Alkaloids of the Ecuadoran Poison frog *Epipedobates boulengeri*

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Background & Significance

Poison frogs are easily recognized for their bright coloration and toxic secretions. These toxins are made up of compounds known as alkaloids, which are organic compounds containing at least one nitrogen. It has been discovered that the frogs gain some of these alkaloids from the arthropods in their diet, but more research needs to be done on the origin of alkaloids.[1] With our collaborators, we are looking at whether color and toxicity are linked, as well as how these frogs do not poison themselves. We use gas-chromatography mass-spectrometry (GC-MS) to analyze our samples and identify alkaloids. To prepare samples, we do both solid-phase extractions and methanol extractions to isolate alkaloids. We want to identify and quantify the alkaloids present in order to create a database of alkaloids present in the skins of poison frogs.

https://amphibiaweb.org/species/1656

Gas Chromatography Mass Spectrometry

GC-MS is a common method of analyzing alkaloids, as many are volatile. Our lab uses the Thermo Trace GC ultra capillary gas chromatograph for the separation of compounds in our samples. This is attached to a Thermo ITQ1100 ion trap MS, which performs both electron impact ionization (EI), which gives us structural data from

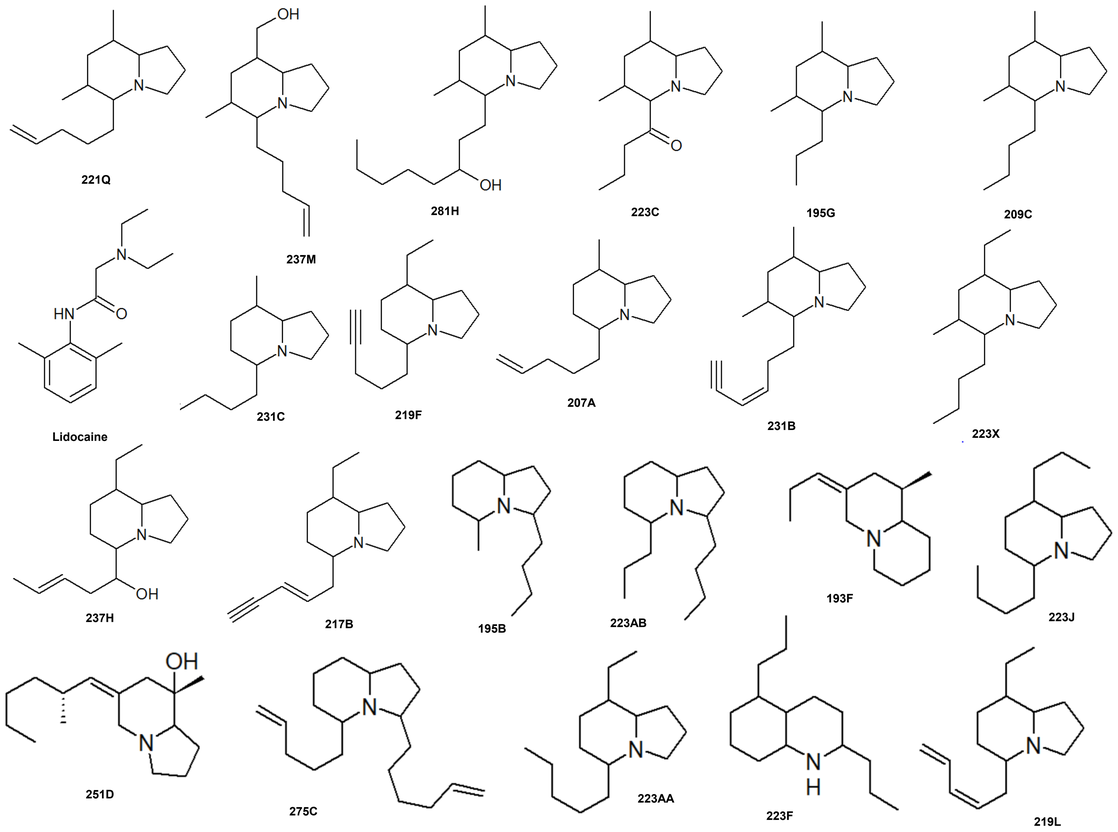
fragmentation patterns for our samples, as well as chemical ionization (CI), which gives us the molecular weights of the compounds by adding a reagent gas to protonatye the original molecule. A solid phase extraction technique was also used to help us separate and identify alkaloids.

Sample and Alkaloid Origins

These samples were extracted and sent to us by our collaborators. The frogs examined in our research were *Epipedobates boulengeri*, a poison frog native to Ecuador. These frogs have been demonstrated to eat arthropods that contain alkaloids, and sequester these alkaloids on their skin, using an uptake system, although more research needs to be done.[2] Some of the frog alkaloids have been found in formicine ants, myrmicine ants, oribatid mites, as well as in varieties of beetles and millipedes.[2,3,4] There are also other pathways that are possible for acquisition of alkaloids in poison frogs, such as the biosynthesis seen in the *Pseudophryne* frogs of Australia, or possibilities of alkaloid modification.[2] In addition, there are some distinction in which structural classes of alkaloids have been found in which arthropods. Classes with branched carbon skeletons have been illustrated to have a probable mite origin, while unbranched classes have been found in ants. Tricyclic structures have been in seen in beetles.[2]

