23. Total Synthesis and Electrophysiological Properties of Natural (−)-Perhydrohistrionicotoxin, its Unnatural (+)-Antipode and their 2-Depentyl Analogs

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Dedicated to Dr. *Willy Leimgruber*, deceased July 8, 1981

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Summary

Natural (−)-perhydrohistrionicotoxin (**6a**), its unnatural (+)-antipode **6b**, (−)-2-depentylperhydrohistrionicotoxin (**7a**) and its (+)-antipode **7b** have been prepared and characterized. *Kishi*'s lactam **8** reacted with optically active isocyanates, and the mixture of diastereomeric carbamates so obtained was separated and hydrolyzed yielding the optical antipodes of *Kishi*'s lactam in optically pure form. Reduction with $LiAlH_4$ yielded the optically active 2-depentyl analogs, while another sequence already developed in the racemic series afforded the natural toxin and its (+)-antipode. Some electrophysiological properties of these compounds are presented.

Natural histrionicotoxin (**1**, (−)-HTX) was isolated from extracts of skins of the Columbian poison frog *Dendrobates histrionicus* by *Witkop et al.* [1]. HTX represents a spiropiperidine, substituted at C(2) and C(7) with side-chains containing *cis*-ene-yne unsaturation *(Scheme 1)*.

The absolute configuration of **1** shown in *Scheme 1*, was deduced from data obtained by a single crystal X-ray analysis of isodihydrohistrionicotoxin hydro-

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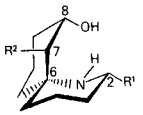
Scheme 1



(2*S*,6*R*,7*S*,8*S*) for **1**–**3**

(2*R*,6*R*,7*S*,8*S*) for **4**–**6**

(6*R*,7*S*,8*S*) for **7a**

1	Histrionicotoxin	$R^1 = CH_2CH{=}CH{-}C{\equiv}CH$ $R^2 = CH{=}CH{-}C{\equiv}CH$
2	Isodihydrohistrionicotoxin	$R^1 = CH_2CH_2CH{=}C{=}CH_2$ $R^2 = CH{=}CH{-}C{\equiv}CH$
3	Neodihydrohistrionicotoxin	$R^1 = CH_2CH{=}CH{-}C{\equiv}CH$ $R^2 = CH{=}CH{-}CH{=}CH_2$
4	Allodihydrohistrionicotoxin	$R^1 = CH_2CH_2CH_2C{\equiv}CH$ $R^2 = CH{=}CH{-}C{\equiv}CH$
5	Octahydrohistrionicotoxin	$R^1 = CH_2CH_2CH_2CH{=}CH_2$ $R^2 = CH_2CH_2CH{=}CH_2$
6a	Perhydrohistrionicotoxin	$R^1 = C_5H_{11}$ $R^2 = C_4H_9$
7a	Depentylperhydrohistrionicotoxin	$R^1 = H$ $R^2 = C_4H_9$

a-Series = (–)-Series

chloride (**2** · HCl) [2][2]). Optical rotations for **1** and its partially hydrogenated natural congeners **2**–**4** are recorded here for the first time *(Table 1)*[3]).

Dodecahydrohistrionicotoxin (**6a**), commonly named perhydrohistrionicotoxin or H_{12}-HTX, is obtained by hydrogenation of **1** or isodihydrohistrionicotoxin (**2**) over Pd/C in THF [4]. H_{12}-HTX, a naturally derived compound[4]), is a reference compound of relevance to many of these alkaloids. It was found that **6a** had biological activity similar to that of **1** in the *in vitro* assay in nerve muscle preparations of frogs, and therefore provides a biochemical standard [3]. A total synthesis of (±)-**6** would be an attractive goal for the synthetic organic chemists, and several completed total syntheses of this compound are the fruits of such efforts [5]. An investigation regarding the structure/activity relationship in this interesting series of spiroamines has also been carried out in the more easily accessible 2-depentyl series in which (±)-**7** showed equal potency in the bioassay as **6a** [6].

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[2]) Careful inspection of the data reported [2] shows, that the stereodesignation given for isodihydrohistrionicotoxin is that of an isomer. This misnomer was recognized, and the IUPAC nomenclature for H_{12}-HTX (**6a**) is now (–)-(2*R*,6*R*,7*S*,8*S*)-7-butyl-2-pentyl-1-azaspiro[5.5]undecan-8-ol. We would like to thank Dr. *J. V. Silverton* for helpful discussions regarding this topic. Because of the complexity of the IUPAC nomenclature, we are using in this report the informal nomenclature, relating to histrionicotoxin, the most unsaturated toxin thus far isolated in this series.

[3]) Compounds **2**–**4** belong to the (–)-series of toxins. There are several other partially hydrogenated HTX-derivatives found in nature [4] including **5**, but there was not enough material available to measure their optical rotations. The members of the (–)-series possess the same absolute configuration as the compounds of the perhydro **a**-series discussed in this paper. We would like to thank Dr. *J. W. Daly* for having given us the natural toxins **1**–**4** and a sample of naturally derived **6a**.

