108. Gephyrotoxins, Histrionicotoxins and Pumiliotoxins from the Neotropical Frog *Dendrobates histrionicus*

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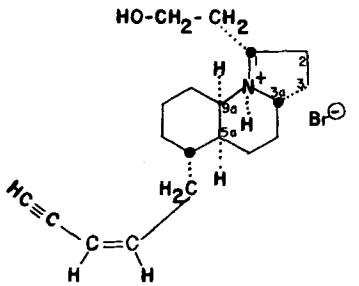
To the memory of Professor *Hans Schmid* who set himself a lasting monument by his pioneering work on natural products

(10. I. 77)

Summary

The structure and absolute configuration of a new acetylenic alkaloid gephyrotoxin isolated from skin extracts of the Colombian frog *Dendrobates histrionicus*, has been determined by Röntgen-ray crystallography. Gephyrotoxin, previously referred to as HTX-D, is a novel tricyclic alkaloid, [1*S*,3a*S*,5a*S*,6*S*(*Z*),9a*R*,10*R*]dodecahydro-6-(2-penten-4-yl)pyrrolo[1,2-*a*]quinoline-1-ethanol. Dihydrogephyrotoxin, a minor skin constituent, contains a 6-(2,4-pentadienyl)substituent. Two further spiropiperidine alkaloids related in structure to histrionicotoxin, (6*R*[6 α[2*S**(*Z*)],[7 β(*Z*), 8 α]]7-(1-buten-3-ynyl)-2-(2-penten-4-ynyl)-1-aza*spiro*[5.5]undecan-8-ol), have been isolated from *Dendrobates histrionicus:* allodihydrohistrionicotoxin which differs from histrionicotoxin in having a 2-(4-pentynyl)-substituent, while allotetrahydrohistrionicotoxin, a minor constituent, has 2-(4-pentynyl)- and 7-(1,3-butadienyl)-substituents. Three alkaloids related in structure to pumiliotoxin C, ([2*S*,4a*S*,5*R*,8a*R*]5-methyl-2-*n*-propyl-*cis*-decahydroquinoline), have been isolated from *Dendrobates histrionicus*. These alkaloids, with molecular weights of 195, 223, and 269, have, respectively, a 2-butylsubstituent, 2-propyl and 5-propyl-substituents, and 2-(3,4-pentadienyl) and 5-(2-penten-4-ynyl)-substituents. The last compound was hydrogenated to a dodecahydro-derivative identical in molecular weight, but not in other properties, with authentic dodecahydro-8-deoxy-histrionicotoxin, which was prepared from histrionicotoxin. Gephyrotoxin, in contrast to histrionicotoxin and pumiliotoxin C, is a muscarinic antagonist.

Introduction. – The neotropical frogs of the family *Dendrobatidae* have elaborated an amazing variety of unique alkaloids including batrachtotoxin [1], histrionicotoxin and congeners [2][3], pumiliotoxin C [4] and pumiliotoxin A and B [4][5]. Six alkaloids related to histrionicotoxin have been reported in extracts of the Colombian frog, *Dendrobates histrionicus* [2][3]. A seventh alkaloid, formerly referred to as HTX-D [3], has now been named gephyrotoxin, and has been crystallized as the hydrobromide salt. Other constituents of skin extracts of this frog are allodihydrohistrionicotoxin and five minor alkaloids, the latter representing members of the gephyrotoxin, histrionicotoxin and pumiliotoxin C classes of dendrobatid alkaloids.



Gephyrotoxin (Chemical Abstract stereodesignation: [1*S*-1α,3aβ,5aα,6α(*Z*),9aα]dodecahydro-6-(2-penten-4-ynyl) pyrrolo[1,2-a]quinoline-1-ethanol hydrobromide. The IUPAC stereodesignation would be: [1*S*,3a*S*, 5a*S*,6S(*Z*),9a*R*,10*R*]).

Results and Discussion. – Column chromatography of alkaloids from skin extracts of the neotropical frog *Dendrobates histrionicus* led to the isolation of a variety of alkaloids including gephyrotoxin. The name gephyrotoxin (Greek: gephyra meaning bridge) is proposed to replace HTX-D (3), since the compound bridges two classes of dendrobatid alkaloids in having the decahydroquinoline system of the pumiliotoxin C class and the vinylacetylene side chain of the histrionicotoxins. In the literal sense, the name refers to the bridge which presumably arises by addition of the nitrogen function to an unsaturated five carbon atoms side chain. Two other new alkaloids from *Dendrobates histrionicus* were elucidated by ^{1}H-NMR. and mass spectroscopy as histrionicotoxins and confirmed by catalytic reduction to perhydrohistrionicotoxin. Three further new compounds, on the basis of ^{1}H-NMR. appear to be members of the pumiliotoxin C class. Thus, three classes of piperidine alkaloids have now been found as skin constituents in a single population of *Dendrobates histrionicus* from southwestern Colombia. The properties of gephyrotoxin and the new alkaloids are summarized in *Tables 1–3*. Preliminary analysis of skin extracts from nine populations of this extremely variable dendrobatid frog by combined gas chromatography-chemical ionization mass spectrometry has provided evidence for more than twenty different alkaloids [6]. The occurrence of nearly *one hundred different alkaloids* in extracts from skins of dendrobatid frogs [7] introduces a chemical parameter for taxonomic and evolutionary studies [6].