https://www.chaosofdelight.org/mites#all-about-mites-oribatida

Structures of Alkaloids Identified in *E. boulengeri* in This Study



Quantities of Identified Alkaloids in *E. boulengeri*

Crude RT	Compound Type	Library ID	EI Base Peak (Parent)	SPE EI RT	CI [M+H]	6803	6804	6805	6806	6807	6808	6809	6810	6811	6812	6813	6814	6815	6816	6817	6818	6819
7.32	Tricyclic	193C	70, 94, 108, 110, **122**, 136, 150, 164, 178, 192, (193)	8.2	194			trace			52217	23502					29194					
7.4	5,6,8-I	193G	70, 110, **152**, (193)	8.29	194	327451		23568	1037613	4324			106548									
7.42	5,6,8-I	195G	70, 110, **152** (195)	8.28	196							108151		65527								
7.66	3,5-I	195B	70, **138**, 180 (195)	8.59	196	126356		trace		trace	56571		172480		49432				14289		12266	
8.14	5,6,8-I	207N	110, 122, 136, 150, **152** (207)	8.98	208			trace			52342	10209		9880	42264		20820	14478	2361			
8.17			70, 112, **138**, 168			29172		15624		trace			67692				41765		14576			
8.53	5,6,8-I	209C	110, **152** (209)	9.4	210	trace		trace			13767			8339	17990		26402	8250	6119			
8.81	5,8-I		96, **138**,			78724		764093	144597	25963	3236779	1066642	17900								19328	
9.49	5,6,8-I	223C	70, 110, **152**, (223)	10.35	224	184886		548128	75191		1403466	876233	39496									
9.75	3,5-I	223AB	70, 96, 124, **166**, 180, (223)	10.61	224	173712			87883	131102	843444	654147										
9.87			**110**, 152					29700			153615	77116										
9.94	5,6,8-I	223X	70, 110, 124, **166** (223)	10.82	224					131102				481338				119267				
10.00	Izidine	223I	166, **180**, 194, (223)	10.86	224	65623	7656	1979	10444			6241	155773									
10.3	5,6,8-I	237M	70, 110, **150**, 168, (237)	11.14	238	31595			49512			10465										
10.33	5,6,8-I		75, 110, 134, **150**, 169	11.15	226								3144	21138								
10.52	unclass	New 235	70, 110, 122, 136, **150**, **164**, 192, 206, 220, (221)	11.39	236	27766		38335	26942	29551	870951	21606	6755				96098		3789			
10.58	unclass	New 235	96, 110, 122, 136, 152, **164**, 179	11.39	236	18677		322457				71531										
10.63	5,8-I		96, 138, **166**					85409				17021										
10.78	5,8-I	223AA	96, **152** (253)	11.67	224									56134	16413				9420			
10.84	5,6,8-I	related to 253H	70, 110, 124, 150, 164, **194**, 252 (253)	11.69	254						7295			4408	4725							
11.05	5,8-I	219F	70, 96, **152** (219)	11.89	220	172101		211032	118317		615630			15387	33693							
11.09	5,8-I	217A OR 217B	96, **152**, (217)	11.92	218	57596		70817	47745	19761	935233	63716										
11.14	5,8-I	237H	96, **152**, (237)	11.98	238	114083		118898	62952	50512		154435	19166	17916	98873	87756				5668		
11.28	5,8-I	231B	110, **152**, (231)	12.14	232			23518					15343					30824	4556			
11.38	PTX		70, 124, 148, **166**, 194, 236			7194		11205	5432		13854	4743	4233						6765			
11.38	DHQ	223F	81, 95, 107, 121, **180** (223)	12.28	224									21235	991809		90980	36251	53396	4072		
11.44	5,8-I	217A OR 217B related to new 235	96, **152**, (217) 84, 95, 124, 136, 150, 164, 166, 178, 192, **206**, 220, 234, (249)	12.27	218						120518											
11.68				12.57	250	377755	3356	355864	268080	96597	442408	118964	51239	5227	4878	170635	2787	14356				
12.06	5,8-I	231C	70, 96, **138**, 202 230 (231)	12.94	232	22224		375689	9921		261364	427350										
12.14	5,6,8-I	231K	110, **166** (231)	13.04	232			3227			34913	6113		24634	6999						3248	
12.23	5,8-I	231C ISOMER (a)?	96, **138**, 164, 188, 202, 230, 231 (231)	13.10	232	20361		110033	9357		310236	136748										
12.35	PTX	251D	70, 95, 122, 148, **166**, 176, 194, 208, 218, 250 (251)	13.22	252	1168103	6193	1008797	1486780	486823	2891502	506792	1125030	3279	14549		16385	2878	15896			
13.05	PTX	251D relative	70, 96, 110, 148, **166**, 251			600024		63259	94483	39303	159894	19818	55446									
13.80	3,5-I	275C	124, **192**, 206 (275)	14.68	276	33099	340144	49304	375856	20021												
14.09	Lidocaine	Lidocaine	58, **86** (234)	14.97	235		198204	3433117	2230676	5851851	10547077	5830730	3867550	7115952	5214746	1242304	9036758		3661427			2656

Quantities reported are the integrated areas of extracted ion chromatograms of the base peak for the identified alkaloid. Quantities are not absolute masses. These cannot be determined without analytically pure calibration standards, none of which are currently available to us. Thus, these are intended as relative quantities as limited by the biases of ion yield and fragmentation probability.

Conclusions

Within our samples of the E. boulengeri frog, we found 133 alkaloids. The majority structural class with 40 alkaloids is 5,8-disubstituted indolizidines. 31 5,6,8-trisubstituted indolizidines were found as well as 15 3,5-disubstituted indolizidines, seven pumiliotoxins, fourteen tricyclic alkaloids, six decahydroquinolines along with two Izidine alkaloids. We also identified one alkaloid from another class, indolizidine. . Lidocaine was found in ten samples. There were 4 alkaloids whose structural class could not be determined, or who are considered unclassified.

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