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An Endogenous Bile Acid and Dietary Sucrose from Skin Secretions of Alkaloid-Sequestering Poison Frogs

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Abstract

The skins of Madagascar poison frogs (*Mantella*) and certain Neotropical poison frogs (*Epipedobates*, *Dendrobates*) secrete the new bile acid tauromantelic acid (**1**), which was found in both wild-caught and captive-born frogs. This is the first molecule of endogenous origin detected in skin secretions from these taxa. Sucrose was also detected in secretions from wild-caught *Mantella* but not in captive-born frogs, suggesting a dietary origin.

Frog skins have proved to be a prolific source of secondary metabolites, and in particular over 800 different lipophilic alkaloids have been detected in skin extracts from six unrelated frog families from five continents.^{1–3} With few known exceptions, however,^{4, 5} these alkaloids are sequestered unchanged into the frogs' granular skin glands from alkaloid-containing arthropod prey, and are thus not poison frog products. Relative to other families

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DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures detailing sample collection; Figures S1–S11, mass spectra of tauromantelic acid (**1**) detected in individual samples; Figure S12, product ion spectrum of tauromantelic acid (**1**); Figure S13–S23, NMR spectra of tauromantelic acid (**1**); Figures S24–S25, HSQC spectra of individual, wild *Mantella* poison frogs with overlay of sucrose standard; Table S1, exact measured masses of different tauromantelic acid (**1**) samples. This material is available free of charge via the Internet at <http://pubs.acs.org>:

of such “tropical poison frogs”,⁶ Madagascar poison frogs (*Mantella*; Mantellidae) and several genera of neotropical dendrobatid poison frogs (Figure 1) contain the greatest variety of alkaloids in skin glands^{2, 7–11} and some of their alkaloid-containing arthropod prey have been identified.¹² No other skin chemistry has yet been documented from these frog taxa.^{2, 13} This report documents the presence of the new bile acid tauromantellic acid (**1**) and sucrose in the skin secretions of these frogs. Tauromantellic acid (**1**) is the first example of a genuine poison frog metabolite from the skin of any tropical poison frog.

RESULTS AND DISCUSSION

Skin secretions were obtained non-lethally from eight wild *Mantella* frogs, four each of *Mantella baroni* and *Mantella betsileo*. Analysis of these secretions by conventional positive ion GC-MS indicated the presence of several known alkaloids previously identified from a *M. baroni* secretion;^{7, 12} these are labeled (Figure 2A) with molecular weight codes as tabulated in Daly’s “frog alkaloid” mass spectroscopic library.²

MS analysis in negative ion mode gave a very different picture, and revealed the presence of a single major peak (Figure 2B) with composition C₂₆H₄₁NO₆S, as determined by accurate mass measurements on in both positive and negative ion modes (Figure 2C,D). This novel compound was detected in each of the 11 frog skin secretion samples, as shown by TIC and ESI spectra (Table S1, Figures S1–S11, Supporting Information).

Sample manipulation in natural products chemistry can result in undesirable chemical modifications and even in total loss of some major components,¹⁴ demonstrating the value of direct analysis of unfractionated material.¹⁵ However, there are no reports of direct NMR spectroscopic analyses of unfractionated skin secretions from poison frogs. The technique of direct NMR analysis was thus applied to the skin secretions of *Mantella* spp., and this analysis indicated that the major constituent of the skin secretions was a bile acid. Purification of this major constituent was accomplished by pooling skin secretions from several frogs, leading to the isolation of compound **1**.

The ¹H NMR spectroscopic data of **1** (Table 1 and Figure S13, Supporting Information) showed signals for two angular methyl groups at δ 0.60 and 1.04 (each 3H, s), a methyl group on a double bond (δ 1.68, 3H, s), an oxygen-bearing methine (δ 3.81, 1H, m), a vinyl proton at δ 5.38 (1H, br t, *J* = 6.9 Hz), and two two-proton AB triplets of a taurine group at δ 3.58 and 2.94 (each 2H, t, *J* = 6.8 Hz). The ¹³C NMR signals generated from HMQC and HMBC experiments revealed the presence of 26 signals including those for a carbonyl group (δ 213.5), one oxygen bearing methine (δ 67.6) and an amide carbonyl group (δ 172.9). The above data coupled with the ¹H- and ¹³C NMR data in Table 1 indicated that tauromantellic acid has a steroid skeleton with a ketone, a hydroxy group and an unsaturated side chain containing an amide group and the carbons of a taurine unit.

