Full Papers

Occurrence and Significance of Decahydroquinolines from Dendrobatid Poison Frogs and a Myrmicine Ant: Use of ^{1}H and ^{13}C NMR in Their Conformational Analysis†

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Structures for 2,5-disubstituted decahydroquinolines (DHQs) are reported for the two diastereomeric pairs *cis*-**275B** (**14**) and *cis*-**275B′** (**15**) and 5-*epi*-*trans*-**269AB** (**18**) and *trans*-**269AB** (**19**), all isolated from skin extracts of dendrobatid frogs, and for 5-*epi*-*cis*-**275B′** (**16**) and 5-*epi*-*trans*-**275B** (**17**) found in the extracts of virgin queens of a myrmicine ant [*Solenopsis (Diplorhoptrum) azteca*]. Detection of such DHQs in an ant, their first reported occurrence, strengthens a dietary hypothesis for the origin of the approximately 30 DHQs that have been detected in extracts of frog skin. NMR data on the two conformers of *cis*-decahydroquinoline permit assignment of ring conformations and stereochemistry to *cis*-DHQs of the "N-endo" type or the "N-exo" type. These conformations are also assigned on whether H-8a is equatorial or axial as determined with E-COSY or 1D-HOHAHA spectra.

2,5-Disubstituted decahydroquinolines (DHQs) represent one of the major classes of amphibian alkaloids.[1] They have been detected in skin extracts of dendrobatid and mantelline frogs[2−4] and bufonid toads.[5] About 30 alkaloids in skin extracts have been assigned to the DHQ class, based in many cases only on GC−MS and GC−FTIR analyses. A code system was devised to designate the various alkaloids detected in amphibian skin that uses a boldface number corresponding to the nominal molecular weight and a distinguishing boldface letter or letters. In the case of DHQs, both cis and trans ring fusions (positions C-4a and C-8a) have been identified as well as diastereomers at C-2 and C-5, and that is also reflected in our DHQ nomenclature. Pertinent structures are indicated in Figure 1. The absolute configurations of *cis*-**195A** (**1**) and *trans*-**219A** (**3**) have been determined by X-ray crystallography.[2,6] A C-5 diastereomer (**4**) of *trans*-**219A** has been detected and characterized by GC−MS and GC−FTIR (unpublished results). ^{1}H NMR analyses, particularly with phase-sensitive COSY (DQF), E-COSY, and 1D-HOHAHA spectra, have determined cis versus trans ring fusions and equatorial or axial orientations for H-2 and H-5 for several additional DHQs, including *cis*-**211A**[7] (**5**), cis-**219A** (**6**), cis- and *trans*-**243A** (**8** and **9**), and 5-*epi*-*trans*-**243A** (**10**).[2,3] The

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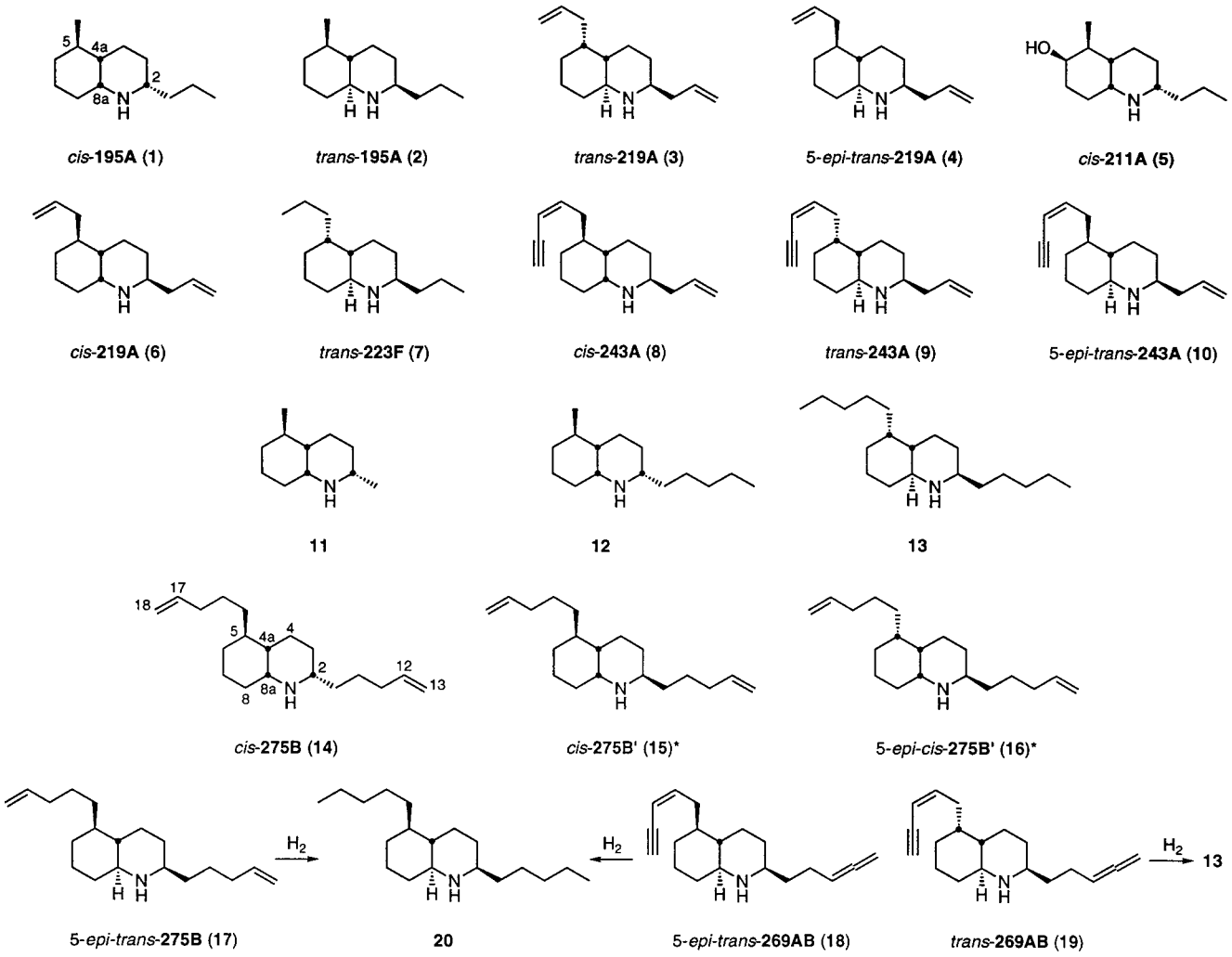


Figure 1. Structures of selected 2,5-disubstituted decahydroquinolines (DHQs) and stereochemical correlations. *The configuration at C-5 is tentative. An alkaloid corresponding to *trans*-**275B** has not yet been detected.

structure of *trans*-**223F** (**7**) was established by comparison with a tetrahydro derivative of **3**.[5] The structure of *trans*-**195A**[8] (**2**) has now been established by comparison with synthetic material.[9] With the exceptions of **1** and **3**, absolute configurations are not known. Those frog DHQs, whose structures have been established, are 13-carbon (**1**, **2, 5**), 15-carbon (**3**, **4**, **6**, **7**), or 17-carbon alkaloids (**8**−**10**). Nineteen-carbon DHQs also occur as discussed below.

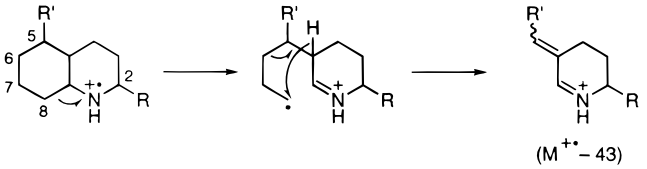
Structures for some DHQs, present as minor components in complex mixtures, have been proposed solely on the basis of GC−MS and GC−FTIR spectroscopy and the following observations: DHQs exhibit a dominant mass spectral fragment ion arising from loss of the C-2 substituent by α-cleavage and a minor fragment from C-5 cleavage. They have an exchangeable hydrogen on nitrogen detectable by chemical ionization MS with ND_3. Bohlmann bands in IR spectra can be used to assign the relative configuration, cis (diaxial) or trans, of H-2 and H-8a,[3,8] while the IR fingerprint region can be used to assign cis or trans ring fusions.[3]

Several such DHQs were 19-carbon alkaloids. One, *cis*-**275B**, found initially in dendrobatid frogs[10] and then detected in a toad of the bufonid genus *Melanophryniscus*, was proposed by IR[5] to be a cis-2,5-disubstituted decahydroquinoline with H-2 and H-8a also cis. Quantities sufficient for NMR analysis have now been isolated, and *cis*-**275B** has been confirmed to be a cis-fused 2,5-disubstituted DHQ (**14**) with H-2 and H-8a cis and diaxial. In other frog skin extracts, both the above *cis*-**275B** (**14**) and an isomeric *cis*-**275B′** (**15**) were detected. Two C_{19} decahydroquinoline alkaloids, diastereomeric with either of the *cis*-**275B** alkaloids from frogs, have now been detected in virgin queens of an ant of the myrmicine genus *Solenopsis* (*Diplorhoptrum*). The major ant alkaloid was a cis-fused DHQ with H-2 and H-8a trans, named 5-*epi*-*cis*-**275B′** (**16**), while the minor diastereomer was trans-fused DHQ with H-2 and H-8a *cis,* named 5-*epi*-*trans*-**275B** (**17**).

Another 19-carbon DHQ, *trans*-**269AB**, whose structure has remained poorly defined for many years,[1,11] has now been isolated also in quantities sufficient for NMR analysis. It proved to be a mixture of two trans ring-fused DHQs, separable by capillary GC only after hydrogenation or N-acetylation. Both had H-2 and H-8a cis. The major diastereomer, hereafter referred to as *trans*-**269AB**, after hydrogenation, proved identical with the synthetic[12,13] 2,5-dipentyl DHQ, **13**, and thus has the structure **19**. The minor diastereomer 5-*epi*-*trans*-**269AB** has the structure **18**.

The present demonstration of two 19-carbon DHQs (**16** and **17**) in an ant suggests a dietary ant source for the many DHQs found in wild-caught dendrobatid frogs but absent in captive-raised frogs.[14−16] Arguments used for the structural assignments for the **275B** and **269AB** series of alkaloids are presented. Hydrogenation correlations are summarized in the lower portion of Figure 1.

Scheme 1. Extrusion of the Radical $C_3H_7^{\bullet}$ from 2,5-Disubstituted DHQs



Results and Discussion

Structures of the 275B DHQs from the Frog *Dendrobates pumilio* Schmidt 1857. Two **275B** decahydroquinolines (~1:1) were detected by GC−MS and GC−FTIR in skin extracts of the dendrobatid frog *D. pumilio* collected near Carbón, Limón Province, Costa Rica, in 1990 and were both characterized as cis ring fused; one isomer (**14**) possessed a *cis* H-2-H-8a configuration and the other (**15**) a trans H-2-H-8a configuration by the IR evidence discussed below. Both isomers showed *m/z* 206 as the base peak in their MS and also exhibited an unexpectedly large loss of 43 amu from the molecular ion, yielding a fragment ion at *m/z* 232 (~25%) that was shown to be a C_3H_7 loss by HRMS. On the basis of the fragmentation pathways reported[17] for *cis*- and *trans*-decahydroquinoline, this loss most probably arises by extrusion of carbons 6, 7, and 8 from the non-nitrogen-containing ring by the mechanism indicated in Scheme 1. CIMS with NH_3 showed an $(M + H)^+$ ion of *m/z* 276, and CIMS with ND_3 showed *m/z* 278, indicating one exchangeable hydrogen. The GC−FTIR spectra of the two components are shown in Figure 2. Both indicate terminal double bonds by the pair of C−H deformation peaks at 913 and 990 cm^{-1}, the C=C stretching frequency at 1640 cm^{-1}, and particularly the vinyl C−H stretching frequency at 3085 cm^{-1}. The H-4a−H-8a cis configuration of the ring junction in both isomers is indicated by broad or double peaks at ~1350 and also at ~1150 cm^{-1}.[3] The significant Bohlmann band at 2803 cm^{-1} in the IR spectrum of the isomer of shorter GC retention time (**14**), characteristic of *cis* but not of trans 2,6-disubstituted piperidines, indicates that H-2 and H-8a are cis, i.e., ring A (N-containing) comprises a cis 2,6-disubstituted piperidine moiety.[8] The absence of any appreciable Bohlmann bands in the isomer of longer GC retention time (**15**), where only a faint shoulder in the 2800 cm^{-1} region is discernible, supports a trans configuration for H-2 and H-8a, i.e., a *trans*-piperidine ring-A moiety.[8] A sample of the DHQ **14** from another Costa Rican population of *D. pumilio* collected by the Río Sarapiqui in Heredia Province in 1989 was purified by a combination of HPLC and silica gel column chromatography. The diastereomer **15** was not detected in this frog population.

