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, CES16, MRC 188, Washington, D.C. 20013-7012, United States

Supporting Information

ABSTRACT: Workers of the ant Carebara 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.

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 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 C19, 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 ants1 or the more complex C19 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 C19 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

Received: July 11, 2012
Published: October 22, 2012
genera Oophaga, Ameerega, and Phyllobates. Their initial occurrence in the eponymous Oophaga histriomaca (originally Dendrobates histriomacae) 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.15 Many DHQs seen so far in ants or frogs are of 19 carbons and are not hydroxylated, although one example of a C19 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 C19 DHQ and HTX structures has been sketched.12

Since our group was fairly certain that the HTXs in various dendrobatids arise from dietary ants,1,2,16 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. auratus15 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 C13 and a C15 HTX, while another site (El Copé) had four of the more common C19 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.13 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 reports21 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 269ABs, 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 νC−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 160.25 Both of these ion pairs were observed in one GC peak (Table 1, 40.5

---

**Figure 1.**

![Diagram of molecular structures](image-url)
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. 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 profile24) 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. 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. C19 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

<table>
<thead>
<tr>
<th>$t_R$ (min)</th>
<th>TIC area (%)</th>
<th>A = C. Ant; F = frog</th>
<th>MW and class</th>
<th>frog alkaloid$^a$</th>
<th>mass spectral ions m/z (intensity, %)</th>
<th>Solenopsis (Diplo) sp.$^b$ (%) $t_R$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>39.1</td>
<td>0.6 F</td>
<td>269 DHQ</td>
<td>269B</td>
<td>268 (8), 226 (5), 202 (100), 134 (10), 96 (10), 91 (22), 67 (40), 65 (10)</td>
<td>9.8 [39.1]</td>
<td></td>
</tr>
<tr>
<td>39.5</td>
<td>1.9 A</td>
<td>271 DHQ</td>
<td>271D</td>
<td>270 (16), 256 (5), 242 (12), 204 (100), 122 (25), 111 (25), 96 (70), 67 (25)</td>
<td>3.4 [39.4]</td>
<td></td>
</tr>
<tr>
<td>39.6</td>
<td>36.8 A</td>
<td>269AB (cis-fused)</td>
<td>269AB</td>
<td>268 (30), 254 (100), 240 (20), 228 (10), 204 (75), 202 (100), 148 (10), 134 (22), 96 (100), 67 (45), 65 (35)</td>
<td>5.8 [39.7]</td>
<td></td>
</tr>
<tr>
<td>39.9</td>
<td>1.6 A</td>
<td>271G (in earlier run)</td>
<td>271G</td>
<td>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)</td>
<td>6.5 [39.1]</td>
<td></td>
</tr>
<tr>
<td>40.5</td>
<td>4.9 A</td>
<td>285/285C (1:1)</td>
<td>285B</td>
<td>284 (10), 268 (10), 256 (5), 242 (10), 228 (15), 220 (15), 202 (10), 190 (25), 162 (12), 160 (12), 96 (100), 91 (32), 67 (18), 65 (15)</td>
<td>5.4 [39.7]</td>
<td></td>
</tr>
<tr>
<td>40.7</td>
<td>0.8 A</td>
<td>287 HTX</td>
<td>287D</td>
<td>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)</td>
<td>5.4 [39.7]</td>
<td></td>
</tr>
<tr>
<td>41.2</td>
<td>9.7 A</td>
<td>285 HTX</td>
<td>285A</td>
<td>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)</td>
<td>5.4 [39.7]</td>
<td></td>
</tr>
<tr>
<td>41.3</td>
<td>6.1 A</td>
<td>287 HTX</td>
<td>287A</td>
<td>287 (12), 286 (10), 272 (25), 220 (25), 202 (15), 162 (40), 176 (25), 148 (30), 134 (40), 122 (42), 109 (78), 96 (100), 91 (55), 67 (42), 65 (12)</td>
<td>5.4 [39.7]</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Extract of a single skin of a male *O. granulifera* (formerly *D. granuliferus*) frog from Rio Grande de Térraba, Palmar Norte, Puntarenas Prov., Costa Rica, June 1990. This is collection #1. $^b$Extract of the ant *Solenopsis* (Diplorhoptrum) sp. from Itabuna, Brazil, 1997 (Jones, Delabie, unpublished data). “The Supporting Information includes TIC GC chromatograms for the *Carebarella* ant and the *O. granulifera* frog with every GC peak identified.
saturated DHQs of MW 279 at \( t_\text{R} \) 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 \( \text{C}_6\text{H}_6 \) (43 amu) from the molecular ion, a process described by Spande et al.,\(^\text{12}\) where the EIMS and IR of \( \text{H}_{10} \) 269AB (trans) were also characterized. Both components lost the fully saturated C-2 side chain, \( \text{C}_6\text{H}_{11}\), by \( \alpha \)-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 283, fitting the EI mass spectrum of a perhydrohistrionicotoxin derivative of the hydrogenation product of the frog extract. 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.\(^\text{12}\) Several new MW 271 DHQs were observed in the present study that exhibit the molecular ion at \( m/z \) 277 and some of these may be dehydro HTX artifacts formed on storage. The mass spectra of the MW 267/269/271 decahydroquinoline complex is discussed in Spande et al., 1999.\(^\text{12}\) Several new MW 271 DHQs were observed in the present study that exhibit the molecular ion at \( m/z \) 277 and some of these may be dehydro HTX artifacts formed on storage.
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 to 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 Carebara 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 ND₂-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 CH₂=CH₂ 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 Carebara 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 Carebara 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 (C₅H₈) moiety being retained at C-5. In the frog, a different isomer is seen at a much greater tₙ and a base peak at m/z 204, indicating a cleavage of a 69 amu fragment from C-2 and a C₅H₈ 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
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 occurrence in this ant of the histriocitoxins 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 Carebaraella 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 x 0.25 mm i.d. column. The GC oven was programmed (data of Tables 1–3) from 60 to 250 °C at 5°C/min and held at this temperature for 20 min. A one skin sample “methylene extract” of the frog O. granulifera (formerly Dendrobates granuliferus) was also run with this program. One analysis of the Carebaraella 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°C/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. granulifer a frog extract from a single male (collection #1) was accomplished with a few milligrams of PtO

### 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.

#### REFERENCES


5. Frost, D. R. http://research.amnh.org/vz/herpetology/amphibia/. An on-line database of the latest taxonomy relevant to this paper.


(14) Daly, J. W.; Hight, R. J.; Myers, C. W. Toxicon 1984, 22, 905–919. A single specimen of this frog was supplied by a commercial dealer and was identified by C. W. Myers at the AMNH, where a voucher is kept. Myers was familiar in 1984 also with M. aurantiaca. A misidentification or the frog being raised outside of Madagascar was proposed by a reviewer to explain this one-time finding of histrionictoxins in a mantellid. A misidentification seems unlikely in view of the expertise of Myers, and the source being a commercial dealer makes it unlikely we will ever know the relevant history of that one specimen. It could well have been raised outside of Madagascar. John Daly in The Alkaloids, Vol. 43, Cordell, G. A., Ed.; Academic Press: New York, 1993; p 206, pointed out that HTXs had never been found as a skin alkaloid in dozens of collections of many mantellid species that we and others had made over the years. The pet trade sample implies however that an uptake mechanism for HTXs does exist in mantellids as a reviewer notes.


(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 might have 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 C19 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 HTXs13 (see text).


(26) The possibility of an enzymatic interconversion of the C19 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.