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Histrionicotoxin Alkaloids Finally Detected in an Ant

Tappey H. Jones,*[,†] Rachelle M. M. Adams,[‡] Thomas F. Spande,[§] H. Martin Garraffo,[§] Tetsuo Kaneko,[§] and Ted R. Schultz[⊥]

[†]Department of Chemistry, Virginia Military Institute, Lexington, Virginia 24450, United States

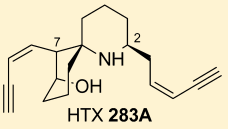
[‡]Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

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

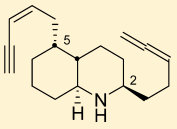
[⊥]Department of Entomology, Smithsonian Institution, POB 37012, NHB, CE516, MRC 188, Washington, D.C. 20013-7012, United States

Supporting Information

ABSTRACT: Workers of the ant *Carebarella bicolor* collected in Panama were found to have two major poison-frog alkaloids, *cis*- and *trans*-fused decahydroquinolines (DHQs) of the **269AB** type, four minor **269AB** isomers, two minor **269B** isomers, and three isomers of DHQ **271D**. For the *first* time in an ant, however, the DHQs were accompanied by six histrionicotoxins (HTXs), viz., **283A**, **285A**, **285B**, **285C**, **287A**, and **287D**. This co-occurrence of the HTX and DHQ alkaloids is the usual pattern seen in dendrobatid frogs. This finding contrasts with our earlier study, where workers of a Brazilian ant, *Solenopsis* (*Diplorhoptrum*) sp., were found to have a very similar DHQ complex but failed to show HTXs. Several new DHQ alkaloids of MW 271 (named in the frog as **271G**) are reported from the above ants that have both *m*/*z* 202 and 204 as major fragment ions, unlike the spectrum seen for the poison-frog alkaloid **271D**, which has only an *m*/*z* 204 base peak. Found also for the first time in skin extracts from the comparison frog *Oophaga granulifera* of Costa Rica is a trace DHQ of MW 273. It is coded as **273F** in the frog; a different isomer is found in the ant.



HTX **283A**



DHQ *trans*-**269AB**

Myrmicine ants have proven to be a significant source of alkaloids accumulated in specialized skin glands of the so-called poison frogs and toads, as reviews, including structural types, provided within the following references attest.[1−4] The poison-frog skin alkaloids arise, in part, from sequestration of ant defensive venoms and trail-marking alkaloids, as a consequence of ants being a major prey item, particularly for dendrobatid frogs. The ant alkaloids are typified by a straight-chain carbon backbone and, as a group, are composed mainly of 3,5-disubstituted indolizidines, 3,5-disubstituted pyrrolizidines, 4,6-disubstituted quinolizidines, 2,5-disubstituted decahydroquinolines (DHQs), 2,5-disubstituted pyrrolidines, and 2,6-disubstituted piperidines. An example of another straight-chain alkaloid class, the lehmizidines, with an azabicyclo[5.3.0]decane ring system originally found in the Colombian frog *Oophaga*[5] *lehmanni* (formerly *Dendrobates lehmanni*), was discovered recently in the ant *Myrmicaria melanogaster* of Brunei.[6] These alkaloids are considered to be synthesized by the ant, although the role of a microsymbiont has not been excluded.[4,7b] Recently, however, two *branched-chain* alkaloids of the pumiliotoxin class were encountered in two tiny Panamanian ant species of the genera *Brachymyrmex* and *Nylanderia* (formerly *Paratrechina*).[7a] A 5,8-disubstituted indolizidine, another branched-chain alkaloid, was also reported in a Madagascan ant of the genus *Tetramorium*.[8] Other than a few such exceptions, a rule of thumb seems to be that monocyclic or bicyclic alkaloids with a branched chain, often incorporating isoprenoid units, likely derive mainly from mites either by biosynthesis or via some symbiont or food source.[4,7b,9] Another straight-chain alkaloid class, evidently related biosynthetically to the DHQs and frequently of the same carbon numbers, especially C_{19}, is the histrionicotoxin (HTX) class, with unusual 2,7-disubstituted-1-azaspiro[5.5] undecanol structures (Figure 1, absolute configurations shown).[10,11] Despite the finding of either simple DHQs such as *cis* **195A** in ants[1] or the more complex C_{19} DHQs typified by **269AB** or **271D**,[12] not a single instance was found over the years of an HTX in any ant.

Incidentally, the nomenclature of the DHQ, **269AB**, reflects the early view that it represented a GC-inseparable 1:1 mixture of two DHQs, one losing a side chain of 65 amu, to give a base peak at *m*/*z* 204, and the other component losing a 67 amu fragment, to give a base peak at *m*/*z* 202. We now know that both fragments, of intensities approaching 100%, are lost from the same molecule. The 67 amu loss occurs by the expected α-cleavage from C-2, and the 65 amu loss by an allylic cleavage from C-5.[12] As of 1999, 17 species of dendrobatids were observed to have C_{19} DHQ alkaloids (MW range 267−271) in skin extracts and were in the genera *Oophaga* (formerly *Dendrobates*), *Ameerega* (formerly *Epipedobates*), and *Phyllobates*.[12] The HTXs are so far only known in New World frogs of the family Dendrobatidae, in particular, certain species of the