Gephyrotoxin. This alkaloid has been detected only in dendrobatid frogs of the species *Dendrobates histrionicus* [7]. In the population under study from southwestern Colombia it is a minor alkaloid (see exper. Part and [3]). Previous studies of gephyrotoxin (HTX-D) had established the empirical formula $C_{19}H_{29}NO$, the presence of a $-CH_2-CH=CH-C\equiv CH$ side chain, the facile formation of an *O*-acetate or *O*-*p*-bromobenzoate and the perhydrogenation to a hexahydro derivative [3] (*Tables 1–3*).

Table 1. *Thin layer and gas chromatographic data for gephyrotoxin, histrionicotoxin and pumiliotoxine class alkaloids.* Solvent chloroform/2-propanol/aqueous ammonia 22:1:0.15. (For additional the layer and gas chromatographic data of dendrobatid alkaloids see [3] [6] [7]).

Compound	Thin layer Rf value[a])	Emergent Temperature (°C)
Histrionicotoxin class		
Histrionicotoxin	0.48	193
Isodihydrohistrionicotoxin	0.34	197
Neodihydrohistrionicotoxin	0.47	195
Allodihydrohistrionicotoxin	0.37	194
Tetrahydrohistrionicotoxin	0.48	194
Isotetrahydrohistrionicotoxin	0.37	199
Allotetrahydrohystrionicotoxin	0.28	197
Octahydrohistrionicotoxin	0.25	193
Dodecahydrohistrionicotoxin	0.23	194
Deoxydodecahydrohistrionicotoxin	0.43	176
Gephyrotoxin class		
Gephyrotoxin	0.12	202
Dihydrogephyrotoxin	0.17	201
Pumiliotoxin C class		
Alkaloid I (Mol.-wt. 195)	0.32	140 (0.9 min)[c]
Alkaloid II (Mol.-wt. 223)	0.13	140 (2.6 min)[c]
Alkaloid III (Mol.-wt. 269)	0.20	191
Perhydro(H_{10})-Alkaloid III	0.18	186

[a]) Silica gel GF, *Merck*. Solvent chloroform/2-propanol/aqueous ammonia 22:1.0:0.08 (see [3]).
[b]) A 2% OV-1 on CH-W column with starting temperature 140°, programmed at 10°/min (see [6] [7]).
[c]) Emergent time at constant temperature of 140°.

The ^{1}H-NMR. spectrum is presented in *Fig. 1*. Gephyrotoxin is accompanied by a minor congener, dihydrogephyrotoxin, which, on the basis of ^{1}H-NMR. spectroscopy, differs from gephyrotoxin only in the presence of a $-CH_2-CH=CH-CH=CH_2$ side chain. On catalytic reduction both compounds yield the same perhydro derivative. Röntgen-ray analysis of a single crystal of gephyrotoxin hydrobromide established the molecular formula, the relative and absolute configuration and conformation. Röntgen-ray analysis has been essential for the elucidation of the structures of the histrionicotoxins [2][8] and pumiliotoxin C [4].

Crystallographic Data and Analysis. Gephyrotoxin crystallizes in space group $P2_12_12_1$ with cell parameters $a = 10.38 \pm 0.01$ Å, $b = 17.53 \pm 0.02$ Å, $c = 9.72 \pm 0.01$ Å, and $V = 1768.7$ Å^3 and a calculated density of 1.383 g/cm^3. Intensity data were collected on a crystal of dimensions $0.40 \times 0.15 \times 0.27$ mm with MoK_α radiation on an automatic four-circle diffractometer to a maximum scattering angle of $2\theta = 50°$. The coordinates of the Br^- ion were determined from a *Patterson* function [9] computed with $(|E_h|^2 - 1)$ coefficients rather than $|F_h|^2$, where the $|E_h|$ are the normalized values for the experimentally derived $|F_h|$. Phases based on the position of the Br^- ion were refined and

Table 2. *^{1}H-NMR. spectral assignments for the protons associated with side chains of allohistrionicotoxins and gephyrotoxin*

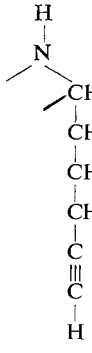
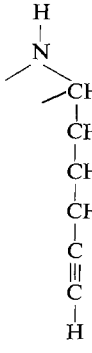
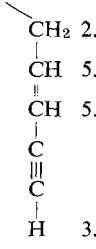
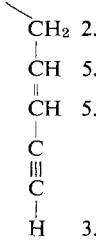
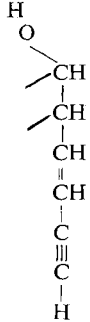
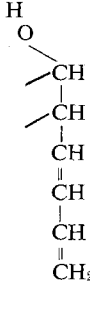
Allodihydrohistrionicotoxin			Allotetrahydrohistrionicotoxin			Gephyrotoxin			Gephyrotoxin *p*-bromobenzoate		
	δ	J		δ	J		δ	J		δ	J
	3.00 (br.)			3.00 (br.)			2.45 ($d\times d$)	8, 8		2.47 ($d\times d$)	8, 8
							5.97 ($d\times t$)	8, 11		5.96 ($d\times t$)	8, 11
							5.46 ($d\times d$)	11, 2		5.43 ($d\times d$)	11, 2
	2.18 ($d\times t$)	2, 7		2.18 ($d\times t$)	2, 7						
	1.91 (t)	2		1.90 (t)	2		3.08 (d)	2		3.04 (d)	2
	3.78 (br.)			3.68 (br.)			2.05 (m), 1.32 (m)				
	3.66 ($d\times d$)			3.88 (d br.)	11		3.98 (m)	3, 11, 11		4.35 (t)	6.3
	5.82 ($d\times d$)	11, 11		5.24 ($d\times d$)	11, 11		3.62 (m)	4, 4, 11			
	5.46 ($d\times d$)	9, 2		5.98 ($d\times d$)	11, 11						
				6.73 ($d\times d\times d$)	11, 11, 16						
	3.14 (d)	2		5.10 (d)	11						
				5.16 (d)	16						

Table 3. *Mass spectral data for gephyrotoxin, histrionicotoxin and pumiliotoxin C class alkaloids.* (For additional mass spectral data see [2–4] [7]. The *m/e* values obtained at 70 ev are presented followed by peak intensity relative to a base peak set equal to 100 in parentheses.

