found in skin extracts of wild-caught frogs from the same leaf-litter site. Ant alkaloids, a beetle alkaloid, and putative millipede alkaloids were among those detected in the skin extracts of the captive-raised frogs. Two ant alkaloids, both pyrrolidines, were detected only in the captive-raised frogs. Pumiliotoxins, an allopumiliotoxin, and histrionicotoxins were major skin alkaloids in the wild-caught frogs but were not detected in captive-raised frogs provided with leaf-litter as the sole source of dietary arthropods. We also report the occurrence of a decahydroquinoline and a pair of diastereomeric pyrrolizidines

TABLE 1. SUMMARY OF ALKALOIDS OF EIGHT STRUCTURAL CLASSES REPORTED FROM ARTHROPODS AND/OR FROG SKIN EXTRACTS[a]

	Reported from		
Structural class	Arthropods and frog skin	Only arthropods	Only frog skin
2,5-Disubstituted pyrrolidines	5	~15[b]	11
2,6-Disubstituted piperidines	3	~17[b]	16
3,5-Disubstituted pyrrolizidines	3	2[b]	18
3,5-Disubstituted indolizidines	4	~6[b]	16
4,6-Disubstituted quinolizidines	1[b]	0	1
2,5-Disubstituted decahydroquinolines	2	2[b]	~40
Spiropyrrolizidines + spiropyrrolidines	2[c]	0	4
Tricyclic coccinelline class	2	>10[d]	15

[a] Includes present report (see Daly et al., 1999).
[b] Myrmicine ants.
[c] Millipedes.
[d] Coccinellid beetles.

that occur in myrmicine ants and in skin extracts of microsympatric poison frogs.

METHODS AND MATERIALS

Tadpoles of the poison frog *Dendrobates auratus* were collected near the entrance to Quarry Heights on Cerro Ancón (Ancon Hill), in the city of Panamá, from late November 1993 until January 15, 1994. Fourteen transformed frogs were obtained from January 26 to March 16, 1994. The frogs were raised for five to seven months as follows at the base of Cerro Ancón behind the Tupper building of the Smithsonian Tropical Research Institute.

Four juvenile frogs were placed on January 26 in an outdoor cage (1.8 m wide × 2.8 m long × 2.3 m high) screened with metallic mosquito netting to impede access of insects from the outside. Three frogs survived until they were killed for analysis of skin alkaloids on September 11, 1994 [weight 2.1–2.3 g, snout–vent length (SVL) 2.8–3.0 cm]. The frogs' diet in this control cage was provided by an excess of fruit flies (*Drosophila*) in the cage, breeding on bananas and *Drosophila* medium. Leaf litter (frozen for two weeks to eliminate insects) placed along the inside borders of the cage was sprayed daily with water to maintain humidity. Although some small insects did appear in the cage during the experiment, the main source of food was the excess of breeding fruit flies.

Six juvenile frogs were placed during the period of February 10 to March 16 in another outdoor cage, also screened with metallic mosquito netting. Four frogs survived until sacrifice for analysis of skin alkaloids on September 11, 1994 (weight 1.4–1.8 g, SVL 2.7–2.9 cm). A fifth also survived but was very emaciated (weight 1.1 g, SVL 2.6 cm) and was not included in the analysis. The frogs' diet in this case was provided each week by fresh leaf litter gathered on Cerro Ancón from the area where *Dendrobates auratus* were common and where the tadpoles were collected. Wingless fruit flies were used to supplement the diet every two weeks. Insects from the area surrounding the cage undoubtedly contributed to the diet to some extent.

Four juvenile frogs were introduced on January 31 into a large glass terrarium (30 gallon) placed in an adjacent cage and sealed to restrict access of insects from the outside. Two frogs survived until sacrifice for analysis of skin alkaloids on September 11, 1994 (weight 1.6 and 1.8 g, SVL 2.5 and 2.5 cm). The frogs' diet in this terrarium consisted entirely of wingless *Drosophila* provided every second day.

Two adult *Dendrobates auratus* from the site on Cerro Ancón were sacrificed on September 29, 1994 (weight 1.7 and 2.7 g, SVL 2.9 and 3.2 cm) for analysis of skin alkaloids.

