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HISTRIONICOTOXINS

CARBON-13 MAGNETIC RESONANCE SPECTRAL ASSIGNMENTS AND STRUCTURAL DEFINITION OF FURTHER ALKALOIDS FROM POISON FROGS (DENDROBATIDAE)

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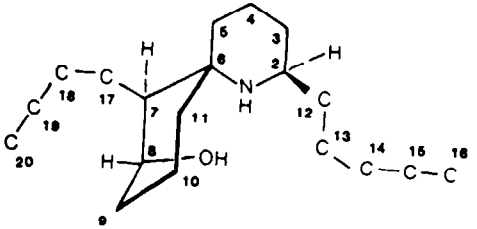
Abstract—Structures of three further members of the histrionicotoxin class (2,7-disubstituted 1-azaspiro[5.5]undecan-8-ols) of dendrobatid alkaloids are defined based on mass and NMR properties. ^{13}C NMR assignments for histrionicotoxin, six natural congeners, perhydrohistrionicotoxin and 8-deoxyperhydrohistrionicotoxin are presented.

Histrionicotoxins, a group of unique 2,7-disubstituted-1-azaspiro[5.5]undecan-8-ol alkaloids occur in skin of poison frogs of the neotropical family Dendrobatidae.[1-4] The structures and absolute configuration of the parent compound histrionicotoxin and of a congener dihydroisohistrionicotoxin were determined by X-ray crystallographic analysis.[1,5] Subsequently, the structures of six further congeners were defined based on PMR spectra.[2,3] These congeners differ only in the degree and nature of unsaturation in the five C side chain at position-2 and the four C side chain at position 7. The empirical formulae ranged from histrionicotoxin $C_{19}H_{25}NO$, to octahydrohistrionicotoxin $C_{19}H_{33}NO$. It would appear likely that all of the histrionicotoxins have the same absolute configuration; histrionicotoxin and four congeners were found to be levorotatory.[6] A survey of alkaloids in dendrobatid frogs revealed the presence of two further compounds which appeared to belong to the histrionicotoxin class of azaspiro[5.5]undecan-8-ols based on mass spectral fragmentation patterns.[4] The empirical formulae of these two compounds were $C_{15}H_{25}NO$ and $C_{17}H_{25}NO$ and they were designated, respectively, as alkaloid **235A** and **259**. It appeared likely that the first, histrionicotoxin **235A**, contained a $-CH_2-CH{=}CH_2$ substituent at position-2 and a $-CH{=}CH_2$ substituent at position-7, while the second, histrionicotoxin **259**, contained a $-CH_2-CH{=}CH_2$ substituent at position-2 and a $-CH_2-CH{=}CH-C{\equiv}CH$ substituent at position-7. ^{13}C and mass spectral assignments for the histrionicotoxins and definitive structures for histrionicotoxin **259** and for two further histrionicotoxins are now reported. The synthesis of 8-deoxyperhydrohistrionicotoxin is presented and the possible occurrence of deoxyhistrionicotoxins in skin of dendrobatid frogs is discussed. Histrionicotoxins because of their potent effects on chemosensitive and voltage dependent channels in nerve and muscle have become invaluable research tools for the investigation of molecular mechanisms involved in the function of such channels.[7,8] Alterations in substituents affect the potency and selectivity of histrionicotoxins at voltage-dependent sodium and potassium channels and acetylcholine receptor controlled channels in neuromuscular preparations.[9] Thus, definition of further structural analogs may provide more selective pharmacological agents and a better understanding of structure activity correlations.

Carbon-13-magnetic resonance spectral assignments for histrionicotoxins. Assignments for carbons 2, 6, 7, 8 and the unsaturated carbons of the side chains of histrionicotoxins are unambiguous. Assignments of the methylene carbons were facilitated by selective proton decoupling experiments. The ^{13}C resonance assignments are provided in Table 1 for histrionicotoxin, dihydrohistrionicotoxin, isodihydrohistrionicotoxin, neodihydrohistrionicotoxin, allodihydrohistrionicotoxin, Δ^{17}-trans-histrionicotoxin, isotetrahydrohistrionicotoxin, octahydrohistrionicotoxin, the semi-synthetic dodecahydrohistrionicotoxin and histrionicotoxin 259. It should be noted that some of the assignments to the methylene carbons are not unambiguous. Dihydrohistrionicotoxin and the Δ^{17}-*trans*-isomer of histrionicotoxin represent the eleventh and twelfth alkaloids of the histrionicotoxin class to be found in skin extracts of dendrobatid frogs (*vide infra*).

