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ORIGINAL PAPER

Contact toxicities of anuran skin alkaloids against the fire ant (*Solenopsis invicta*)

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Abstract Nearly 500 alkaloids, representing over 20 structural classes, have been identified from the skin of neotropical poison frogs (Dendrobatidae). These cutaneous compounds, which are derived from arthropod prey of the frogs, generally are believed to deter predators. We tested the red imported fire ant (*Solenopsis invicta*) for toxicosis following contact with 20 alkaloids (12 structural classes) identified from dendrobatids or other anurans. Individual ants forced to contact the dried residues of 13 compounds exhibited convulsions and/or reduced ambulation. We estimated the cutaneous concentrations of several compounds based on their reported recoveries from skin extracts of free-ranging frogs and our measurements of the skin surface areas of museum specimens. Pumiliotoxin 251D exhibited contact toxicity below its estimated cutaneous concentration in the Ecuadorian frog, *Epipedobates anthonyi*, an observation consistent with the hypothesized role of this compound in anuran chemical defense. Our results and those of a previous study of mosquitoes indicate that some anuran skin compounds function defensively as contact toxins against arthropods, permeating their exoskeleton.

Keywords Alkaloid · Allomone · Ant · Defense · Frog · Toxicity

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Introduction

Studies of alkaloids isolated from the skins of neotropical poison frogs (Dendrobatidae) have led to the discovery of nearly 500 compounds, representing over 20 structural classes, chiefly bicyclic "izidines" with complex side chains (Daly et al. 1999, 2005). The skin alkaloids of dendrobatids, which are acquired from the ants (Saporito et al. 2004), mites (Saporito et al. 2007a), and other leaf-litter arthropods that these frogs eat (Daly et al. 2005; Saporito et al. 2012), generally are believed to deter predators, as suggested by the rejection of dendrobatids by predatory ants (Fritz et al. 1981), spiders (Szelistowski 1985), snakes (Silverstone 1976; Daly et al. 1978), and birds (Silverstone 1975; Darst and Cummings 2006). The biological activities of alkaloids from dendrobatids and other anurans, however, are known primarily from ex vivo neuromuscular preparations

designed to disclose pharmacological modes of action, as, for example, interference with sodium–potassium channels of electrogenic membranes or binding to nicotinic acetylcholine receptors.

A study of the yellow fever mosquito (*Aedes aegypti*), a hematophage and pathogen vector, demonstrated the contact toxicity of a common and, in some anurans, abundant skin alkaloid, pumiliotoxin 251D (PTX 251D) (Weldon et al. 2006). Both PTX (+)-251D and its opposite (unnatural) enantiomer reduced landing and feeding by mosquitoes when applied to artificial feeding membranes. Mosquitoes forced to contact either enantiomer were disabled in flying off the substrate and became moribund or died. The natural enantiomer was more effective in all assays, and it elicited leg autotomy, a dramatic response exhibited by mosquitoes to some noxious compounds.

Ants are widespread omnivores that prey on a variety of small vertebrates, including amphibians. We tested the red imported fire ant (*Solenopsis invicta*), a fierce omnivore, for responses to 20 alkaloids representing 12 structural classes isolated from the skin of dendrobatids and/or other anurans (Daly and Spande 1986). *S. invicta*, which is indigenous to the seasonally flooded Pantanal region of southern Brazil, was accidentally introduced into the United States in Mobile, Alabama, during the 1930s. It has since spread throughout the southern United States, decimating populations of small animals, damaging crops and other plants, and inflicting painful and sometimes lethal venomous stings on humans and livestock. Introductions of *S. invicta* also have occurred in Australia, Taiwan, and China (Ascunce et al. 2011).

Previous studies of the responses of ants to animal-derived alkaloids have focused on feeding deterrents in the defensive secretions of arthropods (e.g., Smolanoff et al. 1975; Hare and Eisner 1993), including heterospecific ant competitors (Andersen et al. 1991). Blum et al. (1991) tested alkaloids in the venom of the ant genera *Solenopsis* and *Monomorium* as feeding deterrents of myrmicine ants. Ants possessing venom alkaloids, including *S. invicta*, were less deterred by these compounds than were ants whose venoms contain small amounts or none of these compounds, a feature that Blum et al. (1991) suggested confers a competitive advantage upon alkaloid-producing ants.

