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ALKALOIDS FROM DENDROBATID POISON FROGS: FURTHER CIS-DECAHYDROQUINOLINES AND 8-METHYLINDOLIZIDINES

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Abstract - Skin extracts from one population of the poison frog *Dendrobates auratus* contain a variety of alkaloids, including 2,5-disubstituted-cis-decahydroquinolines and 5-substituted-8-methylindolizidines. Three of the major alkaloids are cis-decahydroquinolines, whose structures based on NMR spectral analyses are 2,5-diallyl-cis-decahydroquinoline (cis-**219A**), 2-allyl-5(pent-2-en-4-ynyl)-cis-decahydroquinoline (cis-**243A**) and 5-methyl-2-propyl-cis-decahydroquinoline (cis-**195A**); the last is identical with "pumiliotoxin C" previously isolated from the poison frog *Dendrobates pumilio*. Alkaloids cis-**219A** and cis-**243A** differ from cis-**195A** in configuration at the C-2 position. Another poison frog, *Dendrobates histrionicus*, was previously shown to produce nearly exclusively trans-decahydroquinoline isomers of **219A** and **243A**. NMR analyses indicate that the structure of a minor alkaloid from *Dendrobates auratus* is 5-(pent-2-en-4-ynyl)-8-methylindolizidine (**203A**). Two additional 8-methylindolizidines isolated from *Dendrobates pumilio* are 5-(hept-4,6-dienyl)-8-methylindolizidine (**233D**) and 5-(hept-6-hydroxy-4-enyl)-8-methylindolizidine (**251B**). FTIR spectral analyses distinguish cis- and trans-decahydroquinolines and provide evidence for configurations of indolizidines.

Frogs of the neotropical family Dendrobatidae produce a remarkably diverse set of alkaloids, which have been designated using a code introduced in 1978 (1), which employs the alkaloid's molecular weight in boldface with an added letter (or letters) to distinguish alkaloids with the same molecular weight. Over two hundred "dendrobatid alkaloids" have been detected and grouped into classes, based in many instances only on gas chromatographic-mass spectrometric (GC-MS) analyses (see ref. 2,3).

One major class of dendrobatid alkaloids are the 2,5-disubstituted decahydroquinolines. The first of this class named pumiliotoxin C (**195A**) was isolated from a Panamanian population of *Dendrobates pumilio*, and shown by x-ray crystallographic analysis to be 2S,4aS,5R,8aR-5-methyl-2-propyl-cis-decahydroquinoline (4). Recently, trans-decahydroquinolines, namely 2,5-diallyl-trans-decahydroquinoline (**219A**) and 2-allyl-5-(pent-2-en-4-ynyl)-trans-decahydroquinoline (**243A**), were isolated from a Colombian population of *Dendrobates histrionicus* and the absolute stereochemistry defined for the

former, 2S, 4aS, 5R, 8aS, by x-ray crystallographic analysis (5). Alkaloids **219A** and **243A** appeared, based on GC-MS analysis, to be present in various species of dendrobatid frogs. In some species, namely *Dendrobates pumilio* and *Dendrobates histrionicus*, other isomers, designated **219A'** and **243A'**, also were detected (3). The mass spectra for the pairs of isomers are virtually identical in each case (**219A** versus **219A'**, and **243A** versus **243A'**). Related alkaloids isomeric with **219A** and **243A** now have been isolated from a Panamanian population of another frog, *Dendrobates auratus*. NMR spectral analyses reveal that these decahydroquinolines are cis-fused.

The previously described trans-isomers of **219A** and **243A** (5) now will be referred to as trans-**219A** and trans-**243A**. The isomeric cis-isomers from *Dendrobates auratus*, described in the present paper, will be referred to as cis-**219A** and cis-**243A**. "Pumiliotoxin C" will be referred to as cis-**195A**, rather than **195A**. An axial hydroxy congener of cis-**195A**, namely **211A** (6) will likewise be termed cis-**211A**. A congener of trans-**219A**, namely **253D**, containing a 5-(2,3-dihydroxypropyl) substituent instead of the 5-allyl substituent (6) will be termed trans-**253D**. Further analysis of alkaloid fractions from other dendrobatid species by gas chromatography-mass spectrometry and gas chromatographic FTIR spectroscopy on capillary columns will be required to determine the nature of the various isomers of decahydroquinolines **195A**, **219A**, **243A**, etc. that occur in each frog species. Analysis of gas chromatographic FTIR spectra now has been found to provide a means to distinguish between the isomeric cis- and trans-decahydroquinolines. Structures of decahydroquinolines from dendrobatid frogs are given in Fig. 1.

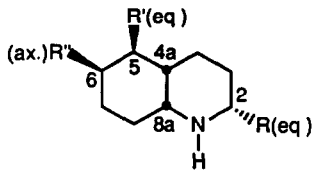
Another major subclass of dendrobatid alkaloids are the 5-substituted 8-methylindolizidines. Four members of this subclass (**205A**, **207A**, **235B**, **235B'**) have been isolated and, based on NMR analyses (6-8), assigned indolizidine structures with an equatorial 8-methyl group and an equatorial 5-substituent (Fig. 1). The present extract of a population of *Dendrobates auratus* yielded another 5-substituted-8-methylindolizidine, **203A**, which, based on NMR and FTIR spectra, has the same relative configuration as other members of this indolizidine subclass. Structures of **203A** and two additional 8-methylindolizidines, **233D** and **251B**, isolated from a Panamanian population of *Dendrobates pumilio* are shown in Fig. 1, along with a tentative structure for a trace 8-methylindolizidine alkaloid, **209B**, detected in certain dendrobatid frogs (3).