From June 20 until August 25, 1996, fifty separate ant colonies were collected near the entrance to Quarry Heights on Cerro Ancón at the site where *Dendrobates auratus* were common. Winged ants were obtained from only 15 of these colonies. On August 29, 1996, eleven separate ant colonies were collected on Isla Taboga near a site where adult *Dendrobates auratus* previously had been collected (Daly et al., 1994b) for analysis of skin alkaloids. No winged ants were collected on Isla Taboga. Ants were placed in a small volume (~0.5 ml) of methanol in a NUNC vial for detection of alkaloids by gas chromatographic–mass spectral analysis with a Finnigan model 800 ion trap detector, interfaced with a Varian model 3400 gas chromatograph fitted with a 30 m × 0.32 mm ID, RTX 5 (Restek) fused silica-bonded column programmed from 100° to 280°C at the rate of 10°C/min.

Frog skins were extracted and alkaloid fractions were prepared and analyzed by gas chromatography–mass spectrometry and vapor-phase infrared spectroscopy (see Daly et al., 1994b and references therein). The gas chromatograms depicted in Figure 2 were obtained with a 6-ft 1.5% OV-1 packed column (2 mm ID) with a flame ionization detector. A sample of 2 μl of a methanolic alkaloid fraction equivalent to 2 mg wet weight skin was injected at a column temperature of 150°C. After the solvent maximum passed (0.3 min), the column was programmed to 280°C at 10°C/min.

Certain methanolic ant extracts and frog alkaloid fractions were analyzed on a chiral Supelco Beta-DEX 120 fused silica capillary column (30 m × 0.25 mm ID, 0.25-μm film thickness) at 150°C.

Voucher specimens of frogs and ants are deposited in the American Museum of Natural History, New York, New York, and in the Los Angeles County Museum of Natural History, Los Angeles, California, respectively.