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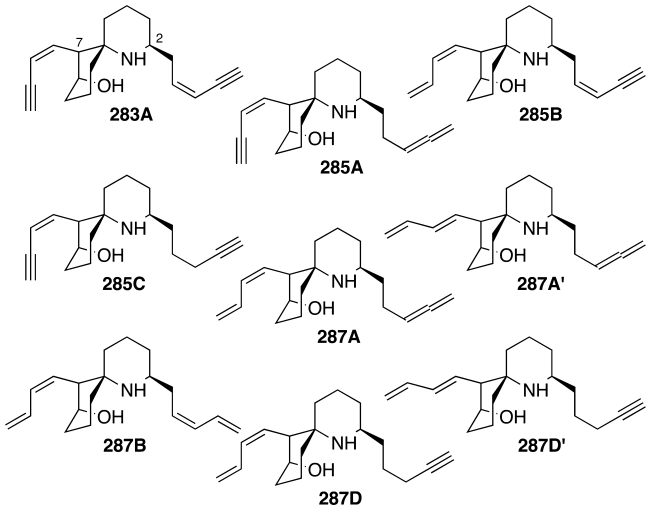

Figure 1.

genera *Oophaga*, *Ameerega*, and *Phyllobates*. Their initial occurrence in the eponymous *Oophaga histrionica* (originally *Dendrobates histrionicus*) appeared to be a useful chemotaxonomic character for this frog species, but this soon proved not to be the case. For example, three widely separated populations of *D. auratus*, two in Panama and one in Costa Rica, all had HTXs, although of differing structures and in different amounts.[13] There is one curious report of the HTXs **283A**, **285A**, and **285C** in a single specimen of the Old World frog *Mantella madagascariensis*, allegedly collected in Madagascar, but the provenance of this frog from the pet trade was never established unambiguously.[14]

The formulas and D-exchange data of HTXs, while apparently related to the fused-ring bicyclic secondary amine DHQs by an additional oxygen, have, however, an unusual spiro bicyclic structure with a strong N---HO internal hydrogen bond. Secondary amines, including piperidines and pyrrolidines, are uncommon in frog skin, although common in ants. It seems that frogs do not accumulate piperidines and pyrrolidines efficiently when present in the diet.[3] Many DHQs seen so far in ants or frogs are of 19 carbons and are not hydroxylated, although one example of a C_{13} 6-hydroxy DHQ (*cis* **211A**) is known in a frog.[15] No deoxy HTX has yet been reported. A pathway, not meant to indicate biosynthesis, relating C_{19} DHQ and HTX structures has been sketched.[12]

Since our group was fairly certain that the HTXs in various dendrobatids arise from dietary ants,[1,12,16] considerable effort has been invested over the years to identify the elusive ant prey in various collections from Central America and the Caribbean, but no HTX link was discovered.[17,18] When eight alkaloid-free captive-raised *D. auratus* frogs were kept in indoor terraria near Ancon Hill, Panama, and then fed leaf litter and the entrained arthropods, the following HTXs were observed in the three surviving frogs: **283A**, **285A**, **285C**, **287A**, and **287D**.[19] The amounts were significant, but less than in wild-caught *D. auratus* from the same area. This result clearly indicated however that a dietary source of the HTXs was in leaf litter. A later attempt to analyze in detail the leaf litter organisms, in hopes of identifying the HTX-producing arthropod, using isolation with forceps was fruitless in this regard, although many other frog-skin alkaloid classes were detected.[19] The early results with Panamanian and Costa Rican *D. auratus*[13] strongly suggested that more than one leaf litter arthropod, likely tiny ants, were the source of HTXs since one site (Isla Taboga, Bay of Panama) had a C_{13} and a C_{15} HTX, while another site (El Copé) had four of the more common C_{19} HTXs. Three of those were also found in Costa Rica. A collection of a Brazilian *Ameerega flavopicta* was recently reported to have a single HTX, **285A**, as a significant skin alkaloid.[20]

HTXs as well as DHQs and pumiliotoxins were found in *D. auratus* from Isla Taboga, Panama, as mentioned above.[13] When a population from this site was relocated to Manoa Valley, Oahu, Hawaii, in 1932, and their progeny reexamined in 1988, HTXs were no longer observed.[13,19] This observation was perplexing at the time, before the dietary hypothesis had been formulated.[3] Now it is known that a dietary arthropod was missing from the Hawaiian menu.

The present work, for the first time, confirms that an ant, *Carebarella bicolor* Emery [Myrmicinae], commonly found in leaf litter of Panama, does indeed contain alkaloids of the HTX class. Little is known about this Panamanian ant species except that workers do show up in leaf litter and queens are known at present only from their having been taken in flight. Eidmann reports[21] colonies of *C. bicolor* in the nest of a termite (*Nasutitermes* sp.) and of the leaf-cutting ant *Acromyrmex subterraneus*, suggesting perhaps a lestobiotic or "thief ant" relationship.[22]

RESULTS AND DISCUSSION

The genus *Carebarella* is a member of the tribe Solenopsidini, but its relationships within the tribe are poorly understood. Phylogenetic evidence based on three genes suggests that *Carebarella* is sister to *Solenopsis*; however, more complete taxon sampling is needed (Adams, unpublished). Morphological features also suggest a close relationship, and some features, such as the clypeal configuration, support this. It has been suggested that *Carebarella* could be a junior synonym of *Solenopsis* (Rodriguez, Pacheco, and MacKay, personal communication, and Fernandez and Rodriguez, personal observations), but that remains to be tested. The genus contains three described species, but workers are known only for *C. bicolor*. These are tiny ants (≈ 1 mm) and light-tan colored.