The isolated DHQ **14** provided the ^1H NMR and ^{13}C NMR data in Table 1 as well as the MS and FTIR data recorded in the Experimental Section. The ^1H NMR data obtained on *cis*-**275B** (**14**), as the free base, indicated the near identity (average deviation = ±0.08 ppm; maximum deviation is seen at H-2, 0.29 ppm) of most of the ring hydrogens with those of *cis*-**195A** (**1**) for which the conformation has been proved[3] by the 1D-HOHAHA spectrum. Thus, the conformation of *cis*-**275B** (**14**) is as shown in Figure 3. This conformation results in energetically favorable equatorial orientations for both C-2 and C-5 substituents. Interestingly, the signals for H-14/H-14′ and H-15/H-15′ showed significant separations, while H-9/H-9′ and H-10/H-10′ had virtually no separation. The signal for H-8a in the DCl salt of **14** was seen as a broad doublet at δ 3.33 (J = 9.2 Hz) significantly downfield shifted from its position in the free base. Irradiation of the broad singlet at δ 8.40, assigned to the NH proton, collapsed this doublet to a broad singlet, indicating the origin of the large coupling to be that between H-8a and NH. No large J value would be expected with either H-8 proton for the conformation depicted in Figure 3, and indeed, none was observed. The ^1H NMR signal of H-4a, seen as a doublet of broadened

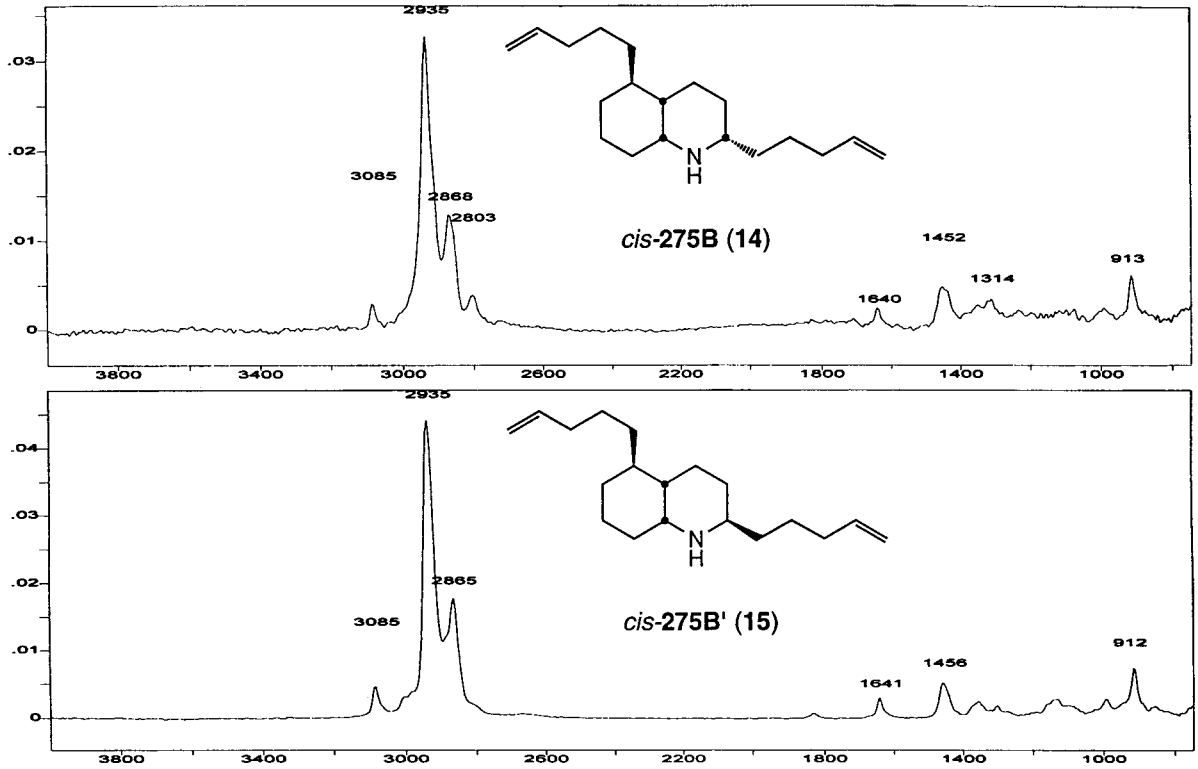
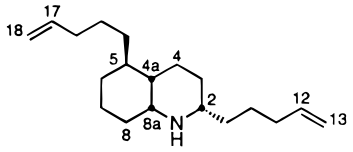


Figure 2. GC−FTIR spectra of DHQs *cis*-**275B** (**14**) and *cis*-**275B′** (**15**) from the frog *Dendrobates pumilio*. Spectra are plotted (*y*,*x*) as absorbance vs wavelength (cm^{-1}).

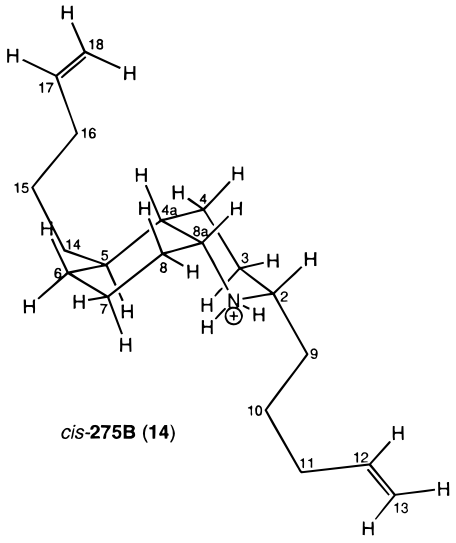
Table 1. ^{13}C and ^{1}H NMR Spectral Data for *cis*-**275B** (**14**) and *cis*-**275B**·DCl[a]



cis-**275B** (**14**)

carbon	δ_C [b, c]	δ_H	C−H coupling[d]	H−H coupling[e]	**14**·DCl δ_H, m[c], (*J*, Hz)
2	58.0 (d)	2.25	3, 3′, 4	3′, 9, 9′	2.98 q (13.6, ≈9.6)
3	27.4 (t)	1.36; 1.06	4′	3′-2; 3′-4; 3′-4′	2.1; 1.8
4	26.8 (t)	1.92; 1.28	3, 3′	4-3′; 4′-3′	2.26; 1.60
4a	40.3 (d)	1.17	3, 4′	5, 8a	2.43 d (14.3)
5	31.7 (d)	1.75	4, 6′, 14, 14′	4a, 14, 14′	1.58
6	32.1 (t)	1.73; 0.96	7′, 14	6-7; 6′-7; 6′-7′	0.95 qd (≈13; 1.5); 1.95
7	21.2 (t)	1.57; 1.39	6′, 8′	7-6; 7-6′; 7-8′; 7′-6′	2.35; 1.95;
8	33.3 (t)	1.51; 1.48		8-8a; 8′-7; 8′-8a	1.63, 1.52
8a	56.3 (d)	2.83	4, 4′, 8′	4a, 8, 8′	3.33 d (10.9)
9	36.9 (t)	1.36; 1.32	10, 11	9-2; 9′-2	ca. 1.50 for both
10	25.4 (t)	1.38	9, 9′, 11, 12	10-11	1.32
11	34.0 (t)	2.00	10	11-10; 11-12; 11-13	2.04
12	138.9 (d)	5.76	11	12-11; 12-13	5.73[f] dddd (17.2, 10.3, 6.6)
13	114.4 (t)	4.96	11	13-11; 13-12	5.00[f] dd (17.2, 1.8); 4.95 dd (10.3, 1.5)
14	32.7 (t)	1.42; 0.98	15, 16	14-5; 14′-5; 14′-15′	1.36
15	25.4 (t)	1.38; 1.25	14, 14′, 16, 17	15-16; 15′-14′	1.32
16	34.3 (t)	2.00	14′, 15, 17, 18	16-15; 16-17; 16-18	2.04
17	139.3 (d)	5.76	16	17-16; 17-18	5.84[f] dddd (17.2, 10.3, 6.6)
18	114.1 (t)	4.89	16	18-16; 18-17	5.00[f] dd (17.2, 1.8); 4.93 dd (9.9, 1.5)

[a] ^{1}H and ^{13}C NMR, phase-sensitive 2D-NMR (H,H-COSY(DQF) and C,H-COSY), HMQC, and HMBC spectra were obtained on the free base **14** in $CDCl_3$ at 400 MHz with a GX400 spectrometer. ^{1}H NMR and H−H COSY spectra were obtained on the deuteriochloride at 300 and 500 MHz (normal and phase sensitive). Chemical shifts (δ) are relative to TMS at 0.00 ppm. [b] ^{13}C NMR spectra on **14** were obtained in $CDCl_3$ at 100 MHz and reported in ppm relative to $CDCl_3$ at 77.0 ppm. [c] Multiplicity of ^{13}C signals in parentheses: d = doublet; t = triplet. Same for proton multiplicity, also with q = quartet, m = multiplet. [d] Long-range C−H coupling. Primed hydrogen signals (e.g., 6′) are upfield of the unprimed (e.g., 6) and have no stereochemical significance. [e] Geminal couplings are omitted. [f] Vinyl hydrogen assignments may be interchanged. J_{vic} = 6.6 Hz, J_{cis} = 10.3 Hz, J_{trans} = 17.2 Hz for H-12 or H-17. J_{vic} = 1.5 or 1.8 Hz for H-13a/b or H-18a/b.



cis-**275B** (**14**)

Figure 3. Conformation of DHQ *cis*-**275B** (**14**). The program Chem3D for the Macintosh computer generated this energy-minimized structure. This conformation is also adopted by *cis*-**195A** (**1**).

peaks at δ 2.45 (J = 14.3 Hz), indicates that there is one large coupling, most likely J_{4a-5} (dihedral angle ca. 180°), since H-4a and H-8a are in a cis relationship and the dihedral angle between H-4a and each one of the H-4 hydrogens is likely to be ca. 60°. The signal for H-2, also shifted downfield from its position in the free base, was observed as a quartet at δ 2.98 ($J \approx 9$ Hz) and originated from three large couplings, one with the NH at δ 8.40 (the same NH irradiation that collapsed H-8a, changed the H-2 quartet signal to a triplet), one with H-9 at δ 1.80, and one with the H-3 proton at δ 1.54. Decoupling of H-2 revealed the other H-3 at δ 1.60. Irradiation of H-8a showed coupling with the two H-8 protons at δ 1.74 and 1.60. A COSY ^{1}H NMR spectrum of **14**·DCl showed a weak cross-peak between H-8a and H-4a and a strong cross-peak between H-8a and one of the H-8 protons, that at δ 1.60. This behavior was reversed for the free base; i.e., a stronger cross-peak was observed between H-8a and H-4a than H-8a and one of the two H-8 protons. Other signals, such as those for H-12, H-13, H-17, and H-18, in either the free base or deuteriochloride had chemical shifts and coupling constants consistent with terminal double-bond hydrogens. The ^{13}C NMR spectrum of **14** showed (Table 1) all of the 19 carbons expected; long-range C−H couplings for C-8 were not detected. A phase-sensitive COSY (DQF) ^{1}H NMR spectrum of the free base revealed a large J value between H-2 and H-3_{ax} and a small J value between H-2 and H-3_{eq}, further supporting an equatorial substituent at C-2. A strong cross-peak was also observed with a large J value between H-5 and H-4a, supporting a trans diaxial orientation of those hydrogens and consequently an equatorial orientation of the C-5 side chain. Unfortunately, H-5 overlapped one of the H-6 signals, and the strong H-6−H-6′ cross-peak consequently obscured the cross-peak between H-5 and the upfield axially oriented H-6′. The phase-sensitive COSY (DQF) cross-peaks for H-8a/H-8 and H-8a/H-8′ provided the clearest indication of the axial or equatorial orientation of proton H-8a and, hence, the predominant conformation adopted by the particular *cis*-DHQ. In the case of *cis*-**275B**, proton H-8a showed weak cross-peaks with small Js for coupling with H-4a, H-8, and H-8′ and is thus consistent with the dihedral angles and conformation ("N-endo") depicted in Figure 3. The DHQs **1** and **6** and all of the other *cis*-DHQs with four assigned

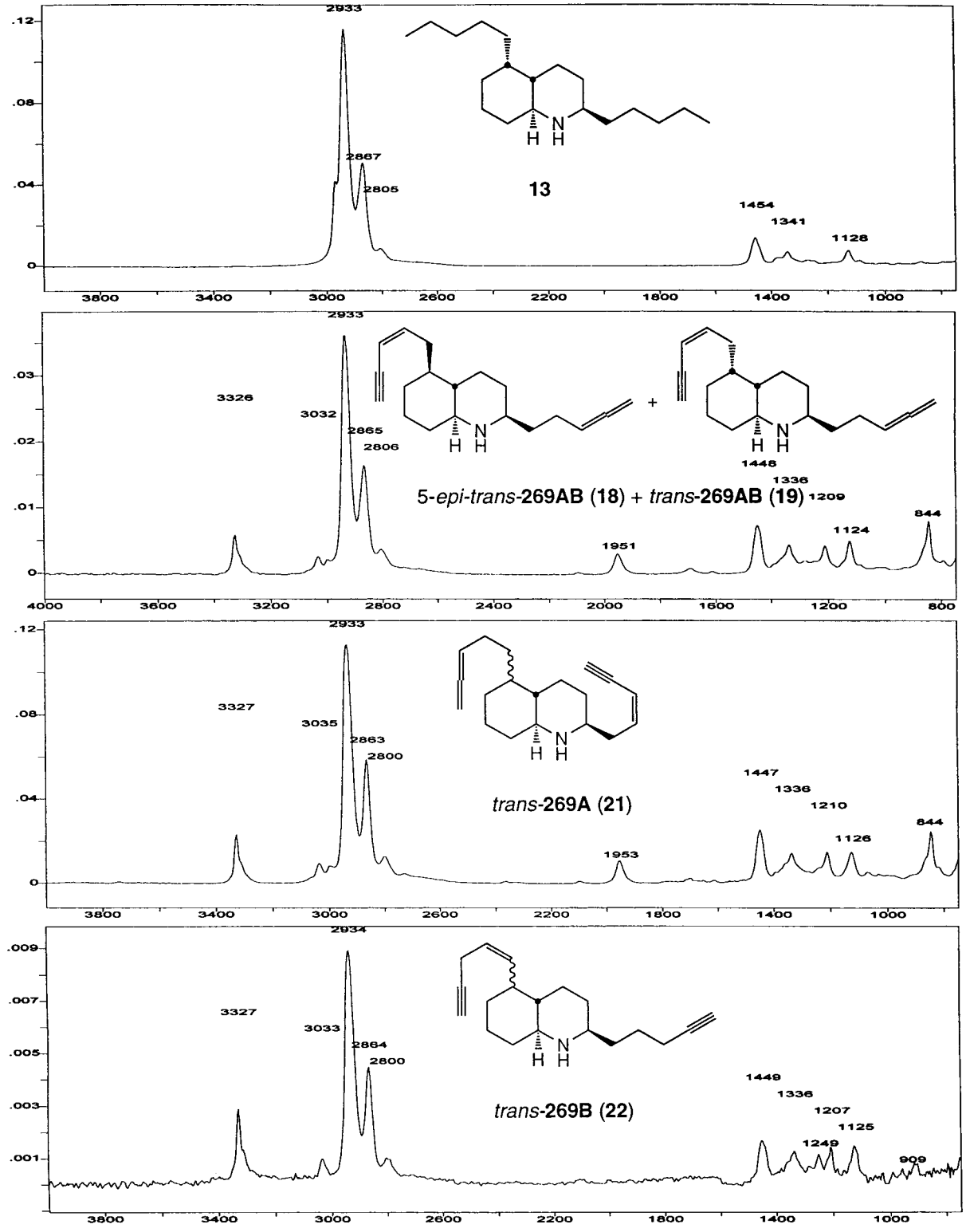
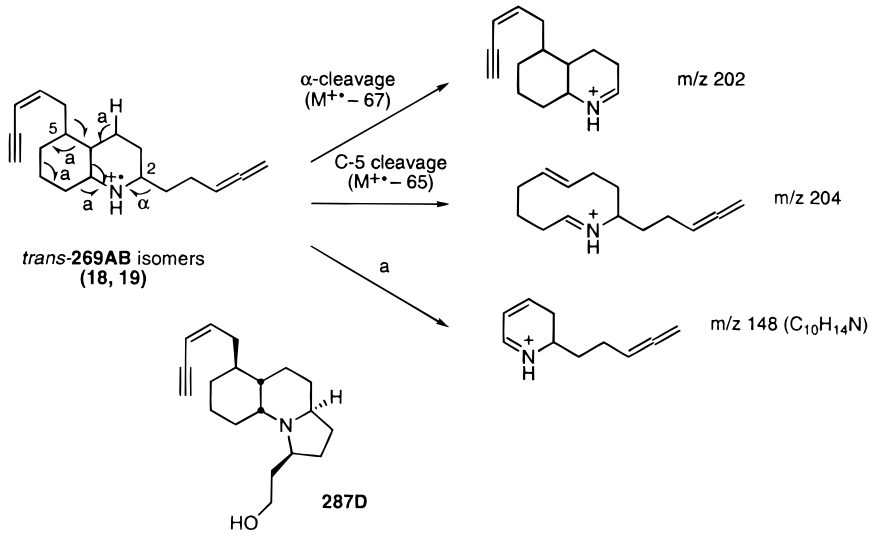