Decahydroquinolines

The mass spectrum of trans-**219A** from *Dendrobates histrionicus* (5) is virtually identical to that of the **219A** alkaloid now isolated from *Dendrobates auratus*, both having a dominant fragment at m/z 178, representing loss of the 2-allyl moiety. The proton and carbon-13 magnetic resonance spectra of the **219A** alkaloids, however, are different. Analysis of phase-sensitive double-quantum filtered COSY spectra indicated that the **219A** alkaloid from *Dendrobates auratus* is a 2,5-diallyl-cis-decahydroquinoline, now to be referred to as cis-**219A**. The ^{1}H- and ^{13}C-NMR assignments for the two **219A** alkaloids and

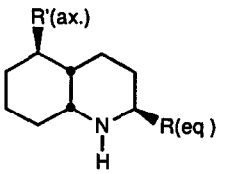
DECAHYDROQUINOLINES

CIS-FUSED:
(preferred conformations)



cis-195A : R = $CH_2CH_2CH_3$, R' = CH_3, R'' = H

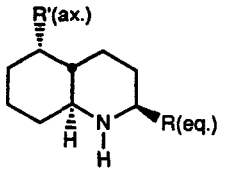
cis-211A : R = $CH_2CH_2CH_3$, R' = CH_3, R'' = OH



cis-219A : R = R'= $CH_2CH{=}CH_2$

cis-243A : R = $CH_2CH{=}CH_2$
R' = $CH_2CH{=}CHC{=}CH$ (Z)

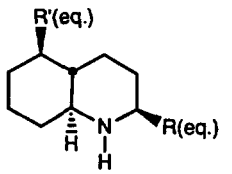
TRANS-FUSED:



trans-219A : R = R' = $CH_2CH{=}CH_2$

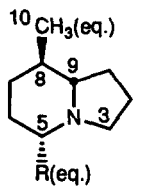
trans-243A : R = $CH_2CH{=}CH_2$
R' = $CH_2CH{=}CHC{=}CH$ (Z)

trans-253D : R = $CH_2CH{=}CH_2$
R' = $CH_2CH(OH)CH_2OH$



5-epi-trans-243A : R = $CH_2CH{=}CH_2$
R' = $CH_2CH{=}CHC{=}CH$ (Z)

5-SUBSTITUTED-8-METHYLINDOLIZIDINES



(5,9-Z)

B

203A : $CH_2CH{=}CHC{=}CH$ (Z)

205A : $(CH_2)_3C{=}CH$

207A : $(CH_2)_3CH{=}CH_2$

209B : *n*-C_5H_{11}

233D : $(CH_2)_3CH{=}CHCH{=}CH_2$ (Z)

235B : $(CH_2)_3CH{=}CHCH_2CH_3$ (Z)

235B' : $(CH_2)_5CH{=}CH_2$

251B : $(CH_2)_3CH{=}CHCH(OH)CH_3$ (Z)

Figure 1. Structures for cis- and trans-decahydroquinolines and 5-substituted-8-methylindolizidines from dendrobatid frogs.

other decahydroquinolines from dendrobatid frogs are given in Tables 1 and 2.

Analyses of FTIR spectra reveal that a trans-decahydroquinoline, such as trans-**219A**, has broader, more significant Bohlmann bands and sharp, single peaks at ~1100 and ~1300 cm^{-1}, while cis-fused decahydroquinolines, such as cis-**195A** and cis-**219A**, have less evident Bohlmann bands and show doublets of absorptions at ~1120 and ~1340 cm^{-1} (Fig. 2). The latter may be a consequence of two cis-fused conformations splitting two of the δCH absorptions (e.g. see cis-**195A**, cis-**219A** conformations in Fig. 4).

Table 1. Proton magnetic resonance assignments for cis- and trans-decahydroquinolines. Assignments were based on phase-sensitive DQF homo-nuclear shift correlation and HOHAHA spectroscopies. (Solvent $CDCl_3$, 40°C, 400 mHz). Abbreviations: a, axial-configuration; e, equatorial configuration; aA, axial-configuration with respect to A-ring (N-containing) of the decahydroquinoline; eA, equatorial configuration with respect to A-ring.