Gephyrotoxin class
Gephyrotoxin: 287(12), 286(11), 243(21), 242(100), 222(50), 192(7), 190(4), 176(5), 148(5), 122(21), 85(27), 83(40).
Dihydrogephyrotoxin: 289(4), 288(3), 245(21), 244(100), 222(49), 204(9), 202(10), 190(3), 176(4), 148(7), 122(20).
Gephyrotoxin p-*bromobenzoate:* 471,469(1); 426,428(3); 406,404(20), 243(24) 242(100), 204(8), 202,200(8), 185,183(28), 176(8), 157,155(8), 148(6), 122(15).
Perhydro(H_6)-*gephyrotoxin:* 293(5), 292(3), 250(16), 249(15), 248(100), 222(32), 192(3), 176(3), 148(4), 122(9).

Histrionicotoxin class
Allodihydrohistrionicotoxin: 285(36), 268(23), 218(46), 202(15), 200(18), 190(27), 176(50), 162(31), 144(33), 122(36), 96(100).
Allotetrahydrohistrionicotoxin: 287(29), 272(10), 270(16), 244(10), 220(24), 202(36), 176(38), 162(49), 148(21), 134(70), 122(24), 120(38), 106(40), 96(100).
Deoxydodecahydrohistrionicotoxin: 279(28), 264(2), 250(12), 237(16), 236(71), 222(10), 209(15), 208(39), 194(12), 181(40), 180(83), 168(100), 152(16), 138(8), 124(9), 123(10), 110(23), 96(80)

Pumiliotoxin C class
Alkaloid I (mol.-wt. 195): 195(1), 180(6.5), 152(1), 138(100), 136(2.5), 124(2), 122(1.5), 110(1.5), 95(7), 82(3.5).
Alkaloid II (mol.-wt. 223): 223(1.2), 222(1.5), 194(3), 181(13), 180(100), 164(1.5), 152(4) 138(2.5), 124(10), 96(4), 70(8).
Alkaloid III (mol.-wt. 269): 269(5), 268(13), 254(7), 240(7), 226(7), 204(85), 202(100), 188(9), 174(11), 160(6), 148(24), 122(15), 109(20), 96(39), 81(18).
Perhydro(H_{10})-*alkaloid III*: 279(1.4), 278(1.8), 248(10), 236(4), 226(6), 209(21), 208(100).

extended with the tangent formula [10], and the *E*-map [11] based on these phases revealed the positions of all the atoms and established the molecular formula and relative stereoconfiguration of gephyrotoxin. The absolute stereoconfiguration was derived from the anomalous scattering of the Br^- ion by MoK_α radiation [12]. The value for the agreement factor $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, where $|F_o|$ are the 1532 experimentally observed values and $|F_c|$ are the derived structure factors after least-squares refinement when the anomalous scattering of the Br^- ion is taken into consideration, is 8.1% for the configuration shown in this paper and 10.5% for the mirror image. The difference in the values of the R factors [13] establishes the configuration shown in *Fig. 2* as the absolute configuration. Coordinates for the absolute configuration of gephyrotoxin are listed in *Table 4*.

The absolute configuration of laevorotatory pumiliotoxin C with the 2-(*S*) configuration has also been established on the basis of the anomalous scattering of the Cl^- ion. Intensity data were recollected with Cu radiation on an automatic diffractometer for hkl and hk$\bar{l}$ as well as h$\bar{k}$l and h$\bar{k}\bar{l}$ reflections for a total of 1663 measurements. The agreement factor R was 8.9% for the configuration shown in Fig. 3. and 9.6% for the mirror image, thus establishing the absolute configuration as that in *Fig. 3*. The coordinates of pumiliotoxin C as listed in the original publication [4] are correct for the absolute configuration presented in *Fig. 3* of [4] while *Fig. 5* of [4]