We examined the locomotory responses of *S. invicta* to anuran-derived alkaloids. We did not assess repellence, as is often done in studies of defensive allomones. Instead, we examined contact toxicity, scoring ambulation and convulsions of ants in an open arena following contact by them with dried alkaloid residues. In addition, we estimated the cutaneous concentrations of several compounds to examine the plausibility of their use by frogs as contact deterrents of ants. Our results demonstrate different contact toxicities of anuran skin alkaloids against *S. invicta*, and they confirm that some pumiliotoxins are highly potent in this regard.

Methods

Insects

Mature, queenright, monogyne *S. invicta* colonies were excavated from field populations or reared from newly mated queens, all collected near Gainesville, Florida. Field-collected colonies were established in the laboratory at least 1 month before testing, and colonies from newly mated queens were at least 1 year old. Laboratory colonies were housed in plastic trays (83×53×13 cm), the inner walls of which were coated with Fluon® to prevent their escape. They were maintained on crickets, a 10 % sucrose solution, and water. Ant subjects were drawn from one to four colonies for tests with any compound.

Bioassay

Tests were conducted from 0900–2300 h at 25±1.0 °C. Each ant was inserted into a 3-cm long glass tube (4 mm=i.d.) that had been heat-sealed at one end and was plugged with cotton at the other. A 1-μl droplet of methanol or methanolic alkaloid solution was applied by microsyringe (±0.1 uL) to the flat tip (diameter=3 mm; area=7.1 mm^2) of a 10-cm metal rod (syringe plunger) and allowed to air-dry for 1 min. The tip of the rod was then inserted into the glass tube, confining the ant to the terminal 5 mm of the tube and forcing it to contact the rod tip. After 3 min, the ant was tapped or prodded out of the tube into the center of a plastic Petri dish (diameter=14 cm, height=2.4 cm), the walls of which were coated with Fluon®.

A paper sheet marked at 2-cm intervals with a series of concentric circles out to 10 cm from the center was placed under the Petri dish. The number of lines crossed by each ant during 2 min (ambulation score) and the number of convulsions (seizure score), where an ant suddenly flexed its abdomen and legs and/or tumbled on its side (Fig. 1), were scored.

Most of the compounds we tested were obtained as purified fractions of frog skin extracts; some compounds were synthesized or obtained commercially (Tables 1, 2, and 3). Most compounds were first tested with ten ants per condition at a concentration of 100 mM, which is equivalent to 1.41 μM/cm^2 applied to the rod tip. This was the highest concentration we tested given the limited availability of most compounds. If ants failed to exhibit lowered ambulation compared with controls and no convulsions, no further tests were conducted with the compound. If, on the other hand, a compound affected ants at 100 mM, it was further presented in 33 % serial dilutions until ambulation scores did not differ significantly from controls or until no convulsions were exhibited. The lowest dilution of a compound to significantly reduce ambulation or elicit a convulsion is referred to as the estimated minimum toxic concentration

Fig. 1 *S. invicta* convulsively flexes its abdomen and legs (*left*) and tumbles (*right*) after contacting pumiliotoxin 251D. Only major workers were used in bioassays (length mean± SEM=6.1±0.1 mm; *N*=10)

(EMTC) for its respective measure. Due to the exceedingly limited quantities of allodihydrohistrionicotoxin 285A available, this compound was tested only for its activity in eliciting convulsions, which preliminary studies indicated are manifest at low dilutions.

Cutaneous alkaloid estimates

We estimated the cutaneous concentrations of PTX 251D, batrachotoxin (BTX), and batrachotoxinin A on the basis of published information on the amounts of these compounds recovered from the skin extracts of free-ranging frogs and our measurements of the surface areas of museum specimens. Our estimate of the cutaneous abundance of PTX 251D is based upon the reported recovery of 21 mg of this compound from 750 *Epipedobates anthonyi*, previously referred to as *Dendrobates tricolor* by Daly et al. (1980), from southwestern Ecuador following the fractionation of methanolic frog skin extracts by column chromatography (Daly et al. 1980). We estimate the total quantity of PTX 251D present in this collection of *E. anthonyi* to be 28 mg, assuming a minimum loss of 25 % during chromatographic isolation (Myers et al. 1978); hence we estimate 37 μg per frog.