[4]) We would like to thank Dr. *J. W. Daly* of the Laboratory of Bio-Organic Chemistry, NIADDK, National Institutes of Health, for having informed us that the catalytic hydrogenation of isodihydrohistrionicotoxin (**2**), a major natural congener of **1** in frog tissues, and used in form of its hydrochloride for the X-ray structure determination, also afforded **6a** under the same conditions. It is thus clearly demonstrated that **1**, **2** and **6a** possess the same absolute configuration at all 4 centers of chirality.

Table 1. *Physical data of* **1–4**, **6a**, **6b**, **7a**, **7b**, **15a** *and* **15b**[a])

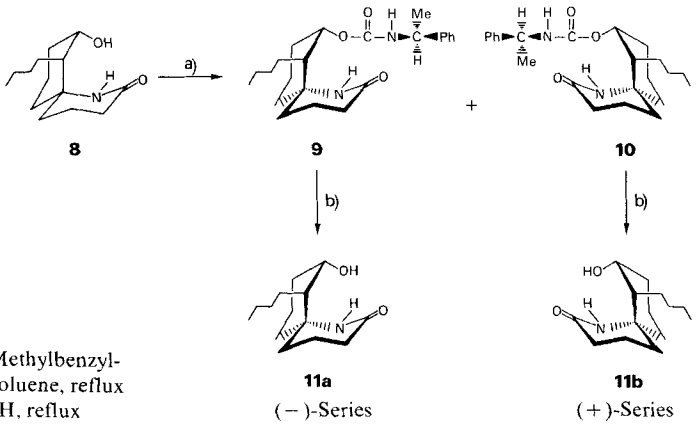
	Compound	$[\alpha]_D^{25}$ (*c*, solvent)	M.p. (°C)
1	Histrionicotoxin HTX	−96.3° (1.04, EtOH)	225–228 (decomp.)
2	Isodihydrohistrionicotoxin Iso-H_2-HTX	−35.3° (0.51, EtOH)	240–243 (decomp.)
3	Neodihydrohistrionicotoxin Neo-H_2-HTX	−125.9° (1.06, EtOH)	195–200 (decomp.)
4	Allodihydrohistrionicotoxin Allo-H_2-HTX	−43.4° (1.18, EtOH)	247–250 (decomp.)
6a	Perhydrohistrionicotoxin H_{12}-HTX (natural)	−34.6° (1.00, EtOH) −36.2° (1.01, $CHCl_3$)	184–186
	Perhydrohistrionicotoxin H_{12}-HTX (synthetic)	−34.5° (1.01, EtOH) −36.0° (1.00, $CHCl_3$)	184–186
6b	Enantiomer of **6a**	+35.8° (1.00, $CHCl_3$)	184–186
15a	2-Epi-perhydrohistrionicotoxin 2-Epi-H_{12}-HTX	−46.4° (1.02, $CHCl_3$)	223–225
15b	Enantiomer of **15a**	+46.6° (1.01, $CHCl_3$)	223–225
7a	Depentylperhydrohistrionicotoxin	−16.6° (1.01, EtOH)	180–181
7b	Enantiomer of **7a**	+16.8° (1.03, EtOH)	180–181

[a]) The data of **1–4**, **6** and **15** refer to their hydrochlorides, the ones of **7a** and **7b** to their 2,4,6-trinitrobenzenesulfonates.

We now would like to report the first total synthesis of naturally derived (−)-H_{12}-HTX (**6a**) and its unnatural enantiomer **6b**. This account furthermore includes the preparation and characterization of the corresponding enantiomers **7a** and **7b** of the 2-depentyl series. A short discussion of the preliminary biological activities measured with all four optical isomers is included.

Synthesis. – *Kishi*'s lactam **8** containing an alcohol function at C(8) was prepared in 20% overall yield from readily available starting materials [6]. Its optical resolution was achieved by transformation of the alcohol moiety, followed by a separation of the two diastereomers formed. The sequence is shown in *Scheme 2*,

Scheme 2



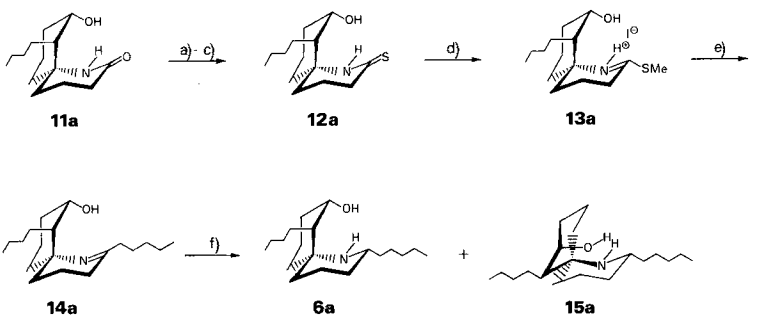
[a]) (+)-(*S*)-α-Methylbenzyl-isocyanate/toluene, reflux
[b]) EtONa/EtOH, reflux

and can be summarized as follows: Condensation of **8** with (+)-(*S*)-α-methylbenzylisocyanate in toluene afforded a mixture of the two carbamates **9** and **10** which were separated by preparative HPLC., affording the two esters in crystalline form. Hydrolysis of **9** and **10** with sodium ethoxide in ethanol gave the optically active lactams **11a** and **11b**, showing identical physical properties except for their opposite optical rotations.