	cis-195A	cis-219A	cis-243A	trans-219A	trans-243A
Free Bases					
2	2.54a	2.90a	2.85a	2.56a	2.59a
3	1.10a, 1.35e	1.24a, 1.76e	1.16a, 1.72e	1.23a, 1.74e	1.23a, 1.75e
4	1.34a, 1.95e	1.78a, 1.42e	1.77a, 1.40e	1.39a, 1.53e	1.44a, 1.53e
4a	1.09eA	1.76aA	1.67aA	1.33a	1.35a
5	1.88a	1.64e	1.70e	1.71e	1.78e
6	0.94a, 1.66e	1.33a, 1.50e	1.26a, 1.52e	1.33, 1.75	1.36, 1.66
7	1.65a, 1.41e	~1.52	~1.54	~1.49	1.60a, 1.53e
8	1.52, 1.59	1.87a, 1.44e	1.86a, 1.35e	1.26a, 1.77e	1.25a, 1.75e
8a	2.85aA	3.17eA	3.16eA	2.48a	2.54a
9	1.34	2.17	~2.11	~2.17	2.17
10	1.35	5.76	5.75	5.74	5.76
11	0.91	5.07, 5.10	5.05, 5.08	5.05, 5.09	5.06, 5.10
12	0.83	~2.15	~2.45	1.97, 2.19	2.35, 2.41
13	——	5.77	6.00	5.70	5.94
14	——	4.98, 5.00	5.49	4.96, 4.99	5.47
16	——	——	3.05	——	3.08
Hydrochlorides					
2	2.94a	3.21a	3.22a	2.96a	2.95a
3	1.54a, 1.73e	1.65a, 1.96e	1.66a, 1.96e	1.76a, 2.00e	1.75a, 2.01e
4	1.41, 2.04	1.82a, 1.53e	1.84a, 1.55e	1.45a, 1.64e	1.48a, 1.65e
4a	1.37eA	2.16aA	2.15aA	2.03a	2.01a
5	2.06a	1.73e	1.82e	1.83e	1.91e
6	0.93a, 1.82e	1.41a, 1.53e	1.37a, 1.56e	1.46, 1.74	1.45, 1.64
7	2.36a, 1.45e	1.54a, 1.70e	~1.69	~1.46	~1.57
8	1.55a, 2.46e	1.81a, 2.08e	1.84a, 2.06e	1.83a, 2.38e	1.83a, 2.40e
8a	2.28aA	3.64eA	3.67eA	2.97a	2.99a
9	~2.14	2.51, 2.86	2.52, 2.87	2.55, 2.97	2.55, 3.00
10	1.23, 1.40	5.77	5.76	5.74	5.72
11	0.89	5.16, 5.19	5.15, 5.18	5.11, 5.15	5.10, 5.14
12	0.86	~2.20	2.35, 2.61	1.91, 2.16	~2.33
13	——	5.75	6.01	5.68	5.89
14	——	5.03, 5.05	5.52	4.99, 5.00	5.50
16	——	——	3.08	——	3.05

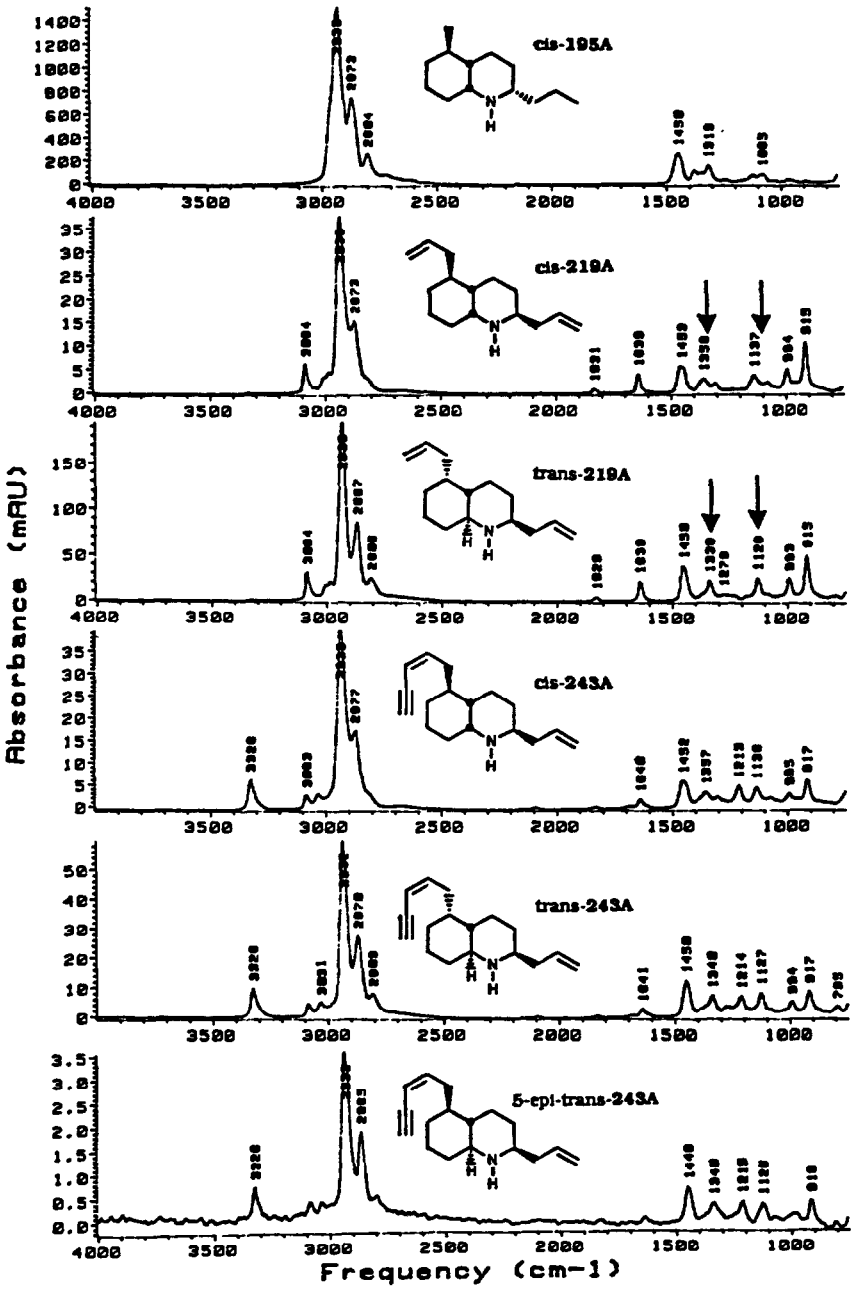


Figure 2. FTIR spectra for cis-195A, cis- and trans-219A, cis-243A and two trans-243A isomers. Characteristic absorptions are indicated.