Figure 4. GC−FTIR spectra of 2,5-dipentyl DHQ (**13**), *trans*-**269AB** DHQs (**18/19**), *trans*-**269A** (**21**), and *trans*-**269B** (**22**). Spectra are plotted (*y*,*x*) as absorbance vs wavelength (cm^{-1}).

stereocenters[3] (Figure 1) have H-5 and H-8a in an *E* relationship, adding additional support to the C-5 configuration assigned by ^{1}H NMR to **14**.

The two *cis*-**275B** isomers in a sample of the original extract were hydrogenated to two perhydro isomers of molecular weight 279, each giving in the MS a base peak at *m*/*z* 208 and exhibiting a fairly significant loss of 43 amu from the parent molecular ion to yield a fragment ion at *m*/*z* 236 (∼10−20% of the intensity of the base peak). Synthetic, saturated DHQs supplied by Overman,[18,19] namely, 2,5-dimethyl-DHQ (**11**) and 2-pentyl-5-methyl-DHQ (**12**), both with cis ring fusions and *cis*-piperidine A rings, as well as a 2,5-dipentyl-DHQ (**13**) synthesized by Schultz and co-workers[12,13] (FTIR top spectrum, see Figure 4), having a trans ring fusion and a *cis*-piperidine A ring, showed similar losses of propyl fragments from their molecular ions (see Table 9 for intensities of $M^{\bullet+} - 43$ fragments). These results, while not ruling out any

Scheme 2. Proposed Mass Spectral Fragmentations for the Frog DHQs 5-*epi*-*trans*-**269AB** (**18**) and *trans*-**269AB** (**19**)



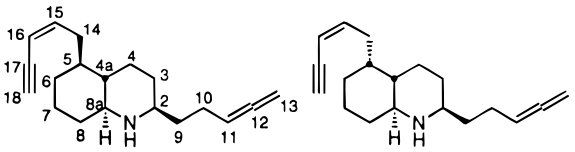
participation of the side chains in producing the 43 amu loss, indicate that it is unlikely and that the propyl loss occurs with trans-fused as well as cis-fused DHQs, agreeing with results reported for *cis*- and *trans*-decahydroquinoline.[17]

Structures of *trans*-269AB DHQs from the Frog *D. pumilio* Schmidt 1857. The extract from the Río Sarapiqui population of *D. pumilio* also provided, by HPLC purification, an apparently homogeneous sample of a 2-(3,4-pentadienyl)-5-(2-penten-4-ynyl)-DHQ, an alkaloid seen frequently accompanying alkaloids of the histrionicotoxin class in dendrobatid skin extracts and referred to[10] as **269AB**. The MS displayed a pair of prominent characteristic fragment ions at *m*/*z* 202 and 204. A weak fragment ion at *m*/*z* 226 ($M^{\cdot+} - 43$) was also seen. CIMS with ND_3 gave an $(M + D)^+$ ion of *m*/*z* 272, indicating one exchangeable hydrogen. A tentative DHQ structure for this alkaloid was first proposed[20] in 1977, but it was then suggested[21] that it could perhaps be a mixture of two inseparable isomeric DHQs, one (**269A**) having an α-cleavage loss of a 65 amu fragment and the other (**269B**) having an α-cleavage loss of a 67 amu fragment to give the twin fragment ions observed for **269AB** at *m*/*z* 204 and 202, respectively. However, further investigation indicated that a single DHQ could give both fragment ions, and the designation *trans*-**269AB** for this alkaloid was introduced, which also conveyed the ring-fusion configuration.[1] The originally hypothetical codes **269A** and **269B** are actually now in use for two other DHQs discussed below.

Recent work with 5 °C/min programs on several different 30 m capillary GC columns also suggested that the HPLC-purified material was a single compound by the criteria of identical FTIR and MS as the GC peak was examined throughout the peak scan by scan. However, one GC column (see the Experimental Section) did partially separate the sample of *trans*-**269AB** into a leading shoulder and a main peak whose mass spectra were virtually identical so far as nearly all fragments were concerned, including the ratio of *m*/*z* 202/204 ions, but differed significantly in the intensity of an *m*/*z* 148 ion (see Scheme 2 for a possible fragmentation to this $C_{10}H_{14}N$ ion). The heterogeneity of the *trans*-**269AB** sample was more clearly demonstrated when it was observed that the *N*-acetyl derivative showed two baseline-separated peaks of identical mass spectra [*m*/*z* 311 ($M^{\cdot+}$), 204 (30), 202 (100)] in a 1:3 ratio on GC−MS. Here, an initial acetyl fragmentation ($M^{\cdot+} - 42$) competes with α-cleavage, and thus, α-cleavage from the molecular ion was minor (see Table 9: $M^{\cdot+} - 67 \rightarrow$ *m*/*z* 244). The FTIR spectrum of the *trans*-**269AB** sample (Figure 4) indicated a trans ring fusion with a *cis*-piperidine A ring. The failure to completely separate by HPLC or GC the *trans*-**269AB** diastereomers (deduced to be epimeric at C-5) was unexpected. It should be noted that the two 17-carbon DHQ C-5 epimers, *trans*-**243A** (**9**) and 5-*epi*-*trans*-**243A** (**10**), are separable by either technique.[3]

The 1D and 2D ^1H NMR spectra had also suggested the possibility that two stereoisomers were present, most probably diastereomers at C-5 (e.g., H-16, in the C-5 enyne side chain appeared as overlapping doublets at δ 5.58 and 5.56 ($J = 10.8$ Hz; $J = 10.9$ Hz) in a 1:3 ratio, respectively). The ^1H NMR data for *trans*-**269AB** (a nonseparable 1:3 mixture of C-5 diastereomers **18** and **19**, respectively) are summarized in Table 2. Table 3 reports ^{13}C NMR data for this sample showing more clearly a minor and a major component with the minor one matching very closely the ^{13}C NMR chemical shifts (data[3] not shown) of 5-*epi*-*trans*-**243A** (**10**) and the major one correlating well with data of *trans*-**243A** (**9**). The NMR data was entirely consistent with a pair of C-5 epimers of *trans*-**269AB** being present and sharing the same configuration at the other three chiral centers as found in the two *trans*-**243A** diastereomers previously studied.[3] The H-14 allylic hydrogens of the enyne side chain were coupled with H-5. H-2 was coupled with more upfield nonallylic hydrogens supporting the side-chain structures inferred from MS data and ruling out an interchange of the side chains as a possible difference between the two isomers. The allene and *cis*-enyne groups, inferred from the IR spectrum, were confirmed. The signal for H-8a at δ 3.23, which overlaps that of H-2, was seen to be a triplet of doublets signal ($J = 11.0$, 3.0 Hz, i.e., two large *J*s and one small *J*), indicating a trans ring fusion. Unfortunately, no clear evidence on the orientation of either the C-2 or C-5 side chain could be discerned from the ^1H NMR spectrum. To assign these, we relied upon IR analysis and the stereochemical correlations. Thus, catalytic hydrogenation of the *trans*-**269AB** sample produced two closely related perhydro compounds of molecular weight 279 (~1:3), both with the same relative stereochemistry at C-2, C-4a, and C-8a as indicated by virtually identical IR spectra. The major isomer was shown to be identical to the synthetic material **13** prepared by Schultz and co-workers[12,13] by its GC retention time, FTIR, and MS.

Table 2. Selected ^{1}H NMR Spectral Data for *trans*-**269AB** (**18**, **19**)



5-*epi*-*trans*-**269AB** (**18**) *trans*-**269AB** (**19**)

carbon	δ_H in D_2O (J, Hz)[a]	δ_H in $CDCl_3$ (J, Hz)[b]	δ_H in D_2O-CD_3OD[c]	H−H couplings (J, Hz)[c]	decoupling (irradiation at $\delta_H \rightarrow$ changes)[c]
2	3.20 m		3.24		1.9 (H-9); 1.82, 1.50 (H-3)
3			1.46_{ax}; 1.70_{eq}		
4			1.77_{ax}; 2.2_{eq}		
4a			1.46		d (7.1) for H-14; t (11) for H-8a, changes at 1.5, 1.7, 1.8
5	1.98		2.08		dd for H-14 (7.1, <1)
6			1.77_{ax}; 2.2_{eq}		
7			1.46_{eq}; 1.70_{ax}		
8			1.46_{ax}; 2.08_{eq}		irr. H-$8_{eq}\rightarrow$t for H-8a changes at 2.1, 1.8, 1.6, 1.4
8a	3.23 td (11, 3)		3.20	td (*ca*. 12, *ca*. 4)	changes at 2.12 (H-8_{eq}), H-8_{ax} 1.50 (H-4a)
9			1.77; 1.86		
10	2.09 m	2.0 m	2.2 m		dd (J = 7.0, <1) for H-13; 1.86 (H-9); 1.70 (H-9); t for H-11
11	5.19 (5 lines, 6.7)	5.06, 5-lines	5.29	5-lines, 6.6	br s for H-13
13	4.70 (overlaps HOD)	4.70 dd (6.1, *ca*. 1)	4.95	5-lines (overlap. dd; 6.6, 3.3)	t for H-11; 2.20 (H-10)
14	2.37 m	2.35 m	2.48	td (7.4, <1)	d for H-15; sharper d for H-16 change at 2.08 (H-5)
15	6.10 dt (10.3, 8.2)	5.91 m	6.20	dt (10.6, *ca*. 7)	s for H-16; d for H-14
16	5.58 d (10.8) (**18**)	5.53 d (10.5)	5.65 (**19**)	d (10.6)	t for H-15; H-14 → t (7.4) since small J removed from H-14
	5.56 d (10.9) (**19**)		5.68 (**18**)	d (*ca*. 10)	
18	3.71	3.11 d (1.95) (**18**) 3.13 d (1.95) (**19**)	3.81		no changes detected

[a] Assignments for **18**/**19**·DCl in ppm are relative to HOD at 4.72 ppm. [b] Assignments for the free base of **18**/**19** in ppm are relative to TMS at 0.00 ppm. H-16 but not H-15 is coupled with H-18. A spectrum in C_6D_6 failed to separate protons of the downfield region. [c] ^{1}H NMR spectra and H−H COSY (relay = 0 and 1) spectra were obtained for **18**/**19** DCl in D_2O-CD_3OD (3:1) at 500 MHz. Minor peaks due to **18** are usually omitted. Abbreviations: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, m = multiplet.

Table 3. ^{13}C NMR Spectral Data for *trans*-**269AB** (**18**, **19**)[a]

carbon	δ_C **18** (minor)	δ_C **19** (major)
2	56.9	56.3
3	32.0	32.9
4	27.9	29.0
4a	46.2	45.5
5	41.2	37.9
6	29.7	29.5
7	24.8[b]	19.7
8	33.4	34.1
8a	61.6	55.7
9	36.2	36.2
10	24.6[b]	24.6
11	89.7	89.7
12	208.6	208.6
13	74.9	74.9
14	33.4	27.9
15	144.4	146.1
16	109.2	108.7
17	80.7	80.7
18	81.3	81.3

[a] Chemical shifts in ppm (δ) in $CDCl_3$ relative to internal $CDCl_3$ (77.2 ppm). Spectra obtained at 100 MHz. [b] May be interchanged.

The minor hydrogenation product must then have structure **20** (see Figure 1).

Interestingly and possibly significant in assigning the C-5 configurations of some DHQs is the observation that **20** showed a very weak loss of 43 amu from the parent molecular ion, while the major hydrogenation product **13** showed a minor but significant loss. The fact that the 43 amu loss is structure dependent may prove useful in assigning configurations to C-5 and other DHQ stereocenters; however, at this time we do not understand the structural parameters involved.