Dihydrohistrionicotoxin. Pharmacological studies with histrionicotoxins required isolation of further amounts of *l*-histrionicotoxin and its congeners from skin extracts of *Dendrobates histrionicus.* The relative amounts of the various histrionicotoxins and gephyrotoxins isolated from the most recent skin extracts from one population of *Dendrobates histrionicus* are provided in Table 2. Two new histrionicotoxins were obtained during this isolation. These were dihydrohistrionicotoxin and $^{17}\Delta$-*trans*-histrionicotoxin (*vide infra*), which are relatively minor

Table 1. Carbon–13 magnetic resonance assignments for histrionicotoxin (HTX) and congeners. Resonance peaks designated with a superscript a may be interchanged as may peaks designated with a superscript b. All spectra were in $CDCl_3$ except for isotetrahydro-HTX which was in CD_3OD



Carbon Number	HTX	Dihydro-HTX	Isodihydro-HTX	Neodihydro-HTX	Allodihydro-HTX	Δ^{17}-trans-HTX	Isotetrahydro-HTX	Octahydro-HTX	Dodecahydro-HTX	HTX 259
2	49.7	50.1	49.2–49.7	50.0	49.6	50.0	51.8	50.0	50.3	49.6
3	36.8	35.3	36.0–36.8	36.8	36.3	37.1	37.0	36.9	36.6	37.0
4	19.5	19.7	18.9–19.5	19.5	19.2	19.1	20.3	19.5	19.5	19.6
5	29.0	29.2	28.5–29.1	28.7	28.8	28.8	29.1	27.7	27.6	29.1
6	54.1	54.4	53.8–54.3	54.4	54.3	54.2	56.1	55.4	55.8	54.4
7	41.4	41.7	40.9–41.6	38.8	41.2	44.9	39.5	37.7	38.1	41.6
8	71.3	71.6	70.8–71.5	72.4	71.2	72.6	73.6	69.6	69.7	71.5
9	32.8	32.6	32.4–33.0	32.9	32.7	32.8	32.8	33.1	30.3	32.5
10	15.0	15.3	14.7–15.3	15.2	15.0	15.2	16.2	15.2	15.2	15.3
11	37.8[a]	37.1	37.5–38.4	37.5	37.8	37.6	37.5	36.9	37.4	38.1
5-Carbon side chain										
12	37.9[a]	38.2	36.2–37.1	38.0	36.5	37.9	36.2	36.9[a]	36.6	41.6
13	141.6[b]	128.1	23.9–24.5	141.7	18.3	141.6	25.7	25.6[a]	25.6	134.6
14	110.0[b]	131.6	89.2–89.8	110.6	24.5	111.2	90.3	32.2	32.7	117.5
15	80.3	132.0	207.9–208.4	80.5	83.9	80.5	209.7	138.5[b]	22.5[a]	—
16	81.7	117.8	74.7–75.2	81.9	68.5	81.9	75.2	114.9[b]	14.1	—
4-Carbon side chain										
17	142.9[b]	143.1	142.3–143.1	129.8	142.6	144.1	129.9[a]	34.0[a]	32.1	143.1
18	110.3[b]	110.2	109.6–110.1	131.8	110.0	110.8	132.8[a]	27.4[a]	27.6	110.2
19	79.7	80.0	79.3–79.9	130.8	79.6	82.1	132.4[a]	138.5[b]	23.0[a]	79.9
20	82.6	82.7	82.3–82.5	118.6	82.6	76.9	119.3	114.6[b]	14.1	82.6

Table 2. Isolation of dendrobatid alkaloids from skin extracts of *Dendrobates histrionicus*

Histrionicotoxin (HTX)	136 mg
Isodihydro-HTX	48 mg
Neodihydro-HTX	10 mg
Allodihydro-HTX	30 mg
Dihydro-HTX	6 mg
Δ^{17}-trans-HTX	1 mg
Gephyrotoxin	24 mg

