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284. Allenic and Acetylenic Spiropiperidine Alkaloids from the Neotropical Frog, *Dendrobates histrionicus*[1])

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(25. II. 74)

Summary. Four analogs of the acetylenic alkaloid, histrionicotoxin ($C_{19}H_{25}NO$) and the allenic alkaloid, isodihydrohistrionicotoxin have been isolated from extracts of skins of the arrow poison frog, *Dendrobates histrionicus* and characterized as neodihydrohistrionicotoxin, tetrahydrohistrionicotoxin, isotetrahydrohistrionicotoxin and octahydrohistrionicotoxin. These spiropiperidine (8-hydroxy-1-azaspiro[5.5]undecane) alkaloids differ only in the degree of unsaturation in the five carbon atoms (position 2) and four carbon atoms (position 7) side chains. A fifth compound, HTX-D, corresponds in empirical formula to a tetrahydrohistrionicotoxin with a 7-(*cis*-1-butenyl-3-ynyl) side chain, but the major mass spectral fragmentation with loss of C_2H_5O is not characteristic of the histrionicotoxins. Reduction of histrionicotoxin with hydrogen and *Lindlar* catalyst affords an isomeric dihydrohistrionicotoxin with the terminal acetylene of the five carbon atoms side chain reduced, tetrahydrohistrionicotoxin and hexahydrohistrionicotoxins, while reduction with hydrogen and palladium on charcoal affords dodecahydrohistrionicotoxin which is readily methylated to the tertiary amine by methyl iodide.

Introduction. – The major alkaloids from skin extracts of the dendrobatid frog *Dendrobates histrionicus* were recently isolated and characterized as histrionicotoxin (HTX, I) and isodihydrohistrionicotoxin (isodihydro HTX, II) [1]. These compounds (see Fig. 1) and their perhydro (dodecahydro) derivative showed unique properties as cholinolytics and antagonists of specific ionic channels in electrogenic membranes [2–4]. A number of minor constituents from the skin extracts of this frog have now been isolated and characterized.

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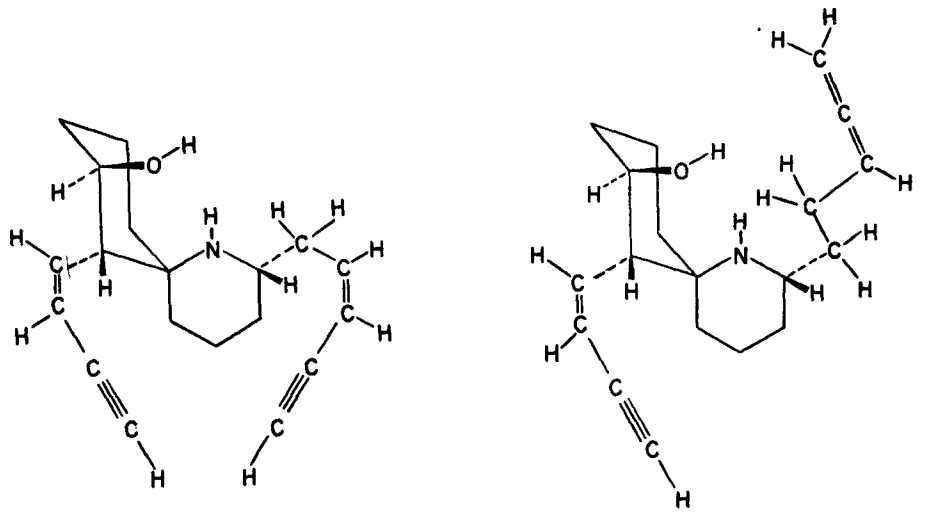
[1]) Presented in part in the 13th Paul Karrer Lecture on 30 June 1971; Experientia *27*, 1121 (1971).