Scheme 2 rationalizes the prominent allylic cleavage of the C-5-enyne side chain that must compete with the facile α-cleavage from C-2. Such allylic cleavage is also seen in the tricyclic gephyrotoxin **287D** (see Scheme 2) where a significant *m*/*z* 222 (45%) fragment results from cleavage of a pent-2-en-4-yne side chain from a ring position equivalent to C-5 in DHQs.[1]

Other 269AB-Series DHQs from the Frog *Dendrobates histrionicus* Bertholdt 1846. Many *D. histrionicus* extracts have only the one peak corresponding to the *trans*-**269AB**s (**18** and **19**), yet after acetylation varying mixtures (e.g., 1:3−1:1) of the separable *N*-acetyl derivatives of **18** and **19**, respectively, were obtained; some extracts, however, gave only the *N*-acetyl derivative of **19** (see Table 4). In some dendrobatid extracts, other isomers accompany the major **18**/**19** peak. In 1987, using a 25 m OV-17 capillary column and a 5 °C/min program, at least five GC−MS peaks with molecular ions of *m*/*z* 269 were detected with varying ratios of *m*/*z* 202 and 204 fragments from skin extracts of *D. histrionicus* collected near Guayacana, Nariño Department, Colombia, in l971 and l974; only three isomers of the five were shared between the 1971 and 1974 extracts. Hydrogenation of the former extract gave on GC−MS three well-separated perhydro compounds of molecular weight 279, having base peaks at *m*/*z* 208.

Table 4. Occurrence of Decahydroquinolines (DHQs) in Skin Extracts of Dendrobatid Frogs[a]

DHQ	formula	mass fragment ions	FTIR	NMR	occurrence
13-carbon					
cis-**195A**	$C_{13}H_{25}N$	**152** > 109	+	+	Relatively common: *D. auratus, D. granuliferus, D. histrionicus, D. imitator, D. pumilio, D. speciosus, E. bassleri, M. minutus, P. lugubris* (9/50). Also in *Mantella*[4]
trans-**195A (2)**	"$C_{13}H_{25}N$"	**152**	+		Rare: *E. bassleri* (1/50)
cis-**211A (5)**	$C_{13}H_{25}NO$	**168**, 152	+	+	Uncommon. Found only with *cis*-**195A**: *D. auratus, D. pumilio, P. lugubris* (3/50)
15-carbon					
cis-**219A (6)**	$C_{15}H_{25}N$	**178**	+	+	**219A**-series. Common. Both cis and trans occur:
trans-**219A (3)**	$C_{15}H_{25}N$	**178**	+	+	*D. auratus, D.azureus, D. granuliferus, D. histrionicus*
5-*epi*-*trans*-**219A (4)**	"$C_{15}H_{25}N$"	**178**	+		*D. imitator, D. pumilio, D. tinctorius, D. truncatus, D. ventrimaculatus, E. bassleri, E. cainarachi, E. macero, E. parvulus, E. pictus, E. trivittatus, M.* new species, *P. aurotaenia, P. bicolor, P. vittatus* (20/50)
cis-**223F**	$C_{15}H_{29}N$	**180**	+		**223F**-series. Uncommon. Usually the *trans* and found only with
trans-**223F (7)**	$C_{15}H_{29}N$	**180**	+	+	**219A**s in dendrobatids: *D. granuliferus, D. pumilio, E. bassleri, E. macero, E. pictus, P. vittatus* (6/50). Also in *Melanophryniscus*[5]
trans-**253D**	$C_{15}H_{27}NO_2$	**212**	+	+	Rare: *D. pumilio* (1/50)
17-carbon					
cis-**243A (8)**	$C_{17}H_{25}N$	**202**	+	+	**243A**-series. Common. Often both cis and trans, usually
trans-**243A (9)**	$C_{17}H_{25}N$	**202**	+	+	with **219A**s: *D. auratus, D. azureus, D. granuliferus,*
5-*epi*-*trans*-**243A (10)**	$C_{17}H_{25}N$	**202**	+	+	*D. histrionicus, D. imitator, D. pumilio, D. reticulatus, D. tinctorius, D. truncatus, D. ventrimaculatus, E. bassleri, E. cainarachi, E. macero, E. trivittatus, M. bombetes, P. aurotaenia, P. bicolor* (20/50)
cis-**245E**	$C_{17}H_{27}N$	**202**, 180	+		Rare: *D. histrionicus* (1/50)
cis-**249D**	"$C_{17}H_{31}N$"	**206** > 180	+		Rare: Only in *Melanophryniscus*[5]
trans-**249D**	"$C_{17}H_{31}N$"	**206** > 180	+		Rare: Only in *Melanophryniscus*[5]
trans-**249E**	"$C_{17}H_{31}N$"	206, **180**	+		Rare: Only in *Melanophryniscus*[5]
19-carbon					
cis-**267L (23)**	"$C_{19}H_{25}N$"	**202**	+		Uncommon: *D. auratus, D. fantasticus, D. histrionicus, D. pumilio* (4/50)
cis-**269AB (24)**	"$C_{19}H_{27}N$"	**204**, **202**	+		**269AB**-series. Common. Usually *trans* or 5-*epi*-*trans*:
trans-**269AB (19)**	$C_{19}H_{27}N$	**204**, **202**	+	+	*D. auratus, D. fantasticus, D. granuliferus,*
5-*epi*-*trans*-**269AB (18)**	$C_{19}H_{27}N$	**204**, **202**	+	+	*D. histrionicus, D. imitator, D. occultator, D. pumilio, D. truncatus, D. variabilis, E. bassleri, E. cainarachi, E. macero, E. pictus, P. aurotaenia, P. bicolor, P. lugubris, P. vittatus* (17/50)
trans-**269A (21)**	"$C_{19}H_{27}N$"	**204** > 202			Uncommon: *D. auratus, D. fantasticus, D. granuliferus, D. histrionicus, D. pumilio* (5/50)
trans-**269B (22)**	"$C_{19}H_{27}N$"	**202** > 204	+		Uncommon: *D. auratus, D. granuliferus, D. histrionicus, D. pumilio, E. bassleri* (5/50)
cis-**271D (25)**	"$C_{19}H_{29}N$"	**204** > 228	+		**271D**-series. Uncommon: *D. granuliferus, D. histrionicus,*
trans-**271D (26)**	"$C_{19}H_{29}N$"	**204** > 228	+		*D. pumilio* (3/50)
iso-5-*epi*-*trans*-**271D (27)**	"$C_{19}H_{29}N$"	**204** > 228	+		
cis-**275B (14)**	$C_{19}H_{33}N$	**206** > 232	+	+	**275B**-series. Uncommon: *D. auratus, D. granuliferus,*
cis-**275B (15)**	$C_{19}H_{33}N$	**206** > 232	+		*D. pumilio* (3/50). Also in *Melanophryniscus*[5]
		Tentative DHQs			
13-carbon					
209A	"$C_{13}H_{23}NO$"	**168**			Rare: *D. new species* (1/50)
211K	"$C_{13}H_{25}NO$"	**168** > 196	+		Rare: *D. pumilio* (1/50)
14-carbon					
209J	"$C_{14}H_{27}N$"	**166**	+		Rare: *D. imitator, E. bassleri* (2/50)
15-carbon					
219C	$C_{15}H_{25}N$	**152**			Rare: *D. pumilio* (1/50)
219D	$C_{15}H_{25}N$	**180**			Rare: *D. new species* (1/50)
221C	$C_{15}H_{27}N$	**152**			Rare: *D. pumilio* (1/50)
221D	$C_{15}H_{27}N$	**180**			Rare: *D. pumilio* (1/50)
cis-**223Q**	"$C_{15}H_{29}N$"	208, 180, **152**	+		Rare: *D. pumilio* (1/50)
17-carbon					
251A	"$C_{17}H_{33}N$"	**152** > 208			Rare: *D. histrionicus* (1/50)

[a] All these alkaloids appear to be bicyclic and to have an exchangeable hydrogen on nitrogen. Significant mass spectra fragment ions are reported as follows: The base peak and peaks >80% of the intensity of the base peak are boldface, while peaks 25−80% of the base peak are in regular font, and diagnostic peaks less than 25% of the base peak are indicated following the > sign. Empirical formulas within quotes have not been confirmed by HRMS. FTIR data are diagnostic for stereochemistry, while NMR permits more rigorous definition of structures (see the text). Certain alkaloids, in particular the **219A**, **223F**, **243A**, **269AB**, **271D**, and **275B** series, have not always been analyzed in extracts by FTIR spectroscopy, and their occurrence in *Dendrobates* (*D.*), *Epipedobates* (*E.*), *Minyobates* (*M.*) and *Phyllobates* (*P.*), therefore, is tabulated by the series rather than by the individual diastereomers. Fifty dendrobatid frogs species have been examined, and the fraction of total species containing each DHQ or DHQ series is indicated by (*n*/50).

This establishes that at least two stereocenters differ among these five **269AB**-type isomers. Accompanying these **269AB** isomers were minor amounts of related congeners of molecular weight 267 and 271 and various histrionicotoxins (see below), making even GC−MS analysis difficult. Larger amounts of these materials, enough for analysis by GC−FTIR, would be required to define ring junctions and the stereochemistry at C-2 before tentative

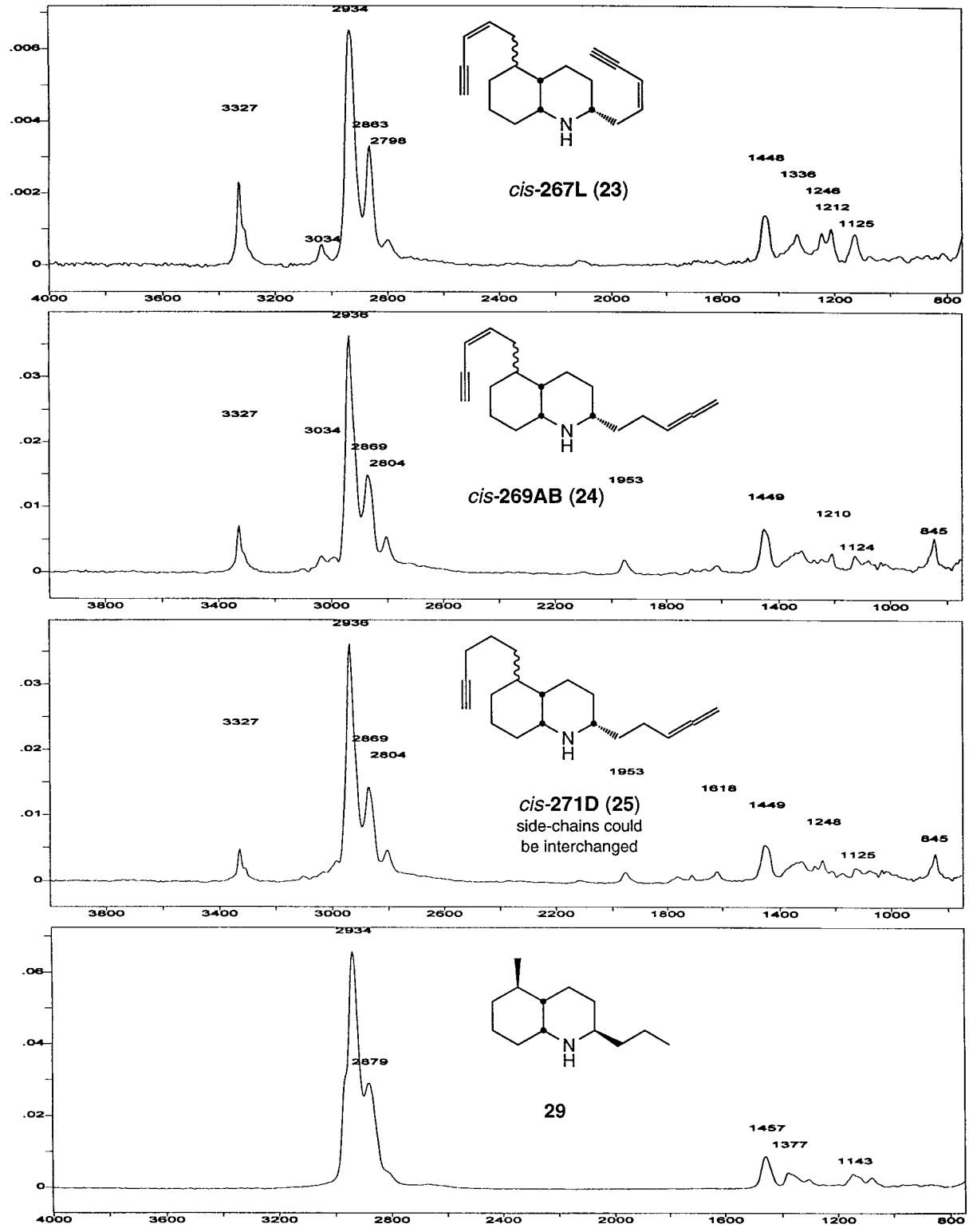


Figure 5. GC−FTIR spectra of *cis*-**267L (23)**, *cis*-**269AB** (**24**), *cis*-**271D** (**25**), and the 2-epimer of *cis*-**195A** (**29**). Spectra are plotted (*y*,*x*) as absorbance vs wavelength (cm^{-1}).

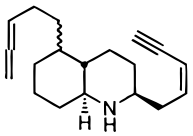
structures can be proposed. Another skin extract from *D. histrionicus* collected at the Quebrada Docordo in the Río San Juan drainage, Choco Department, Colombia, in 1983 had the GC−MS peak corresponding to the **18**/**19** mixture, as well as two peaks for additional minor isomers, one of which was not present in the extracts from *D. histrionicus* collected near Guayacana. GC−FTIR spectra confirmed that the major isomer had a trans ring fusion and a *cis*-piperidine ring-A moiety (i.e., a spectrum virtually identical to that of **18/19**); one of the minor isomers for which an FTIR spectrum was possible had a trans ring fusion and what appeared to be a *trans*-piperidine ring-A configuration. The relative configuration of the latter DHQ will have to be confirmed by other methods, as it is unprecedented in frog skin DHQs.