Alkaloid fractions were prepared from one thousand skins of frogs collected near Guayacana, Narino, Colombia (for methodology see ref. 3). The alkaloids were isolated from a reversed phase silica gel column (Merck, RP-8 size B) using mixed solvent of acetonitrile:tetrahydrofuran:methanol:triethylamine:water (45:12:24:0.6:40). For isolations of alkaloids from the same population of frogs collected in other years see ref. 1,2,3. The histrionicotoxin was obtained crystalline, mp 79-80° and gave a satisfactory analysis for carbon, hydrogen, and nitrogen. The gephyrotoxin was levorotatory $[\alpha]_D^{25}$ -51.5° (see ref. 10).

constituents. The mass spectrum and proton and ^{13}C NMR of dihydrohistrionicotoxin confirm its structure (Table 3). Dihydrohistrionicotoxin like other histrionicotoxins[6] is the *l*-enantiomer. The optical rotation (HCl salt) was $[\alpha]D^{25}$ $-122°$ ($c = 1.0$, C_2H_5OH). Dihydrohistrionicotoxin was previously obtained as a partial reduction product from histrionicotoxin using Lindlar's Pd catalyst with quinoline.[2] The mass spectral data and chromatographic properties of synthetic dihydrohistrionicotoxin[2] proved identical to the natural compound.

Δ^{17}-trans-*Histrionicotoxin.* An isomer of histrionicotoxin was obtained as a trace constituent from the most recent isolation of alkaloids from skin extracts of *Dendrobates histrionicus* (Table 2). This compound was shown by analysis of PMR and ^{13}C NMR to be the *trans*-isomer of histrionicotoxin at the double bond of the four C side chain. The ^{13}C and PMR assignments are in Tables 1 and 3. The optical rotation of Δ^{17}-*trans*-histrionicotoxin was not measured because of the limited amount available.

The mass spectrum (30 eV) of Δ^{17}-*trans*-histrionicotoxin was as follows: *m/z* 283(26), 282(12), 266(11), 264(5), 218(60), 200(21), 190(8), 188(11), 174(14), 160(40), 124(14), 96(100). The R_f on silica gel thin-layer chromatography was 0.80 compared to an R_f for histrionicotoxin of 0.71 with a mixed solvent of

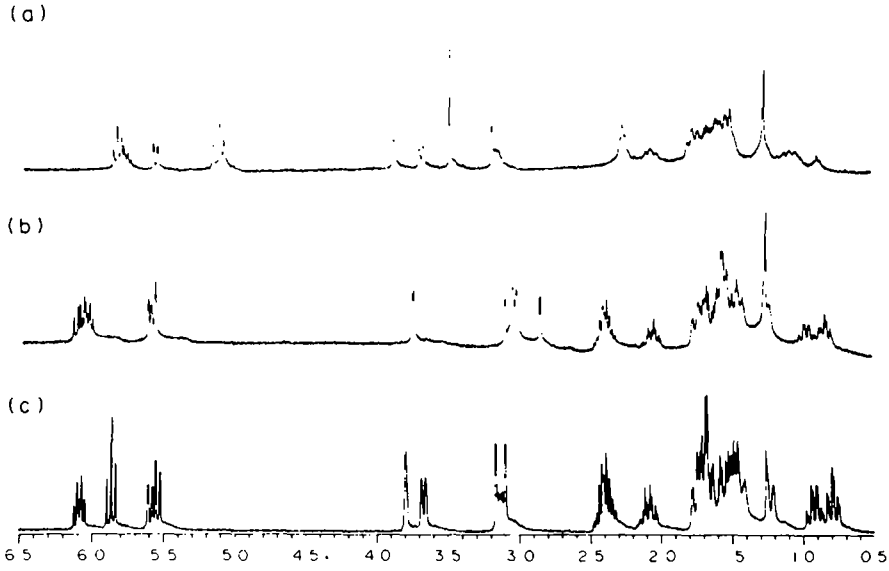
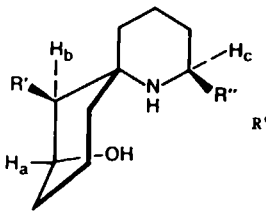


Fig. 1. Proton magnetic resonance spectra (360 MHz) for (a) Alkaloid 259, (b) Δ^{17}-*trans*-Histrionicotoxin, (c)-Histrionicotoxin. Chemical shifts in δ. Solvent $CDCl_3$ with a tetramethylsilane standard.