Conversion of **11a** and **11b** followed routes already established in the racemic series [5] and afforded, as shown in *Scheme 3*, the optically active thiolactam **12a** and its thiomethyl ether derivative **13a**[5]). *Grignard* reaction of **13a** with pentylmagnesium bromide in methylene chloride gave the ketimine **14a**, isolated as the crystalline hydrobromide salt. We were unable to achieve a highly stereoselective reduction of **14a** to **6a**, but could easily separate **6a** from its epimer **15a**[6]) by chromatography of the reaction mixture over silica gel. HPLC. and NMR. analysis of the mixtures obtained by reducing **14a** with AlH_3 at room temperature in cyclohexane revealed the presence of **6a** besides **15a** in a ratio of 7:3. Both compounds could be separated by chromatography and gave crystalline and stable hydrochloride salts. The sample of **6a** · HCl obtained by total synthesis was identical in every respect with natural material. Except for optical properties and m.p., our sample was also identical with synthetic (±)-**6** prepared elsewhere[7]).

Reduction of **11a** with $LiAlH_4$ or catalytic desulfurization of **12a** with *Raney*-nickel catalyst afforded the (−)-2-depentyl compound **7a** which could not be obtained as a crystalline hydrochloride salt, but was characterized as its 2,4,6-tri-

Scheme 3[8])



[a]) Ac_2O/Py, 25°. [b]) P_2S_5/benzene, reflux. [c]) MeONa/MeOH, 25°. [d]) MeI/CH_2Cl_2, 25°. [e]) $C_5H_{11}MgBr$/CH_2Cl_2, 0°→ reflux. [f]) AlH_3/cyclohexane, 25°.

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[5]) The reaction sequence carried out in the enantiomeric (+)-series (starting with **11b**) took a similar course and shall therefore not be discussed in detail. The physical data of the compounds of the (+)-series are listed in the exper. part.

[6]) The structure shown for epimer **15a** is one of possible conformers.

[7]) We would like to thank Prof. *Y. Kishi* for having provided us with a sample of synthetic (±)-**6** · HCl.

[8]) The same sequence, but starting with **11b** ((±)-series), afforded the enantiomers of the compounds mentioned in *Scheme 3*.

nitrobenzene sulfonate[9]). Physical measurements carried out with **7a** and **7b** proved these compounds to be chemically and optically pure.

Effects of (+)- and (−)-perhydrohistrionicotoxin (6b and 6a), and (+)- and (−)-dependylperhydrohistrionicotoxin (7b and 7a) on the neuromuscular transmission of the frog sartorius muscle. – 1. *Electrophysiological Techniques.* All experiments were performed at room temperature (20–22°) on sciatic sartorius muscle preparations of the frog, *Rana pipiens*. The physiological solutions used had the following composition (concentrations given in mmol/l): NaCl 116, KCl 2.0, $CaCl_2$ 1.8, Na_2HPO_4 1.3 and NaH_2PO_4 0.7. Through the solutions 100% O_2 was bubbled; they had a pH of 6.9–7.1.

For twitch tension studies, the nerve was stimulated with supramaximal pulses having a duration varying from 0.05 to 0.1 ms *via* an Ag/AgCl bipolar platinum electrode [3]. Direct stimulation of the muscle was accomplished by applying supramaximal rectangular pulses of 1.0–2.0 ms duration at a rate of 0.05 Hz through a bipolar platinum electrode placed around the middle portion of the muscle. The muscle tension generated by both direct and indirect stimulation was measured by a *Grass* FT03 force-displacement transducer, and the twitches tensions were reported on a *Grass* polygraph.

Solutions ($(1–2) \cdot 10^{-2}$ M) were made in 95% ethanol and stored at −10°. They were diluted with physiological NaCl-solution immediately before use.

2. *Discussion of biological data.* Isometric contraction of the frog sciatic nerve sartorius muscle preparations elicited by indirect stimulation was blocked by both (+)- and (−)-perhydrohistrionicotoxin (H_{12}-HTX; **6b** and **6a**, resp.) and their (+)- and (−)-dependyl analogs **7b** and **7a**, respectively. The onset of this blockade began immediately after the addition of the toxin to the preparation. Both (+)- and (−)-H_{12}HTX completely blocked neuromuscular transmission, although the (+)-H_{12}-HTX blocked the twitch 10 min earlier than did (−)-H_{12}-HTX in all three muscles (*Fig. 1* and *Table 2*). Though suggestive, this difference is not statistically significant. Equimolar concentrations ($2 \cdot 10^{-5}$ M) of the (+)- and (−)-dependyl analogs blocked the indirectly elicited twitch with indistinguishable time courses (*Fig. 1* and *Table 2*). Thus, recognition of optical isomers is not noticeable in this system and seems less important than other, probably hydrophobic, effects [6].