I II

Fig. 1. *Structure of histrionicotoxin* (I) *and isodihydrohistrionicotoxin* (II). Configurations depicted are based on Roentgen ray analysis of the crystalline hydrochloride salts

Results and discussion. – Methanolic extracts were obtained from 1100 skins of *Dendrobates histrionicus* in Guayacana, Narino, Colombia, by *J. W. Daly* and Dr. *Charles W. Myers* of the American Museum of Natural History, New York, at the end of November 1971. These methanol extracts were diluted with water and extracted twice with equal volumes of chloroform. Partition of combined chloroform extracts with 0.1 N HCl, followed by addition of excess aqueous ammonia to the aqueous phase and reextraction into chloroform to afford an alkaloid fraction (1.2 g) followed the published procedures [1]. This alkaloid fraction was chromatographed on silica gel and Sephadex LH-20 as shown in the flow sheet of Fig. 2. From these extracts a total of 226 mg of histrionicotoxin and 320 mg of isodihydrohistrionicotoxin were isolated. In addition, the following minor constituents were isolated and characterized: neodihydrohistrionicotoxin (19 mg); tetrahydrohistrionicotoxin (2 mg), isotetrahydrohistrionicotoxin (6 mg), octahydrohistrionicotoxin (9 mg) and HTX-D (47 mg).

Histrionicotoxin was reduced with hydrogen and palladium on charcoal to dodecahydro derivative, while reduction with hydrogen and *Lindlar*'s palladium catalyst for 1 hour afforded a mixture of products. These were identified as a dihydrohistrionicotoxin different from the naturally occurring neodihydrohistrionicotoxin, a tetrahydrohistrionicotoxin identical with the naturally occurring tetrahydrohistrionicotoxin and a small amount of hexahydrohistrionicotoxin. The dodecahydrohistrionicotoxin was easily converted to the N-methyl derivative with methyl iodide in methanol or acetonitrile and methylated to the quaternary salt in dimethylsulfoxide. Reduction of HTX-D with hydrogen and palladium on charcoal afforded a hexahydro derivative. Acetylation of HTX-D yielded an O-acetyl derivative. The properties of these compounds are given in the experimental section. Thin-layer chromatographic data are summarized in Table 1. Since dodecahydrohistrionicotoxin