The methanol extract of the ants revealed very few gas chromatographic peaks that were considered neutral such as fatty acid esters or terpenes. Instead, 22 components were identified as alkaloids, most commonly typified, as here, in their electron-impact mass spectra having odd-mass molecular ions and mainly even-mass fragments. Two major ant alkaloids were identified by comparison of mass spectra and retention times with poison-frog skin alkaloids of the **269AB** type, one with a *cis*-fusion (2.3 parts) and the other (1 part) with a *trans*-fusion. Along with these were trace amounts of four other **269AB**s, not seen before in frogs, two DHQs of MW 269 and five of MW 271 along with a trace of a DHQ of MW 273. Tentative generic structures are proposed in Figure 2. The configurations shown there of the ring-junction hydrogens and C-2 and C-5 hydrogens are relative and are based mainly upon certain characteristic ν_{C-H} frequencies and Bohlmann band patterns in vapor-phase infrared spectra. No absolute configurations are known for any of the natural DHQs of MW 267−273.

Intermixed with the DHQs were clearly six representatives of the elusive HTX class, characterized by a diagnostic m/z 96 ion (often seen as the base peak) and pairs of diagnostic ions that include m/z 220 or 218 with either m/z 162 or 160.[23] Both of these ion pairs were observed in one GC peak (Table 1, 40.5

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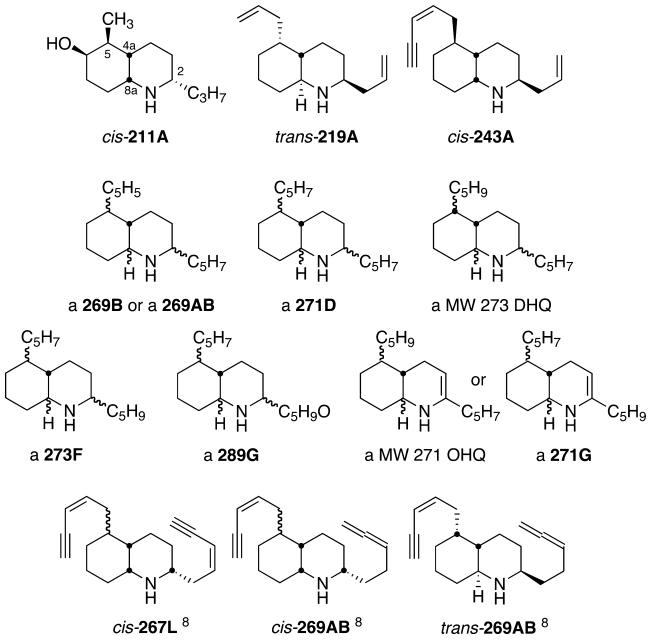

Figure 2.

min), common to ant and frog, indicating a mixture of histrionicotoxins **285B** and **285C**. The proportions differed between ant and frog. The DHQ and HTX alkaloids shared between a reference specimen of the dendrobatid frog *O. granulifera* (collection #1) collected in Costa Rica and the *C. bicolor* ants are displayed in Table 1 in order of increasing retention time (t_R) on the GC column with diagnostic mass spectrometric ions underlined. Table 2 includes mainly DHQs found in the ant but not found in the frog single-skin reference extract. Table 3 includes data from four skins of another frog collection (#2) from four years later. In the last columns of Tables 1 and 2 are some of the unpublished results obtained in 1996 with an extract of a Brazilian ant collected by J. H. C. Delabie but identified only to the subgenus level. These unpublished data are included here, as it was a first encounter in an ant with the MW 267−271 DHQ complex seen in many poison dendrobatid frogs.[12] However, this ant had no trace of the HTX class of alkaloids, nearly always accompanying the DHQs in poison frogs. The data of 1996 were accumulated with an earlier model of the Shimadzu GC-MS instrument, a different column, and a 10 °C/min ramp temperature program. Although the 1996 conditions of GC-MS analysis of the four-skin sample (see packed column GC chromatogram "2C" for an earlier alkaloid profile[24]) gave a poorer separation, some of the major GC peaks were identical with those of the single skin (population #1) of *O. granulifera* used as a reference for Table 1.[24] In this single skin as currently examined, we detected 14.5% histrionicotoxins in the total alkaloid mixture, considerably less than the overall amount in ants (27%). Even though the four-skin and one-skin collections of *O. granulifera* were from the same site, they were obtained four years apart. The observation that few alkaloids are shared between skins of these two collections from the same location attests to the fact that temporal variations in arthropod prey availability and/or prey choices will be manifested in differing alkaloid profiles.