The mass spectrum of trans-**243A** from *Dendrobates histrionicus* is virtually identical to that of the **243A** alkaloid isolated from *Dendrobates auratus* and both are dominated by a fragment at m/z 202, corresponding to loss of the 2-allyl moiety. An analysis of the ^{1}H- and ^{13}C-NMR spectra (Tables 1,2), as described above for cis-**219A**, showed that the **243A** alkaloid from *Dendrobates auratus* is a 2-allyl-5-(pent-2-en-4-ynyl)-cis-decahydroquinoline, now to be referred to as cis-**243A**. The ^{1}H-NMR spectra of cis-**243A** is shown in Fig. 3.

A coupling constant of about 4 Hz between 4aH and 8aH of decahydroquinolines indicates a cis ring-junction, e.g. the cis-fused decahydroquinoline cis-**195A** from *Dendrobates pumilio* (4,6) has a coupling constant of 4.5 Hz, while cis-**219A** and cis-**243A** both have constants of 4.0 Hz. The corresponding trans-decahydroquinolines, trans-**219A** and trans-**243A** from *Dendrobates histrionicus* (5), have coupling constants of about 10.5 Hz. The coupling constants, $J_{4a,8a}$, for the cis-decahydroquinolines were determined directly from the network of coupling constants deduced from 1D-HOHAHA and phase-sensitive double quantum filtered COSY spectroscopies and did not rely on indirect assignments using analogous compounds (9).

Further analysis of NMR spectra established the structures of cis-**219A** and cis-**243A** isolated from *Dendrobates auratus*. A 1D-HOHAHA NMR experiment (10) provides a simple method for conformational analysis of a complex matrix of homo-scalar couplings. The cis-decahydroquinoline cis-**195A**, whose structure was established by x-ray crystallographic analysis (4), served as a model for subsequent conformational analysis of cis-**219A** and cis-**243A**. The results of the 1D-HOHAHA experiments indicated that the hydrochloride of cis-**195A** in chloroform solution has an equatorial 2-propyl and an equatorial 5-methyl substituent (Fig. 4) since upon irradiation of 8aH, the magnetization was propagated evenly to 8H, 8'H (the primed hydrogen is upfield of the unprimed; this nomenclature has no configurational significance) and 4aH during the short mixing time, indicating very similar coupling constants between these hydrogens and 8aH and that the configuration of 8aH, therefore, is axial on the N-containing ring. Upon irradiation of 2H, the magnetization was propagated more effectively to 3'H than to 3H during the short mixing time, indicating that the configuration of 2H is axial and that the 2-propyl side chain, therefore, is equatorial. A 2H (eq.) hydrogen, equally coupled ($J \approx 3.5$ Hz) to both 3H (ax.) and 3H (eq.), would not show any difference in the propagation of magnetization (see Fig. 4).

For the hydrochlorides of cis-**219A** and cis-**243A** the results of the 1D-HOHAHA experiments indicated that an equatorial configuration is adopted by the 8a proton on the A ring (N-containing ring) (Fig. 4). Upon irradiation of 8aH, magnetization was propagated more readily to 8'H than to 8H and 4aH during the short mixing time. Upon irradiation of 2H in cis-**219A** and cis-**243A**, the magnetization was propagated more effectively to 3'H than to 3H, as was the case for cis-**195A**. Thus, an axial 2H and an equatorial 2-allyl substituent are indicated for cis-**219A** and cis-**243A**. Upon irradiation of 12'H, the magnetization was relayed evenly to 6H and 6'H through 5H, indicating that 5H

Table 2. Carbon-13 magnetic resonance assignments for cis- and trans-decahydroquinolines (free bases). Assignments were based on hetero- and homonuclear shift correlation spectroscopies (solvent $CDCl_3$, 40°C, 100 MHz). Values marked with an asterisk may be transposed. Certain assignments for carbons 3, 4, 6 and 8 of the trans-isomers have been revised from the earlier assignments (5). Both cis-219A and cis-243A gave spectra in which some signals were not detected, evidently due to the molecular motion intrinsic to this class of decahydroquinolines. The greater sample size of cis-219A permitted a CH-COSY spectrum to complete the assignments below.

Carbon	cis-195A	cis-219A	trans-219A	trans-243A	5-epi-trans-243A
2	57.8d	49.3d	56.1d	56.2d	56.0d
3	27.5t	31.4t	33.2t	33.0t	32.7t*
4	27.2t	25.4t	29.1t	29.1t	28.8t
4a	42.8d	39.2d	45.5d	45.4d	45.9d
5	27.5d	38.6d	37.8d	38.0d	41.0d
6	36.1t	25.1t	29.1t	29.7t	31.9t
7	21.3t	20.5t	19.3t	19.7t	24.5t
8	33.6t	27.1t	34.2t	34.0t	33.6t*
8a	56.1d	50.7d	55.5d	55.6d	61.5d
9	39.9t	40.6t	41.6t	41.4t	41.4t
10	19.2t	135.3d	135.7d	135.6d	135.5d
11	14.3q	117.3t	117.1t	117.2t	117.2t
12	19.9q	37.2t	31.2t	27.9t	33.3t
13	----	137.9d	138.8d	146.1d	144.2d
14	----	115.6t	115.1t	108.7d	109.0d
15	----	----	----	80.7s	80.6s
16	----	----	----	81.3d	81.3d