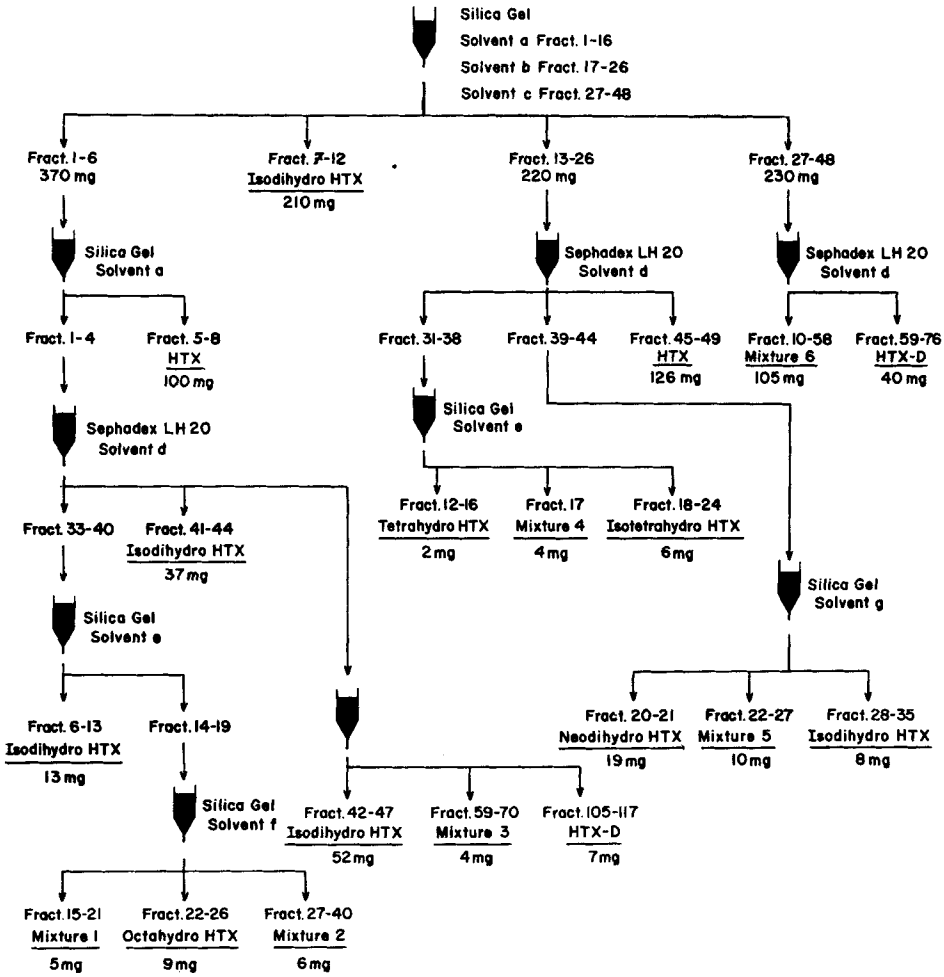


Fig. 2. *Chromatographic separation of alkaloids from skin extracts from* 1100 Dendrobates histrionicus. Total weight of alkaloids was 1.2 g. Fractions were approximately 2 ml. Solvents: a, chloroform; b, chloroform plus 2% methanol; c, chloroform/methanol 1:1; d, benzene/cyclohexane/ethanol/triethylamine 35:8:4:1; e, chloroform/2-propanol/aqueous ammonia 20:1:0.1; f, chloroform/cyclohexane/methanol/triethylamine 4:10:1:1; g, cyclohexane/2-propanol-aqueous ammonia 20:1:0.1. Chemical ionization mass spectral analysis indicated that the nonhomogeneous fractions contained alkaloids with the following apparent molecular weights: Mixture 1, mainly 291; Mixture 2, mainly 291, also 349, 351; Mixture 3, 285, 279, 267, 259, 235; Mixture 4, mainly 287; Mixture 5, mainly 285; Mixture 6, mainly 287, also 289, 269, 267

represents a reference compound of relevance to many of these alkaloids, its NMR. spectrum is presented in Fig. 3.

Mass and NMR. spectral analyses of the various naturally occurring compounds revealed that all, except HTX-D, were closely related to histrionicotoxin (I) and differed only in the nature of the side chains. Detailed analysis of spin decoupled NMR. spectra allowed the assignment of the structures of the side chains as presented

Table 1. *Rf values of histrionicotoxins on thin-layer silica gel plates. Solvent I, chloroform/2-propanol/aqueous ammonia 9:1:0.08. Solvent II, chloroform/methanol 9:1*

	Solvent I	Solvent II
HTX	0.69	0.52
IsodihydroHTX	0.50	0.44
NeodihydroHTX	0.62	0.50
TetrahydroHTX	0.64	0.48
IsotetrahydroHTX	0.55	0.48
OctahydroHTX	0.34	0.42
'Synthetic' DihydroHTX	0.57	–
HexahydroHTX	0.52	–
DodecahydroHTX	0.35	0.38
N-MethyldodecahydroHTX	–	0.80
N-MethyldodecahydroHTXmethiodide	–	0.05
HTX-D	0.22	0.18
HexahydroHTX-D	0.27	–
O-AcetylHTX-D	–	0.50