In the **269AB** series it was noted that the *m*/*z* 202/204 ratios were often inversely related to the ratios of *m*/*z* 65 and 67 hydrocarbon fragment ions; i.e., a larger 65/67 ratio was reflected in a smaller *m*/*z* 202/204 ratio. It was concluded that one 67 amu side chain at C-2, either 3,4-

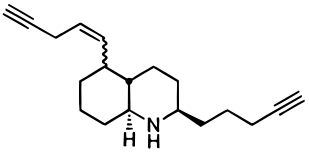
pentadienyl, 2,4-pentadienyl, or 4-pentynyl, in combination with a pent-2-en-4-ynyl (65 amu) side chain at C-5 of the DHQ nucleus, could account for the observed fragment ions.

Structure of a 269A DHQ from the Frog *Dendrobates auratus* Girard 1855. A DHQ with molecular weight 269 and a *m/z* 204 peak greatly dominating that at *m/z* 202 was considered to be of the **269A** type.[1] Such an alkaloid presumably would have a pentenynyl side chain at C-2 rather than C-5, augmenting the facile α-cleavage by producing an allylic radical, and consequently, the competing cleavage at C-5 is minor. Decahydroquinolines of the **269A** type have rarely been detected,[10] although a recent example is provided in an extract from *D. auratus* collected at Río Sand Box, Limón Province, Costa Rica, in 1990, which showed a major GC−MS peak with ion trap fragment ions *m/z* 204 ≫ *m/z* 202. A significant (10%) fragment ion at *m/z* 226 corresponds to the loss of 43 amu. The GC−FTIR spectrum (see Figure 4) indicated a trans ring-fused DHQ with a *cis*-piperidine A ring and the enyne and allene moieties analogous to **18/19**. DHQ **269A** must be an isomer (**21**) of **18/19** with the side chains interchanged.

Structure of a 269B DHQ from the Frog *D. auratus* Girard 1855. Accompanying the *trans*-**269AB** GC−MS peak in some dendrobatid extracts were isomeric DHQs that showed base peaks at *m/z* 202 and very weak *m/z* 204 peaks. When the *m/z* 202 peak greatly dominates the *m/z* 204 peak (∼9:1), the DHQ was considered to be of the **269B** type.[1] At least one of the isomers from the extract of *D. histrionicus* collected near Guayacana (see above) was of the **269B** type; i.e., cleavage at C-5 is not significant (see Scheme 2). A representative of this type was present in skin extracts from a *D. auratus* population collected on Isla Pastores, Bocas Province, Panama, in 1983.



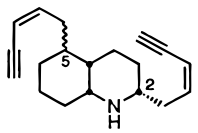
trans-**269A (21)**



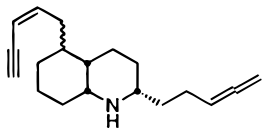
trans-**269B (22)**

The FTIR spectrum (Figure 4) showing a trans ring fusion and a *cis*-piperidine A ring, the absence of any allenic group (e.g., no 1952 and 845 cm^{-1} absorptions), but instead enhanced acetylenic absorption (3328 cm^{-1}) and an internal *Z*-double bond (3033 cm^{-1}, no 964−968 cm^{-1}), suggested structure **22** for this particular **269B**-type isomer, one of several that exist. In the case of **22**, only α-cleavage is expected to be significant. At least two other minor isomers accompanied **22** in the extract of *D. auratus* but were present in amounts insufficient for FTIR spectra.

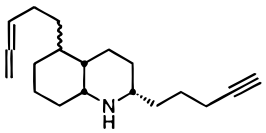
The 267L and 271D DHQs. Nineteen-carbon DHQs with molecular weights of 267 (*m/z* 202 base peak on MS) and 271 (*m/z* 204 base peak on MS) have also been detected and coded as **267L** and **271D**. An FTIR spectrum of a **267L** isomer (Figure 5) in skin extracts of *D. pumilio*, collected on Isla Colón, Bocas Province, Panama, in 1992, was obtained and indicated a cis ring fusion and *cis*-piperidine A ring. Both side chains appeared to be pent-2-en-4-ynyls as indicated in the tentative structure **23**. Analysis of skin extracts of *D. granuliferus* Taylor, 1958, collected in 1992 near Palmar Norte, Puntarenas Province, Costa Rica, revealed on GC−MS five DHQ isomers of molecular weight 271, in addition to peaks corresponding to the *trans*-**269AB**s and a *cis*-**269AB** isomer (**24**), the latter shown by its FTIR spectrum (Figure 5) to be cis ring fused with a *cis*-piperidine ring-A moiety, i.e., stereochemistry at C-2, C-4a, and C-8a analogous to *cis*-**275B** (**14**). Four of the DHQs of molecular weight 271 (ratio 1:2.3:4:14) had, in addition to *m/z* 228 fragments ($M^{\bullet+}$ − 43; 5−10%), only *m/z* 204 fragments (negligible *m/z* 202) and are thus of the **271D** series with two side chains of 67 amu. One apparently homogeneous GC peak of molecular weight 271 had both *m/z* 202 and 204 fragments (∼1:1). No structure was assigned to this peak as it was not established to be a single component. GC−FTIR spectra (Figures 5 and 6) were possible for the other three DHQs of the **271D** series and indicated that the minor component (**25**) had a cis fusion and *cis*-piperidine moiety as seen in *cis*-**275B** (**14**), while the two more prominent components had trans ring fusions and *cis*-piperidine A-ring moieties, analogous to these stereocenters in *trans*-**269AB**. FTIR spectra of these latter two DHQs (Figure 6) led to the tentative structures **26** and **27**, respectively. DHQ **27** has two 3,4-pentadienyl side chains, while isomers **25** and **26** (complete structures unassigned) had either a 3,4 pentadienyl side chain at C-2 and a 4-pentynyl side chain at C-5, or the reverse.



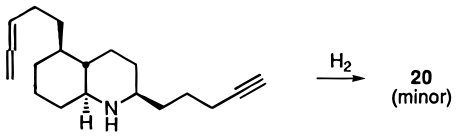
cis-**267L (23)**



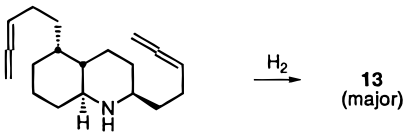
cis-**269AB (24)**



cis-**271D (25)**
side-chains may be interchanged



trans-**271D (26)**
side-chains may be interchanged



iso-5-*epi*-*trans*-**271D (27)**

Hydrogenation of the *D. granuliferous* extract provided a 1:20:2 mixture of **20**, **13**, and a perhydro **14**, respectively. The preponderance of **13** indicated that a substantial amount of the **271D** isomers had a trans ring fusion and the same configurations at all four stereocenters as *trans*-**269AB** (**19**) and differed only in the nature of the unsaturation in their side chains. Table 4 lists the DHQs detected so far in frog skin extracts, including the **271D** series discussed above. At least 11 are of the 19-carbon type.

NMR Data on a Synthetic 2-Epimer of Decahydroquinoline *cis*-195A. We present in Table 5, ^1H NMR

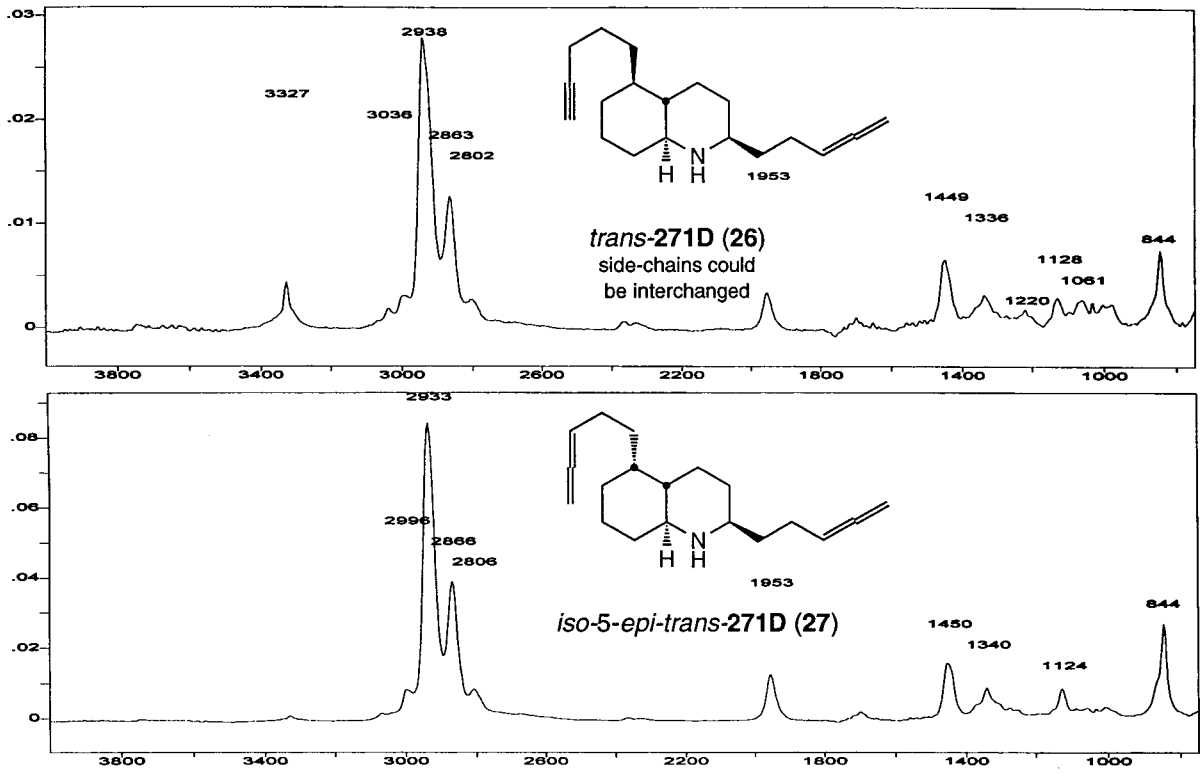
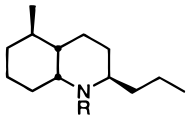


Figure 6. GC−FTIR spectra of *trans*-**271D** DHQs **26** and **27**. Spectra are plotted (*y*,*x*) as absorbance vs wavelength (cm^{-1}). Spectra are plotted (*y*,*x*) as absorbance vs wavelength (cm^{-1}).

spectral assignments for the *N*-methylated C-2 epimer of *cis*-**195A** (**28**), synthesized by Polniaszek and co-workers[23] as an intermediate to the C-2 epimer of *cis*-**195A** (**29**). Neither has yet been found in Nature nor, indeed, has any *N*-methyl DHQ.



28 R = CH_3
29 R = H

These assignments were verified by COSY and NOESY spectra (Table 5, footnote b) as well as decoupling experiments. This is previously unpublished data and it is included for purposes of assigning NMR signals in other DHQs. TOCSY spectra showed a rapid (0.015 s) transfer of magnetization from H-2 to H-3_{ax}, indicating that these hydrogens were in a trans diaxial orientation and, consequently, establishing an equatorial orientation of the C-2 propyl group. The hydrogens H-8a and H-8_{ax} had a similar rapid transfer of magnetization and must also be in a trans diaxial orientation supporting conformation **B** (see the next section). Table 6 presents ^{13}C NMR data for **28** and **29**. Figure 5 includes the FTIR spectrum of **29**. The C-2 epimer of *cis*-**195A** (**29**) has not been detected in frogs, although *trans*-**195A** (**2**)[8] was detected in skin extracts of *Epipedobates bassleri* Melin, 1941, collected in San Martín Province, Perú, in 1988.

^{13}C NMR Data on *cis*-Decahydroquinoline Conformers. *cis*-Decahydroquinoline, prepared by catalytic reduction of quinoline with PtO_2/HOAc, was purified by HPLC, and the ^{13}C NMR spectra of the base in $CDCl_3$ and the HCl salt in CD_3OD were studied over temperature ranges of −80 to −30 °C and −70 to −30 °C, respectively, in increments of 10 °C, and the ^{13}C NMR chemical shifts were extrapolated to 25 °C. Two equilibrating species (**A**, **B**) were detected and assigned conformations on the basis of phase-sensitive 2D C,H COSY spectra and 2D HOHAHA C,H COSY spectra (mixing times of 8 and 17 ms at 100 MHz). Surprisingly, the δ_C values of the ring-junction pairs were virtually identical in **A** and **B** (Table 7).

These data have been used to assign the ring conforma-

Table 5. ^1H NMR Data (500 MHz) for the *N*-Methyl C-2 Epimer of *cis*-**195A** (**28**)[a,b]

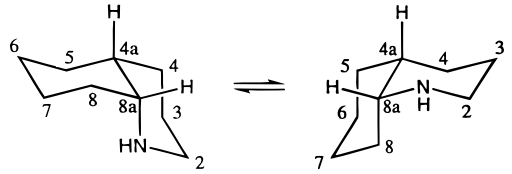
proton(s)	δ (C_6D_6)	m (*J*, Hz)	δ (acetone-d_6, 55 °C)	m (*J*, Hz)
2	2.35	ddd (11.7, 8.1, 3.9)	2.52	br s
3_{ax}	1.2		1.30	
3_{eq}	1.48		1.61	
4_{ax}			1.7	qd (9.2, ∼4)
4_{eq}			1.39	
4a	1.32		1.39	
5	1.70	m	1.82	m
6_{ax}	1.50		1.61	m
6_{eq}	0.97		1.10	
7_{ax}	1.10		1.34	
7_{eq}	1.38		1.50	
8a	2.75	dt (9.3, 3.8)	2.74	br s
8_{ax}	1.58		1.7	qd (9.2−9.5, ∼4)
8_{eq}			1.39	
9,9′	1.18, 1.28		1.30, 1.39	
10,10′	1.08		1.20, 1.30	
CH3−11	0.76	t (7.1)	0.88	t
CH_3-12	0.83	d (7.1)	0.96	d
N-CH_3	2.24	s	2.23	s

[a] Abbreviations: s = singlet; d = doublet; dt = doublet of triplets; ddd = doublet of doublet of doublets; t = triplet; q = quartet; qd = quartet of doublets; br = broad; m = multiplicity or multiplet; ax = axial configuration; eq = equatorial configuration. Spectrum is in C_6D_6 or acetone-d_6 with δ relative to TMS = 0.00. [b] Selected NOE interactions from a 2D NOESY spectrum (500 Mz, acetone-d_6, 1.3 s mixing time): *N*-CH_3 with H-2, H-9 (or H-9′), H-10 (or H-10′), and H-8_{ax}; H-2 with *N*-CH_3, H-8_{ax} (or H-4_{ax}); H-3 with H-9 (or H-9′); H-8a with *N*-CH_3, CH_3-12, and H-7_{ax}; CH_3-12 with H-4a, H-6_{eq}, H-7_{ax}, and H-8a.