Table 3. Proton magnetic resonance spectral assignments for histrionicotoxin (HTX) and congeners. Chemical shifts in δ solvent $CDCl_3$ with a tetramethylsilane standard. Coupling constants (J) in parentheses. br, broad; m, multiplet; d, doublet; t, triplet; d, d, doublet of doublets



	HTX	Δ^{17}-trans-HTX	DihydroHTX[a]	HTX-259
H_a	3.76[br]	3.79[br]	3.78[br]	3.78[br]
H_b	3.66[d,br](11)	3.03[d,br]	3.68[d,br](11)	3.70[d,br]
R'	CH 5.87[d,d](11,10) ‖ CH 5.52[d,d](11,2) | C ≡ C | H 3.20[d](2)	HC 6.12[d,d](16,10) ‖ CH 5.58[d,d](16,2) | C ≡ C | H 2.84[d](2)	CH 5.87[d,d](11,10) ‖ CH 5.52[d,d](11,2) | C ≡ C | H 3.17[d](2)	CH 5.87[d,d](11,11) ‖ CH 5.52[d,d](11,2) | C ≡ C | H 3.15[d](2)
H_c	3.10[br]	3.0[br]	3.10[br]	3.00[br]
R''	CH_2 2.38[d,d](8,8) | CH 6.08[d,t](8,11) ‖ CH 5.57[d,d](11,2) | C ≡ C | H 3.13[d](2)	CH_2 2.37[d,d](8,8) | CH 6.05[d](8,11) ‖ CH 5.58[d,d](11,2) | C ≡ C | H 3.09[d](2)	CH_2 2.26[d,d](7,7) | CH 5.50[d,t](7,11) ‖ CH 6.14[d,d](11,11) | CH 6.63[d,d,d](11,10,16) ‖ CH_2 5.13[d](10) 5.22[s](16)	CH_2 2.11[m] | CH 5.4[m] ≡ CH_2 5.13[d,br] 5.04[d,br]

Chloroform:isopropanol:aqueous ammonia (14:1:0.1). It is unknown whether this *trans*-isomer occurs naturally or represents an isomerization product formed from histrionicotoxin during isolation.

Histrionicotoxin **259**

An alkaloid $C_{17}H_{25}NO$ was detected in extracts from various *Dendrobates* species (*D. histrionicus, D. auratus, D. granuliferous, D. occultator, D. tinctorius, D. trivittatus, D. truncatus*) and was proposed to be a histrionicotoxin based primarily on its mass spectrum containing a major fragment at *m/z* 218 (loss of $-C_3H_5$) and a base peak at *m/z* 96 ($C_6H_{10}N$).[4] The compound formed an octahydro-derivative which now showed a major fragment in its mass spectrum at *m/z* 224 (loss of $-C_3H_7$). This alkaloid was isolated in sufficient quantities from skin extracts of *Dendrobates auratus* (Isla Taboga, Panama) to permit analysis of PMR and ^{13}C NMR and confirm the proposed structure as 2-allyl-7-(1,3-butadienyl)-1-azaspiro[5.5]undecan-8-ol. The PMR assignments and the ^{13}C assignments are in Tables 3 and 1, respectively. Details of isolation of histrionicotoxin **259** and other alkaloids from skin extracts of *Dendrobates auratus* will be published elsewhere. Histrionicotoxin **259** represents the first member of the histrionicotoxin alkaloids to contain a three rather than a five C side-chain at position-2. Histronicotoxin **235A** also appears to contain a three C side-chain at position-2[4] but sufficient material for confirmation of the proposed structure by magnetic resonance spectroscopy has not been obtained.

Deoxyhistrionicotoxins. Five major classes of alkaloids have been defined from dendrobatid frogs: (i) batrachotoxins (complex steroidal alkaloids); (ii) pumiliotoxin C classś (2, 5-disubstituted decahydroquinolines); (iii) histrionicotoxins (2,7-disubstituted 1-azaspiro [5.5]undecan-8-ols); (iv) tricyclic gephyrotoxins (6-substituted dodecahydropyrrolo[1, 2α]quinoline-1-ethanols); (v) bicyclic gephyrotoxins (3, 5-disubstituted indolizidines); (vi) pumiliotoxin-A class (8-hydroxy-8-methyl-6-alkylidene-1-azabicyclo[4.3.0]nonanes).[10] It appears likely based on biosynthetic considerations that deoxyhistrionicotoxins might be present in skin of dendrobatid frogs. In order to provide a reference for such compounds 8-deoxyperhydrohistrionicotoxin has been prepared and characterized by magnetic resonance spectroscopy and mass spectrometry. Histrionicotoxin was reduced, dehydrated and reduced again to yield 8-deoxyperhydrohistrionicotoxin as described in the Experimental.