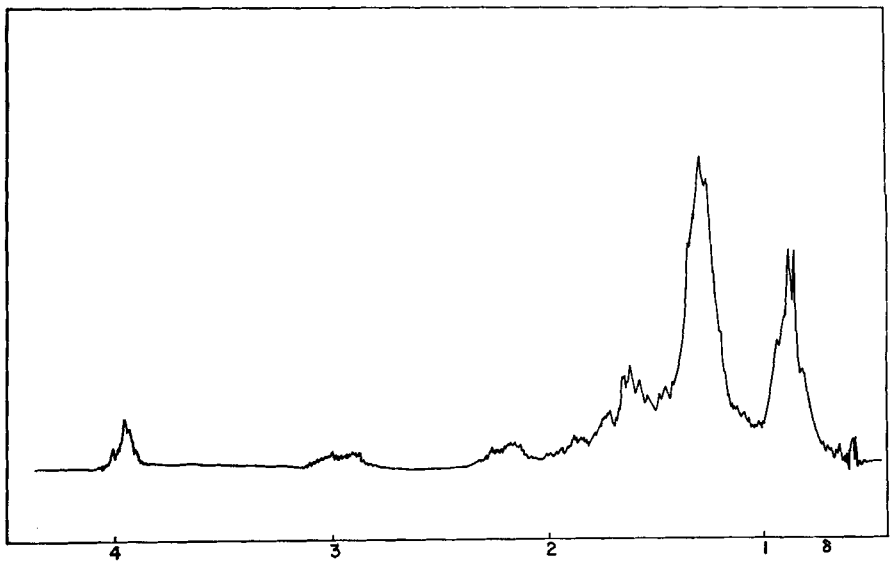


Fig. 3. *NMR. spectrum* (100 MHz) *of Dodecahydrohistrionicotoxin.* Values (δ) are in ppm relative to an internal standard of tetramethylsilane ($\delta = 0$). The solvent was chloroform

in Fig. 4. Possible mass spectral fragmentation pathways for histrionicotoxin, and octahydrohistrionicotoxin are suggested in Fig. 5. Similar fragmentations are observed for the other compounds. The mass spectral data are listed in the experimental section.

The structure of HTX-D is as yet unresolved. Although it contains the same five carbon atoms side chain of histrionicotoxin (Fig. 4), other data, including the reduction to a hexahydro derivative, would suggest that it is a tricyclic alkaloid containing a C_2H_5O moiety which can be O-acetylated and which is readily lost during mass spectral fragmentation. Further studies on this alkaloid are in progress.

Table 2. *NMR. spectral assignments for the protons associated with the side chains of histrionicotoxins* [a])

			Histrionicotoxin		Dihydroiso HTX		neo-Dihydro HTX		syn Dihydro HTX		Allenic-tetrahydro HTX		Tetrahydro HTX		Octahydro HTX		HTX-D	
			δ	J	δ	J	δ	J	δ	J	δ	J	δ	J	δ	J	δ	J
Five-Carbon Side Chain	A		3.04 br		3.00 br		3.15 br		3.15 br		3.04 br		3.11 d,br	11				
	B	CH_2	2.40 d,d	8,8	–		2.39 d,d	8,8	2.37 d,d	7,7	–		2.30 d,d	7,7	–		2.45 d,d	8,8
	C	CH	6.06 d,t	8,11	2.18 m		6.06 d,t	8,11	5.45 d,t	7,11	2.1 m	(3.5)	5.49 d,t	7,11	–		5.97 d,t	8,11
	D	CH	5.60 d,d	11,2	5.16 t,t	7,7	5.56 d,d	11,2	6.12 d,d	11,11	5.15 t,t	(7)	6.14 d,d	11,11	2.04 m		5.46 d,d	11,2
	E								6.64 d,d,d	11,10,16			6.64 d,d,d	11,11,17	5.77 d,d,t	8,11,17	3.08 d	2
	F	CH	3.08 d	2	CH_2	4.69 d,t 7,3.5	CH	3.07 d	2	CH_2	5.13 d	10	CH_2	4.68 d,t 7, 3.5	CH_2	5.15 d	11	CH	
											5.20 d	16				5.21 d	17		
Four-Carbon Side Chain	α		3.80 br		3.82 br		3.71 br		3.88 br		3.76 br		3.76 br		3.94 br			
	β		3.70 d,br	11	3.73 d,br	10	3.41 d,br	10	3.67 d,br	11	3.46 d,br	11	3.45 d,br	11				
	γ	CH	5.88 d,d	11,11	5.89 d,d	10,10	CH	5.28 d,d	10,10	CH	5.83 d,d	11,11	CH	5.33 d,d	11,11	CH	5.29 d,d	11,11	CH	–	
	δ	CH	5.55 d,d	11,2	5.54 d,d	10,2	CH	6.04 d,d	10,10	CH	5.56 d,d	11,2	CH	6.08 d,d	11,11	CH	6.08 d,d	11,11	CH_2	2.13 m	
	ε						CH	6.76 d,d,d	10,10,16				CH	6.80 d,d,d	11,11,17	CH	6.80 d,d,d	11,11,17	CH	5.80 d,d,t	8,11,7
	ζ	CH	3.19 d	2	CH	3.17 d	2	CH_2	5.16 d	10	CH	3.17 d	2	CH_2	5.20 d	11	CH_2	5.19 d	11	CH_2	4.99 d	11
									5.20 d	16					5.24 d	17		5.24 d	17		5.07 d	17