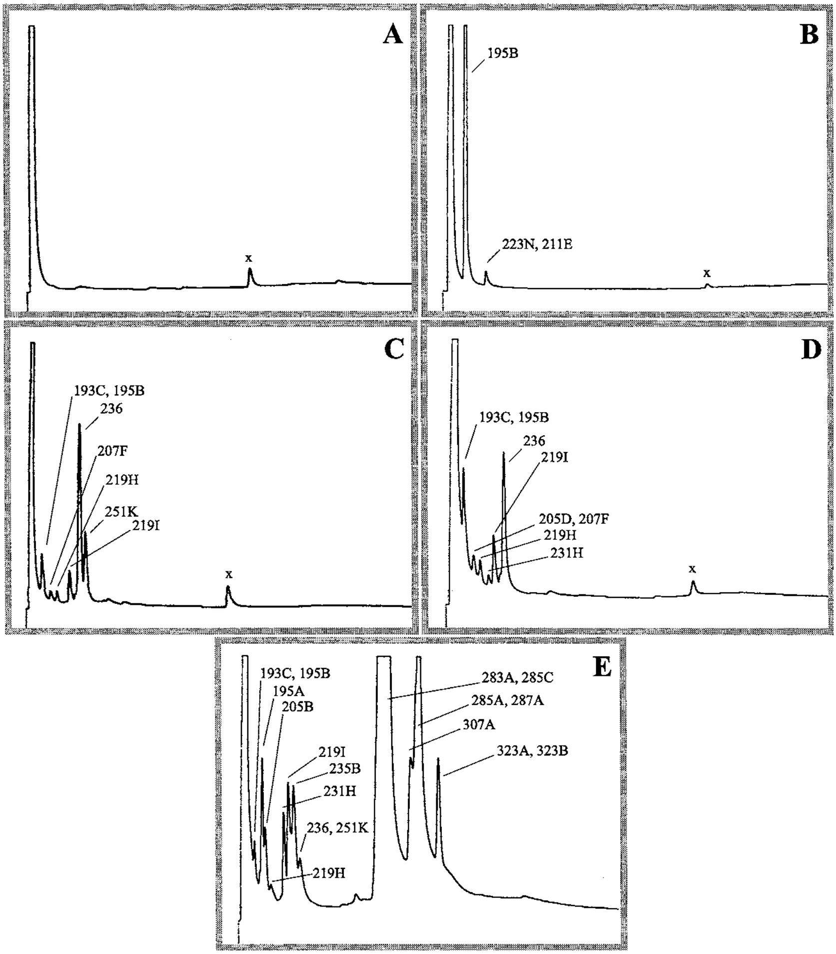

FIG. 2. Gas chromatographic profiles for alkaloids in skins of poison frogs (*Dendrobates auratus*). (A) Terrarium raised frog [snout–vent length (SVL) 25 mm] with diet of wingless fruit flies. (B) Outdoor cage-raised frog (SVL 30 mm) on diet mainly of fruit flies. (C) Outdoor cage-raised frog (SVL 26 mm) on diet from fresh leaf litter. (D) Outdoor cage-raised frog (SVL 27 mm) on diet from fresh leaf litter. (E) Wild-caught frog (SVL 32 mm) from Cerro Ancón. Peaks marked with x are nonalkaloidal artifacts.

RESULTS AND DISCUSSION

One of the frogs raised in the large outside glass terrarium with a diet of only wingless fruit flies had no detectable alkaloids in the skin extract (see Figure

2A), as expected from previous studies (Daly et al., 1992, 1994a,b). However, the other frog did have a trace amount of indolizidine **195B** (data not shown), an alkaloid presumably obtained from a myrmicine ant. It has been noted that exclusion of small insects from the terrarium was not completely successful.

Frogs raised in the screened outdoor cage in which fruit flies were by far the most abundant food source contained as a major alkaloid in skin extracts the 3-butyl-5-methyl indolizidine **195B**, an alkaloid known to occur in myrmicine ants as monomorine I. Traces of another isomer of **195B** and another indolizidine **211E** were present, along with trace amounts of two 2,5-disubstituted pyrrolidines, **197B**, and **223N**, all of which presumably are derived from small myrmicine ants, which, along with other small insects, were able to penetrate the control cage despite the mosquito screen. In addition, such ants may have been targeted to some extent as prey items, since the frogs in the control cage were provided with an abundance of fruit flies as food. The flame ionization gas chromatogram for one of three frogs is shown in Figure 2B. The chromatograms for the other two frogs were similar. The occurrence of alkaloids in skin extracts from these captive-raised frogs is presented in Table 2.

The frogs raised in the screened outdoor cage that was provided each week with fresh leaf litter from Cerro Ancón as a source of dietary arthropods had at least 16 different alkaloids in skin extracts. The chromatograms for two frogs are shown in Figures 2C and 2D. The chromatograms for the other two frogs were similar to these, but with lesser amounts of alkaloids. The major alkaloid in all four frogs was the spiropyrrolizidine oxime ether **236**, an alkaloid presumably obtained from a millipede. In lesser amounts there were the indolizidine **195B** and an isomer and two diastereomers of the pyrrolizidine **251K**, presumably all from myrmicine ants; the tricyclic **193C** (precoccinelline), presumably from a coccinellid beetle; another tricyclic **219I**; and four alkaloids, **205D**, **207F**, **219H**, and **231H**, of unknown structural class. Trace amounts of another indolizidine **211E**, two pyrrolidines **197B** and **223N**, and two other spiropyrrolizidine oximes **222** and **252A** were present (see Table 2).

One wild-caught frog from Cerro Ancón had over 40 alkaloids in its skin extract (Figure 2E). The other wild-caught frog had a similar profile, but at about twofold higher levels (data not shown). Thirty six of the major, minor, and trace alkaloids from these frogs are tabulated in Table 2. Other alkaloids were present in such trace amounts that they could not be well characterized. The major alkaloids were histrionicotoxins. These were not detected in the frogs raised in outside cages provided with fresh leaf litter from Cerro Ancón, nor were the pumiliotoxins **307A** and **323A**, which were significant alkaloids from skin of the wild-caught frogs. The spiropyrrolizidine **236**, which was the major alkaloid in captive-raised frogs, was a minor alkaloid in the wild-caught frogs. The two isomers of pyrrolizidine **251K**, the indolizidine **195B**, the tricyclics **193C** and **219I**, and alkaloid **219H**, all of which were minor alkaloids in the captive-raised