The assignment of the ketone carbonyl to C-3, the hydroxy group to H-7, the taurine group to an amide bond at C-24, and the *E* configuration of the double bond were substantiated by analysis of the HSQC, COSY, HMBC and NOESY spectra. The COSY experiment (Figures S14–S15, Supporting Information) showed the spin systems H-1 to H-2; H-4, H-5, H-6, H-7, H-8, H-9, H-11, H-12; H-14, H-15, H-16, H-17; H-22, H-23; and H-25, H-26 (Figure 3A).

The presence of three methyl groups in **1** was indicated by crosspeaks in its ¹H-¹³C HSQC spectrum (Figures S16–S17, Supporting Information), and two of these resonated at δ 0.60 and 1.04 ppm, indicating the presence of two angular methyl groups characteristic of a steroid skeleton. The nature of the steroid skeleton and the stereochemistry of the A/B, B/C, and C/D ring junctions were indicated by a comparison of its ¹³C NMR signals with those of 7α-hydroxycholestan-3-one¹⁶ (Table 1) and by the interpretation of the NOESY

spectroscopic data (Figures S18–S19, Supporting Information). The A/B, B/C, and C/D ring junctions were all deduced to be *trans* from the observation of NOESY correlations between CH₃-19_{ax} and H-1_{eq}, H-4_{ax}, H-6_{ax}, and H-8_{ax}, between H-5_{ax} and H-4_{eq}, H-6_{eq}, and H-9, between H-9 and H-14, and between H-8 and CH₃-18 and CH₃-19. A correlation from the C-21 methyl group to CH₃-18 assigned the *R* configuration to C-17. A clear NOESY correlation was observed from the proton at δ 5.38 (br t, H-22) to that of H-16 β , allowing assignment of the *E* configuration to the double bond (Figure 3B). The axial orientation of the hydroxy group at C-7 was substantiated by the NOESY correlation between H-7_{eq} (δ 3.81, m) and H-8_{ax} (δ 1.46, td, J = 11.2, 2.5), and by the inferred coupling constant of 2.5 Hz between H-8_{ax} and H-7_{eq}.

The assignment of the AB ring junction of **1** as *trans*, instead of the more common *cis* stereochemistry for bile acids, was confirmed by a comparison of the NMR spectra of tauromantellic acid (**1**) with the spectra of the A and B rings of 7 α -hydroxycholestan-3-one (Table 1).¹⁶ Both the ¹H and ¹³C NMR signals of the A/B ring system of **1** agreed much better with the corresponding signals of 7 α -hydroxycholestan-3-one than with those of 7 α -hydroxy-24-nor-5 β -cholan-3-one.¹⁷ In particular, the ¹H NMR spectrum of 7 α -hydroxy-24-nor-5 β -cholan-3-one has a signal assigned to an H-4 proton at 3.39 ppm (t, J = 15);¹⁸ the H-4 protons in **1** resonate at 1.93 and 2.35 ppm and those in 7 α -hydroxycholestan-3-one at 1.95 and 2.36 ppm (Table 1). The α -configuration of H-5 was supported by a NOESY correlation observed from H-6 axial (δ 1.57, td, J = 14.5, 2.5 Hz) and the C-19 methyl group (δ 1.04); such a correlation would not be possible if H-5 had the β -configuration. The large coupling constant observed for H-6 also supported this assignment. Finally, the ¹³C NMR chemical shift of the C-19 methyl in AB-*trans*-fused 3-keto-7 α -hydroxy steroids appears around 10.4 ppm, while in the corresponding *cis*-fused series it appears between 12 and 22 ppm.¹⁸ A calculation of the ¹³C NMR shifts of both **1** and 5-*epitauromantellic* acid using commercially available software gave results in reasonably good agreement between the calculated and observed values for **1**, while the calculated values for 5-*epitauromantellic* acid differed widely from the observed values for C-9 (calculated 33.2 ppm, observed 45.2 ppm) and C-19 (calculated 21.9 ppm, observed 10.4 ppm).