a) NMR. in chloroform solution at 100 MHz with δ values in ppm relative to an internal standard of tetramethylsilane ($\delta = 0$). Decoupling performed on a JEOLCO Model JNM-PS100. Coupling constants (J) are reported in Hz: br. = broad; d = doublet; t = triplet; $d \times d$ = doublet of doublets, etc. Protons A, α and β are at the ring positions shown. The other protons are on carbons B, C, D, E, and F of the five carbon side chain or γ, δ, ε, and ζ of the four carbon side chains, respectively. The assignment of protons of δ, ε, ζ and D, E, F, of octahydrohistrionicotoxin is arbitrary and may be reversed.

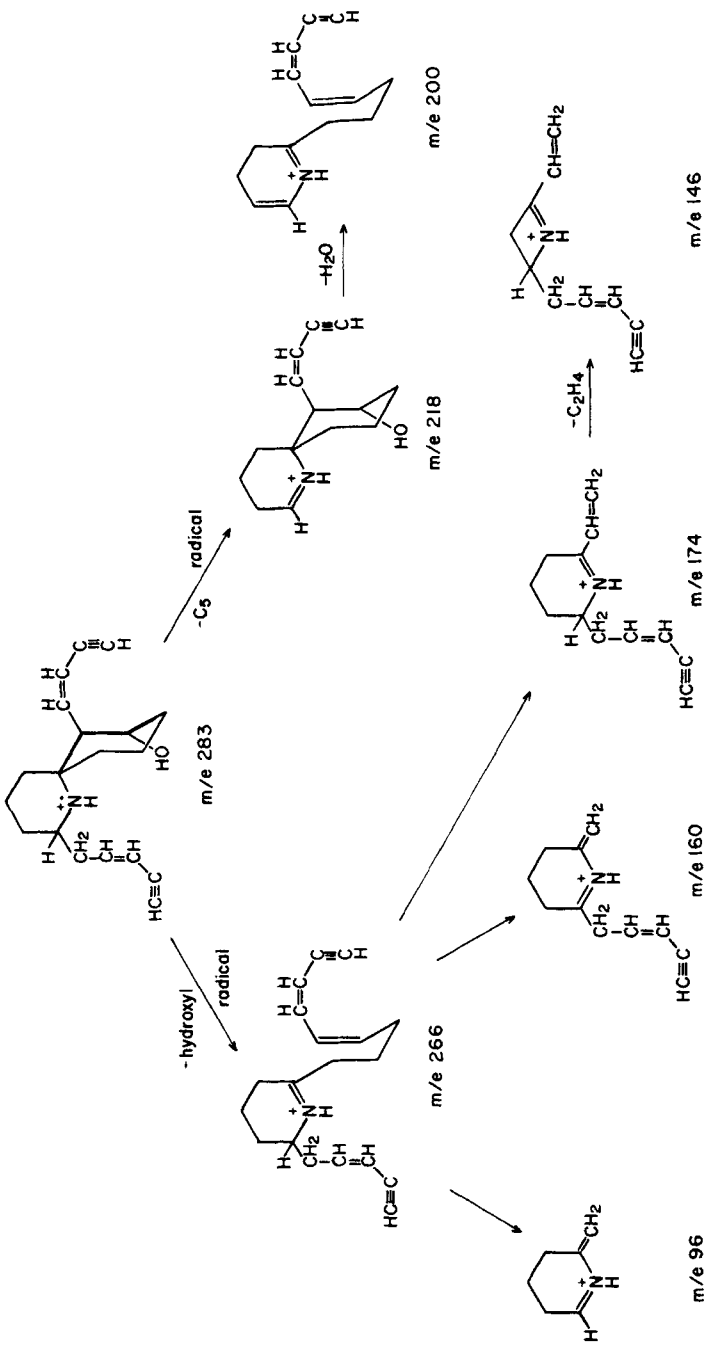


Fig. 4. *Possible Fragmentation Pathways for Histrionicotoxin* (m/e *283*). Similar fragmentations were observed for the various di-, tetra-, octa- and dodecahydro-(iso)histrionicotoxins. An alternate major pathway for octahydrohistrionicotoxin (*m/e* 291) is illustrated

Analogs of histrionicotoxin of the type described in this paper are proving their usefulness as tools in the study of ion conductance changes in electrogenic membranes [2–5]. Pharmacological evaluation of these analogs revealed the importance of the nature of the side chain with respect to cholinolytic activity or antagonism to the transport of sodium or potassium through their respective channels in electrogenic membranes [4] [5]. Such differences in relative reactivity of these alkaloids at membrane sites may reflect differences in i) steric parameters as illustrated in Fig. 1 for conformations of the side chains of crystalline histrionicotoxin and isodihydrohistrionicotoxin and ii) differences in electronic properties of the saturated, olefinic, allenic and acetylenic side chains. Synthetic efforts aimed at the elaboration of these and other spiropiperidine alkaloids have been initiated and further studies on the alkaloids found in skin extracts of other dendrobatid frogs are in progress.