TABLE 2. ALKALOIDS IN SKIN EXTRACTS OF PANAMANIAN POISON FROG (*Dendrobates auratus*)

	I Captive-raised in outdoor cages on *Drosophila*	II Captive-raised in outdoor cages on leaf litter arthropods	Wild-caught	
Alkaloid[a]			III, Ancon Hill	IV, Isla Taboga[b]
Histrionicotoxins				
235A				+
259A			+	+
261A			+	
283A			+++	+
285A			+++	
285C			+++	
287A			++	
Pumiliotoxins				
251D			+	+
277B			+	
307A			++	+
307B			+	+
323A			++	++
Allopumiliotoxins				
253A				+
267A				++
305A[c]				+
323B			++	+
339A				+
2,5-Decahydroquinolines				
cis-**195A**			++	++
211A			+	
219A				+++, +
243A			+	++, +
269AB			+	
3,5-Pyrrolizidines				
249I			+	
cis-**251K**		++	++	++
trans-**251K**		+	+	+
265J[c]				+
3,5-Indolizidines				
195B	+++, +	++, +	++, +	
211E	+	+		
5,8-Indolizidines				
203A				+
235B			++	+
5,6,8-Indolizidines				
195G				+
223A				+

TABLE 2. CONTINUED

Alkaloid[a]	I Captive-raised in outdoor cages on *Drosophila*	II Captive-raised in outdoor cages on leaf litter arthropods	Wild-caught	
			III, Ancon Hill	IV, Isla Taboga[b]
233G			+	
249H			+	++
263D				+
277C				+
Gephyrotoxins				
287C			+	
Spiropyrrolizidines				
222		+	+	+
236		+++	++	+
252A		+	+	+
Tricyclics				
193C		++	+	+
205B			++	
207J[c]				+
219I[c]		++	++	
2,5-Pyrrolidines				
trans-**197B**	+	+		
223N	+	+		
Unclassified				
205D[c]		+	+	
207F[c]		++	+	
217C[c]				+
219H[c]		++	+	
231H[c]		+	+	
243E[c]				+
271C[c]			+	

---

[a] For structures see Figure 1 and Daly et al. (1999). The numbers preceding the ring system indicate the positions of substitution. The relative amounts (+++ = major, ++ = minor, + = trace) follow the notation used in prior publications. For example, the major alkaloid **195B** in column I (Figure 2B) was present in amounts of about 80 μg/frog. The major alkaloid **236** of column II (Figure 2C) was present in amounts of about 20 μg/frog. The major alkaloids **283A**, **285A**, **285C** of column III (Figure 2E) were present in amounts of 150–200 μg/frog. The major alkaloid **219A** of column IV was present in amounts of about 50 μg/frog (see Daly et al., 1994b). The trace alkaloids were present in amounts of 1 μg or less per frog, while the minor alkaloids were present in significantly lower amounts than the major alkaloid(s) in each extract. Where two entries are given and separated by commas, this indicates two isomers in the order of elution from the gas chromatograph.

[b] Data are from Daly et al. (1994b). Some of the alkaloids have been reclassified in light of additional data.

[c] Alkaloids of undefined or incompletely defined structures.

frogs, were also minor alkaloids in wild-caught frogs. However, several of the minor alkaloids of the wild-caught frogs, most notably the decahydroquinoline **195A**, the tricyclic **205B**, the allopumiliotoxin **323B**, and the 5,8-disubstituted indolizidine **235B** were not detected in the captive-raised frogs (see Table 2). Conversely, the two pyrrolidines, **197B** and **223N**, detected as trace alkaloids in extracts from both groups of frogs raised in the outdoor cages, were not detected in the wild-caught frogs.