Table 4. *Fractional coordinates and thermal parameters for gephyrotoxin*

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Br	0.0664	0.1020	0.1323	4.00	3.14	3.37	−0.06	−0.17	0.45
O	0.8659	0.0167	0.9220	6.75	6.26	7.90	−0.59	−0.38	0.03
C(1)	0.6088	0.0701	0.8210	3.10	2.71	2.41	−0.37	−0.56	0.26
C(2)	0.5719	0.0194	0.9447	2.51	5.12	3.45	0.87	0.64	1.79
C(3)	0.4267	0.0233	0.9520	7.57	2.69	2.14	−1.23	0.97	0.73
C(3a)	0.3808	0.0819	0.8434	2.83	3.72	2.84	0.04	1.17	0.40
C(4)	0.2519	0.0704	0.7749	2.66	4.02	2.22	−1.38	0.56	0.15
C(5)	0.2359	0.1279	0.6565	2.52	3.79	2.49	−0.04	0.35	0.22
C(5a)	0.3440	0.1177	0.5529	2.27	2.47	2.21	−0.44	0.06	−0.17
C(6)	0.3296	0.1737	0.4293	2.20	2.52	1.31	0.34	0.03	0.30
C(7)	0.3618	0.2544	0.4747	1.75	2.10	3.46	0.60	−0.92	−0.30
C(8)	0.4917	0.2630	0.5401	3.15	2.20	2.76	−0.19	0.49	0.13
C(9)	0.5065	0.2095	0.6634	2.79	2.76	2.85	−0.41	−0.60	0.53
C(9a)	0.4798	0.1275	0.6189	1.88	2.38	2.63	−0.38	−0.06	0.76
N(10)	0.4868	0.0710	0.7388	2.93	3.11	1.42	−0.16	−0.67	1.03
C(11)	0.7230	0.0399	0.7373	1.58	5.60	2.42	0.77	−0.12	0.73
C(12)	0.8462	0.0658	0.8123	3.06	15.23	3.08	1.51	0.80	3.11
C(13)	0.4114	0.1465	0.3094	3.31	2.84	2.51	0.77	0.57	0.78
C(14)	0.4040	0.2003	0.1847	3.78	3.91	2.93	0.54	−0.43	−0.92
C(15)	0.5015	0.2264	0.1090	4.35	3.45	3.12	−0.24	0.91	−1.13
C(16)	0.6295	0.2012	0.1280	3.62	3.32	3.96	−0.64	0.28	−0.65
C(17)	0.7364	0.1825	0.1405	4.63	3.89	5.94	−0.34	1.11	−1.13

pictured the optical antipode. In the meantime 2-(*S*)-pumiliotoxin C has been synthesized by two different routes in Darmstadt [14] and Geneva[1]).

A comparison of *Figs. 2* and *3* shows that gephyrotoxin and pumiliotoxin C both contain a *cis*-decahydroquinoline ring system. It should be noted that for a *cis*-decahydroquinoline ring system with each individual ring in the chair conformation there are two *distinct conformations* for the same absolute configuration, *Fig. 4*, **a** and **b**. The two conformers are not superimposable, although they are interconvertible. It is interesting that conformer **a** is contained in gephyrotoxin and conformer **b** is contained in pumiliotoxin C. (The numbering in conformers **a** and **b** corresponds to the numbering of atoms in gephyrotoxin and pumiliotoxin C, respectively.) The configuration is the same in both molecules at ring atom 6 in gephyrotoxin and the equivalent atom 5 in pumiliotoxin C although the side chain in pumiliotoxin C is equatorial, whereas the side chain in gephyrotoxin is axial. At ring atom 2 in pumiliotoxin C and the equivalent ring atom 3a in gephyrotoxin, although side chains are equatorial in both compounds, the configurations are opposite.

Gephyrotoxin is a congener of histrionicotoxin [2] and dihydrohistrionicotoxin [8]. It does not contain the spiro ring system of histrionicotoxin but does retain one side chain with the vinylacetylene moiety, while the other unsaturated side chain in histrionicotoxin has been transformed to a five-membered ring plus a $-CH_2CH_2OH$

[1]) *W. Oppolzer* in an independent elegant synthesis starting with *R*-norvaline ($[\alpha]^{20}$ −29°) also arrived at 2-*S*-pumiliotoxin C, $[\alpha]^{20} = -13°$ (personal communication); *W. Oppolzer & E. Flaskamp*, Helv. *60*, 204 (1977).

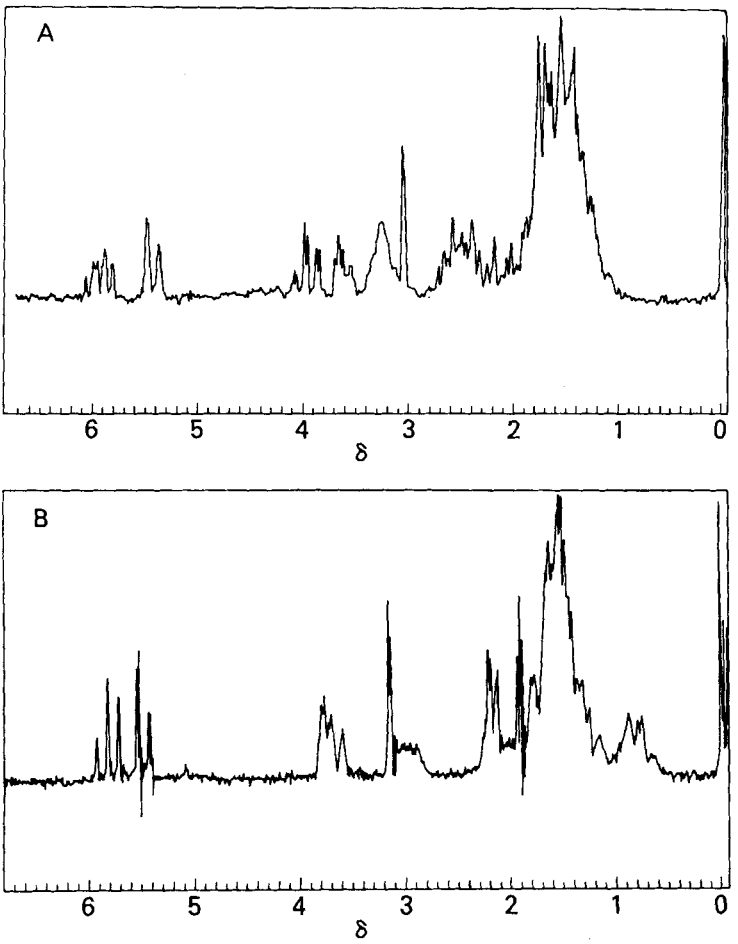


Fig. 1. ^{1}*H-NMR. spectrum* (100 mHz) *of Gephyrotoxin* (A) *and Allodihydrohistrionicotoxin* (B). Values are in ppm relative to an internal standard of tetramethylsilane ($\delta = 0$). The solvent was chloroform.