Estimates of the cutaneous abundances of BTX and batrachotoxinin A are based upon the reported recovery of these compounds from 100 *Phyllobates terribilis* from western Colombia, where again we estimate a 25 % loss of compounds during chromatography (Myers et al. 1978). From recovery values of 500 μg per frog of BTX and 200 μg per frog of batrachotoxinin A, we calculate 670 and 270 μg of these compounds per frog, respectively.

Frog specimens for surface area estimates were obtained from the American Museum of Natural History (AMNH), New York, New York; the specimens chosen were the size of typical adults. Surface areas were determined for one *E. anthonyi* (snout-vent length [SVL]=2.1 cm) collected in El Oro, Ecuador (AMNH A104931), and a captive-reared *P. terribilis* (SVL=4.3 cm) (AMNH A162742).

Images of frogs were obtained using a 1,388×1,038-pixel-resolution CCD camera and were analyzed with IMAQ Vision Builder 5 software (Hoffmann and Hewitt 2005). Images of the dorsal and ventral aspects of each frog were captured, where the frogs' legs were extended to obtain definitive outlines. A corresponding image of a ruler was captured to scale each image. Because of the size differences in the two specimens, two scales were used. The image resolutions for the *E. anthonyi* and the *P. terribilis* were 24 and 58 μm/pixel, respectively.

The silhouettes of the frogs were outlined around the borders of the ventrum, dorsum, and appendages, including the regions typically excised in skin removal, using the trace feature of the software. The forelegs were measured up to the distal tip of the radio-ulna; the hindlegs were measured up to the distal tip of the tibiofibula. The trace feature allowed continuous lines to be drawn around each specimen, which created enclosed areas, i.e., number of pixels enclosed by the continuous line. Areas were calculated using the appropriate image resolutions.

Results

Bioassay

Comparisons of ambulation scores for 100 mM concentrations versus control conditions (Mann–Whitney *U* test, one-tailed) failed to detect differences for batrachotoxinin A, PTX 237A, (5*E*, 9*E*) 3-butyl-5-propylindolizidine 223AB, decahydroquinoline *cis*-223F, pseudophrynaminol, spiropyrrolizidine oxime, and (+)-gephyrotoxin (Table 1). Comparisons of ambulation scores indicated EMTC for the following compounds, listed in increasing order of efficacy: 5,8-disubstituted indolizidine 205A, 5,8-disubstituted indolizidine 235B', and octahydrohistrionicotoxin 291A; 2-methyl-6-undecylpiperidine, PTX 267C, and BTX; histrionicotoxin 259A and nicotine; PTX 323A; PTX 251D; allopumiliotoxin 267A; and PTX 307A (Table 2).

We failed to observe convulsions during any control session. The absence of variability in the results for the controls obviates the use of statistical tests, like the Mann–Whitney *U* test, that assume similar sampling distributions of experimental and control conditions. Hence, we identify the EMTC of compounds that elicited convulsions as the lowest concentration that elicited any convulsions. Comparisons of convulsion scores indicated EMTC for the following compounds, also listed in increasing order of efficacy: BTX and octahydrohistrionicotoxin 291A; allodihydrohistrionicotoxin 285A; histrionicotoxin 259A and

Table 1 Anuran alkaloids that failed to induce contact toxicosis in *S. invicta*

Alkaloid	Control	100 mM
Batrachotoxinin A[a]	44.5±18.0	42.2±19.6
Pumiliotoxin 237A[b, h]	27.1±10.1	23.3±11.1
(5*E*, 9*E*) 3-Butyl-5-propylindolizidine 223AB[c, h]	24.4±7.9	19.2±5.6
Decahydroquinoline *cis*-223F[d]	33.4±19.2	30.4±13.1
Pseudophrynaminol[e, h]	34.1±18.7	29.1±12.4
Spiropyrrolizidine oxime[f]	28.0±8.4	20.6±8.5
(+)-Gephyrotoxin[g, h]	19.7±9.8	24.4±10.7

The mean ambulation scores (± standard deviation) in response to control and 100 mM (1,400 nmol/cm^2) concentrations of each compound are statistically indistinguishable (Mann–Whitney *U* test, one-tailed *P* values>0.05). Convulsions were not observed during any trial. Representative anuran sources (taxonomy according to Frost 2011) are indicated by superscript letters a–g