The directly elicited twitch was not significantly blocked by $2 \cdot 10^{-5}$ M concentrations of (+)- or (−)-H_{12}-HTX, or (+)- or (−)-dependyl-H_{12}-HTX *(Table 2)*. None of these compounds potentiated the directly or indirectly elicited twitches at this concentration. There was very little if any recovery after (+)- or (−)-H_{12}-HTX but partial recovery (40 to 60% of control) was observed with the (+)- and (−)-dependyl analogs following washing with normal physiological *Ringer's* solution (60 min).

The finding that the natural (−)-isomers were almost equipotent with the unnatural (+)-isomers in this assay is interesting but has to be substantiated in other biological systems. It is also noteworthy that the 2-pentyl group in H_{12}-HTX does not seem to be crucial [6].

(−)-H_{12}-HTX (**6a**) which is of considerable importance in studying cholinergic receptor mechanism in the neuromuscular system [7] has now become available in quantity.

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[9]) For biological testing the trinitrobenzene sulfonates were converted in the usual way into their free bases which were purified by chromatography over silica gel, dissolved in the stoichiometric amount of aqueous 1 N HCl, and adjusted, to afford a 1% solution of **7a** · HCl and **7b** · HCl.

Table 2. *Effects of (+)- and (−)-perhydrohistrionicotoxin (H_{12}-HTX; **6b** and **6a**, resp.) and (+)- and (−)-dependylperhydrohistrionicotoxin (dependyl-H_{12}-HTX; **7b** and **7a**, resp.) on the sciatic nerve sartorius muscle preparation of the frog*[a])

Compound	Twitch tension as % of control at time shown Indirect (min)							
	0	5	10	15	20	25	30	60
(+)-H_{12}-HTX	100	47±10	21±7	7±5	0	0	0	0
(−)-H_{12}-HTX	100	75±12	44±10	23±8	11±5	5±2	0	0
(+)-Depentyl-H_{12}-HTX	100	60±5	48±7	37±4	29±5	24±8	24±8	21±6
(−)-Depentyl-H_{12}-HTX	100	67±8	48±6	42±6	33±6	29±5	24±3	17±2
	Direct (min)							
	0	5	10	15	20	30	60	
(+)-H_{12}-HTX	100	96±5	91±6	87±6	87±9	85±7	84±10	
(−)-H_{12}-HTX	100	93±2	86±3	79±10	77±11	75±13	75±13	
(+)-Depentyl-H_{12}-HTX	100	98±2	97±2	92±2	84±5	81±4	73±2	
(−)-Depentyl-H_{12}-HTX	100	100±2	92±7	91±5	85±4	80±6	70±6	

[a]) Muscles were exposed to toxins ($2 \cdot 10^{-5}$ M) for 60 min. The values are means ± S.E.M. of three muscles. Toxins were added to the bath following 30–40 min after setting up the muscles. There was no recovery of the twitch after H_{12}-HTX while partial recovery (40–60%) was observed after depentyl-HTX following washout with normal physiological *Ringer*'s solution for 60 min.

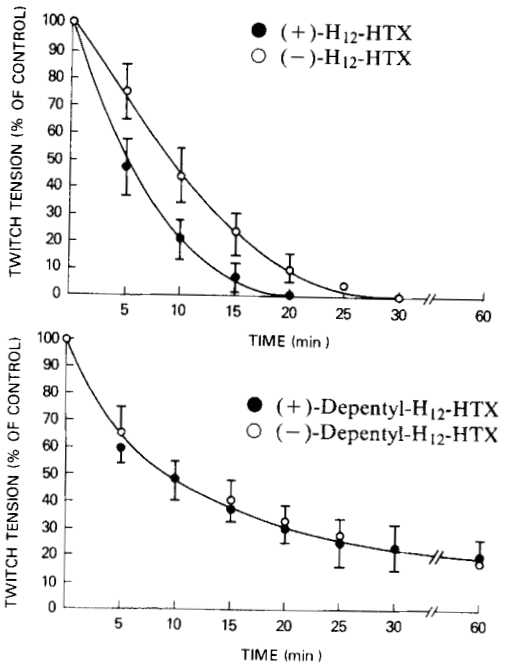

Fig. 1. *Time course of the block of the indirectly elicited isometric twitch tension of the frog sartorius muscles produced by $2 \cdot 10^{-5}$ M (+)- and (−)-perhydrohistrionicotoxin (**6b** and **6a**, resp.) and (+)- and (−)-depentyl analogs **7b** and **7a** at 22°.* Toxin was added to the bath after the muscle twitch had stabilized (30–40 min). Results are shown as mean ± S.E.M. of 3 muscles.

Experimental Part

General remarks. Melting points were taken on a *Fisher-Johns* melting point apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation of this Laboratory. Optical rotations were determined by using a *Perkin-Elmer* Model 241 MC polarimeter. IR. spectra (in cm^{-1}) were obtained on a *Beckman* 4230 instrument. ^{1}H-NMR. spectra were determined by using a *Varian* HR-220 spectrometer or a *Jeol* FX-100 spectrometer with Me_4Si as an internal reference (δ in ppm, J in Hz). Chemical ionization (CI.) mass spectra (m/z) were obtained by using a *Finnigan* 1015D spectrometer. Electron impact (EI.) mass spectra (m/z) were obtained with a *V.G. Micromass* 7070F spectrometer with a *Perkin-Elmer* Sigma 3 gas chromatograph equipped with 2% *OV-1* column, and with a *Hitachi-Perkin-Elmer* RMU-6E spectrometer (70 eV). Analytical high performance liquid chromatography (HPLC.) was carried out with a *Waters* 6000 A solvent delivery system equipped with a 30 cm × 3.9 mm column of *μ-Porasil.* Preparative HPLC. was performed with a *Waters* Prep LC/System 500 equipped with a 30 × 5.7 cm *Silica Cartridge.* Thin-layer chromatography (TLC.) plates were purchased from *Analtech, Inc.*, and silica gel 60 for column chromatography (70–230 mesh or 230–400 mesh) were from *EM Laboratories.*