Effects of Hydrogenation. Hydrogenation of a small sample of the *C. bicolor* extract in methanol provided three alkaloidal products as observed on GC-MS. Two were the fully

Table 1. C_{19} Alkaloids of the Decahydroquinoline (DHQ) and Histrionicotoxin (HTX) Classes Found in the Ant *Carebarella bicolor* from Panama, a Collection (#1) of the Frog *Oophaga granulifera* from Costa Rica, and a *Solenopsis* (*Diplorhoptrum*) Ant from Brazil

t_R (min)	TIC area (%) A = C. Ant; F = frog[c]	MW and class	frog alkaloid[a]	mass spectral ions *m/z* (intensity, %)	*Solenopsis (Diplo)* sp.[b] TIC area, (%) [t_R (min)]
39.1	1.3 A 0.6 F	269 DHQ	**269B**	268 (8), 226 (5), 202 (100), 134 (10), 96 (10), 91 (22), 67 (40), 65 (10)	9.8 [39.1]
39.5	1.9 A 1.00 F	271 DHQ	**271D**	270 (16), 256 (5), 242 (12), 204 (100), 122 (25), 111 (25), 96 (70), 67 (25)	3.4 [39.4]
39.6	36.8 A 37.5 F	269 DHQ	**269AB** (*cis*-fused)	268 (30), 254 (100), 240 (20), 228 (10), 204 (75), 202 (100), 148 (10), 134 (22), 96 (100), 67 (45), 65 (35)	
39.9	1.6 A 2.1 F	271 OHQ	**271G** (in earlier run)	270 (15), 246 (8), 242 (10), 230−226 (8), 216 (8), 204 (100), 202 (100), 174 (12), 162 (12), 148 (35), 136 (49), 122 (60), 109 (40), 96 (85) 67 (50), 65 (15)	5.8 [39.7]
40.5	4.9 A 5.5 F	285 HTX	**285B**/**285C** (1:1 A; 3:1 F)	284 (10), 268 (10), 256 (5), 242 (10), 228 (8), 220 (15), 218 (15), 202 (10), 190 (25), 162 (12), 160 (12), 96 (100), 91 (32), 67 (18), 65 (15) ant data	
40.7	0.8 A 9.5 F	287 HTX	**287D**	287 (10), 272 (15), 258 (10), 248 (10), 220 (25), 202 (15), 162 (40), 148 (19), 134 (100), 121 (50), 107 (50), 96 (100), 91 (40), 67 (30), 65 (15)	
41.2	9.7 A 4.9 F	285 HTX	**285A**	285 (10), 284 (10), 268 (10), 256 (10), 242 (10), 230 (10), 218 (15), 190 (25), 176 (25), 162 (30), 148 (20), 134 (25), 122 (35), 109 (52), 96 (100), 67 (25), 65 (25)	
41.3	6.1 A 3.4 F	287 HTX	**287A** + photoprod. **287A′** (see Table 2)	287 (12), 286 (10), 272 (25), 220 (10), 202 (15), 162 (40), 176 (25), 148 (30), 134 (40), 122 (42), 109 (78), 96 (100), 91 (55), 67 (42), 65 (12)	

[a]Extract of a single skin of a male *O. granulifera* (formerly *D. granuliferus*) frog from Río Grande de Térraba, Palmar Norte, Puntarenas Prov., Costa Rica, June 1990. This is collection #1.[24] [b]Extract of the ant *Solenopsis* (*Diplorhoptrum*) sp. from Itabuna, Brazil, 1997 (Jones, Delabie, unpublished data). [c]The Supporting Information includes TIC GC chromatograms for the *C. carebarella* ant and the *O. granulifera* frog with every GC peak identified.

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Table 2. DHQ and HTX Alkaloids Unique to the Ant *Carebarella bicolor* (not in Table 1) or Occurring in the Brazilian Ant *Solenopsis (Diplorhoptrum)* sp.

t_R (min)	ant *C.* TIC area (%)	MW and class	mass spectral ions *m/z* (intensity, %)	ant *S. (Diplo)* sp. of Brazil TIC area (%)[b] [t_R (min)]
[38.9][a]		**269AB** (*cis*) DHQ	268 (23), 254 (9), 240(12), 226 (11), 214 (18), 204 (70), 202 (100), 186 (11), 172 (11), 160 (16), 148 (18), 132 (18), 122 (36), 117 (14), 109−8 (30), 96 (100), 91 (57), 79 (68), 77 (41), 67 (80), 65 (43)	19
38.8	0.7	a 273 DHQ	272 (20), 244 (10), 232 (10), 218 (8), 206 (100), 204 (55), 176 (10), 150 (10), 136 (10), 122 (35), 111 (40), 96 (85), 67 (55), 65 (5)	
38.9	0.7	a **269AB** DHQ	271 (5), 230 (8), 204 (25), 202 (100), 124 (10), 98−96 (18), 91 (30), 69 (40), 67 (37), 65 (30).	
39.0	1.6	a **269B** DHQ	268 (3), 254 (3), 202 (100), 91 (18), 67 (12), 65 (10)	
39.3	0.7	a **269AB** DHQ	269 (3), 226 (5), 204 (73), 202 (100), 96 (15), 91 (39), 79 (28), 67 (30), 65 (12)	17
39.4	1.1	a **269AB** DHQ	268 (5), 228 (3), 204 (30), 202 (100), 117 (12), 96 (10), 91 (15), 67 (20), 65 (10)	
	not in *C. bicolor*	a **267L** DHQ	266 (5), 228 (5), 202 (100), 174 (7), 158 (7), 143 (7), 129 (10), 117 (10), 91 (23), 79 (16), 67 (23), 65 (23).	12 [39.2][a]
	not in *C. bicolor*	a **271G** OHQ	270 (8), 235 (5), 216 (11), 204 (38), 202 (53), 148 (15), 125 (10), 110 (34), 98(46), 97 (57), 96 (76), 69 (100), 67 (100).	6.2 [39.8]
39.7	4.7	a **271G** OHQ	270 (10), 242 (10), 228 (3), 214 (10), 204 (100), 202 (65), 150 (10), 122 (40), 96 (60), 91 (36), 67 (40), 65 (10)	3.4 [39.9]
40.1	16.3	**269AB** (*trans*) DHQ	268 (20), 254 (10), 240 (10), 226 (12), 214 (10), 204 (65), 202 (100), 150 (80), 117 (35), 96 (70), 91 (55), 67 (40), 65 (30)	41% (IR on this GC peak)
40.2	1.7	a **271D** DHQ	270 (3), 256 (2), 242 (2), 228 (3), 220 (15), 204 (100), 148 (15), 130 (15), 122 (20), 96 (40), 91 (25), 67 (33)	
40.3	4.2	283 HTX **283A**	282 (10), 266 (5), 218 (32), 160 (20), 96 (100), 91 (30), 65 (22)	
40.7	2.0	a **271D** DHQ	270 (10), 256 (5), 242 (5), 228 (8), 220 (15), 204 (100), 124 (30), 122 (30), 96 (100), 91 (36), 67 (36)	
40.8	0.6	a **269AB** DHQ	268 (5), 254 (5), 240 (5), 230 (5), 226 (5), 204 (35), 202 (100), 148 (20), 136 (25), 96 (25), 67 (30), 65 (45)	
41.7	1.3	**287A′** HTX (photoproduct)	see 41.3 min peak in Table 1 for virtually identical EIMS	