Experimental Part. – All m.p. are uncorrected. NMR. spectra were obtained in chloroform solution on JEOLCO model JNM-PS100, *Varian* A-60 or *Varian* HR-100 spectrometers. Electron impact mass spectra were determined on a *Hitachi* RMU-6C mass spectrometer at 70 ev. The identities of parent ions were confirmed by chemical ionization spectra obtained on a *Finnegan* 1015 spectrometer with isobutane as the reactant gas. High resolution spectra for the determination of empirical formulae of parent ions and fragments were determined at 70 ev on a AEI MS9 spectrometer and were determined by Dr. *R. Foltz, Battelle* Research Institute, Columbus, Ohio. Roentgen ray analysis of crystals of reduced histrionicotoxin was carried out by Dr. *I. L. Karle*, Naval Research Laboratory, Washington, D.C.

Histrionicotoxin. The name of histrionicotoxin according to the nomenclature adopted by Chemical Abstracts (courtesy of Dr. K. L. Loening, Director of Nomenclature) is [6*R*-[6α(2*S**-(*Z*)], 7β(*Z*), 8α]]-7-(1-buten-3-ynyl)-2-(2-penten-4-ynyl)-1-azospiro[5.5]undecan-8-ol. IR., UV., NMR. and mass spectral data on this alkaloid have been reported [1]. For NMR. spectral data see also Fig. 4. – MS. (major peaks; *m/e* with relative intensity in parentheses): 283 (19), 282 (11), 266 (14), 218 (58), 200 (21), 190 (10), 188 (10), 174 (14), 172 (12), 160 (28), 146 (10), 124 (14), 108 (16), 96 (100). – UV. (EtOH): 224 nm, ε 22,300. – IR. ($HCCl_3$): 2100 cm^{-1} (acetylene).