Table 6. ^{13}C NMR Spectral Data (125 MHz) for DHQs **28** and **29**

carbon	**28**, δ (acetone-d_6)	**28**, δ (C_6D_6, 50 °C)	***29***, δ ($CDCl_3$)	**29**·DCl, δ (D_2O)
2	58.75[a]	57.8[a]	51.4[a]	51.1[a]
3	(3,5,6 obscured by acetone at 28−32 ppm	30.1		34.2
4	25.9	25.2		26.8
4a	39.6	38.9	39.6	38.9
5			18.8	31.7
6		25.2	~34	23.8
7	21.6		19.6	22.4
8	33.0	32.3	~32	26.8
8a	57.8[a]	57.0[a]	51.5[a]	52.0[a]
9				18.0
10	20.2	20.9	19.5	19.2
11	15.0	14.6	13.8	13.0
12	20.2	19.5	18.8	18.2
N-CH_3	43.3	42.2		

[a] Signals may be interchanged. (Not all signals are seen, perhaps due to conformational equilibria. Some assignments were verified with ADEPT experiments, but most assignments rest upon analogy with published[3] data for DHQs.) Chemical shifts (δ) are in ppm with respect to $CDCl_3$ at 77.2 or TMS at 0.00.



A **B**

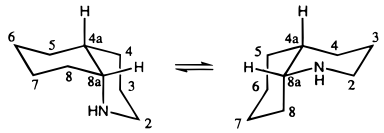
tions of cis 2,5-disubstituted DHQs. The major conformer **A** ("N-endo") has an H-8a axial conformation with respect to the N-containing ring, while conformer **B** ("N-exo") has an equatorial H-8a configuration with respect to that ring. The signals for C-7, C-8, and C-8a in the N-endo conformation (**A**) of the free base were in close agreement with those carbons in *cis*-**275B** (**14**) (Tables 1 and 7), whereas only C-3 of **14** was in close agreement with that carbon signal in the N-exo conformer. The largest deviations with the signals of *cis*-**275B**, outside of those expected for C-2 and C-5 (α-effect), were for the carbons flanking these, i.e., C-3, C-4a, and C-6 (β-effect; for substituent-effects, see refs 31 and 32). Table 7 includes differences ($\Delta\delta_C$) for ring carbons in the N-endo conformer and **14**, which indicate that **14** adopts the former conformation when an appropriate allowance is made for the predicted α- and β-substituent effects and γ_{gauche} effects from the C-5 substituent at C-4 and γ_{gauche} effects from H-4_{eq} at C-5. Table 7 also includes (footnote e) the $\Delta\delta_C$ for a model synthetic compound, 5-β-propyl-*cis*-decahydroquinoline, with respect to **A**, showing a good correlation and indicating that a DHQ with a single β substituent at C-5 will adopt the **A** conformation, a conformation that places that substituent in an equatorial orientation. Thus, ^{13}C NMR represents a very useful method to assign stereochemistry for *cis*-DHQs with equatorial substitutents at C-2 and/or C-5, which, so far, all adopt the "N-endo" ring conformation **A**. This method confirms the correctness of the ^1H NMR assignments. Earlier work[22] on *cis*-decahydroquinoline established many of the assignments. Good agreement was found with signals reported earlier for the N-endo conformer; however, no signals for C-3, C-6, C-7, and C-8 were reported for the N-exo conformer, and signals reported at −50 °C for C-2, C-4, and C-4a were 1.2, 1.2, and 1.7 ppm greater, respectively, than those detected in the present study. The C-4a signals reported previously should apparently be reversed between the N-endo and N-exo conformations.

^{13}C NMR Data for *cis*-243A (8) from the Frog *D. auratus* Girard 1855. ^{13}C NMR chemical shifts have been reported[3] for the DHQs, **1**, **3**, **6**, **9**, and **10**. At that time, insufficient amounts of purified **8** were available for a ^{13}C NMR spectrum. Such data, including C−H multiplicity, have been obtained recently and are presented in Table 8. The free base *cis*-**243A** (**8**), isolated from extracts of *D. auratus* collected on Isla Taboga, Panama Province, Panama, in 1971, showed chemical shifts for carbons C-2−C-11 to be very similar (average deviation ±0.43 ppm) to those of the ring and C-2 allyl side chain of *cis*-**219A** (**6**), supporting an identical conformation for these two alkaloids, i.e., the same conformation as shown for the N-exo **B** conformer of Table 7. For comparison purposes, Table 8 shows the carbon chemical shift differences, $\Delta\delta_C$, between DHQ **6** as well as DHQ **8** and certain carbons of the N-exo conformer (**B**) of *cis*-decahydroquinoline discussed above. In these cases, when the appropriate corrections are made for the effect of the C-2 and C-5 substituents on the N-exo conformer **B** (see Table 8 for a tabulation of substituent corrections required), DHQs **6** and **8** are found to adopt the N-exo conformation. These DHQs have an equatorial substituent at C-2 and an axial substituent at C-5, and it is assumed that the N-exo ring conformation will be adopted by other DHQs of this stereochemistry. Note the close correspondence between all of the ring-carbon signals of **6** and **8** and the parallel downfield shifts of C-5 and C-8, probably resulting from steric compression. Five signals (C-3, C-5, C-6, C-8, and C-9) were broadened, probably reflecting conformational equilibria.

Two DHQs in the Ant *Solenopsis (Diplorhoptrum) azteca* Forel (Formicidae). During an examination of methylene chloride extracts of ants of the myrmicine species *Solenopsis* (*Diplorhoptrum) azteca* collected in Puerto Rico, where these small thief ants are found nesting in decayed wood or decayed coconut husks, it was noted that extracts of virgin queens, but not workers or males, contained two alkaloids (~1:9) of molecular weight 275, each with a base peak at *m*/*z* 206 in the MS and also showing significant *m*/*z* 232 ($M^{\cdot+}$ − 43) (12−15%) fragment ions. This *Solenopsis* species is polygynous, and many wingless queens are commonly found along with workers when forest leaf litter is extracted with Berlese funnels. One such collection yielded 62 workers and 26 dealate queens. The molecular ion of *m*/*z* 275 was confirmed by CIMS with NH_3, and CIMS with ND_3 indicated one exchangeable hydrogen. HRMS indicated for both, a $C_{19}H_{33}N$ formula for the molecular ion, $C_{16}H_{26}N$ for the *m*/*z* 232 fragment, and $C_{14}H_{24}N$ for the *m*/*z* 206 base peak. Neither ant alkaloid was identical in GC retention time (Experimental Section) or in its FTIR spectrum to either of the two frog skin DHQ alkaloids of the same molecular weight (**14**, **15**). Nevertheless, there were great enough similarities in FTIR spectra so that it was obvious that the ant alkaloids were indeed DHQs, *the first ever detected in ants*. The major ant DHQ (**16**), now called 5-*epi*-*cis*-**275B′**, was shown by its FTIR spectrum (Figure 7) to have a cis ring fusion and *trans*-piperidine A ring, while the minor ant DHQ (**17**), now called 5-*epi*-*trans*-**275B**, was shown by its FTIR spectrum (Figure 7) to have a trans ring fusion and a *cis*-piperidine A ring. In our nomenclature for **17** we do not wish to imply the existence of a *trans*-**275B** (corresponding in configuration to **3**, **7**, **9**, and **19**), for it has not yet been detected in any frog skin extract.

Hydrogenation of the ant extract in methanol with a 10%

Table 7. ^{13}C NMR Chemical Shifts of *cis*-Decahydroquinoline and Its HCl Salt



A **B**

	N-endo conformation[a] (**A** type)		*N*-exo conformation[a] (**B** type)		δ_C comparison with cis-**275B** (**14**)	
carbon	δ_C free base[b]	δ_C HCl salt[c]	δ_C free base[b]	δ_C HCl salt[c]	$\Delta\delta_C$(**14-A**)[d,e]	substituent effects obsd
2	47.5	46.6	39.1	39.7	10.5	2-equatorial substituent α-effect
3	20.9	18.8	25.7	23.8	6.5	2-equatorial substituent β-effect
4	30.6	29.3	23.4	22.9	−3.7	5-equatorial substituent γ_{gauche} effect
4a	35.3	34.7	34.7	34.4	5.0	5-equatorial substituent β-effect
5	25.2	25.6	31.4	31.5	6.6	5-equatorial substituent α-effect plus 4-equatorial H γ_{gauche} effect
6	26.4	26.3	20.3	20.7	5.7	5-equatorial substituent β-effect
7	20.4	20.4	25.7	26.0	0.8	
8	32.4	30.2	24.4	23.3	0.8	
8a	55.1	56.7	54.1	55.9	1.2	

[a] The **A**-type conformer has an axial H-8a relative to the nitrogen-containing ring. The **B**-type has an equatorial H-8a relative to this ring. [b] The chemical shifts are in ppm in $CDCl_3$ at 25 °C relative to TMS at 0.00 ppm. [c] The chemical shifts are in ppm in CD_3OD at 25 °C relative to TMS at 0.00 ppm. [d] Difference in ppm between δ_C of free base of *cis*-**275B** (**14**) in $CDCl_3$ (see Table 1) and signals of *cis*-decahydroquinoline, N-endo conformation (**A** type). [e] In the same way as determined for **14**, the $\Delta\delta_C$ values obtained for (5-β-propyl-*cis*-decahydroquinoline **A**) were: C-2, 0.4; C-3, 0.1; C-4, −4.0; C-4a, 4.8; C-5, 6.2; C-6, 5.0; C-7, 0.6; C-8, 0.6; C-8a, 0.8. Deviations from the model are explained by the same substituent effects as given for **14**. See refs 31 and 32 for the derivation of these substituent effects. ^{13}C NMR data ($CDCl_3$, ref = 77.03 ppm) was obtained at −55 and +50 °C (data for 50 °C in parentheses) for 5-β-*n*-propyl-*cis*-decahydroquinoline, prepared via 5β-*n*-propyl-*cis*-octahydroquinolone obtained by Overman's procedure,[18] then reduced with BH_3·SMe_2: C-2, 47.9 (47.2 broad); C-3, 21.0 (22.3 broad); C-4, 26.6 (26.9); C-4a, 40.1 (40.8); C-5, 31.4 (32.9 broad); C-6, 31.4 (31.3 broad); C-7, 21.0 (21.2); C-8, 33.0 (32.6 broad); C-8a, 55.9 (55.6 broad); C-9 35.2 (35.6); C-10, 19.1 (19.5); C-11, 14.7 (14.4) ppm. These data were obtained and assignments made by phase-sensitive H,H COSY, C,H COSY, and HOHAHA C,H COSY spectroscopies at both temperatures.

Table 8. ^{13}C NMR Data of *cis*-**243A** (**8**) and δ_C Comparisons of **8** and *cis*-**219A** (**6**) with *cis*-DHQ Conformation **B**

carbon	*cis*-**243A** (**8**)[a]	$\Delta\delta_C$ (**8**-**B**[b])	*cis*-**219A** (**6**)[c]	$\Delta\delta_C$ (**6**-**B**)	substituent effects obsd
2	48.9 (d)	9.8	49.3	10.2	2-equatorial substituent α-effect
3[d]	32.2 (t)	6.5	31.4	5.7	2-equatorial substituent β-effect
4	25.6 (t)	2.2	25.4	2.0	
4a	40.1 (d)	5.4	39.2	4.5	5-axial substituent β-effect
5[d]	38.8 (d)	7.4	38.6	7.2	5-axial substituent α-effect
6[d]	25.3 (t)	5.1	25.1	4.8	5-axial substituent β-effect
7	20.7 (t)	−5.0	20.5	−5.2	5-axial substituent γ_{gauche}-effect
8[d]	27.7 (t)	3.3	27.1	2.7	5-axial substituent γ-effect
8a	50.6 (d)	−3.6	50.7	−3.4	5-axial substituent γ_{gauche}-effect
9[d]	41.1 (t)		41.6		
10	135.9 (d)		135.7		
11	117.0 (t)		117.1		
12	33.6 (t)		31.2		
13	145.4 (d)		138.8		
14	108.9 (d)		115.1		
15	80.7[e]				
16	81.3 (d)				

[a] δ_C and multiplicity (in parentheses) of free base in $CDCl_3$ at 75 MHz. In ppm (δ) relative to $CDCl_3$ at 77.2 ppm. Multiplicity (d = doublet; t = triplet) determined by DEPT experiment. [b] See Table 7 for structures of **A** (N-endo) and **B** (N-exo) conformations of *cis*-decahydroquinoline and references for substituent effects. [c] See ref 3 for original ^{13}C NMR data on *cis*-**219A** (**6**). [d] Peak weak and broadened. [e] Not detected.