[a]The retention times in brackets are estimated as described in the Experimental Section. [b]The integrations are approximate, as they are based upon the total ion current only of the base peak, usually *m/z* 67, 96, 202, or 204. They are expressed as a percent of the sum of the TICs.

saturated DHQs of MW 279 at t_R 22.9 and 23.1 min (*cis*- and *trans*-fused, respectively, 5 parts to 1), which differed little in their mass spectra. The *trans* isomer did show a slightly greater fragment ion at *m/z* 236, which corresponded to the loss of C_3H_7 (43 amu) from the molecular ion, a process described by Spande et al.,[12] where the EIMS and IR of H_{10} **269AB** (*trans*) were also characterized. Both components lost the fully saturated C-2 side chain, C_5H_{11}, by α-cleavage to afford base peaks at *m/z* 208. The third component at 24.6 min (3 parts) exhibited a single sharp peak on GC-MS of MW 295, fitting the EI mass spectrum of a perhydrohistrionicotoxin derivative of **283A**.[25] It likewise showed a major loss of C_5H_{11} and afforded a base peak at *m/z* 224 and fragments at *m/z* 190, 180, and 96.

Hydrogenation of a sample of the *O. granulifera* frog extract from a single male (collection #1) yielded three perhydro DHQs of MW 279 at t_R 22.3, 22.9, and 23.1 min with 1 part:8 parts:33 parts by total ion current integration. The latter two peaks had the same mass spectra and t_R value as observed with the hydrogenated ant sample. The 22.3 min peak is likely a 2-*epi* isomer, epimeric at C-2 with respect to the usual stereochemistry observed at C-2 in the **269AB** series, and is considered to derive from the hydrogenation of two **271G** components, present in greater amounts in the frog extract. The 5-epi **269AB** (*trans* ring fusion) is known from other work[12] to have a longer t_R than the normal 5-configuration, and this may also hold true for the perhydro derivatives. In addition to the above perhydroquinolines, a perhydro HTX (14 parts) from hydrogenation of the frog extract was observed at 24.7 min and had the same complex mass spectrum as observed for the hydrogenation product of the *C. bicolor* ant extract. Only one-tenth of the material of the ant extract was available for the frog extract hydrogenation, and this may account for the slight difference (+0.12 min) in t_R observed for the perhydro HTX from the frog. A trace of another alkaloid was observed at 23.2 min. It is evidently not a DHQ, and from the apparent molecular ion at *m/z* 277 it might represent the hydrogenation of traces of a dehydro HTX artifact formed on 21-year storage of the frog extract.

The mass spectra of the MW 267/269/271 decahydroquinoline complex is discussed in Spande et al., 1999.[12] Several new MW 271 DHQs were observed in the present study that exhibit both *m/z* 202 and 204 in EIMS and for which isomers having an *octa*hydroquinoline nucleus are proposed (see Figure 2). These alkaloids could be diastereomers, but more likely they possess different side-chain unsaturation patterns, as demonstrated by the hydrogenation study. Since these alkaloids had been seen earlier in *O. granulifera* but not reported until now, we give this group of alkaloids the new code designation **271G**, even though they occur also in both ant species of the present study. Both C-2 and C-5 side chains are of 67 amu in the usual isomers related to **271D**. However, in Figure 2 it is proposed for the **271G** group that the substituent at C-2 has 69 amu; that is, this side chain has one unsaturation, whereas the substituent at C-5 has the usual 67 amu side chain with two unsaturations, such as a 2,4-diene or any other side chain allowing allylic cleavage.