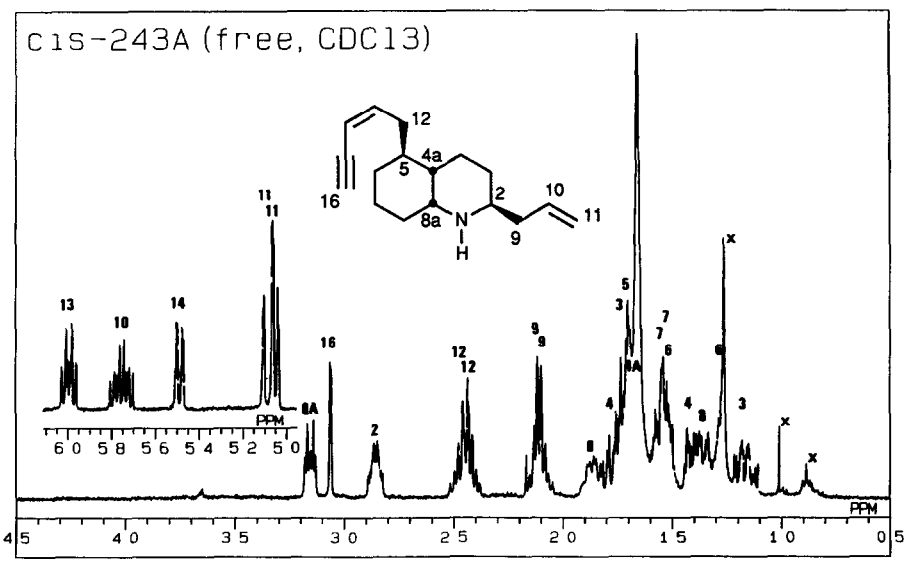
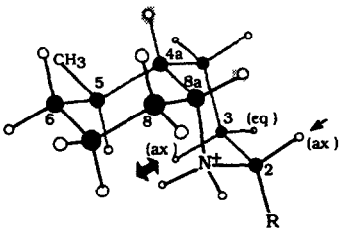


Figure 3. Proton-NMR spectrum of cis-243A. (Solvent $CDCl_3$, 40°C, 400 MHz). Resonance peaks marked X are due to impurities.

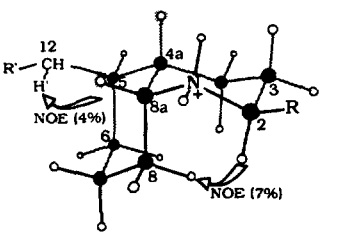
is equatorial and, therefore, that the 5-enyne side chain has an axial configuration. The structures in Fig. 4 are proposed for the hydrochlorides of cis-**219A** and cis-**243A**. Nuclear Overhauser effects (NOE) between 2H and 8'H, 12'H and 8aH on phase-sensitive NOESY spectra support these structures. There is a 4% signal enhancement of 12'H upon irradiation of 8aH (distance between 8aH and 12'H is about 1.8 A with computer molecular models) and a 7% signal enhancement of 8'H upon irradiation of 2H (distance is about 1.4 A). It should be noted that the configurations of cis-**219A** and cis-**243A** coincide with all relevant centers of the tricyclic dendrobatid alkaloid, gephyrotoxin **287C** (11). A GC-FTIR spectrum of cis-**243A** shows the two split bands at ~1120 and ~1340 cm^{-1} typical for the cis-fused decahydroquinoline class (Fig. 2).



cis-**195A** R = $-CH_2CH_2CH_3$

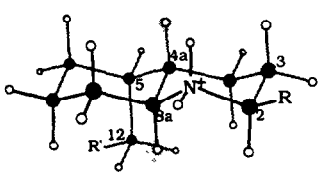
$J_{2ax,3ax} \cong 14Hz$
$J_{2ax,3eq} \cong 3.5Hz$

HOHAHA: irradiation at 2ax causes 3ax to increase rapidly then decrease rapidly



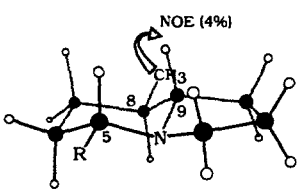
cis-**219A** R = $-CH_2CH{=}CH_2$
R' = $-CH{=}CH_2$

cis-**243A** R = $-CH_2CH{=}CH_2$
R' = $-CH{=}CHC{\equiv}CH$ (Z)

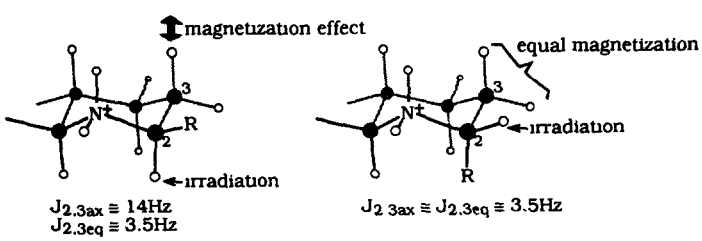


trans-**219A** R = $-CH_2CH{=}CH_2$
R' = $-CH{=}CH_2$

trans-**243A** R = $-CH_2CH{=}CH_2$
R' = $-CH{=}CHC{\equiv}CH$ (Z)



203A, **233D**, **235B**, **251B**, etc. (see Fig. 1)



$J_{2,3ax} \cong 14Hz$
$J_{2,3eq} \cong 3.5Hz$

$J_{2\ 3ax} \cong J_{2,3eq} \cong 3.5Hz$

Figure 4. Configurations of cis- and trans-decahydroquinolines and 5-substituted-8-methylindolizidines from dendrobatid frogs. 1D-HOHAHA experiments were used as indicated to determine configurations.