Synthesis of (−)-(S)-[(6S,7S,8S)-7-butyl-2-oxo-1-azaspiro[5.5]undec-8-yl] N-(1-phenylethyl)carbamate (**9**) *and (+)-(S)-[(6R,7R,8R)-7-butyl-2-oxo-1-azaspiro[5.5]undec-8-yl] N-(1-phenylethyl)carbamate* (**10**). A mixture of the hydroxy lactam **8** (5.75 g, 24 mmol), (+)-(*S*)-α-methylbenzylisocyanate (95%, 5.0 g, 32 mmol), and toluene (50 ml) was heated under reflux for 15 h in a current of Ar. After cooling, the mixture was evaporated under reduced pressure, and the residue was purified by prep. HPLC. with hexane/iso-PrOH 9:1 (200 ml/min) using the recycle method, to afford **10** (3.21 g, 35%), m.p. 142–143° (iso-Pr_2O). Anal. HPLC. with hexane/iso-PrOH 95:5 (2 ml/min): t_R 5.4 min. $[\alpha]_D^{25} = +45.9°$ ($c = 1.02$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +48.0°$ ($c = 1.02$, $CHCl_3$). – IR. ($CHCl_3$): 3450, 3370, 3280, 1710, and 1630. IR. (KBr): 3350, 3270, 1680, and 1660. – ^{1}H-NMR. ($CDCl_3$): 7.45–7.15 (*m*, 5 H, C_6H_5); 6.64 and 5.70 (2 br. *s*, 2 HN); 5.00–4.80 (*m*, 2 H, $C_6H_5CHCH_3$ and H–C(8)); 2.45–2.15 (*m*, 2 H, 2 H–C(3)); 1.50 (*d*, $J = 7$, 3 H, $C_6H_5CHCH_3$); 1.80–1.00 (*m*, 17 H); 0.90 (br. *s*, 3 H, $CH_3(CH_2)_3$). – CI./MS. (NH_3): 387 ($(M+1)^+$). – EI./MS.: 386 (M^+).

$C_{23}H_{34}N_2O_3$ (386.53) Calc. C 71.47 H 8.87 N 7.25% Found C 71.22 H 8.88 N 7.15%

Second fraction gave **9** (3.25 g, 35%), m.p. 177–178° (iso-Pr_2O). Anal. HPLC. (same condition as for **10**): t_R 7.6 min. $[\alpha]_D^{25} = -26.4°$ ($c = 1.02$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -27.2°$ ($c = 1.02$, $CHCl_3$). – IR. ($CHCl_3$): 3450, 3370, 3280, 1710, and 1630. IR. (KBr): 3365, 3250, 1690, and 1665. – ^{1}H-NMR. ($CDCl_3$): 7.45–7.15 (*m*, 5 H, C_6H_5); 6.45 and 5.50 (2 br. *s*, 2 HN); 4.95–4.75 (*m*, 2 H, $C_6H_5CHCH_3$ and H–C(8)); 2.45–2.15 (*m*, 2 H, 2 H–C(3)); 1.50 (*d*, $J = 7$, 3 H, $C_6H_5CHCH_3$); 1.80–1.00 (*m*, 17 H); 0.85 (br. *s*, 3 H, $CH_3(CH_2)_3$). – CI./MS. (NH_3): 387 ($(M+1)^+$). – EI./MS.: 386 (M^+).

$C_{23}H_{34}N_2O_3$ (386.53) Calc. C 71.47 H 8.87 N 7.25% Found C 71.74 H 9.14 N 7.02%

Synthesis of (6S,7S,8S)-7-butyl-8-hydroxy-1-azaspiro[5.5]undecan-2-one (**11a**). A mixture of **9** (3.86 g, 10 mmol), NaOEt (1.02 g, 15 mmol), and EtOH (99%, 50 ml) was heated under reflux for 20 h. The mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with NaCl-solution, dried ($MgSO_4$) and evaporated to leave an oil, which was chromatographed on silica gel (70–230 mesh, 60 g) with CH_2Cl_2/MeOH 95:5 to give **11a** (1.99 g, 83%), m.p. 102–103° (CH_2Cl_2/hexane). Anal. HPLC. with hexane/iso-PrOH/EtOH 85:7.5:7.5 (2 ml/min): t_R 8.8 min. $[\alpha]_D^{25} = -65.3°$ ($c = 1.01$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -68.2°$ ($c = 1.01$, $CHCl_3$). – IR. (KBr): 3265, 3190, and 1635. – ^{1}H-NMR. ($CDCl_3$): 8.28 and 5.60 (2 br. *s*, each 1 H, HN and HO); 4.04 (br. *s*, 1 H, H–C(8)); 2.40–2.10 (*m*, 2 H, 2 H–C(3)); 2.10–1.00 (*m*, 17 H); 0.88 (br. *t*, $J = 6$, 3 H, $CH_3(CH_2)_3$). – CI./MS. (NH_3): 240 ($(M+1)^+$).