A vapor-phase infrared spectrum (unpublished data) was obtained from the Brazilian *Solenopsis* (*Diplorhoptrum*) sp. ant for the *trans*-ring-fused **269AB** that was identical in all respects with that reported in Spande et al., 1999.[12] It exhibited absorptions at 3327 and 3033 cm^{-1} (*cis* enyne) and 1954 and 844 cm^{-1} (terminal allene). It also showed absorptions at 1337

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Table 3. DHQ and HTX Alkaloids Found in the Frog *Oophaga granulifera* (Collections #1 and #2[a])

t_R (min) coll. #2	area (%)	MW and class	mass spectral ions m/z (intensity, %)[b]	occurrence in collection #1, t_R (min)
[38.2]	0.5	a **269AB** DHQ	254 (30), 204 (94), 202 (63), 188 (20), 148 (27), 96 (24), 67 (100), 65 (28)	
[38.4]	0.4	a **271G** OHQ	243 (9), 228 (27), 216 (16), 204 (68), 202 (100), 190 (23), 166 (25), 164 (16), 148 (14), 146 (20), 96 (27), 91 (45), 67 (43), 65 (52)	
[38.5]	2.4	a **271G** OHQ	270 (20), 243 (7), 228 (7), 216 (14), 204 (100), 202 (20), 188 (7), 162 (7), 150 (11), 148 (11), 146 (7), 122 (45), 96 (75), 91 (34), 79 (48), 67 (41),	
[38.8]	4.2	a **269AB** DHQ	268 (20), 254 (7), 226 (7), 214 (9), 204 (73), 202 (82), 186 (11), 174 (11), 160 (11), 148 (20), 146 (11), 122 (34), 96 (100), 91 (64), 79 (82), 67 (57), 65 (41)	
[39.1]	5.7	a **271G** OHQ	270 (14), 256 (5), 242 (5), 228 (5), 218 (9), 216 (9), 204 (73), 202 (20), 188 (7), 174 (7), 163 (7), 162 (7), 150 (11), 148 (11), 124 (27), 122 (41), 96 (100), 91 (45), 79 (68), 67 (20), 65 (14)	39.2
[39.5]	37.5	a **269AB** DHQ	268 (20), 254 (7), 240 (9), 226 (7), 214 (7), 204 (100), 202 (100), 188 (7), 174 (14), 162 (11), 160 (11), 148 (34), 134 (16), 122 (16), 117 (20), 109 (36), 96 (82), 91 (59), 79 (75), 67 (52), 65 (34)	
[39.7]	3.7	a **271G** OHQ	271 (7), 270 (11), 256 (5), 243 (7), 230 (7), 228 (9), 216 (11), 204 (100), 202 (100), 188 (7), 174 (11), 162 (12), 148 (23), 136 (11), 122 (18), 109 (18), 96 (41), 91 (34), 79 (55), 67 (43), 65 (11)	
[39.9]	9.4	a **271D** DHQ	271 (3), 270 (14), 256 (2), 242 (9), 228 (5), 216 (7), 204 (100), 188 (5), 174 (5), 162 (7), 150 (9), 148 (7), 124 (36), 122 (27), 110 (36), 96 (100), 91 (39), 79 (61), 67 (45), 65 (16)	40.1
[40.2]	35.3	a **271G** OHQ	270 (11), 256 (2), 243 (7), 228 (5), 216 (5), 204 (66), 202 (14), 188 (5), 174 (7), 162 (5), 148 (32), 124 (36), 122 (25), 110 (34), 96 (100), 91 (39), 79 (64), 67 (43), 65 (14)	40.2
[40.3]	1	a **269AB** DHQ + **287** HTX	287 (7), 270 (5), 269 (7), 268 (11), 254 (9), 240 (11), 230 (11), 220 (11), 218 (18), 204 (48), 202 (95), 188 (7), 187 (7), 186 (7), 185 (7), 174 (23), 160 (27), 148 (23), 146 (57), 134 (20), 124 (12), 123 (11), 122 (13), 121 (11), 120 (20), 109 (25), 108 (25), 96 (100), 91 (52), 79 (59), 67 (45), 65 (32)	
	0.4	a **273F** DHQ	272 (5), 260 (2), 246 (2), 230 (2), 204 (100), 193 (8), 176 (5), 166 (25), 150 (5), 134 (12), 119 (8), 105 (8), 91 (12), 70 (21), 67 (9)	43.1
	0.8	a 287 DHQ	287 (5), 259 (10), 244 (10), 220 (100), 202 (40), 181 (10), 150 (8), 136 (15), 96 (45), 91 (30), 82 (20), 79 (25), 67 (20), 65 (15)	42.7
	0.4	a **289G** DHQ	288 (5), 260 (6), 246 (5), 220 (20), 204 (100), 119 (5), 105 (10), 91(20), 67 (15)	43.6

[a]An extract examined in 1996 was obtained in 1994 from pooled skins of four *O. granulifera*[5] (formerly *D. granuliferus*) frogs from Río Grande de Térraba, Palmar Norte, Puntarenas Prov., Costa Rica. This is collection #2.[24] See footnote to Table 1 for collection #1 data. Retention times for alkaloids from this frog are estimated and in brackets. [b]Electron-impact mass spectra are not normalized with the same base peak; sometimes it is m/z 96, sometimes m/z 204. Retention times in brackets are estimated as described in the Experimental Section.

and 1211 cm^{-1} that have been assigned to the DHQ *trans*-ring fusion. The known structures for the *cis*- and *trans*-fused **269AB** DHQs are shown in Figure 2.