Alkaloid fractions of *Dendrobates histrionicus* were previously shown (5) to contain, in addition to trans-**219A** and trans-**243A**, minor isomers (**219A'**, **243A'**) that emerge slightly before trans-**219A** and trans-**243A** on the packed OV-1 gas chromatographic columns used for routine analysis of dendrobatid alkaloids since the early seventies. Another alkaloid of molecular weight 243 with a mass spectrum identical to cis- or trans-**243A** was present in sufficient quantities in *Dendrobates histrionicus* to allow isolation by HPLC and was shown by NMR to be a trans-fused decahydroquinoline (5). It was initially but mistakenly assumed to be **243A'**, the **243A** isomer of shorter GC retention time. A more recent study of this same extract by capillary gas chromatography (HP-5 column, 25 m x 0.32 mm) indicates that there are <u>two</u> major trans-**243A** isomers, <u>both</u> of which emerge at longer retention times than a third, minor **243A** isomer, which undoubtedly corresponds to the **243A'** observed earlier (5). The two major trans-**243A** isomers have nearly identical FTIR spectra with significant Bohlmann bands and single absorptions at ~1300 and ~1100 cm^{-1} (Fig. 2), confirming the trans ring-fusion assigned by ^{1}H-NMR analyses. Barely separated on capillary GC, they chromatograph as a single peak on packed columns.

Analysis of ^{1}H- and ^{13}C-NMR data for the two trans-**243A** isomers and trans-**219A** (Tables 1,2) indicated that one of the trans-**243As**, the isomer eluting last from the capillary GC columns, and trans-**219A** were very similar. In particular, the δ_C for C(5), C(7) and C(8a) of this isomer and trans-**219A** were all shifted upfield significantly, most probably by the operation of a γ-effect manifested by a common 5-axial substituent. The δ_H for H(2), H(5), H(4a) and H(8a), among others, were also very similar. Consequently we assign to this trans-**243A**, the same 2S,5R configuration as established for trans-**219A** by X-ray diffraction (5). It is tacitly assumed that the 4aS and 8aS configurations established for trans-**219A** are maintained in the trans-**243A** decahydroquinolines. The earlier eluting trans-**243A** isomer of the barely-resolved pair is assigned the 2S,5S configuration. To avoid any further confusion, we will henceforth refer to these materials as trans-**243A** and 5-epi-trans-**243A** (the latter originally and incorrectly termed **243A'**), respectively for the longer and the shorter retention time compounds, retaining the nomenclature **243A'** for the time being for the minor isomer of much shorter retention time, which has yet to be isolated.

<u>5-Substituted-8-methylindolizidines</u>

A major subclass of indolizidine alkaloids from dendrobatid frogs is that of the 5-substituted-8-methylindolizidines (2,3). 8-Methylindolizidines with a five carbon linear chain at the 5-position occur in many species of dendrobatid frogs. Structures for indolizidines **205A** and **207A** have been proposed, based on NMR analyses, as 5-(pent-4-ynyl)-8-methylindolizidine (6) and 5-(pent-4-enyl)-8-methylindolizidine (7,8), respectively, both with 5-equatorial substituents and an 8-equatorial methyl group (Fig. 1). The structures have been confirmed by synthesis (12,13). Indolizidine **203A**, now isolated from *Dendrobates auratus*, is clearly closely related in structure to **205A** and **207A**. The base

peak at m/z 138 in the mass spectra of **203A**, **205A** and **207A** is due in each case to α-cleavage of a five-carbon side chain at the 5-position. The ^{1}H-NMR spectrum of **203A** indicated that the side chain at C(5) was a cis-pent-2-en-4-ynyl moiety (data not shown).

The ^{13}C-NMR chemical shifts of the ring carbons and the 8-methyl group of **203A** coincide with those of another 5-substituted 8-methylindolizidine, **235B** (Table 3), for which a structure (Fig. 1) having an equatorial 5-hept-4-enyl side chain and an equatorial 8-methyl group has been proposed (6) and confirmed by synthesis (12,13). 1D-HOHAHA and NOE experiments also confirmed an equatorial configuration for the 8-methyl in **235B** hydrochloride: Upon irradiation of the 8-methyl, magnetization was transferred to 7H and 9H and with a slight delay to 7'H (Fig. 4). A 4% signal enhancement of 9H occurred upon irradiation of the 8-methyl. The unusually low δ value for 8H (1.8 ppm, free base in chloroform) and small $J_{C,H}$ values for 5C and 10C (128 Hz and 129 Hz, respectively, free base in chloroform) are consonant with values reported as characteristic of indolizidines with a trans (E) arrangement of 5H and 8H (14). Confirmation of the equatorial conformation of the side chain at C(5) of **235B**·HCl was obtained through 2D ^{13}C-^{1}H HOHAHA experiments in which 5H magnetization was transferred to axial 6H (δ1.24) and with a slight delay to equatorial 6H (δ1.74), thereby indicating a 5-axial hydrogen. 1D-HOHAHA spectroscopy was not possible due to overlapping of the 3'H and 5H signals. The correspondence of chemical shifts of the ring carbons and the 8-methyl groups of **235B** and **203A** established that these alkaloids had the same relative configuration and further NMR experiments with **203A** were not considered necessary.