$C_{14}H_{25}NO_2$ (239.36) Calc. C 70.25 H 10.53 N 5.85% Found C 70.11 H 10.30 N 5.62%

Synthesis of (6R,7R,8R)-7-butyl-8-hydroxy-1-azaspiro[5.5]undecan-2-one (**11b**). This compound was prepared by the same method as above and was identical with **11a** except for optical rotation, $[\alpha]_D^{25} = +65.1°$ ($c = 1.00$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +68.2°$ ($c = 1.00$, $CHCl_3$).

$C_{14}H_{25}NO_2$ (239.36) Calc. C 70.25 H 10.53 N 5.85% Found C 70.05 H 10.72 N 5.64%

Synthesis of (−)-2-depentylperhydrohistrionicotoxin (**7a**). a) To a stirred suspension of $LiAlH_4$ (114 mg, 3 mmol) in THF (10 ml) was added dropwise a solution of **11a** (239 mg, 1 mmol) in THF (10 ml). After the mixture was heated under reflux for 15 h in a current of Ar and cooled in an ice-bath, 10% NaOH-solution was added slowly. The mixture was filtered through *Celite*, and the filter cake was washed with CH_2Cl_2. The combined organic layer was dried ($MgSO_4$) and evaporated to leave an oil, which was chromatographed on silica gel (70–230 mesh) with CH_2Cl_2/MeOH/NH_4OH 84:15:1. Appropriate fractions afforded **7a** as an oil. Anal. HPLC. with hexane/iso-PrOH/Et_3N 95:5:0.1 (2 ml/min): t_R 5.5 min. $[\alpha]_D^{25} = -45.3°$ ($c = 7.8$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -47.8°$ ($c = 7.8$, $CHCl_3$). – IR. (neat): 3280. – ^{1}H-NMR. ($CDCl_3$): 3.86 (br. *s*, 1 H, H–C(8)); 3.10–2.60 (*m*, 2 H, 2 H–C(2)); 2.10–1.00 (*m*, 19 H); 0.90 (br. *t*, $J = 6$, 3 H, $CH_3(CH_2)_3$). – CI./MS. (NH_3): 226 ($(M+1)^+$). – EI./MS.: 225 (M^+).

To a solution of **7a** in MeOH was added HCl-saturated Et_2O. The mixture was evaporated to dryness under reduced pressure to leave a foam, attempted crystallization of which was unsuccessful. – ^{1}H-NMR. ($CDCl_3$): 8.95 and 6.80 (2 br. *s*, 2 H and 1 H, resp., HN, HO, and HCl); 4.27 (br. *s*, 1 H, H–C(8)); 3.55–2.95 (*m*, 2 H, 2 H–C(2)); 2.45–1.00 (*m*, 19 H); 0.92 (br. *s*, 3 H, $CH_3(CH_2)_3$).

2,4,6-Trinitrobenzenesulfonate of **7a** (190 mg, 37%), m.p. 180–181° (EtOH/Et_2O), $[\alpha]_D^{25} = -16.8°$ ($c = 1.01$, EtOH), $[\alpha]_{Hg\,578}^{25} = -17.3°$ ($c = 1.01$, EtOH).

$C_{20}H_{30}N_4O_{10}S$ (518.55) Calc. C 46.33 H 5.84 N 10.66% Found C 46.11 H 5.89 N 10.66%

b) A mixture of the thiolactam **12a** (51 mg, 0.2 mmol; s. below), *Raney*-nickel (excess, suspension in EtOH), and EtOH (3 ml) was heated under reflux for 1 h. The mixture was filtered through *Celite*, and the filter cake was washed with EtOH. The filtrate was evaporated to give **7a** (29 mg, 64%), identical with the sample described in a).

Synthesis of (+)-2-depentylperhydrohistrionicotoxin (**7b**). It was prepared from **11b** as above and identical with **7a** in every respect except for optical rotation. **7b**: $[\alpha]_D^{25} = +45.8°$ ($c = 8.8$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +48.0°$ ($c = 8.8$, $CHCl_3$). 2,4,6-Trinitrobenzenesulfonate of **7b**, m.p. 180–181° (EtOH/Et_2O), $[\alpha]_D^{25} = +16.8°$ ($c = 1.03$, EtOH), $[\alpha]_{Hg\,578}^{25} = +17.7°$ ($c = 1.03$, EtOH).