The site of amidation in **1** was assigned to C-24 (δ 172.9), based on the observation of HMBC cross-peaks from H-22 to C-24, H-23 (δ 2.98, d, 6.9 Hz) and from one of the methylene groups of taurine (δ 3.584, t, 6.8 Hz H-25) to C-24 (Figure S21, Supporting Information). Long-range correlations were observed in the HMBC spectrum from the C-21 methyl protons (δ 1.68) and C-17, C-22 and C-20 (Figure S22, Supporting Information), and from H-1 (δ 2.04), H-4 and H-5 to the ketone carbonyl at C-3 (Figure S23, Supporting Information).

The structure of the unsaturated taurine-conjugated side chain in **1** was confirmed by its detailed MS/MS fragmentation (Figure S12, Supporting Information). The structure of the new bile salt **1** was thus assigned as ((*E*)-4-((5*S*,7*R*,8*R*,9*S*,10*S*,13*S*,14*S*,17*R*)-7-hydroxy-10,13-dimethyl-3-oxohexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)pent-3-enamido)methanesulfonic acid or 3-oxo-7 α -hydroxy-5 α -chol-20(22)-enoyltaurine, following bile acid nomenclature.¹⁹ The common name tauromantellic acid is assigned to this novel taurine-conjugated bile acid in honor of *Mantella*, the first poison frog genus in which the new compound has been detected.

In addition to its occurrence in wild specimens of *Mantella baroni* and *M. betsileo*, tauromantellic acid (**1**) was also detected in skin secretions collected from captive-born *Mantella* poison frogs, and from captive-born *Dendrobates* and *Epipedobates* spp. Wild dendrobatids were not sampled (Table 2).

In addition to tauromantelic acid (**1**), sugars were detected and sucrose was identified by 2D-NMR spectroscopic methods in skin secretions collected from *Mantella* poison frogs living in Madagascar rainforests, but sugars were not detected in any of the captive-born poison frogs (Table 2), thus suggesting a dietary origin of sucrose within the habitat of the frogs. As seen in the HSQC spectra of each of the eight individual wild *Mantella*, similar amounts of sucrose and tauromantelic acid were detected in concentrations greater than that of the dietary skin alkaloids of the frogs (Figure 4, Figures S24–S25, Supporting Information). It appears that additional sugars were present in amounts too minute to permit their identification.

The fact that the skins of Madagascar poison frogs (*Mantella*) and certain neotropical poison frogs (*Epipedobates*, *Dendrobates*) secrete the new bile acid tauromantelic acid (**1**) is significant. Although bile acids have been isolated and chemically characterized from the bile of most vertebrate taxa²⁰ including several frog families^{20–25} and their tadpoles,²⁶ they have not been reported from frog skin secretions. In addition, unlike many of the alkaloids sequestered by poison frogs,² **1** must be an endogenous metabolite, since it is found in both wild and captive-bred frogs. It is interesting to speculate on the reason for the presence of this unusual bile acid in poison frog skin, and it is possible that this compound plays a key role in sequestering alkaloids and protecting the frogs from their own toxicity. Thus, tauromantelic acid (**1**) may form micelles that facilitate the uptake of alkaloids into the skin glands and subsequent storage, and it may also inhibit the precipitation of proteins by the basic alkaloids,²⁷ perhaps by forming salts with them or sequestering them in micelles.

Sucrose, a sugar known only to be produced by plants and not by animals, was likely obtained indirectly via the diet of the frogs. Over half of the >600 contents previously identified from *Mantella* stomachs were ants of the genus *Pheidole*,¹² the most abundant genus of ants in Madagascar. All above-ground foraging *Pheidole* ants, including over 100 species in Madagascar, tend honeydew-concentrating coccids (Hemiptera).²⁸ Ants are well documented to obtain sucrose by tending to hemipterans that themselves acquire sugars from the phloem sap of their host plants.^{29, 30} Thus, considering the abundance of sugars in the diet of wild *Mantella* spp. and the lack of sucrose in the diet of captive-born *Mantella* spp., it appears that frogs in this genus sequester sucrose from ants that sequester it from hemipterans that sequester it from plants. These putative roles and the distribution of tauromantelic acid (**1**) and sucrose in amphibian skin secretions require further investigation.