Table 3. Carbon-13 magnetic resonance assignments for 5-substituted-8-methyl-indolizidines. Assignments were based on hetero- and homo-nuclear shift correlation spectroscopies. (Solvent $CDCl_3$, 40°C, 100 MHz).

Carbon No.	**203A**	**205A**	**233D**	**235B**	**251B**
1	29.3t	28.9t	29.7t	29.1t	30.9t
2	20.7t	20.4t	20.2t	20.4t	20.3t
3	52.1t	51.6t	51.6t	51.8t	51.7t
5	63.0d	63.2d	63.7d	63.4d	63.5d
6	31.7t	31.3t	31.9t	31.3t	32.3t
7	33.8t	33.3t	33.4t	33.7t	33.5t
8	36.6d	36.0d	35.7d	36.5d	36.2d
9	71.5d	71.6d	71.8d	71.3d	71.5d
10 (CH_3)	19.1q	18.9q	18.8q	18.8q	18.8q
11	35.9t	33.5t	34.2t	34.2t	33.9t
12	142.6d	24.8t	25.8t	25.9t	25.3t
13	109.5d	18.8t	27.8t	27.4t	28.9t
14	80.5s	84.4s	129.7d	128.9d	130.6d
15	81.6d	68.6d	132.2d	131.7d	134.6d
16	----	----	132.2d	20.5t	68.9d
17	----	----	117.1t	14.3q	23.4q

The ^{13}C-NMR chemical shifts for two other 5-substituted-8-methyl indolizidines, **233D** and **251B**, isolated from further fractionation of extracts from *Dendrobates pumilio* (Isla Bastimentos, Bocas, Panama 1983, see ref. 6) are included in Table 3. The 5,9-Z configuration for these 5-substituted-8-methylindolizidines is supported also by GC-FTIR analyses. Indolizidines **203A**, **235B** and **251B** all show the same pattern of significant Bohlmann bands in the 2900-2600 cm^{-1} region in their FTIR spectra (see Fig. 5 for FTIR of **203A**). This is typical of the 5,9-Z configuration but not the 5,9-E configuration as shown in Fig. 5 for two synthetic 5-hexylindolizidines (15). The structures of **233D** and **251B**, based on mass spectra, FTIR spectra and NMR spectra, and on analogy to **235B** (see above), are shown in Fig. 1. An isomer of indolizidine **235B** with a hept-6-enyl side chain has also been isolated and characterized by NMR analysis (7,8). The structure of this isomer, **235B'** also is shown in Fig. 1, as is the structure of indolizidine **209B**, a trace alkaloid isolated in quantities insufficient for NMR analysis. Both **235B'** and **209B** have been synthesized (12,13).

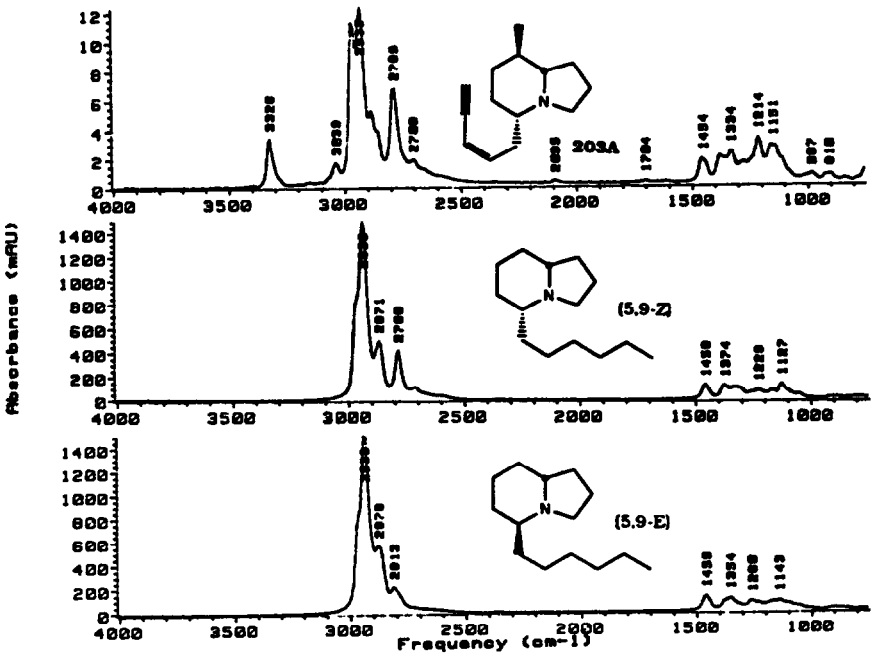


Figure 5. FTIR spectra of indolizidine **203A** and of synthetic cis- and trans-isomers of 5-hexylindolizidine (15).

EXPERIMENTAL

High-resolution mass spectral data were obtained on JEOL D-300 mass spectrometer (electron impact, 70 eV). Gas chromatography-mass spectral analyses were with a 2% OV-225 Chromosorb G AW-DMCS column programmed from 100° to 250° at a rate of 10°/min (electron impact, 30 eV). Some analyses were on capillary columns (25 m x 0.32 mm, either OV-1 or HP-5). Nuclear magnetic resonance spectra were obtained on JEOL FX-100 and GX-400 spectrometers. Phase-sensitive 2D-NMR, homo- and hetero-nuclear shift correlation spectroscopies were routinely used for structure analysis. HOHAHA and NOE effects were observed through 2D-spectra and 1D-differential spectra. Gas chromatographic infrared spectra were obtained on a Hewlett-Packard model 5965A FTIR instrument with a narrow band (4000-750 cm^{-1}) detector. A 59970 IRD ChemStation was used to record FTIR spectra of GC peaks. A Hewlett-Packard model 5890 gas chromatograph fitted with an HP-5 (bonded 5% diphenylsiloxane : 95% dimethylsiloxane) fused silica capillary column (30 m x 0.32 mm) programmed from 100° to 280° at 10°/min was used to generate the total response chromatogram.