$C_{20}H_{30}N_4O_{10}S$ (518.55) Calc. C 46.33 H 5.84 N 10.66% Found C 46.17 H 5.56 N 10.76%

Synthesis of (6S,7S,8S)-7-butyl-8-hydroxy-1-azaspiro[5.5]undecane-2-thione (**12a**). A mixture of **11a** (1.0 g, 4.18 mmol), acetic anhydride (5 ml), and pyridine (5 ml) was set aside at 25° for 15 h. The mixture was evaporated under reduced pressure and the residue heated under reflux with P_2S_5 (0.44 g, 2 mmol) in benzene (30 ml) for 40 min. The mixture was cooled to 25°, diluted with CH_2Cl_2, washed with aq. $NaHCO_3$-solution, and dried ($MgSO_4$). To the solution was added a solution of NaOMe (0.54 g, 10 mmol) in MeOH (50 ml) and the mixture was stirred for 30 min. The mixture was washed with sat. NaCl-solution, dried ($MgSO_4$) and evaporated to leave a solid, which was recrystallized from CH_2Cl_2/Et_2O to yield **12a** (0.89 g, 84%), m.p. 170–171°, $[\alpha]_D^{25} = -174.7°$ ($c = 1.00$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -183.5°$ ($c = 1.00$, $CHCl_3$). – IR. (KBr): 3380, 3170, 3050, and 1545. – ^{1}H-NMR. ($CDCl_3$): 10.45 and 3.50 (2 br. *s*, each 1 H, HO, HN); 4.10 (br. *s*, 1 H, H–C(8)); 3.05–2.50 (*m*, 2 H, 2 H–C(3)); 2.00–1.00 (*m*, 17 H); 0.90 (br. *s*, 3 H, $CH_3(CH_2)_3$). – EI./MS.: 255 (M^+).

$C_{14}H_{25}NOS$ (255.42) Calc. C 65.83 H 9.87 N 5.48% Found C 65.48 H 9.87 N 5.43%

Synthesis of (6R,7R,8R)-7-butyl-8-hydroxy-1-azaspiro[5.5]undecane-2-thione (**12b**). This compound was prepared as above from **11b** and is identical in every respect except for optical rotation, $[\alpha]_D^{25} = +174.0°$ ($c = 1.02$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +183.6°$ ($c = 1.02$, $CHCl_3$).

$C_{14}H_{25}NOS$ (255.42) Calc. C 65.83 H 9.87 N 5.48% Found C 65.66 H 9.99 N 5.42%

Synthesis of (6S,7S,8S)-7-butyl-8-hydroxy-2-methylthio-1-azaspiro[5.5]undec-1-ene hydroiodide (**13a**). A solution of **12a** (430 mg, 1.69 mmol), and MeI (0.25 ml, 4 mmol) in CH_2Cl_2 (10 ml) was stirred at 25° for 15 h. Evaporation of the mixture afforded a solid, the recrystallization of which from CH_2Cl_2/Et_2O gave **13a** (536 mg, 80%), m.p. 151–153°, $[\alpha]_D^{25} = -113.2°$ ($c = 1.01$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -118.8°$ ($c = 1.01$, $CHCl_3$). – IR. (KBr): 3200 and 1615. – ^{1}H-NMR. ($CDCl_3$): 5.79 (br. *s*, 1 H, HO); 4.48 (br. *s*, 1 H, H–C(8)); 3.25–2.75 (*m*, 2 H, 2 H–C(3)); 2.86 (*s*, 3 H, CH_3S); 2.25–1.00 (*m*, 17 H); 0.90 (br. *s*, 3 H, $CH_3(CH_2)_3$). – CI./MS. (NH_3): 270 ($(M-HI+1)^+$). – EI./MS.: 269 ($M^+ - HI$).

$C_{15}H_{28}INOS$ (397.35) Calc. C 45.34 H 7.10 N 3.52% Found C 45.64 H 6.98 N 3.39%

Synthesis of (6R,7R,8R)-7-butyl-8-hydroxy-2-methylthio-1-azaspiro[5.5]undec-1-ene hydroiodide (**13b**). This compound was prepared from **12b** as above and was identical with **13a** in every respect except for optical rotation, $[\alpha]_D^{25} = +113.6°$ ($c = 1.02$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +119.6°$ ($c = 1.02$, $CHCl_3$).

$C_{15}H_{28}INOS$ (397.35) Calc. C 45.34 H 7.10 N 3.52% Found C 45.33 H 6.99 N 3.37%

Synthesis of (6R,7S,8S)-7-butyl-8-hydroxy-2-pentyl-1-azaspiro[5.5]undec-1-ene (**14a**). To a stirred solution of **13a** (536 mg, 1.35 mmol) in CH_2Cl_2 (30 ml) was added pentylmagnesium bromide (1.9M in Et_2O, 15 ml, 28.5 mmol) slowly at 0–5° in a current of Ar. After the ice-bath was removed, the mixture was heated at reflux for 15 h. The mixture was cooled with an ice-bath and sat. NH_4Cl-solution (5 ml) was added dropwise. The resulting mixture was filtered through *Celite*, the organic layer was dried ($MgSO_4$) and evaporated to leave an oil, which was chromatographed on silica gel (230–400 mesh, 40 g) with CH_2Cl_2/MeOH 92:8 and then on aluminum oxide (neutral, grade III) with hexane/Et_2O 1:1 to afford **14a** as an oil (214 mg, 54%). Anal. HPLC. with hexane/THF/Et_3N 90:10:0.1: $t_R = 3.8$ min. – IR. (neat): 3240 and 1655. – ^{1}H-NMR. ($CDCl_3$): 3.95 (br. *s*, 1H, H–C(8)); 2.36–1.00 (*m*, 27H); 0.87 (br. *s*, 6H, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$). – CI./MS. (NH_3): 294 ($(M+1)^+$). – EI./MS.: 293 (M^+).