There are significant differences between the present results in which poison frogs (*Dendrobates auratus*) from Cerro Ancón were raised in large, screened, outdoor cages on arthropods provided each week on fresh leaf litter from Cerro Ancón and previous results in which frogs of the same species were raised on arthropods driven into indoor terraria with heat lamps from Berleze funnels containing leaf litter from Cerro Ancón (Daly et al., 1994b). In both studies the putative millipede alkaloid **236** was the major alkaloid in skin extracts of captive-raised frogs. The tricyclic beetle alkaloid **193C** (precoccinelline) was a minor alkaloid in both studies, as were the tricyclic **219I** and alkaloid **219H**. The ant alkaloids **195B** and **251K** of the present study were not detected in the Berleze funnel paradigm, suggesting that myrmicine ants are not readily obtained from leaf litter with that paradigm and/or that the myrmicine ants were able to enter the outdoor cages despite the screening. The latter explanation in all likelihood accounts for the presence of indolizidine **195B**, which was the major skin alkaloid in frogs raised in the screened outdoor cage on an abundance of fruit flies (Figure 2B). The most inexplicable difference between the two studies is that the 19-carbon histrionicotoxins, a 19-carbon gephyrotoxin, and a 19-carbon decahydroquinoline were detected as minor alkaloids in one frog and as trace alkaloids in two other frogs raised in terraria with the Berleze funnel paradigm but were not detected at all in captive-raised frogs of the present study. It might be noted that the earlier results were with frogs raised in terraria on arthropods from leaf litter obtained from June onwards until death of the frogs seven months later in December, 1992 (Daly et al., 1994b). The present results were with frogs raised in outdoor cages provided with fresh leaf litter from February through August 1994. Thus, in addition to the difference of Berleze funnel extraction versus outdoor cages, the arthropods of the earlier study came more from the rainy season than those of the present study. The levels and range of alkaloids found in the skin of dendrobatid frogs presumably reflect many factors, such as the availability and suitability of alkaloid containing prey items, prey selection by the frog species, and the selectivity and degree of expression of the alkaloid sequestering system in each frog species.

In an effort to identify possible sources of the 16 alkaloids found in skin of the captive-raised frogs, ants from a total of 61 terrestrial nests were analyzed, both from the leaf litter area on Cerro Ancón, where the poison frog *Dendrobates auratus* is common, and from an island, Isla Taboga, in the nearby Bay of Panamá, where *Dendrobates auratus* is also common. The profiles of alkaloids in wild-caught *Dendrobates auratus* from these two sites differ considerably (see Daly et al., 1994b, and Table 2). From the 50 ant nests collected on Cerro Ancón, the alate queens, but not the wingless workers, from one nest were found to contain the decahydroquinoline *cis*-**195A**, which was also a minor alkaloid component in skin extracts from the wild-caught *Dendrobates auratus* from Cerro Ancón (Figure 2D). Both ant and frog contained the (−)-enantiomer of *cis*-**195A** as shown by gas chromatographic analysis on a chiral capillary column that separates the two enantiomers. The ant was identified as a *Solenopsis* (*Diplorhoptrum*) sp. Alate queens of the same species from another nest also contained decahydroquinoline *cis*-**195A**. Ant extracts from the remaining 48 nests did not contain alkaloids. The species were not identified. This and the following example represent the first reported instances of an alkaloid present in skin of a dendrobatid frog and in a microsympatric arthropod available to the frog as a prey item. The other example is from Isla Taboga, where wingless ants, identified as *Megalomyrmex silvestri* Wheeler, from two of 11 nests were found to contain two diastereomers of the 3,5-disubstituted pyrrolizidine **251K** in the same ratio (*cis*-**251K**–*trans*-**251K**, 3 : 1) as in skin extracts from microsympatric *Dendrobates auratus* on Isla Taboga. 5*E*,9*E*-3-Butyl-5-hexylpyrrolizidine (*trans*-**251K**) has been previously reported from a myrmicine ant, *Megalomyrmex foreli*, of Costa Rica (Jones et al., 1996) at a site at which dendrobatid frogs do not occur. Extracts from ants of the other nine nests on Isla Taboga contained no alkaloids, nor were the ants identified.

In summary, fresh leaf litter, collected mainly during the dry season in Pacific Panamá, apparently can provide dietary sources for some of the many alkaloids detected in skin extracts of poison frogs (*Dendrobates auratus*) found at the same site. The major classes of alkaloids provided by the leaf-litter arthropods are pyrrolizidines and indolizidines known from myrmicine ants, a tricyclic known from coccinellid beetles, and spiropyrrolizidines, putatively from millipedes. In addition, one species of myrmicine ants collected at the leaf-litter site was found to contain the decahydroquinoline (−)-*cis*-**195A**, which was also present as a minor alkaloid in skin of the microsympatric population of the poison frog *Dendrobates auratus*, while another species of myrmicine ants from a small island contained a *cis*- and a *trans*-pyrrolizidine **251K** in the same 3 : 1 ratio that was present in skin of a microsympatric population of the poison frog *Dendrobates auratus*.