The discovery of endogenous tauromantelic acid (**1**) and diet-sequestered sucrose in certain tropical poison frog skin secretions demonstrates that a non-lethal approach to frog skin secretion collection can result in particularly useful samples. Additionally, minimizing sample manipulation including the direct collection of secretion from living frogs³¹ combined with analysis via new combinations of modern technologies can reveal novel classes of compounds in amphibians that have already been studied in detail, even when only limited amounts of natural samples are available.

EXPERIMENTAL SECTION

General Experimental Procedures

The UV spectrum was measured on a Shimadzu UV-1201 spectrophotometer. ¹H and ¹³C NMR spectra were recorded on Bruker 600 MHz spectrometers in CD₃OD with TMS as internal standard. Calculated NMR shifts were obtained using ACD Laboratories Release 11.00; Product Version: 11.01. Mass spectra were acquired using LTQ Orbitrap XL or LTQ Orbitrap Velos instruments (Thermo Fisher Scientific, Bremen, Germany) operated in both negative and positive ion modes, as detailed in the Supporting Information. Preparative

HPLC was performed using Shimadzu LC-10AT pumps coupled with a semi-preparative Varian Dynamax C₁₈ column (5 μ m, 250 $\times$ 10 mm), a Shimadzu SPD M10A diode array detector (DAD), and a SCL-10A system controller.

Sample Collection and Preparation

Poison frog secretion samples were collected in the field (Madagascar rainforests) and laboratory (captive-bred and born) by non-lethal methods in accordance with IACUC protocol 2006-0067 to V.C.C. at Cornell University³¹ which allowed the harvesting of skin secretions from living frogs. Wild *Mantella* species from Madagascar were sampled individually whereas captive-born *Mantella* species and secretions of dendrobatid poison frogs were pooled into one sample per species. In some cases, the secretion appeared as a milky substance on the skin surface, but was usually clear or invisible. As detailed in the Supporting Information, samples were collected by wiping skin secretions off their backs, both with and without electrical stimulation induced by a Transcutaneous Amphibian Stimulator (TAS).³² After each of two 10 sec stimulation events, their backs were wiped with MeOH-laced Kimwipes[®],³¹ and these Kimwipes[®] were stored in tubes or vials until being filtered away as described in Supporting Information. After being wiped with MeOH, each frog was immersed in water and monitored for at least 10 min prior to release at point of capture. Frogs monitored in captivity were feeding, and in some cases calling, within 4 h of these sampling events.

Purification and Chemical Analyses

Filtered and dried secretion samples from wild *Mantella* spp. were used for the initial structural studies at the University of Florida, and additional captive-bred *M. betsileo* samples were used to purify about 1 mg of taumantellic acid (**1**) at Virginia Tech. The secretions collected from six captive-born *M. betsileo* frogs were combined, and the material was purified by C₁₈ reversed-phase HPLC at a flow rate of 2 mL/min and UV detection at 262 nm. The solvent system was a gradient starting with 50% MeOH in H₂O containing 1% of HCOOH for 10 min, then from 50% to 90% MeOH over 10 min, from 90% to 100% MeOH over 5 min, concluding with 100% MeOH for 15 min. Approximately 1.0 mg of taumantellic acid (**1**) was obtained, and was shown to be approximately 90% pure by ¹H NMR spectroscopy.

Mass Spectrometry

A 2 μ L sample of diluted extract was separated for each run via a Surveyor MS pump plus LC equipped with a MicroAS autosampler (all Thermo Fisher Scientific, San Jose, CA), using a Hypersil Gold column (C₁₈ reversed-phase material, 1 mm inner diameter, 10 cm length, 1.9 μ m particle size) at a flow rate of 50 μ L/min. A gradient of 0 – 40% MeCN containing 0.1% HCOOH in 40 min followed by a wash step was used for analysis on the LTQ Orbitrap Velos. On the LTQ Orbitrap XL, a gradient of 0–30% MeCN containing 0.1% HCOOH in 18 min, a gradient of 30–70% MeCN from 18 to 22 min, and maintained at 70% MeCN until 26 min, followed by a wash step was used. The mass spectrometer performed a full MS scan with resolving power 30,000 full-width at half maximum at m/z 400, followed by six data-dependent HCD (higher energy collisional dissociation) MS/MS scans with resolving power 7,500 full width at half maximum at m/z 400. An Automatic Gain Control (AGC) target value of 1e6 ions was used for full FTMS survey scans and 1e5 ions for HCD MS/MS scans. The threshold for triggering MS/MS scans was set to 40,000 counts. Normalized collision energy was 37 eV with an activation time of 0.1 msec. ToxID 2.1.1 and XCalibur (Thermo Fisher Scientific) were used for data analysis and MassFrontier 6.0 (Thermo Fisher Scientific) was used to assign fragment ions.