[a] Dendrobatidae: *Phyllobates* spp. (Colombia, Costa Rica, Panama) (Daly and Spande 1986)

[b] Bufonidae: *Melanophryniscus montevidensis* (Uruguay) (Mebs et al. 2005); Dendrobatidae: *Adelphobates quinquevittatus* (Peru) (Daly and Spande 1986), *Ameerega parvula* (Peru) (Daly and Spande 1986), *Epipedobates* spp. (Ecuador) (Daly and Spande 1986, Saporito et al. 2004), *Minyobates steyermarki* (Venezuela) (Daly and Spande 1986), *Oophaga histrionica* (Ecuador) (Daly and Spande 1986), *Ranitomeya* spp. (Ecuador, Peru) (Saporito et al. 2004); Mantellidae: *Mantella* spp. (Madagascar) (Clark et al. 2005)

[c] Bufonidae: *Melanophryniscus stelzneri* (Argentina) (Garraffo et al. 1993a); Dendrobatidae: *O. histrionica* (Colombia) (Daly et al. 1978; Spande et al. 1981)

[d] Bufonidae: *Melanophryniscus stelzneri* (Argentina) (Garraffo et al. 1993a); Dendrobatidae: *Adelphobates quinquevittatus* (Peru) (Daly and Spande 1986), *Dendrobates auratus* (Panama) (Daly and Spande 1986)

[e] Myobatrachidae: *Pseudophryne* spp. (Australia) (Daly et al. 2005)

[f] Dendrobatidae: *O. pumilio* (Panama) (Daly et al. 2005)

[g] Dendrobatidae: *O. histrionica* (Colombia) (Daly and Spande 1986)

[h] Synthetic

PTX 267C; and PTX 251D (Table 3). Most compounds that impaired ambulation and elicited convulsions exhibited a lower EMTC for convulsions (Tables 2 and 3).

Cutaneous alkaloid estimates

Measurements of *E. anthonyi* revealed a total surface area of 4.3 cm^2. Thus, we estimate the cutaneous concentration of PTX 251D in this species to be 8.6 μg/cm^2 (37 μg/4.3 cm^2). Measurements of *P. terribilis* revealed a total surface area of 23.8 cm^2. Thus, we estimate the cutaneous concentrations of BTX and batrachotoxinin A in this species to be 28.2 μg/cm^2 (670 μg/23.8 cm^2) and 11.3 μg/cm^2 (270 μg/23.8 cm^2), respectively.

Discussion

Our study indicates the contact toxicities of 13 anuran skin alkaloids. Most of the compounds we tested were purified from methanolic frog skin extracts, thus they were available in exceedingly limited quantities. The presentation of micro-quantities of compounds to individual ants was essential to our investigation. However, the small sample sizes (ten subjects per condition) we used make it difficult to differentiate the behavioral effects of compounds statistically. More precise quantification of behaviors also may better differentiate the effects of compounds. For example, our observations suggest that ambulation is reduced both when ants are afflicted with seizures during extreme toxicosis and when they engage in protracted grooming.

Notable variation was observed in the performance of *S. invicta* in assay trials, as can be seen by the wide range of ambulation scores to the controls, with mean values from 19.6 to 50.6 lines crossed in the 2-min trials. This variation may be due to behavioral differences among the colonies from which subjects were drawn; subjects from one to four colonies were used in tests with different compounds. Diurnal variation in ant activity over the 14-h span during which tests were conducted also may have contributed to inter-trial variation.