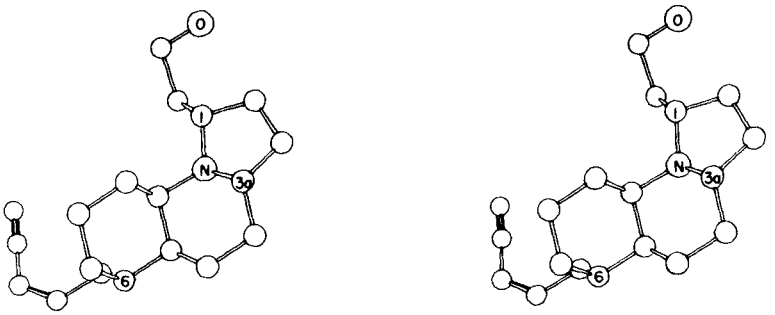
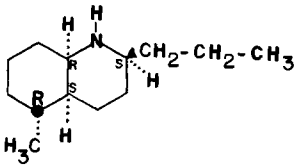


Fig. 2. *A stereodiagram of gephyrotoxin depicting the absolute configuration as determined by x-ray diffraction analysis*



2-(S)-*Pumiliotoxin C*

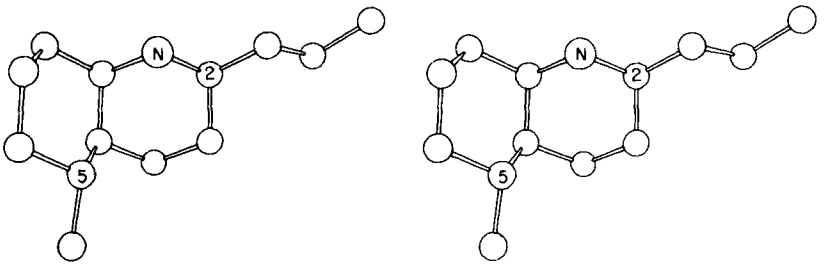
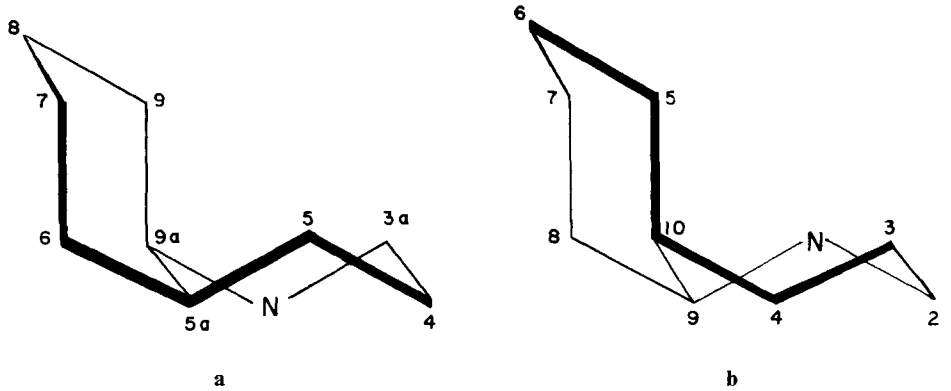


Fig. 3. *A stereodiagram of the absolute configuration of pumiliotoxin C as determined by x-ray diffraction analysis*



a b

Fig. 4. *Two different conformations for* cis*-decahydroquinoline with the same absolute stereoconfiguration.* **a** as in geophyrotoxin and **b** as in pumuliotoxin C. The numbering coincides with the numbering of the atoms in the individual molecules.

chain in gephyrotoxin. Bond lengths and angles, listed in *Table 5*, are within expected values. Values for the torsional angles, *Fig. 5*, show that both six-membered rings are in the chair conformation and that the five-membered ring is in the envelope conformation with the *N*-atom 0.66 Å out of the plane formed by the remaining four atoms. The vinyl acetylene moiety, C(13) to C(17), is in the *cis*-configuration and nearly planar. The $-CH_2CH_2OH$ chain is extended from the ring system with the oxygen atom approximately *gauche* rather than *trans* to C(1).

The orientation of the hydroxyl oxygen atom in the *gauche* position in the crystal is probably constrained by the formation of a hydrogen bond with the Br^- ion. The