NMR Spectroscopy

Dried samples were reconstituted in deuterated methanol (CD₃OD) and analyzed by ¹H NMR spectroscopy to determine which samples merited additional NMR experiments, as evidenced by signature methyl group signals (i.e. the C-18, C-19, and C-21 methyls, with shifts at 0.60, 1.04, and 1.68 ppm, respectively). Standard pulse sequences and methods were used to acquire 1D ¹H spectra, 2D ¹H/¹H spectra (including, as appropriate, COSY, TOCSY, NOESY and ROESY experiments), and gradient-based 2D ¹H/¹³C spectra (multiplicity-edited HSQC and magnitude-type HMBC experiments). These NMR experiments were performed on: (a) both unfractionated/crude and purified samples on a 600 MHz Bruker spectrometer at the VirginiaTech NMR facility, and (b) unfractionated/crude samples using a Bruker Avance-II-600 spectrometer equipped with a 5-mm CryoProbe™ at the AMRIS Facility of the University of Florida. At the AMRIS Facility, dried samples of the crude secretions were each reconstituted in 100 μl of 99.96% methanol-*d*₄ (Cambridge Isotope Laboratories, Inc.), and those solutions were transferred to 2.5 mm × 100 mm capillary NMR tubes (Norell, Inc.) for data acquisition. The capillary tubes were suspended in the 5-mm probe using a Bruker MATCH™ device. All spectra were acquired at a regulated probe temperature of 24 °C. Chemical shift axes were referenced to the ¹H and ¹³C solvent signals of HCD₂OD. All spectra were analyzed using MNOVA software.

Tauromantellic acid (1)—White solid; [α]_D²⁵ +23.3 (c 0.06, MeOH); UV (MeOH) end absorption only; ¹H NMR (600 MHz, CD₃OD), and ¹³C NMR (151 MHz, CD₃OD), see Table 1 and Figure S13 (Supporting Information, ¹H NMR only); positive ion HRESIMS *m/z* 496.2722 [M + H]⁺ (calcd for C₂₆H₄₂NO₆S⁺, 496.2727); negative ion HRESIMS *m/z* 494.2576 [M + H][−] (calcd for C₂₆H₄₀NO₆S[−], 494.2582).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Mantellid and dendrobatid poison frogs that provided skin secretions. (A) *Mantella baroni*. (B) *Mantella betsileo*. (C) *Dendrobates auratus*. (D) *Epipedobates tricolor*. Photograph credits: A, B, D by V. C. Clark; C by R. Skylstad.

Figure 2.

Orbitrap MS data acquired using a secretion sample collected from an individual wild *Mantella baroni* sample 'Live Frog #3' is shown here, and was replicated for other samples as seen in Figures S1–S11. (A) Positive ion mode total ion chromatogram (TIC) reveals peaks of previously identified alkaloids (see Refs.^{7,12}). (B) Negative ion mode TIC contains a major peak representing tauromantellic acid. (C) Electrospray ionization (ESI) spectrum of the quasi-molecular ion $[M+H]^+$ region of tauromantellic acid in positive ion mode. (D) ESI spectrum of the quasi-molecular ion region $[M-H]^-$ of tauromantellic acid in negative ion mode.

Figure 3.
(A) COSY correlations for **1**. (B) NOESY correlations for **1**.

Figure 4.

HSQC spectrum of skin secretions of one individual *Mantella baroni* frog. The signals from the HSQC spectrum of a sucrose standard are overlaid as red circles. This sample is referred to as “Live Frog #3” in reference 12. A larger version of this Figure is included as Figure S24 (Supporting Information).