Acknowledgments—The Instituto Nacional de Recursos Naturales Renovables of Panama is gratefully acknowledged for permission to collect specimens necessary for these studies. The authors thank Roberto Ibáñez for help in collecting the tadpoles and for suggestions during the course of the experiments.

REFERENCES

CALDWELL, J. P. 1996. The evolution of myrmecophagy and its correlates in poison frogs (family Dendrobatidae). *J. Zool.* (*London*) 240:75–101.

DALY, J. W. 1998. Thirty years of discovering arthropod alkaloids in amphibian skin. *J. Nat. Prod.* 61:162–172.

DALY, J. W., MYERS, C. W., WARNICK, J. E., and ALBUQUERQUE, E. X. A. 1980. Levels of batrachotoxin and lack of sensitivity of its action in poison-dart frogs (*Phyllobates*). *Science* 208:1383–1385.

DALY, J. W., SECUNDA, S. I., GARRAFFO, H. M., SPANDE, T. F., WISNIESKI, A., NISHIHARA, C., and COVER, J. F., JR. 1992. Variability in alkaloid profiles in neotropical poison frogs (Dendrobatidae): Genetic versus environmental determinants. *Toxicon* 30:887–898.

DALY, J. W., SECUNDA, S. I., GARRAFFO, H. M., SPANDE, T. F., WISNIESKI, A., and COVER, J. F., JR. 1994a. An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). *Toxicon* 32:657–663.

DALY, J. W., GARRAFFO, H. M., SPANDE, T. F., JARAMILLO, C., and RAND, A. S. 1994b. Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? *J. Chem. Ecol.* 20:943–955.

DALY, J. W., GARRAFFO, H. M., and MYERS, C. W. 1997a. The origin of frog skin alkaloids: An enigma. *Pharm. News* 4:9–14.

DALY, J. W., GARRAFFO, H. M., HALL, G. S. E., and COVER, J. F., JR. 1997b. Absence of skin alkaloids in captive-raised Madagascan mantelline frogs (*Mantella*) and sequestration of dietary alkaloids. *Toxicon* 35:1131–1135.

DALY, J. W., GARRAFFO, H. M., and SPANDE, T. F. 1999. Alkaloids from amphibian skins, pp. 1–161, *in* S. W. Pelletier (ed.). Alkaloids, Chemical and Biological Perspectives, Vol. 13. John Wiley & Sons, New York.

DONNELLY, M. A. 1991. Feeding patterns of the strawberry poison frog, *Dendrobates pumilio* (Anura: Dendrobatidae). *Copeia* 3:723–730.

JONES, T. H., TORRES, J. A., SPANDE, T. F., GARRAFFO, H. M., BLUM, M. S., and SNELLING, R. R. 1996. Chemistry of venom alkaloids in some *Solenopsis* (*Diplorhoptrum*) species from Puerto Rico. *J. Chem. Ecol.* 22:1221–1236.

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., and SPANDE, T. F. 1999. Further alkaloids common to ants and frogs: Decahydroquinolines and a quinolizidine. *J. Chem. Ecol.* 25:1179–1193.

MEINWALD, J., SMOLANOFF, J., MCPHAIL, A. T., MILLER, R. W., EISNER, T., and HICKS, K. 1975. Nitropolyzonamine: A spirocyclic nitro compound from the defensive glands of a milliped (*Polyzonium rosalbum*). *Tetrahedron Lett.* 1975:2367–2370.

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., and JONES, T. H. 1999. Structures of decahydroquinolines from dendrobatid poison frogs and a myrmicine ant: Use of ^{1}H- and ^{13}C-NMR in conformational analysis. *J. Nat. Prod.* 62:5–21.

TOFT, C. A. 1980. Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. *Oecologia* 45:131–141.

TOFT, C. A. 1995. Evolution of diet specialization in poison-dart frogs (Dendrobatidae). *Herpetologica* 51:202–216.