Table 5. *Bond lengths and angles in gephyrotoxin*

Bond	A	Angle	Deg.
C(1)–C(2)	1.54	N(10C(1)C(2)	102
C(1)–N(10)	1.50	N(10)C(1)C(11)	112
C(1)–C(11)	1.53	C(2)C(1)C(11)	114
C(2)–C(3)	1.51	C(1)C(2)C(3)	105
C(3)–C(3a)'	1.55	C(2)C(3)C(3a)	108
C(3a)–C(4)	1.51	C(3)C(3a)C(4)	119
C(3a)–N(10)	1.51	C(3)C(3a)N(10)	99
		C(4)C(3a)N(10)	109
C(4)–C(5)	1.54	C(3a)C(4)C(5)	110
C(5)–C(5a)	1.52	C(4)C(5)C(5a)	110
C(5a)–C(6)	1.56	C(5)C(5a)C(6)	112
C(5a)–C(9a)	1.56	C(5)C(5a)C(9a)	112
		C(6)C(5a)C(9a)	110
C(6)–C(7)	1.52	C(5)C(6)C(7)	110
C(6)–C(13)	1.52	C(5a)C(6)C(13)	110
		C(7)C(6)C(13)	113
C(7)–C(8)	1.50	C(6)C(7)C(8)	114
C(8)–C(9)	1.53	C(7)C(8)C(9)	111
C(9)T-C(9a)	1.53	C(8)C(9)C(9a)	110
C(9a)–N(10)	1.53	C(9)C(9a)C(5a)	113
		C(9)C(9a)N(10)	113
		C(5a)C(9a)N(10)	107
		C(1)N(10)C(3a)	105
		C(1)N(10)C(9a)	117
		C(3a)N(10)C(9a)	113
C(11)–C(12)	1.54	C(1)C(11)C(12)	109
C(12)–O	1.39	C(11)C(12)O	108
C(13)–C(14)	1.54	C(6)C(13)C(14)	113
C(14)–C(15)	1.33	C(13)C(14)C(15)	127
C(15–C(16)	1.41	C(14)C(15)C(16)	122
C(16)–C(17)	1.16	C(15)C(16)C(17)	177

packing of GTX^+ ions and the Br^- ions is shown in *Fig. 6* where the two possible hydrogen bonds, OH...Br^- and NH...Br^-, of length 3.28 Å and 3.24 Å, respectively, are indicated by light lines. There are no solvent molecules in the crystal.

Allodihydrohistrionicotoxin. Two major and four minor alkaloids of the histrionicotoxin-class had been isolated in earlier studies of extracts from *Dendrobates histrionicus* [2][3]. The major alkaloids were histrionicotoxin and isodihydrohistrionicotoxin and the minor ones neodihydrohistrionicotoxin, isotetrahydrohistrionicotoxin, tetrahydrohistrionicotoxin, and octahydrohistrionicotoxin. Allodihydrohistrionicotoxin represents another minor alkaloid in extracts of this frog. Its presence had been suspected, but until now it was not isolated free of other histrionicotoxins. H-NMR. (*Table 2* and *Fig. 1*) and mass spectrometry (*Table 3*) firmly establish a 2-pentyl side chain with a terminal acetylene. By catalytic reduction it is converted to perhydrohistrionicotoxin (*cf.* [3]).

In addition to allodihydrohistrionicotoxin a minor congener, allotetrahydrohistrionicotoxin, was isolated. It differs from allodihydrohistrionicotoxin only by the presence of a $-CH=CH-CH=CH_2$ side-chain (*Table 2*). Thus three major and five minor alkaloids of the histrionicotoxin class have now been characterized. Allodihydrohistrionicotoxin has been found to occur in the nine populations of *Dendrobates*

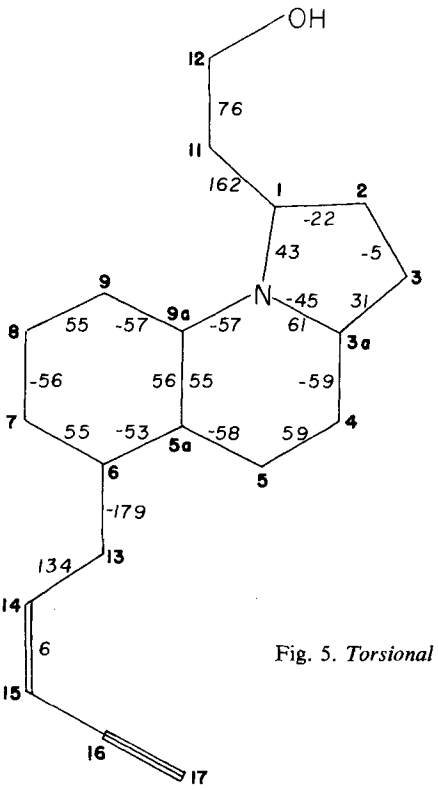


Fig. 5. *Torsional angles in geophyrotoxin*

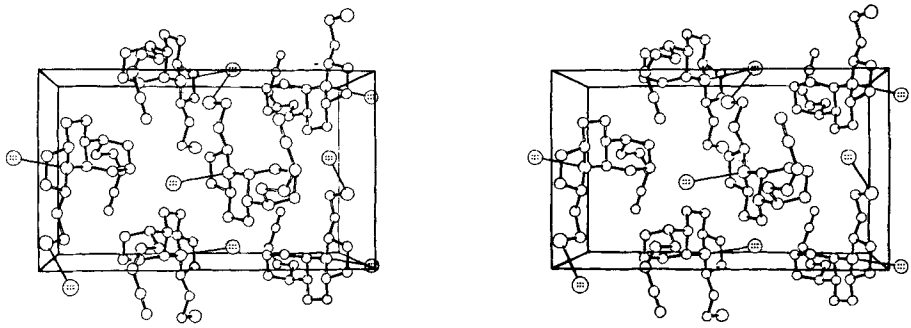
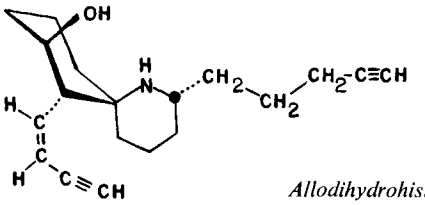


Fig. 6. *Stereodiagram of the packing in the gephyrotoxin · HBr crystal.* The Br^- ions are indicated by the dotted circles and the NH ... Br^- and OH ... Br^- hydrogen bonds are indicated by light lines. The axial directions are a ↓, b → and c directed out of the paper.