Seven of the compounds had no effect on *S. invicta* at the highest concentration we tested. These compounds may be nontoxic. As Daly and Spande (1986) stated, the designation of some dendrobatid skin alkaloids as “toxins” is misleading; some compounds, e.g., (+)-gephyrotoxin, which was not effective in our tests, are not toxic in vivo or only weakly so in commonly used ex vivo assays. These alkaloids originally had been isolated from extracts along with other alkaloids that are toxic, and they were assumed to be

Table 2 Anuran alkaloids that elicited reduced ambulation in *S. invicta* following contact

Alkaloid	Control	100 mM (1,400)	33 mM (462)	4 mM (56)	11 mM (154)	1 mM (14)	0.33 mM (4.6)	0.11 mM (1.53)	0.04 mM (0.56)	0.01 mM (0.14)	0.004 mM (0.056)	0.001 mM (0.014)
5,8-Disubstituted indolizidine 205A[a]	43.8±18.3	21.0±13.9[o]	27.4±19.1									
5,8-Disubstituted indolizidine 235B'[b]	36.8±13.7	23.0±7.1 [o]	27.5±15.4									
Octahydrohistrionicotoxin 291A[c]	32.7±13.4	22.3±13.7 [o]	27.1±10.6									
2-Methyl-6-undecylpiperidine[d]	35.8+8.0		20.3+9.7 [o]		31.0+12.9							
Pumiliotoxin 267C[e]	20.0±8.8		12.5±10.7 [o]		17.6±11.1							
Batrachotoxin[f]	30.3±18.3		13.9±7.8 [o]		28.4±16.8							
Histrionicotoxin 259A[g, m]	35.8±9.8					23.7±11.7 [o]	36.9±15.2					
Nicotine[h, n]	50.6±26.6					7.3±10.8 [o]	43.8±18.4					
Pumiliotoxin 323A[i]	24.2±17.9						7.6±9.3 [o]	20.2±9.4				
Pumiliotoxin 251D[j]	26.2±9.4							14.6±13.2 [o]	19.4±9.6			
Allopumiliotoxin 267A[k]	19.6±4.1								10.0±5.9 [o]	20.9±18.4		
Pumiliotoxin 307A[l]	35.2±13.6										17.2±7.5 [o]	32.3±15.6

Mean ambulation scores (± standard deviation) are given. Concentrations are shown as millimolar and nanomoles per square centimeter. Superscript letters a–l indicate representative anuran sources

[a] Dendrobatidae: *O. pumilio* (Panama) (Daly et al. 2003); Mantellidae: *Mantella madagascariensis* (Madagascar) (Garraffo et al. 1993b)

[b] Dendrobatidae: *Oophaga* spp. (Colombia, Panama) (Daly and Spande 1986)

[c] Dendrobatidae: *O. histrionica* (Colombia) (Daly and Spande 1986)

[d] Dendrobatidae: *O. pumilio* (Costa Rica) (Jones et al. 1999; Daly unpubl. obser.)

[e] Bufonidae: *Melanophryniscus moreirae* (Brazil) (Saporito et al. 2004); Dendrobatidae: *D. auratus* (Hawaii) (Saporito et al. 2004); Mantellidae: *Mantella* spp. (Madagascar) (Saporito et al. 2004); Myobatrachidae: *Pseudophryne bibroni* (Australia) (Saporito et al. 2004)

[f] Dendrobatidae: *Phyllobates* spp. (Colombia, Costa Rica, Panama) (Daly and Spande 1986)

[g] Dendrobatidae: *O. histrionica* (Colombia) (Daly et al. 2005)

[h] Bufonidae: *Melanophryniscus stelzneri* (Argentina) (Garraffo and Daly, unpubl. obser.), Dendrobatidae: dendrobatid spp. (Daly et al. 2005), Mantellidae: *Mantella baroni* (Madagascar) (Clark et al. 2005)

[i] Bufonidae: *Melanophryniscus* spp. (Argentina, Brazil, Uruguay) (Grant et al. 2012); Dendrobatidae: *Epipedobates tricolor* (Ecuador) (Daly et al. 1980), Mantellidae: *Mantella* spp. (Madagascar) (Garraffo et al. 1993b); Myobatrachidae: *Pseudophryne* spp. (Australia) (Daly et al. 1990)

[j] Bufonidae: *Melanophryniscus montavidensis* (Uruguay) (Saporito et al. 2004), *M. stelzneri* (Argentina) (Saporito et al. 2004); Dendrobatidae: *Adelphobatus quinquevittatus* (Peru) (Daly and Spande 1986), *Ameerega erythromos* (Ecuador) (Saporito et al. 2004), *A. silverstonei* (Peru) (Saporito et al. 2004), *D. auratus* (Hawaii) (Saporito et al. 2004), *Epipedobates* spp. (Ecuador) (Saporito et al. 2004), *Minyobates steyermarki* (Venezuela) (Saporito et al. 2004), *Oophaga* spp. (Colombia, Panama) (Saporito et al. 2004), *Ranitomeya* spp. (Colombia, Ecuador, Panama) (Daly and Spande 1986; Saporito et al. 2004); Mantellidae: *Mantella* spp. (Madagascar) (Saporito et al. 2004)