The only two decahydroquinolines in common between the Brazilian and Panamanian ants are the **269AB** alkaloids, although the ratio of *cis/trans* isomers is ca. 2:1 for the Panamanian ant and 1:2 for the Brazilian ant. None of the MW 271 DHQs appear to be identical between the two ant extracts, although the fragmentation patterns are similar and similar substituents are proposed (see Figure 2).

The hydrogenation experiment demonstrated that (1) all of the DHQs of the *Carebarella* ant and nearly all those of the *O. granulifera* frog differ only at the ring fusion, and the configurations at C-2 and C-5 in all the isomers are likely the same, with differences only in the unsaturation patterns of the side chains; (2) all of the ant HTXs are converted to a single perhydro derivative that still has the hydroxyl group (cf. m/z 295→278 (M − OH)), and that group is very likely of the same α-orientation as seen with the frog-skin alkaloids. Thus, complex mixtures of DHQ and HTX unsaturation analogues that are seen with either the ant or the frog simplify after hydrogenation to mainly two perhydro DHQs and one perhydro HTX.

New Structures. Figure 2 indicates probable generic structures for the new MW 271 DHQs, isomers of the frog alkaloid **271G**, which show significant fragment ions at both m/z 204 and 202, as well as the known series related to **271D**, where only a base peak at m/z 204 is observed. The structure deduced for the **271G** alkaloids necessitates an octahydroquinoline (OHQ) to account for the fact that the normal facile alpha cleavage at C-2 is retarded and is competitive with the C-5 cleavage (likely allylic in this case). There is known at present only one frog-skin OHQ, code named **193D**,[25] for which a tentative 3-propyl-5-methyloctahydroquinoline structure is proposed based upon ND_3-chemical ionization exchange data showing one H atom being exchanged by D and a vapor-phase FTIR spectrum. A weak absorption at 3040 cm^{-1} is assigned to the presence of a vinyl ν_{C-H}, and a significant absorption at the frequency (1641 cm^{-1}), expected for an enamine, indicates the unsaturation is at C-2,3. In the absence of other data, we propose such a provisional enamine structure for the various isomers of **271G** detected in the present studies. The *Carebarella* ant has two isomers of this structure (total 6.3% of alkaloids), whereas another two are present in the frog (collection #1) (29.5%), but neither is shared with the ant. The Brazilian *Solenopsis* (*Diplorhoptrum*) ant has three of these MW 271 OHQs that are not seen in the frog or the *Carebarella* ant (Table 1). The MW 273 DHQ is seen in both ant and frog; in the latter it is given the code **273F** for this new generic structure. In the ant it has a base peak at m/z 206, where it is likely that a 67 amu fragment is cleaved from C-2 with a 69 amu (C_5H_9) moiety being retained at C-5. In the frog, a different isomer is seen at a much greater t_R and a base peak at m/z 204, indicating a cleavage of a 69 amu fragment from C-2 and a C_5H_7 moiety at C-5 that is not cleaved.

Unexpected Complexity of Ant/Frog DHQs. In early investigations of frog-skin DHQs, it was logically assumed that only a few isomers of any DHQ would be encountered, likely a C-2 or C-5 epimer or a *cis/trans* pair of ring-fusions, and that side-chain unsaturation would be restricted to just a few

patterns, as seen with the HTXs, e.g., enyne, terminal acetylene, or terminal allene. However, the present work with DHQs from two ant species and two collections of the same frog species from the same site has forced a change in that simplistic view. There are just too many variations of the original **269AB**, **271D**, and **271G** structural types (or "classes") to be accommodated unless more side-chain unsaturation patterns are invoked. Our alkaloid extracts are usually complex mixtures with too many components eluting at very similar GC or HPLC retention times for any isolations to be attempted, so ^{1}H NMR is not feasible to assign side-chain unsaturation types. Even vapor-phase IR has been used in this study only once, where a single fortuitously dominant component, (*trans*) **269AB**, was observed in the Brazilian ant. This observation was important however for demonstrating conclusively that an ant and a frog shared an identical DHQ structure. Otherwise, given our usually tiny samples, we are forced to rely upon mass spectrometry and microchemical methods alone with gas chromatography. The hydrogenations used in the present study gave a surprisingly unambiguous result and indicated that C-2 or C-5 epimers of the DHQs were at most two (the second appearing as very minor only in the frog extract and likely differing at C-2, arising from the putative enamines of the **271G** structures) and the diastereomers arising from *cis* and *trans* ring junctions were more important. Hydrogenation of the DHQ enamines would likely produce both epimers at the 2-position. If we can extrapolate from these two simple experiments, we could conclude that the observed complexity of the ant and frog DHQs stems mainly from the side-chain unsaturation patterns. Defined structures may consequently prove an open-ended quest, not impossible but just very difficult with current GC techniques. For example for the usual DHQ five-carbon side chains, the following unsaturation patterns are theoretically possible: four ene structures (69 amu); six diene structures (67 amu); or four enynes (65 amu). We could assume that acetylenes and allenes will be terminal and that double bonds, whether conjugated or not, will be of the *Z*-configuration as that has been our invariant experience; however the enes, dienes, or enynes could occur as many possible structures, and *E* double bonds are not absolutely excluded. Consequently, we have abandoned, for the present, our traditional nomenclature that would imply a specific and discrete structure, as for example does exist for the originally studied **271D**,[12] and instead we refer now to such an alkaloid as "a **271D**", as we have done in Figure 2. This is a provisional nomenclature proposal, as we are of the opinion that the use of primes and or additional bolded letters to distinguish among them at present would prove unwieldy and misleading. The indefinite article implies that no discrete structure can be written for **271D** but that it instead represents a group of alkaloids of MW 271 typified by a base peak at *m*/*z* 204. This mass spectrum might result from C_5H_7 cleavage at C-2 but not C-5 or a C_5H_7 cleavage from C-2 and/or C-5. The substituents at C-2 and C-5 will have the same C_5H_7 formula but not necessarily the same unsaturation pattern. Among probable side-chain unsaturations are 2,4-pentadiene, pent-4-yne, and pent-3,4-diene (an allene). The occurrence of pent-4-yne and pent-3,4-diene at C-2/C-5 in a published structure of *cis* **271D** was demonstrated in earlier work by GC-FTIR.[12] Now it appears to represent just one of many possible **271D** structures. Incidentally, it should be stressed that the various isomers of a 2,5-disubstituted DHQ such as **271D** are thermally stable on GC and the retention times are reproducible.