Pd/C catalyst for 1 h provided two DHQs of molecular weight 279. The minor isomer, a tetrahydro derivative of **17**, had a GC retention time, mass spectrum (with only a feeble loss of the propyl fragment), and FTIR spectrum identical to that of **20**, the minor isomer produced from the *trans*-**269AB** mixture on catalytic hydrogenation, thus confirming a 2,5-disubstituted DHQ structure for one of the ant alkaloids (see Figure 1). The only other occurrences of this relative stereochemistry at the four DHQ stereocenters are that of the frog skin alkaloid 5-*epi*-*trans*-**243A** (**10**) and, of course, 5-*epi*-*trans*-**269AB** (**18**). Since the minor frog DHQ **15** and the major ant DHQ **16** have virtually the same FTIR spectra, indicating a cis ring junction and a *trans*-piperidine A ring, yet differing GC retention times, it was concluded that the configuration at C-5 of **16** is epimeric to that of **15**. Thus, if DHQ **15** is considered likely to have an H-5α configuration, then the ant alkaloid **16** would have the H-5β configuration as indicated in Figure 1.

As yet, no natural 2,5-disubstituted DHQ has been characterized in ants or frogs with a trans ring fusion *and* a *trans*-piperidine ring-A moiety. Any such DHQ would be a higher energy diastereomer with an axial 2-alkyl substitutent. As mentioned above, an apparent example of this configuration was seen by FTIR spectroscopy in the extract of *D. histrionicus* collected at Quebrada Docordo, but this will have to be confirmed. Cis ring-fused DHQs having an axial C-2-substituent are common. Here, however, one conformation with the usual C-5-equatorial substituent may be in equilibrium with a second cis-fused conformer with C-2-equatorial and C-5-axial configurations. This equilibrium may account for the split IR absorption bands observed at ∼1350 and ∼1150 cm^{-1} (probably δ_{C-H} and ν_{C-N} absorptions, respectively) in cis-fused DHQs.[3]

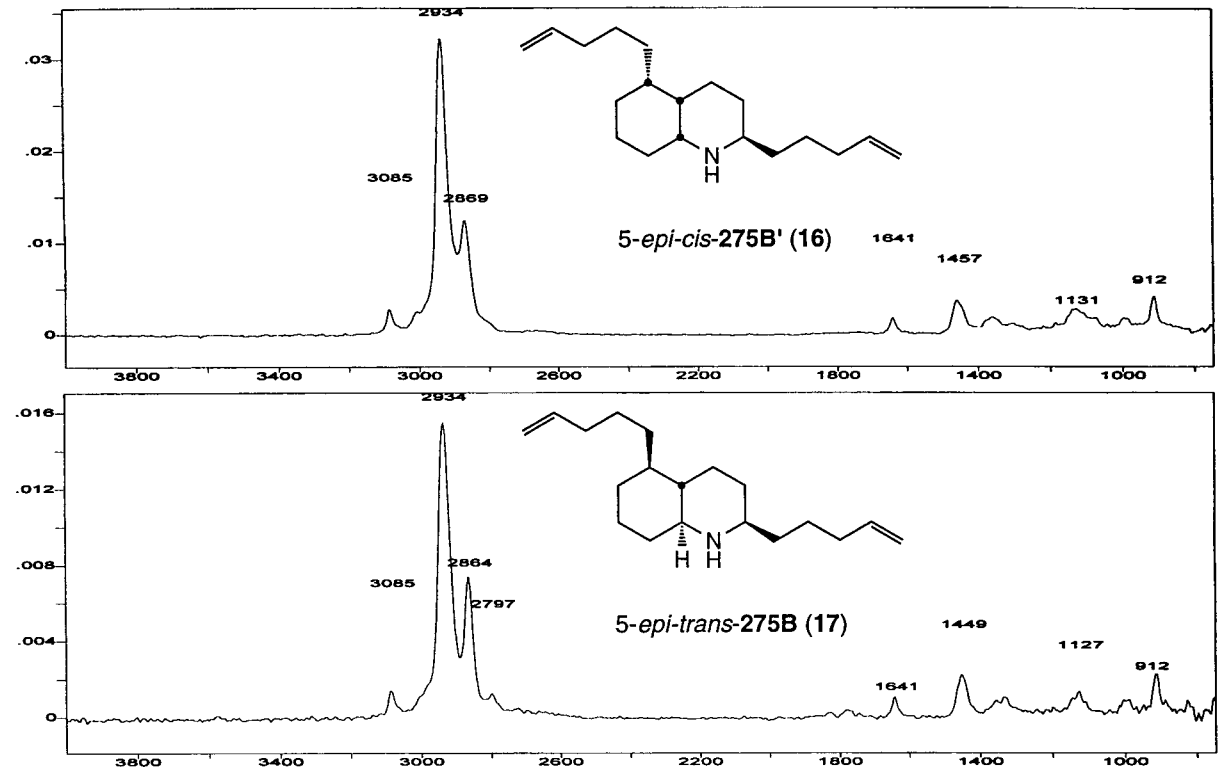
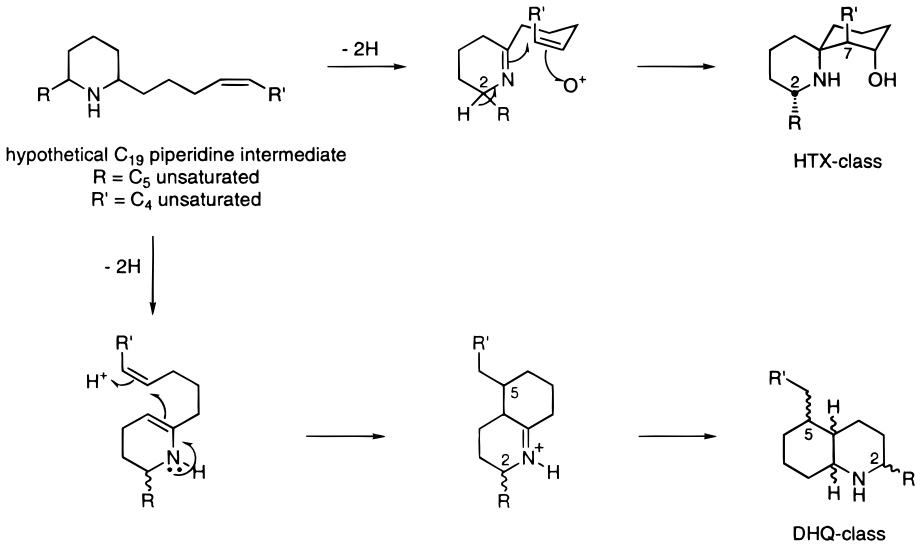


Figure 7. GC−FTIR spectra of DHQs 5-*epi-cis*-**275B′** (**16**) and 5-*epi-trans*-**275B** (**17**) from the ant *Solenopsis* (*Diplorhoptrum*) *azteca*. Spectra are plotted (*y*,*x*) as absorbance vs wavelength (cm^{-1}).

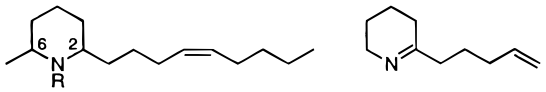
Scheme 3. Possible Biosynthetic Pathways to C_{19}-HTX and C_{19}-DHQ Classes of Alkaloids



The Relationship of the Histrionicotoxin and DHQ Classes. Another class of secondary amine alkaloids from dendrobatid frogs are the histrionicotoxins (HTXs).[1] Nine of these azaspiro[5.5]undecanes are nineteen-carbon alkaloids and have two side chains, one at C-2 of five carbons and one at C-7 of four carbons. The unsaturation patterns (e.g., enynic, acetylenic, allenic, dienic, and olefinic) are similar to those of the C-2 and C-5 DHQ side chains. Members of the HTX class are often found in frog skin extracts accompanied by the 19-carbon DHQs (typified by the *trans*-**269AB**s).[10] The HTXs of molecular weight 283, 285, and 287 may represent a mono-oxygenation metabolic pathway alternative to that producing the DHQs of molecular weight 267, 269, and 271, respectively, and thus could arise from a common 19-carbon straight-chain precusor (Scheme 3). At this stage, it appears that the 19-carbon 2,5-disubstituted DHQs comprise a much more complex pattern of stereocenter configurations than is seen in the putatively related histrionicotoxin class, where so far all of the many members have had the same relative (and presumably absolute) stereochemistry at all asymmetric centers. It may be relevant in this context that the absolute configuration at C-2 in *trans*-**219A** (**3**) is the same as C-2 in the HTXs and, indeed, is also the same as that of C-2 and C-6 (*R*,*R*) in the only 2,6-disubstituted ant piperidine whose absolute stereochemistry has been determined, viz., *trans*-2-methyl-6-tridecylpiperidine (solenopsin B).[24] Histrionicotoxins have never been detected in Nature outside of skin extracts of neotropical frogs of the family Dendrobatidae, except for one instance of detection in a single mantelline frog of unknown origin, obtained via the pet trade.[4] Low levels of HTXs and *trans*-**269AB**s were

present in skins of captive-raised *D. auratus* that had been fed leaf-litter arthropods in Panama.[16] It is possible that HTXs detected in dendrobatid skin extracts arise, like DHQs, from dietary ants.

A hypothetical biosynthetic pathway from a 19-carbon 2,6-disubstituted piperidine to the two different ring systems is proposed in Scheme 3. DHQs and HTXs of 15 and 17 carbons also exist[1] and likewise could arise from unbranched precursors. Possibly relevant to this proposal is the recent discovery[25] that a Puerto Rican ant of a *Solenopis* (*Diplorhoptrum*) species has a 2-methyl-6-nonenyl piperidine (**30**) with a $\Delta^{4,5}$ *Z*-double bond. An unusual 10-carbon 2-(4-pentenyl)piperideine (**31**), analogous to the proposed imine intermediate of Scheme 3, was also detected during the present study in workers and queens of *S.* (*Diplorhoptrum*) *azteca* (data not presented) and, in earlier studies,[26] with an ant (sp A) of the *Solenopsis* (*Diplorhoptrum)* genus (species not determined, but very likely *S. azteca*) from Puerto Rico. It seems reasonable that isomeric Δ^4-pentenyl piperideines might also exist and have undergone substitution at C-6 to provide intermediates to 2-(4-pentenyl)-6-alkyl piperidines. Such piperidines have been detected in the South African ant *Monomorium delagoense* Forel (Formicidae).[27] After several more steps involving imine and enamine intermediates as indicated in Scheme 3, such 2,6-disubstituted piperidines could yield alkaloids of the DHQ class.



30 1:4 mixture of 2,6-*Z* to 2,6-*E* isomers **31**

Caste Differences in *S. (Diplorhoptrum) azteca* Alkaloids. The presence of dissimilar alkaloids in different castes has been reported in a previous study on *Solenopsis* (*Diplorhoptrum*) spp. from Puerto Rico.[26] In *S. (Diplorhoptrum) azteca*, both workers and queens contain piperideine **31**, while the queens also contain the decahydroquinolines **16** and **17**. Since the workers are more likely to be involved in defense and predation, the presence of decahydroquinolines only in the queens suggests a pheromonal role for these compounds in this species.

The Role of Diet in Frog-Skin Alkaloids. While the DHQ class is commonly encountered in skins of dendrobatid frogs, the present paper documents the first instance of a DHQ in an ant. This discovery lends additional support to the hypothesis[14−16] that certain frogs or toads may be sequestering, for defensive purposes, alkaloids from a diet of small arthropods, of which ants are a major item. In the case of the species such as the *S.* (*Diplorhoptrum*) *azteca* ants reported here, the fact that they are often found nesting in decaying leaf litter where they could become easy prey for frogs would further strengthen a proposed dietary link. One of the two ant diastereomers of the **275B** DHQ (**17**) has the same relative stereochemistry at all four stereocenters as the minor diastereomer (**18**) of *trans*-**269AB** and of 5-*epi*-*trans*-**243A** (**10**) found in dendrobatid frogs. It will be noted that the major ant DHQ of molecular weight 275 (**16**) could arise biosynthetically from a trans 2,6-disubstituted piperidine, whereas the minor ant DHQ (**17**) may arise from a cis 2,6-disubstituted piperidine. *trans*-Piperidines are known as major venom components and to dominate *cis*-piperidines in workers of most species of the myrmicine ants of the genus *Monomorium* and subgenus *Solenopsis* (*Solenopsis*).[28,29]

Relevant to the present work is the recent report[30] on the isolation of two new 2-methyl-5-octadienyl cis-fused DHQs, oxygenated in the 3-position, from the distinctively colored marine flatworm, *Prostheceraeus villatus*. These two DHQs and a related DHQ reported previously, as well as two 1,2-disubstituted pyrrolidines found in the flatworm, were also shown to be present in the prey of this flatworm, namely, the tunicate *Clavelina lepadiformis*, and are presumably being sequestered in the biological defense of the flatworm.

Experimental Section

General Experimental Procedures. (A) A Hewlett-Packard model 5890 gas chromatograph having a 25 m × 0.32 mm i.d. HP-5 fused silica bonded capillary column programmed from 100 to 280 °C at a rate of 10 °C/ min, interfaced with a Hewlett-Packard model 5971 mass selective detector and a Hewlett-Packard model 5965B IR instrument with a narrow band (4000−750 cm^{-1}) detector and a Hewlett-Packard ChemStation (DOS based), were used to generate the chromatograms, EIMS, and FTIR spectra of GC peaks. EIMS or modified EIMS (ion trap) of GC peaks were generated with the instruments B−D. (B) A Finnigan Model 800 ion trap mass detector interfaced with a Varian Model 3400 gas chromatograph fitted with a 30 m × 0.32 mm i.d. RTX-5 (Restek) fused silica bonded column and using the same temperature program as A above was used. (C) A Finnigan 4500 mass spectrometer with a 25 m × 0.25 mm i.d. OV-17 fused silica-bonded column (Supelco) with a 60−280 °C program (10 or 5 °C/min) and an INCOS data system was used. (D) A Shimadzu QP-5000 GC/MS fitted with an RTX-5, 30 m × 0.25 mm i.d. fused silica-bonded column and programmed from 80 to 280 °C at either 10 or 5 °C/min was used. (E) HRMS were measured with a JEOL SX 102 instrument fitted with a 15 m × 0.20 mm i.d. HP-5 column. The maximum error was −12.3 ppm in the mass determination of the neutral *m*/*z* 304 material below. All other measurements were within 5 ppm. Chemical ionization used either instruments B or C and NH_3 or ND_3 reagent gases. Instrument C was also used with a D_2O bleed to obtain deuterium-exchanged EIMS. Table 9 includes mass spectral data for the various DHQs and *N*-acetyl derivatives of **18**, **19**, and **27**.