Isodihydrohistrionicotoxin. IR., UV., NMR. und mass spectral data on this alkaloid have been reported [1]. For NMR. data see also Fig. 4. – MS. 285 (24), 284 (12), 268 (26), 246 (14), 230 (14), 228 (16), 228 (16), 218 (26), 200 (16), 192 (14), 190 (27), 176 (45), 162 (37), 148 (21), 134 (24), 122 (27), 120 (21), 109 (46), 108 (34), 106 (30), 96 (100). – UV. (EtOH): 225 nm, ε 8100, 235 nm, ε 7200. – IR. ($HCCl_3$): 1958 cm^{-1} (allene), 2100 cm^{-1} (acetylene).

Neodihydrohistrionicotoxin. NMR. spectrum simular to histrionicotoxin except as shown in Fig. 4. – MS.: 285 (60), 284 (18), 268 (20), 220 (100), 202 (35), 190 (15), 176 (20), 160 (65), 132 (25), 96 (50). – UV. (EtOH): 224 nm, ε 17,300. – IR. ($HCCl_3$): 2100 cm^{-1} (acetylene), 1670 cm^{-1} (diene).

Tetrahydrohistrionicotoxin. NMR. spectrum similar to histrionicotoxin except as shown in Fig. 4. – MS.: 287 (20), 286 (8), 270 (8), 220 (80), 202 (35), 176 (15), 96 (100). – UV. (EtOH): 228 nm, ε 3900. – IR. ($HCCl_3$): 1670 cm^{-1} (diene).

Isotetrahydrohistrionicotoxin. NMR. spectrum similar to isodihydrohistrionicotoxin except as shown in Fig. 4. – MS.: 287 (28), 286 (14), 270 (24), 220 (30), 202 (32), 176 (50), 162 (40), 96 (100). – UV. (EtOH): 228 nm, ε 19,200. – IR. ($HCCl_3$): 1950 cm^{-1} (allene), 1665 cm^{-1} (diene).

Octahydrohistrionicotoxin, m.p. 180–181° (HBr). – NMR spectrum similar to histrionicotoxin except as shown in Fig. 4. – MS.: 291 (28), 274 (21), 250 (88), 236 (15), 232 (13), 222 (50), 194 (36), 192 (22), 178 (100), 165 (36), 98 (13), 96 (34). – UV. (EtOH): end absorption.

HTX-D, m.p. 231–232 (dec.). NMR. spectrum, see Fig. 4 for assignments of C(5) chain. Mass spectrum with empirical formula and relative intensity of fragments in parentheses: 287 ($C_{19}H_{29}NO$, 12), 270 ($C_{19}H_{28}N$, 2), 242 ($C_{17}H_{24}N$, 100), 222 ($C_{14}H_{24}NO$, 50), 190 ($C_{12}H_{16}NO$, 2), 176 ($C_{12}H_{18}N$, 3) and 148 ($C_{10}H_{14}N$, 3). – UV.: (EtOH): 225 nm, ε 8400. – IR. ($HCCl_3$): 2120 cm^{-1} (acetylene).

Reaction of 300 μg HTX-D with 25 μl of Ac_2O in 0.5 ml of acetone containing 2 mg of NaOAc for 1 h, followed by addition of methanol, water and extraction with chloroform yielded a single basic compound: O-Acetyl-HTX-D. – MS.: 329 (3), 264 (45), 242 (100).

Reduction of HTX-D with hydrogen and Pd/C in ethanol for 6 h yielded a single compound, hexahydro-HTX-D, which was isolated by column chromatography on silica gel with chloroform/2-propanol/aqueous ammonia 15:1:0.1. – MS.: 293 (5), 292 (3), 250 (16), 248 (100), 222 (32).