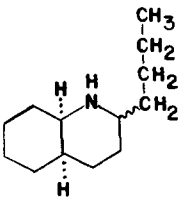
Allodihydrohistrionicotoxin

histrionicus which have been investigated by combined gas chromatography/mass spectrometry [6].

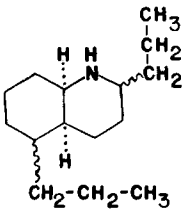
Pumiliotoxin C class Alkaloids. Three additional minor alkaloids were isolated from skin extracts of *Dendrobates histrionicus*, which are referred to as Alkaloids I (mol.-wt. 195), II (mol.-wt. 223), and III (mol.-wt. 269). Their relatively simple mass spectra (*Table 3*), their ^{1}H-NMR. spectra (data not shown) and their behavior on catalytic reduction indicate that they are members of the pumiliotoxin C class of alkaloids which in various dendrobatid frogs consists of at least 25 different compounds [7].

Alkaloids I and II do not undergo catalytic reduction and are formulated as 2-butyl- and 2,5-dipropyl-*cis*-decahydroquinolines, respectively. Compound III can be hydrogenated to a decahydro-derivative. Analysis of the ^{1}H-NMR. spectra provides evidence for two different unsaturated 5-carbon atoms side chains. Since the side chains are similar to those of isodihydrohistrionicotoxin, it appeared possible that III was a deoxyisodihydrohistrionicotoxin.

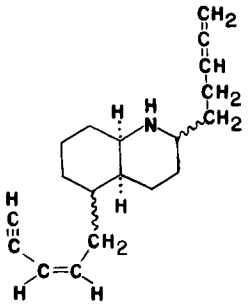
Deoxyperhydrohistrionicotoxin was therefore prepared. Its ^{1}H-NMR. (not shown) and mass spectra (*Table 3*) differ markedly from those of the perhydro-derivative of III. Thus, III is most likely a member of the pumiliotoxin C class; *i.e.*, a *cis*-decahydroquinoline. Assignment of the substituent positions for III is tentative, but, based on biosynthetic considerations, it appears reasonable to assign the allene $-CH_2-CH_2-CH=C=CH_2$ moiety to the 2-position where it occurs in the iso-compounds of the histrionicotoxin series. Further studies on I, II, and III are in progress. It is possible that compound III actually represents a mixture of two isomeric compounds (*cf.* [7]). The absolute configuration of these higher homologs of pumiliotoxin C are of particular interest in view of the difference between the absolute configuration at C(2) of pumiliotoxin C (2β) and (isodihydro)histrionicotoxin (2α) [2] [4] [8] and gephyrotoxin (3a-α).



I



II



III

Pharmacology. The pharmacological activity of dendrobatid alkaloids has proven of considerable interest. The steroidal and highly toxic batrachotoxins selectively increase the permeability of electrogenic membranes to sodium ions thereby eliciting depolarization in a variety of nerve and muscle preparations [5]. The histrionicotoxins antagonize the conductance changes which normally follow activation of nicotinic receptors by acetylcholine in striated muscle [6]. Histrionicotoxins, in addition, antagonize potassium conductance changes associated with action and end plate potentials and bind selectively to the ion conductance modulator unit of the acetylcholine receptor of *Torpedo electroplax* [17]. Preliminary experiments with gephyrotoxin indicate that it, unlike histrionicotoxin, does not posses potent "anticholinergic" properties in nervestriated muscle preparations. Instead, in preliminary experiments, with guinea pig ileum and atrium preparations, gephyrotoxin is a relatively potent muscarinic antagonist. Histrionicotoxins and pumiliotoxin C do not show anticholinergic effects in the latter preparations.

Experimental Part

^{1}H-NMR. spectra were obtained in chloroform solution on a *JEOLCO* Model JNM-PS100 spectrometer. Electron impact spectra were obtained on a *Hitachi* RMU-6C mass spectrometer at 70 ev. The identities of parent ions were confirmed on a *Finnegan* 1015 spectrometer with isobutane as the reactant gas. Combined GC./MS. was with a *Hitachi* M-52 mass spectrometer with a 2% OV-1 on CH-W column with helium as the carrier gas and programmed from 140° at 10°/min.

Initial Purification of Alkaloids. Methanolic extractions of 3200 skins of *Dendrobates histrionicus* (*cf.* [3]) were concentrated *in vacuo* and diluted with three volumes of water and extracted with cold 0.2N HCl. The acidic extract was made alkaline with 1N aqueous ammonia and reextracted into chloroform to yield an alkaloid fraction. Concentration of the chloroform extract *in vacuo* yielded 2.52 g of crude alkaloids.