Separation and Purification of Alkaloids

A methanol extract from skins of *Dendrobates auratus* (1000 skins, Isla Taboga Panama, 1976) was concentrated *in vacuo* and made acidic (pH ca. 1.5) with dilute hydrochloric acid. The solution was passed through an XAD-2 column, which was washed with water. The effluent and wash were combined, made alkaline with aqueous ammonia, and extracted first with hexane and then with chloroform. The hexane extract was evaporated *in vacuo* to yield 0.21 g, which was chromatographed by HPLC on a DIOL column using hexane: chloroform:triethylamine (80:20:1) to yield eight fractions. Each fraction was examined by GC-mass spectrometry to determine constituent alkaloids: fraction 1 (28 mg) **195A**, **223A** and **223B**; fraction 2 (54 mg) **195A**, **203A**; fraction 3 (7 mg) **195A**, **307A**; fraction 4 (32 mg) **219A**, **235A**; fraction 5 (13 mg) mainly **259**; fraction 6 (22 mg) **253A**, **267A**; fraction 7 (14 mg) mainly the O-methyl ether of **323B**, mol. wt. 337 (16); fraction 8 (40 mg) a complex mixture. (For structures and/or properties of dendrobatid alkaloids see ref. 3). Rechromatography of fraction 2 on a DIOL-column using hexane containing 0.3% triethylamine gave pure cis-**195A** (22 mg) and **203A** (8 mg). Rechromatography of fraction 4 on a DIOL column using hexane:chloroform:triethylamine (95:5:1) gave pure cis-**219A** (14 mg) and **235A** (13 mg). The chloroform extract was evaporated *in vacuo* to yield a residue (0.30 g), which afforded a mixture of **339A** and **339B** (80 mg) and **323A** (84 mg) after chromatography on an RP-8 column with acetone:water:triethylamine (30:70:1). The original XAD-2 column was washed further with methanol and then with methylene chloride to remove additional alkaloids. These eluents were combined and evaporated *in vacuo*. The residue was partitioned between 0.1 N HCl and methylene chloride. The acidic layer was made alkaline and extracted with hexane. The hexane extract was evaporated *in vacuo* to yield 0.13 g, which was chromatographed on a DIOL-column with hexane:dioxane:triethylamine (95:5:1) to afford almost pure alkaloids: cis-**195A** (31 mg), cis-**219A** (36 mg) and cis-**243A** (35 mg).

Fractionation of methanol extract from skins of *Dendrobates pumilio* has been described (6). Further purification of fractions on a DIOL column led to the isolation of indolizidines **233D** (3 mg) and **251B** (8 mg).

Alkaloids of Dendrobates auratus

The structures and properties of decahydroquinoline cis-**195A**, pumiliotoxins **307A** and **323A**, allopumiliotoxins **267A**, **323B**, **339A** and **339B** and histrionicotoxin **259**, have been previously reported (4,6,17). The structure of histrionicotoxin **235A**, previously proposed based on mass spectral analysis (2), has been confirmed by NMR analysis as will be reported separately. A tentative structure for allopumiliotoxin **253A** has been proposed (2). The structure of **223A** is still under investigation. The structure of **223B**, a 3,5-dibutylpyrrolizidine, will be reported elsewhere.

The empirical formulae as determined by high resolution mass spectrometry, the electron impact mass spectra with intensities relative to a base peak set equal to 100 in parentheses and other data on the cis-**219A**, cis-**243A**, **203A**, **233D** and **251B** are as follows:

Decahydroquinolines

cis-**219A**, $C_{15}H_{25}N$, m/z 219 (<1), 218 (<1), 179 (15), 178 (100), 67 (10). $[\alpha]_D$, +5.8 (c 0.31, $CHCl_3$).
cis-**243A**, $C_{17}H_{25}N$, m/z 243 (1), 242 (2), 203 (10), 202 (100), 178 (27), 91 (28). $[\alpha]_D$, +10.1 (c 2.4, $CHCl_3$, HCl salt).

8-Methylindolizidines

203A, $C_{14}H_{21}N$, m/z 203 (<1), 138 (100). $[\alpha]_D$, -23.3° (c 0.30, $CHCl_3$).
233D, $C_{16}H_{27}N$, m/z 233 (5), 232 (2), 176 (2), 164 (8), 151 (22), 138 (100). $[\alpha]_D$, -3.4° (c 0.16, CH_3OH, HCl salt).
251B, $C_{16}H_{29}NO$, m/z 250 (<1), 234 (<1), 138 (100). $[\alpha]_D$, + 25.9° (c 0.8, $CHCl_3$).

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16. The allylic hydroxyl in the side chain of pumiliotoxin **307A** and allopumiliotoxin **323B** has been found to undergo facile racemization and facile methanolysis under acidic conditions. Thus, the O-methyl ethers of these two alkaloids with mol. wts of 321 and 337, respectively are artefacts formed during isolation. Properties of the 15-O-methyl ether of **307A** (originally referred to as pumiliotoxin 321) have been reported (6), while those of the 15-O-methyl ether of **323B** will be reported separately.

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