[k] Dendrobatidae: *Ameerega erythromos* (Ecuador), *Adelphobates quinquevittatus* (Brazil), *Dendrobates* spp. (Hawaii, Panama, Surinam, Venezuela), *Epipedobates tricolor* (Ecuador), *Minyobates steyermarki* (Venezuela), *Oophaga* spp. (Colombia, Costa Rica, Panama), *Ranitomeya* spp. (Colombia, Panama, Peru) (Saporito et al. 2004)

[l] Dendrobatidae: *Epipedobates anthonyi* (Ecuador) (Daly et al. 2005), *Oophaga* spp. (Colombia, Panama) (Saporito et al. 2004); Eleutherodactylidae: *Eutherodactylus iberia* (Cuba) (Rodriguez et al. 2011); Mantellidae: *Mantella* spp. (Madagascar) (Saporito et al. 2004)

[m] Synthetic

[n] Commercially available (Sigma)

[o] Indicates that scores are significantly different from controls (Mann–Whitney *U* test, one-tailed *P* values<0.05)

Table 3 Anuran alkaloids that elicited convulsions in *S. invicta* following contact

Alkaloid	11 mM (154)	4 mM (56)	1 mM (14)	0.33 mM (4.6)	0.11 mM (1.53)
Batrachotoxin[a]	0.2±0.6				
Octahydrohistrionicotoxin 291A[b]	3.8±5.8				
Allodihydrohistrionicotoxin 285A[c]			0.3±0.9		
Histrionicotoxin 259A[d, g]				0.3±0.9	
Pumiliotoxin 267C[e]				1.2±1.5	
Pumiliotoxin 251D[f]					2.8±7.9

Mean convulsion scores (± standard deviation) are given at the lowest concentration to elicit convulsions. Concentrations are shown as millimolar and nanomoles per square centimeter. Superscript letters a–f indicate representative anuran sources

[a] Dendrobatidae: *Phyllobates* spp. (Colombia, Costa Rica, Panama) (Daly and Spande 1986)

[b] Dendrobatidae: *O. histrionica* (Colombia) (Daly and Spande 1986)

[c] Dendrobatidae: *Ameerega flavopicta* (Brazil) (Mortari et al. 2004), *O. histrionica* (Colombia) (Daly and Spande 1986)

[d] Dendrobatidae: *O. histrionica* (Colombia) (Daly et al. 2005)

[e] Bufonidae: *Melanophryniscus moreirae* (Brazil) (Saporito et al. 2004); Dendrobatidae: *D. auratus* (Hawaii) (Saporito et al. 2004); Mantellidae: *Mantella* spp. (Madagascar) (Saporito et al. 2004); Myobatrachidae: *Pseudophryne bibroni* (Australia) (Saporito et al. 2004)

[f] Bufonidae: *Melanophryniscus montavidensis* (Uruguay) (Saporito et al. 2004), *M. stelzneri* (Argentina) (Saporito et al. 2004); Dendrobatidae: *Adelphobatus quinquevittatus* (Peru) (Daly and Spande 1986), *Ameerega erythromos* (Ecuador) (Saporito et al. 2004), *A. silverstonei* (Peru) (Saporito et al. 2004), *D. auratus* (Hawaii) (Saporito et al. 2004), *Epipedobates* spp. (Ecuador) (Saporito et al. 2004), *Minyobates steyermarki* (Venezuela) (Saporito et al. 2004), *Oophaga* spp. (Colombia, Panama) (Saporito et al. 2004), *Ranitomeya* spp. (Colombia, Ecuador, Panama) (Daly and Spande 1986; Saporito et al. 2004); Mantellidae: *Mantella* spp. (Madagascar) (Saporito et al. 2004)

[g] Synthetic

toxic before their structures and individual activities were elucidated. Nontoxic compounds may nonetheless function defensively as distasteful agents, as postulated for histrionicotoxins (Daly et al. 2005).