^1H and ^{13}C NMR Spectra (600 MHz and 151 MHz, respectively) for Tauromantellic Acid (**1**) from Captive-born *Mantella betsileo*, and for 7 α -Hydroxycholestan-3-one in CD_3OD .^{a,b,c}

Table 1

Carbon No.	Proton No.	δ , multiplicities, Coupling (in Hz)		δ (ppm)	
		1^d	7 α -hydroxy-cholestan-3-one	1	7 α -hydroxy-cholestan-3-one
C-1	H _{ax} -1	1.39 m	1.38 m	39.5	39.6
	H _{eq} -1	2.04 m	2.04 m		
C-2	H _{eq} -2	2.23 br d (15)	2.23 brd (15)	38.3	38.9
	H _{ax} -2	2.48 td (14.6, 6.6)	2.48 td (14.6, 6.6)		
C-3	-	-	-	213.5	214.7
C-4	H _{eq} -4	1.93 dt (15, 2.7)	1.95 m	43.7	45.0
	H _{ax} -4	2.35 t (15)	2.36 t (15)		
C-5	H-5	2.05 m	2.06 m	39.5	40.7
C-6	H _{eq} -6	1.43 m	1.42 m	37.6	37.9
	H _{ax} -6	1.57 dt (14.5, 2.5)	1.57 m		
C-7	H-7	3.81 m ($w_{1/2} = 8.5$)	3.79 q (2.5)	67.6	68.0
C-8	H-8	1.46 td (11.2, 2.5)	1.43 overlapped	41.0	41.9
C-9	H-9	1.32 overlapped	1.47 overlapped	46.2	46.5
C-10	-			35.0	36.8
C-11	H _{eq} -11	1.62 overlapped	1.54 overlapped	22.1	22.3
	H _{ax} -11	1.42 m			
C-12	H _{ax} -12	1.20 m	2.05 overlapped	39.2	40.8
	H _{eq} -12	1.80 dt (12.8, 3.3)	1.96 m		
C-13	-	-	-	43.7	43.5
C-14	H-14	1.51 td (11.2, 4.7)	1.48 overlapped	49.9	51.7
C-15	Ha-15	1.23 m	1.14 m	24.2	24.5
	Hb-15	1.83 m	1.75 m		
C-16	Ha-16	1.69 br m	1.27 m	25.3	30.2
	Hb-16	1.88 m	1.71 overlapped		

Carbon No.	Proton No.	δ , multiplicities, Coupling (in Hz)	δ (ppm)
		1^d	7α -hydroxy-cholestan-3-one
C-17	H-17	2.13 t (9.4)	58.7
C-18	CH ₃ -18	0.60 s	13.3
C-19	CH ₃ -19	1.04 s	10.4
C-20	-	-	139.1
C-21	CH ₃ -21	1.68 s	18.2
C-22	H-22	5.38 br t (6.9)	117.3
C-23	Ha-23	2.98 d (6.9)	35.2
	Hb-23	2.98 d (6.9)	
C-24	-	-	172.9
C-25	CH ₂ -25	3.58 t (6.8)	35.3
C-26	CH ₂ -26	2.94 t (6.8)	50.0

^aData for 7 α -hydroxycholestan-3-one were obtained on a sample kindly provided by Dr. B. S. Selinsky.

^bAssignments based on HMQC, COSY and HMBC spectra.

^cbr: broad, m: multiplet, d: doublet, t: triplet, q: quadruplet, s: singlet.

^dOnly peaks corresponding to the bile salt were observed in the NMR spectra; thus, the nature of this compound as a protonated species or as an anion is not yet clear.

Table 2

Occurrence of Tauro mantellic Acid (1), Sucrose, and Various Alkaloids in Samples Collected from Frogs Living in the Wild in Madagascar and from Captive-bred Frogs.

poison frog species	sample size ^a	source	1	sucrose	alkaloids
<i>Mantella baroni</i>	4	SE Madagascar	+	+	+
<i>Mantella betsileo</i>	4	NW Madagascar	+	+	+
<i>Mantella betsileo</i>	4	captive-bred	+	-	-
<i>Mantella betsileo</i>	6	captive-bred	+	-	-
<i>Dendrobates auratus</i>	7	captive-bred	+	-	-
<i>Epipedobates tricolor</i>	1	captive-bred	+	-	-

^aWild frog secretions were analyzed individually, whereas captive-bred frog secretions were pooled. Sample collection, purification, and analysis are detailed in the Supporting Information.