Thus it appears that even greater complexity in the C_{19} DHQs lies ahead, as the current study is only a small sampling representing two frog collections and two ant species. The ecological and biosynthetic significance of so many DHQs (and some OHQs) is totally mystifying, as is the surprisingly rare co-occurrence in this ant of the histrionicotoxins with such hydroquinolines. Why in the dendrobatid frogs are the DHQs invariably accompanied by HTXs but, so far, only in the single instance we report on here are they found together in an ant?[26]

EXPERIMENTAL SECTION

General Experimental Procedures. Two samples of roughly 20 workers each of *Carebarella bicolor* Emery 1906 were prepared in the field in vials containing MeOH. The collection was made from a single nest at El Llano, Panama (lat./long. 9.27956° N/78.96115° W), on May 21, 2010. Voucher specimens of these collections were deposited in the collection of the Department of Entomology of the Smithsonian National Museum of Natural History, Washington DC.

Gas chromatography–mass spectrometry was carried out in the EI mode using a Shimadzu QP-2010 GC/MS equipped with an RTX-5, 30 m × 0.25 mm i.d. column. The GC oven was programmed (data of Tables 1–3) from 60 to 250 °C at 5°/min and held at this temperature for 20 min. A one skin sample "methanolic extract" of the frog *O. granulifera* (formerly *Dendrobates granuliferus*) was also run with this program. One analysis of the *Carebarella* ant, earlier work on a *Solenopsis* (*Diplorhoptrum*) sp. ant, and a four-skin extract of the frog *O. granulifera* used a more rapid temperature program: 60 to 250 °C at 10°/min and a hold time at that temperature for 20 min. A correction factor based upon the co-occurrence of *cis* and/or *trans* **269AB** in ant and frog allowed most retention times to be estimated and data to be compared. A frog voucher, under the species name *Dendrobates granuliferus*, is deposited with the American Museum of Natural History, New York, NY.

Hydrogenation in methanol of small samples of the *C. bicolor* ant extract or the *O. granulifera* frog extract from a single male (collection #1) was accomplished with a few milligrams of PtO_2 and a gentle stream of hydrogen for approximately 5 min.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: (540)-464-7422. Fax: (540)-464-7261. E-mail: jonesth@vmi.edu.

Notes

The authors declare no competing financial interest.

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(19) Daly, J. W.; Garraffo, H. M.; Spande, T. F.; Jamarillo, C.; Rand, A. S. *J. Chem. Ecol.* **1994**, *20*, 943−955.
(20) An extract of 55 combined skins of adults of the Brazilian dendrobatid frog *Ameerega flavopicta* was found to have pumiliotoxin **251D** and a *single* HTX, **285A**, as major alkaloids and two DHQs (**219A** and **243A**) as minor ones [Mortari, M. R.; Ferroni-Schwartz, E. N.; Schwartz, C. A.; Pires, O. R., Jr.; Santos, M. M.; Bloch, C., Jr.; Sebben, A. *Toxicon* **2004**, *43*, 303−310]. The occurrence of a sole HTX is unusual and particularly one that differs in carbon number from the co-occurring DHQs. Ants (unidentified) were a minor part (ca. 12%) of the stomach contents by volume; the remainder were mainly termites. A reviewer has suggested that frogs selectively consuming only one ant species perhaps having one or only a few HTXs could be responsible for a finding like the above and that a larger number of HTXs would result when more ant species were consumed, as would be likely in an extract of frogs from a large collection. This situation cannot be ruled out, but our finding of the present work that a single ant species can have most of the known C_{19} HTXs found in frogs (**285E** and **287B** are the only known HTXs not present) diminishes the likely generality of such a proposal. Our finding that collections of *Dendrobates auratus* differed in HTXs certainly does indicate ant species having a variety of HTXs *are* being consumed as prey. These collections in Panama and Costa Rica had two to four HTXs[13] (see text).
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(26) The possibility of an enzymatic interconversion of the C_{19} DHQs to HTXs in frogs was at one time speculated on in our group (Daly et al., unpublished) before the dietary hypothesis had been established. This could account for the fact that DHQs in frogs were often found with HTXs, but only DHQs were found in ants. A reviewer raises this issue. The bioprospecting results and the occurrence of HTXs in the *Carebarella* ant of the present work now make such a complex biotransformation essentially a moot point.