A potentially important aspect of assessing the toxicity of our compounds, given our methods, is the impediment to chemical uptake posed by the integument of *S. invicta*. The generally lipophilic nature of anuran skin alkaloids may permit them to permeate the waxy layer of the insect cuticle, but whether most compounds in our array do so is unknown. However, nicotine, which occurs in trace amounts in dendrobatid, mantellid, and bufonid frogs and was moderately active in our assay, is known to permeate insect cuticle (e.g., Noble-Nesbitt 1970).

A study of the toxicity of pumiliotoxins that were injected into tobacco budworms (*Heliothis virescens*) demonstrated an approximately 20-fold increase in efficacy of PTX 251D over PTX 307A (PTX-A) and PTX 323A (PTX-B) (Bargar et al. 1995). Our results attest to the high toxicity of PTX 251D and indicate an even greater efficacy of PTX 307A. Daly et al. (2003) observed that allopumiliotoxin 267A is approximately five times more toxic than PTX 251D when subcutaneously injected into mice. The former compound was slightly more effective than the latter in our tests. The inactivity of PTX 237A (synthetic), however, is surprising in view of the fact that this compound lacks only the C-15 terminal methyl group of PTX 251D.

Our investigation of the contact toxicity of alkaloids provides results more relevant to the defensive use of these compounds for anurans than do those involving subcuticular or subcutaneous injection because anurans do not inject toxins. Some ants topically apply alkaloid-laden secretions on offending arthropods (e.g., Andersen et al. 1991), thus rendering contact toxicity an appropriate allomonal parameter to assess in these interactions as well. One compound that we tested, 2-methyl-6-undecylpiperidine, occurs in the skin secretions of *Oophaga pumilio* (Jones et al. 1999; Daly pers. comm.) and in the venom of *Solenopsis* spp.; it deters termites (*Reticulotermes* sp.) (Blum 1988; Escoubas and Blum 1990) and, as we show here, *S. invicta*.

To assess the results of toxicity assays in the context of anuran defense against mosquitoes, Weldon et al. (2006) considered the efficacy of PTX 251D in light of its estimated concentration in the skin of *E. anthonyi* from Ecuador. Because the cutaneous concentration of PTX 251D in this frog was estimated to be higher than the lowest concentration observed to adversely affect mosquitoes, a deterrent function of this compound against these hematophages was deemed plausible.

The cutaneous concentrations of most of the anuran compounds we tested are unknown. Thus, we cannot relate our results to the plausible defensive value of all compounds in our study. However, the efficacy of some compounds whose concentrations we have estimated can be considered. These estimates should be viewed in light of several caveats. The cutaneous alkaloids of dendrobatids are stored in granular glands situated below the skin surface, and these glands may be distributed unevenly in the integument (Saporito et

al. 2012 and references therein). Hence, it is difficult to infer the concentrations of compounds routinely encountered on the skin surface, even when their overall abundances are known. Moreover, dendrobatid skin alkaloids occur in multicomponent mixtures that vary according to individual, sex, locale, and other factors (Saporito et al. 2006, 2007b, 2010).

Although we presented batrachotoxinin A to ants at a concentration (586.6 μg/cm^2) that far exceeds its estimated concentration on the skin of *P. terribilis* (11.3 μg/cm^2), it was ineffective in our assay. The EMTC of BTX inducing convulsions in ants (249 μg/cm^2) also exceeds its estimated cutaneous concentration of this compound in this frog (28.2 μg/cm^2). These results fail to support the plausible use of these compounds by frogs as contact toxins against ants. PTX 251D, on the other hand, reduced ambulation and elicited convulsions in ants at a concentration (386.5 ng/cm^2) that is lower than its estimated cutaneous concentration in *E. anthonyi* (8.6 μg/cm^2). This result and those obtained in a study on the responses of mosquitoes to PTX 251D (Weldon et al. 2006) are consistent with the use of this compound as a contact deterrent of offending insects and possibly other leaf-litter arthropods. Skin toxins also may protect foraging dendrobatids against defensive attacks by the ants upon which they prey, as has been suggested for the skin secretions of the myrmecophagous narrowmouth toad, *Gastrophryne carolinensis* (Microhylidae) (Garton and Mushinsky (1979).

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