The mass spectrum of the deoxyperhydrohistrionicotoxin was dominated by fragments at *m/z* 180 and 167 undoubtedly $C_{12}H_{22}N$ and $C_{11}H_{21}N$ as in perhydrohistrionicotoxin itself.[3] A major fragment at *m/z* 96, $C_6H_{10}N$, typical of histrionicotoxins was also present. Further studies will be required to demonstrate whether or not any dendrobatid alkalids with unassigned structures exhibit these spectral properties.

The ^{13}C NMR properties and tentative assignments for the synthetic deoxy compounds are as follows:

8-deoxydecahydrohistrionicotoxin (8,9-ene). ^{13}C NMR ($CDCl_3$, δ values). C-2, 50.2; C-3, 34.2; C-4, 20.0; C-5, 32.8; C-6, 53.5; C-7, 35.8; C-8, 129.0; C-9, 126.6; C-10, 21.7; C-11, 32.2; C-12, 37.0; C-13, 25.6; C-14, 31.8; C-15, 22.7; C-16, 14.2; C-17, 31.5; C-18, 29.9; C-19, 23.2; C-20, 14.2 (see Table 1 for assignments of histrionicotoxins).

8-deoxyperhydrohistrionicotoxin. ^{13}C NMR ($CDCl_3$, δ values). C-2, 49.0; C-3, 35.9; C-4, 21.0; C-5, 33.4; C-6, 53.4; C-7, 32.7; C-8, 19.8; C-9, 22.5; C-10, 19.7; C-11, 35.2; C-12, 37.9; C-13, 25.9; C-14, 32.1; C-15, 22.7; C-16, 14.3; C-17, 26.9; C-18, 30.7; C-19, 23.1; C-20, 14.2. Assignments for C-16 and C-20 and for C-8 and C-10 may be interchanged.

EXPERIMENTAL

Mass spectra-gas chromatographic analyses were on a JEOLCO D-300 in electron impact mode at 30 eV. Gas chromatographic analyses were with a 2% OV-1 on Chromosorb WAW DMSC column with programing at 10° per min from 150°. High-resolution mass spectral data were obtained on JEOL D-300 mass spectrometer electron impact (70 eV). NMR were obtained on JEOL FX-100 or FX-60 spectrometer. PMR were determined at 90.60 MHz using a 16 K Fourier transform and 1 KHz spectra range for a digital resolution of 0.12 Hz. Typically, free induction decays from a 45° pulse were collected at 6 sec intervals. Carbon-13 spectra were determined at 25.05 MHz using a 16 K or 8 K Fourier transform and 5 KHz spectra range for a digital resolution of 0.61 Hz or 1.22 Hz. Typically, 2000 free induction decays from a 45° pulse were collected at 15 sec intervals to obtain a completely decoupled spectra.

8-deoxydecahydrohistrionicotoxin (8, 9-*ene*). To an anhyd pyridine soln of perhydrohistrionicotoxin (48 mg) $SOCl_2$ (0.42 ml) in benzene was added dropwise under cooling at −23° (dry ice-CCl_4 bath). After 15 min at −23° and 20 h at −10°, dilute aqueous ammonia was added to the mixture followed by extraction with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by chromatography on a short column of silica gel with $CHCl_3$. A yield of 39 mg of 8-deoxydecahydrohistrionicotoxin (free base) was obtained. Mass spectrum (30 eV), relative intensities in parentheses: *m/z* 277(6), 234(4), 206(5), 168(85), 110(13), 96(100), α_D^{25} = −102°(*c* = 1.0, MeOH).

8-deoxyperhydrohistrionicotoxin. 8-deoxydecahydrohistrionicotoxin (39 mg) in MeOH (1 ml) was hydrogenated with PtO_2 (5 mg) and hydrogen at 8 atm pressure (115 lb/in^2) for 20 h. After filtration and concentration the crude product was purified by chromatography on a short column of silica gel with $CHCl_3$. A yield of 45 mg of 8-deoxyperhydrohistrionicotoxin was obtained after conversion to the HCl salt. Mass spectrum (30 eV), *m/z* 279(14), 264(1), 250(4), 236(20), 222(6), 208(14), 194(4), 180(100), 167(55), 110(7), 96(25). $[\alpha]_D^{25}$ = −44.3° (*c* = 1.0, MeOH).

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