A portion (0.93 g) of the crude alkaloids was chromatographed on a Sephadex LH-20 column with benzene/cyclohexane/2-propanol/triethylamine 50:15:5:1 (*cf.* [3]). Fractions (10 ml) were analysed by thin-layer chromatography (silica gel, *cf. Table 1*) and by combined GC./MS. Based on these analyses nine combined fractions (A-I) were made: A) (Fraction 18–27) 25 mg (primarily non-alkaloids); B) (Fraction 28–31) 28 mg (major component: An alkaloid with mol. wt. 223 (II)); C) (Fraction 32–35) 80 mg (octahydro- and isotetrahydrohistrionicotoxins, and alkaloids with mol. wt. 223 (II) and 195 (I)); D) (Fraction 36–37) 80 mg (isotetrahydrohistrionicotoxin (43 mg) and tetrahydrohistrionicotoxin (5 mg) were isolated by further chromatographic separation (*cf.*, [3])); E) (Fraction 38–40) 88 mg (major components: isotetrahydro- and tetrahydrohistrionicotoxin; minor components: octahydro- and allotetrahydrohistrionicotoxins, dihydrogephyrotoxin and an alkaloid with mol.-wt. 269 (III)); F) (Fraction 41–42) 70 mg (isodihydrohistrionicotoxin (25 mg) and neodihydrohistrionicotoxin (25 mg) were isolated by further chromatographic separation (*cf.*, [3])); G) (Fraction 43–45) 185 mg (isodihydrohistrionicotoxin (130 mg), allodihydrohistrionicotoxin (10 mg) and neodihydrohistrionicotoxin (10 mg) were isolated by further chromatographic separation (*cf.* [3])); H) (Fraction 46–48) 108 mg (major components: isodihydro- and allodihydrohistrionicotoxins and gephyrotoxin); I) (Fraction 46–60) 183 mg (Histrionicotoxin).

Isolation of gephyrotoxin and allodihydrohistrionicotoxin. Fraction H was rechromatographed on Sephadex LH-20. Fraction 44–47 yielded isodihydrohistrionicotoxin (26 mg). Fraction 49–52 (57 mg) yielded after chromatography on a silica gel column (15 g) with chloroform/2-propanol/aqueous ammonia 30:1:0.15 allodihydrohistrionicotoxin (39 mg) and gephyrotoxin (15 mg). Fraction 55–57 yielded histrionicotoxin (8 mg).

Isolation of allotetrahydrohistrionicotoxin, dihydrogephyrotoxin and an alkaloid with molecular weight 269 (III). Fraction E was chromatographed on a silica gel column (30 gr) with chloroform/2-isopropanol/aqueous ammonia 30:1:0.15. Fractions of 3.8 ml were taken. Fraction 18 yielded tetrahydrohistrionicotoxin (20 mg). Fraction 19–25 yielded after rechromatography on a silica gel column (12 g) with the same solvent tetrahydrohistrionicotoxin (7 mg), isotetrahydrohistrionicotoxin

(30 mg) and allotetrahydrohistrionicotoxin (3 mg). Fraction 26–28 yielded octahydrohistrionicotoxin (3 mg). Fraction 30–32 yielded dihydrogephyrotoxin (1 mg).

Isolation of alkaloids with molecular weights of 195 and 223. Fraction C was chromatographed on a silica gel column (56 g) with hexane/tetrahydrofuran/ aqueous ammonia 4:1:0.15 and fractions of 5 ml. Fraction 14–19 yielded an alkaloid with mol.-wt. 223 (II) (15 mg). Fraction 20–26 yielded an alkaloid with mol.-wt. 195 (I) (12 mg). Fraction 27–40 yielded after rechromatography on a silica gel column octahydrohistrionicotoxin (32 mg) and isotetrahydrohistrionicotoxin (13 mg).

Deoxydodecahydrohistrionicotoxin. Thionyl chloride (50 μl) was added with vigorous agitation at $-20°$ to a pyridine solution (220 μl) containing 5.7 mg of dodecahydrohistrionicotoxin (3). After 30 min at $-20°$ and 2 h at $-10°$ the solution was evaporated to dryness *in vacuo*. The residue was dissolved in $HCCl_3$, the chloroform solution washed with dilute aqueous ammonia, dried with Na_2SO_4 and evaporated to dryness. The residue was purified on a thick silica gel thin-layer chromatoplate with chloroform/2-propanol/aqueous ammonia 22:1:0.15 and eluted from a zone (variable Rf) detected with iodine vapor. This product (mol.-wt. 267) showed a vinyl proton on ^{1}H-NMR. spectroscopy ($\delta = 5.63$, 1 H, $J = 2$) and thus appeared to be a dehydro-($\Delta^{7,8}$)-derivative. Catalytic reduction with platinum oxide and hydrogen (5 atm) in methanol yielded deoxydodecahydrohistrionicotoxin (mol.-wt. 269), homogeneous by gas chromatography (see *Table 1*).

*Gephyrotoxin-*p*-bromobenzoate.* Dicyclohexylcarbodiimide (12.9 mg) in dichloromethane (0.2 ml) was added in portions at 0° to a dichloroethane solution of *p*-bromobenzoic acid (11 mg). After 30 min a dichloromethane solution (0.3 ml) of gephyrotoxin (13 mg) was added. After 16 h the solution was filtered and the filtrate chromatographed on thick silica gel chromatoplates with chloroform/2-isopropanol/aqueous ammonia 22:1:0.15 to yield 10 mg of gephyrotoxin *p*-bromobenzoate.

Catalytic reductions. Reduction of alkaloids was carried out in methanol with platinum oxide and 5 atm. of hydrogen. Allodihydrohistrionicotoxin, allotetrahydrohistrionicotoxin and histrionicotoxin yield perhydro(H_{12}) histrionicotoxin (*cf.* [3]). Gephyrotoxin and dihydrogephyrotoxin yield hexahydrogephyrotoxin (*cf.* [3]). Alkaloid III (mol.-wt. 269) yields a decahydroderivative, while I (mol.-wt. 195) and II (mol.-wt. 223) are fully saturated and are not hydrogenated.

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