Hydrogenation of Hystrionicotoxin. A. With Pd/C: Histrionicotoxin (40 mg) was dissolved in 10 ml of tetrahydrofuran containing 100 mg of 5% Pd/C and reduced with hydrogen for 4 h followed by filtration and evaporation of the solvent. The residue which showed one component by thin layer chromatography on silica gel was purified by chromatography on a small silica gel column with chloroform containing 2% methanol to yield approximately 30 mg of dodecahydrohistrionicotoxin. One hydrogenation with smaller amounts of catalyst afforded material which deposited crystals of the hydrochloride salt from acetone. Roentgen ray analysis of one of these crystals established that it contained co-crystallized tetrahydro- and octahydro-compounds. The tetrahydro-compound was isomeric with naturally occurring tetrahydrohistrionicotoxin, but the unsaturated moieties of the five carbon atoms side chain had undergone partial reduction and isomerization to a 1,3-pentadienyl system. The octahydro-compound contained a saturated four carbon atoms side chain and the same 1,3-pentadienyl system.

B. With Lindlar's *catalyst.* Histrionicotoxin (28 mg) was dissolved in 2 ml of ethanol with 3 mg of *Lindlar*'s palladium catalyst and 2 drops of quinoline. In the course of 1 h 3.5 ml of hydrogen was consumed. Following filtration and concentration, the residue was chromatographed on a Sephadex LH-20 column in benzene/cyclohexane/ethanol/triethylamine (solvent d, Fig. 2) with 2.7 ml fractions. Fractions 45–48 contained 15 mg of a mixture of tetrahydro- and hexahydrohistrionicotoxin. Fractions 50–58 contained 10 mg of a 'synthetic' dihydrohistrionicotoxin. The mixture (fractions 45–48) was chromatographed on a silica gel column in chloroform/2-propanol/aqueous ammonia 15:1:01 with 1.5 ml fractions. Fractions 14–19 contained 9 mg of a tetrahydrohistrionicotoxin identical in properties with natural tetrahydrohistrionicotoxin. Fractions 21–33 contained 3 mg of hexahydrohistrionicotoxins.

Dodecahydrohistrionicotoxin. NMR. spectrum, see Fig. 3. – MS.: 295 (22), 278 (16), 252 (25), 238 (15), 224 (68), 196 (36), 180 (100), 167 (40), 96 (65).

'Synthetic' Dihydrohistrionicotoxin. NMR. spectrum similar to histrionicotoxin except as shown in Fig. 4. – MS.: 285 (26), 284 (8), 267 (35), 252 (26), 238 (8), 218 (100), 200 (84), 176 (13), 145 (43), 96 (78). – UV. (EtOH): 226 nm, ε 24,700. – IR. ($CHCl_3$): 2100 cm^{-1} (acetylene), 1670 cm^{-1} (diene).

Hexahydrohistrionicotoxins. More than one isomer was contained in this product. – MS.: 289 (48), 272 (23), 254 (23), 222 (20), 220 (45), 204 (20), 202 (26), 178 (60), 165 (25), 96 (100). The peaks at 222 and 220, due to loss of five carbon side chain, are indicative of the presence of at least two hexahydro-compounds with dihydro- and tetrahydro-five carbon side chains, respectively.

N-Methyldodecahydrohistrionicotoxin. Dodecahydrohistrionicotoxin (6 mg) was dissolved in 0.5 ml of acetonitrile or methanol containing a 20 fold excess of methyliodide. After 6 days at room temperature in the dark, the secondary amine had been converted quantitatively to the tertiary N-methyl base. The tertiary amine was isolated by preparative thin layer chromatography (silica gel, chloroform/methanol 9:1) to yield approximately 4 mg. – MS.: 307 (45), 290 (18), 264 (24), 252 (14), 238 (100), 210 (11), 194 (42), 181 (48), 114 (63), 110 (47).

The methiodide of the above compound was obtained when the methylation was carried out in dimethylsulfoxide. The mass spectrum was virtually identical with that of N-methyldodecahydrohistrionicotoxin.

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