The 1D or 2D (COSY) ^1H NMR spectra in D_2O−DCl, acetone-d_6, methanol-d_4, benzene-d_6, or $CDCl_3$ were measured with a Varian XL-300, a Varian VXR-500S, or a JEOL GX400 spectrometer. Chemical shifts (δ, ppm) in methanol-d_4 or D_2O are referred to HOD at 4.78 and in other solvents to TMS at 0.00 ppm. ^{13}C NMR spectra were obtained at 125 MHz or as indicated and used $CDCl_3$ at 77.2 ppm as the reference. Care was taken with temperature control in acquiring ^{13}C NMR data for decahydroquinoline·HCl. CD_2HOD was used as a lock signal; its stability was confirmed relative to TMS (0.00), e.g., δ_C, 49.02 ppm at 35 °C; 48.97 ppm at −50 °C. Phase-sensitive 2D-NMR programs for DQF−COSY, TOCSY, or NOESY were used with the JEOL spectrometer. For the ^{13}C NMR spectrum of **18/19**, 2.7 mg was used in a special 5 mm microcell made by JEOL, allowing a good spectrum from 12 000 transients.

Biological Materials. Frogs were collected at the locations and dates cited in the text. Skins were placed in methanol for transport to NIH. Profiles of alkaloids in the extracts from these dendrobatid frogs have been reported[10] or will be the subject of future papers. In most cases, extracts were obtained from only 1−10 frogs. Voucher specimens of frog species described in this work are deposited with the American Museum of Natural History, New York.

Ants were collected in the vicinity of Guaynabo, Puerto Rico, in May−June 1995. Four collections of 10−20 virgin queens (separate colonies) were made. Ants were immediately placed in vials containing methylene chloride. These extracts were

Table 9. Electron-Impact or Ion-Trap Mass Spectral Data on 2,5-Disubstituted Decahydroquinolines and Derivatives[a]

compound	instrument[b]	MS, m/z (intensity)
11	A	167 (14), 152 (53), 138 (1), 124 (100), 99 (5), 82 (8)
12	A	223 (1), 222 (1), 208 (1), 180 (14), 152 (100), 109 (9), 81 (3), 55 (7)
19	D	269 (3), 268 (16), 254 (8), 240 (8), 230 (3), 228 (5), 226 (8), 214 (9), 205 (17), 204 (100), 203 (17), 202 (100), 200 (8), 188 (7), 186 (7), 174 (13), 162 (12), 160 (12), 148 (37), 146 (12), 136 (9), 137 (10), 134 (16), 133 (9), 132 (10), 131 (14), 129 (11), 122 (20), 117 (21), 109 (37), 108 (22), 105 (20), 96 (83), 91 (59), 79 (88), 67 (53), 65 (40)
trans-**269A** (**21**)	B	270 (100), 268 (30), 254 (5), 240 (8), 226 (2), 204 (30), 175 (2), 162 (2), 160 (2), 148 (8), 146 (8), 134 (10), 131 (10), 117 (20), 108 (18), 105 (20)
trans-**269B** (**22**)	B	268 (16), 254 (2), 240 (4), 228 (10), 210 (7), 202 (100), 188 (2), 174 (15), 162 (15), 160 (16), 148 (15), 146 (16), 144 (16), 138 (18), 134 (27), 131 (20), 129 (18), 117 (32), 105 (22), 98 (38), 96 (50), 91 (80)
trans-**269AB** (**18/19**)	B	270 (77), 268 (27), 254 (7), 240 (7), 204 (80), 202 (41), 174 (11), 162 (11), 160 (11), 148 (25), 134 (18), 117 (34), 108 (36), 96 (52), 79 (82), 67 (60), 65 (100)
	D	269 (4), 268 (25), 254 (8), 240 (9), 230 (3), 228 (5), 226 (7), 214 (9), 204 (100), 202 (100), 200 (8), 188 (7), 186 (7), 174 (12), 162 (11), 160 (11), 148 (36), 146 (12), 134 (16), 131 (13), 129 (11), 122 (19), 120 (15), 117 (21), 109 (35), 96 (79), 94 (38), 91 (58), 81 (42), 79 (84), 77 (74), 67 (50), 65 (38)
N-acetyl-**18**	A	311 (<1), 268 (11), 244 (29), 202 (100), 167 (5), 149 (13)
N-acetyl-**19**	A	312 (22), 296 (8), 270 (12), 268 (57), 244 (43), 204 (40), 202 (100), 174 (20)
iso-5-*epi*-*trans*-**271D** (**27**)	D	270 (34), 268 (12), 254 (3), 240 (3), 204 (35), 202 (18), 174 (5), 162 (5), 160 (6), 148 (11), 134 (8), 117 (15), 108 (16), 105 (13), 96 (23), 91 (23), 79 (36), 67 (26), 65 (43)
N-acetyl-**27**	B	314 (30), 298 (16), 270 (47), 258 (40), 244 (40), 228 (8), 216 (5), 204 (100), 202 (48), 190 (5), 174 (10), 162 (12), 160 (10), 148 (17), 136 (22), 134 (18), 131 (15), 117 (20), 105 (30), 91 (48), 79 (82)
H_{10}-*trans*-**269AB** (**13**)	A	279 (<1), 278 (<1), 236 (4), 209 (100), 208 (100), 178 (<1), 165 (1), 150 (1), 136 (2), 121 (2), 95 (<1), 81 (<1)
cis-**275B** (**14**)	B	276 (12), 274 (5), 232 (23), 220 (5), 206 (100), 190 (5), 164 (7), 152 (9), 136 (9), 124 (37), 111 (35)
cis-**275B′** (**15**)	B	276 (23), 260 (<1), 232 (23), 220 (2), 206 (100), 190 (7), 178 (7), 164 (12), 162 (5), 138 (19), 124 (16), 111 (21)
5-*epi*-*cis*-**275B′** (**16**)[c]	B	276 (11), 232 (11), 206 (100), 190 (6), 164 (5), 147 (3), 136 (7)
	D	275 (<1), 260 (<1), 246 (<1), 232 (11), 218 (1), 206 (100), 190 (<1), 176 (<1), 164 (1), 150 (1), 124 (2), 121 (3), 111 (2), 107 (3)
5-*epi*-*trans*-**275B** (**17**)[d]	B	276 (2), 232 (14), 218 (2), 206 (100), 162 (2), 149 (7), 136 (7), 124 (16), 111 (11)
	D	275 (1), 246 (<1), 232 (11), 219 (5), 206 (100), 192 (<1), 176 (<1), 164 (<1), 150 (<1), 124 (9), 111 (9), 98 (9)
20	A	279 (2), 236 (1), 222 (2), 209 (100), 208 (70), 165 (1)
	B	278 (2), 236 (2), 235 (2), 221 (6), 208 (100), 180 (2), 166−4 (2), 151 (6), 150 (6), 137 (7), 135 (7), 121 (17), 109 (15), 98 (24), 97 (25), 96 (38), 95 (36), 87 (22), 83 (34), 81 (58), 79 (32), 69 (50), 67 (76)
13	B	280 (17), 278 (7), 236 (5), 208 (100), 180 (1), 166 (2), 150 (3), 136 (6), 134 (6), 121 (16), 109 (10), 95 (12), 93 (10), 81 (18), 79 (18), 70 (20), 67 (28)

[a] Ion trap spectra (B) with alkaloids generally gave an $M^{\cdot+} + 1$ ion due to self-chemical ionization. This is concentration-dependent. Sometimes, dependent upon instrumental parameters, $M^{\cdot+}$, or $M^{\cdot+} - 1$ may also be observed as the apparent molecular ion. CIMS was used to verify the molecular weight. [b] See discussion in first paragraph of Experimental Section for code to instrumentation used. [c] **16** (HRMS): calcd for $C_{19}H_{33}N$: 275.2605; found, 275.2625; calcd for $C_{16}H_{26}N$: 232.2059; found, 232.2059; calcd for $C_{14}H_{24}N$: 206.1903; found, 206.1903. [d] **17** (HRMS): found, 275.2607; 232.2070; 206.1906.

analyzed directly or, in some cases, after a 10:1 concentration. Voucher specimens of the ant *S.* (*Diplorhoptrum*) *azteca* are in the Los Angeles County Museum of Natural History. GC retention times (instrumental conditions B) were 18.26 min (**16**) and 17.90 min (**17**).

Preparation of Alkaloid Extracts. Alkaloid fractions were prepared as described[4] from methanol skin extracts of dendrobatid frogs using a partition from 50% CH_3OH-H_2O into $CHCl_3$ (3×), extraction of the alkaloids from $CHCl_3$ into 0.1 N HCl (3×), and after basification with 1 N NH_4OH, a final extraction (3×) of alkaloids into $CHCl_3$. After drying with Na_2SO_4 and careful removal of $CHCl_3$ in vacuo, the alkaloid fraction was dissolved in sufficient CH_3OH so that 100 μL corresponded to 100 mg wet weight of skin. In 1992, the procedure was modified, to prevent losses of $CHCl_3$-soluble alkaloid hydrochlorides, by concentration of the initial $CHCl_3$ extract to a small volume followed by reconstitution with *n*-hexane before extraction with 0.1 N HCl.

Purification of Alkaloids *cis*-275B (14) and *trans*-269AB (18, 19). A methanol extract from 502 skins of *D. pumilio* collected by the Río Sarapiqui, Heredia, Costa Rica, in 1989, was concentrated in vacuo and then partitioned between 50% CH_3OH-H_2O and hexane. The hexane phase was extracted with dilute HCl (pH 1.5), and the alkaloids recovered by basification with NH_4OH and extraction into $CHCl_3$. The $CHCl_3$ layer on evaporation afforded 0.31 g of a crude alkaloid mixture, which was chromatographed on a DIOL column (Merck prepacked Lobar column, size B) with hexane−$CHCl_3$−Et_3N (80:20:1) to yield three main fractions (A, B, and C) as monitored by refractive index. Fraction A (136 mg) was rechromatographed on the same column with hexanes−Et_3N (100:0.5) into two main fractions of 41 mg and 26 mg. The former was chiefly *cis*-**275B** (**14**), which was further purified by HPLC using a Merck LiChrosphere 100RP Select B column (5 μm) using $CH_3CN-H_2O-Et_3N$ (84:16:1) at a flow rate of 1.0 mL/min (pressure 75 kg/cm^2) to afford 4 mg of nearly pure *cis*-**275B** (**14**). Compound **14** was still contaminated with a neutral material (*m/z* 304, $C_{17}H_{36}O_4$) that gave major fragments at *m/z* 261, $C_{14}H_{29}O_4$ ($M^{\cdot+} - 43$), and *m/z* 235, $C_{12}H_{27}O_4$ ($M^{\cdot+} - C_5H_9$), and a highly unsaturated alkaloid, isomeric with but evidently not of the HTX class (*m/z* 287, $C_{19}H_{29}NO$), giving a base peak at *m/z* 232 [$C_{15}H_{22}NO$ ($M^{\cdot+} - 55$)] and also weak losses of 43 and 83 amu fragments that suggested a 9-keto analogue of a molecular weight 275 DHQ that were removed by chromatography on a small column of silica gel 60 (Merck) using first CH_2Cl_2, then $CHCl_3$, and finally 2.5−5.0% $CH_3OH-CHCl_3$ to give fractions that were homogeneous in **14** by GC−MS. GC retention times (instrumental conditions B) were 17.36 min (**14**) and 18.50 min for the isomer (**15**) detected in other extracts.

Fraction B (93 mg) was rechromatographed on the DIOL column with hexane−$CHCl_3$−Et_3N (90:5:1) to yield three main fractions that comprised 45 mg of octahydrohistrionicotoxin, 17 mg of a mixture (B-2), and 6 mg, consisting mainly of

isodihydrohistrionicotoxin. HPLC purification of B-2 on an RP-8 select B column with $CH_3CN-H_2O-Et_3N$ (84:16:1) yielded 3 mg of *trans*-**269AB** (**18**, **19**) and 6 mg of octahydrohistrionicotoxin.

One thousand skins of *D. auratus* from Isla Taboga, Panama, were collected in 1976, and the alkaloids were separated and purified as described.[3] The fraction containing *cis*-**243A** was freed of contaminating cholesterol. Additional silica gel column chromatography using a Merck Lobar Li-Chroprep Si60 (size A) column and hexane−$CHCl_3$−*i*-PrOH−NH_4OH (70:20:10:1) provided a pure, crystalline sample (25 mg) of *cis*-**243A**.

Chemical Reactions. Microhydrogenations were carried out in CH_3OH over 30 min to 1 h in either 1.5 or 3 mL glass vials with an 8 mm stirring bar under ~2 atm of H_2 generated by an electrolytic generator (Alltech, Deerfield, IL). Typically, ca. 0.1 mg samples of extracts or purified compounds in 50−100 μL of CH_3OH containing a trace of 5% Pd/C (Degussa, Plainfield, NJ) or PtO_2 (Aldrich, Milwaukee, WI) catalysts were used. Solutions were then filtered with a 4 mm syringe filter (Alltech) and the catalyst washed several times with CH_3OH. The combined filtrates were concentrated under a N_2 stream.

Acetylation was performed at room temperature using a 1:1 (v/v) mixture of Ac_2O−pyridine and an alkaloid fraction corresponding to ca. 0.1 mg of skin extract containing *trans*-**269AB** mixtures for ca. 18 h. Reagents were removed using a stream of N_2. GC−MS indicated some 20% unreacted starting material. *N*-acetyl **19**: FTIR 3326, 3033, 2940, 2873, 1955, 1669, 1450, 1406, 1361, 1306, 1214, 1171, 845 cm^{-1}.

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