Hydrobromide of **14a**, m.p. 152–154° (MeOH/EtOAc), $[\alpha]_D^{25} = -116.2°$ ($c = 1.02$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -121.8°$ ($c = 1.02$, $CHCl_3$). – IR. (KBr): 3360 and 1670. – ^{1}H-NMR. ($CDCl_3$): 5.40 and 2.76 (2 br. *s*, each 1H, HO and HBr); 4.16 (br. *s*, 1H, H–C(8)); 3.11–2.60 (*m*, 4H, CH_2–C=N and 2 H–C(3)); 2.36–1.15 (*m*, 23H); 0.88 and 0.90 (2 br. *s*, each 3H, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$).

$C_{19}H_{35}NO \cdot HBr$ (374.42) Calc. C 60.95 H 9.69 N 3.74% Found C 60.81 H 9.75 N 3.53%

Synthesis of (6S,7R,8R)-7-butyl-8-hydroxy-2-pentyl-1-azaspiro[5.5]undec-1-ene (**14b**). This compound was prepared as above from **13b** and is identical with **14a** in every respect except for optical rotation, $[\alpha]_D^{25} = +116.1°$ ($c = 1.05$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +122.0°$ ($c = 1.05$, $CHCl_3$), m.p. 154–156° (MeOH/EtOAc), mixed m.p. with **14a** 134–136°.

$C_{19}H_{35}NO \cdot HBr$ (374.42) Calc. C 60.95 H 9.69 N 3.74% Found C 61.02 H 9.88 N 3.61%

Synthesis of (–)-perhydrohistrionicotoxin (**6a**) *and (–)-2-epi-perhydrohistrionicotoxin* (**15a**). To a stirred solution of **14a** (190 mg, 0.65 mmol) in cyclohexane (40 ml) was added $AlH_3 \cdot 1/3\ Et_2O$ (110 mg, 2 mmol). and the mixture was allowed to stir at 25° for 15 h. Aq. sat. solution of sodium potassium tartrate was added slowly to the mixture with ice-bath cooling. The layers were separated, and the aq. layer was extracted with CH_2Cl_2. The combined organic layer was dried ($MgSO_4$) and evaporated to leave an oil. Anal. HPLC. with hexane/iso-PrOH/Et_3N 97:3:0.1 shows a ratio **15a**/**6a** of 27:73; t_R of **6a** 4.3 min, t_R of **15a** 2.4 min (2 ml/min). Column chromatography on silica gel (230–400 mesh, 0.5 × 5 in., CH_2Cl_2/MeOH/NH_4OH 95:5:0.5, 2.5 in./min) afforded **15a** as an oil. – ^{1}H-NMR. ($CDCl_3$): 3.85 (br. *s*, 1H, H–C(8)); 2.65–2.45 (*m*, 1H, H–C(2)); 2.10–1.00 (*m*, 27H); 0.88 (br. *s*, 6H, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$).

Hydrochloride of **15a** (62 mg, 29%), m.p. 223–225° (MeOH/Et_2O), $[\alpha]_D^{25} = -46.3°$ ($c = 1.05$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -48.4°$ ($c = 1.05$, $CHCl_3$). – IR. (KBr): 3420, 3240, 3090, and 1595. – ^{1}H-NMR. ($CDCl_3$): 9.80, 8.30 and 5.30 (3 br. *s*, each 1H, HN, HO, and HCl); 4.20 (br. *s*, 1H, H–C(8)); 3.20–2.70 (*m*, 1H, H–C(2)); 2.60–2.30 (*m*, 1H, H–C(7)); 2.30–1.00 (*m*, 26H); 0.85 (br. *s*, 6H, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$).

$C_{19}H_{37}NO \cdot HCl$ (331.97) Calc. C 68.74 H 11.54 N 4.22% Found C 68.86 H 11.45 N 3.86%

Further elution gave **6a**. – ^{1}H-NMR. ($CDCl_3$): 3.89 (br. *s*, 1H, H–C(8)); 3.00–2.80 (*m*, 1H, H–C(2)); 2.20–1.00 (*m*, 27H); 0.92 and 0.89 (2 br. *s*, each 3H, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$).

Hydrochloride of **6a** (113 mg, 52%), m.p. 184–186° (MeOH/Et_2O), $[\alpha]_D^{25} = -36.0°$ ($c = 1.00$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = -37.5°$ ($c = 1.00$, $CHCl_3$), $[\alpha]_D^{25} = -34.5°$ ($c = 1.01$, EtOH), $[\alpha]_{Hg\,578}^{25} = -36.0°$ ($c = 1.01$, EtOH)[10]. – IR. (KBr): 3200, 3050, and 1540. – ^{1}H-NMR. ($CDCl_3$): 9.18, 8.58, and 5.82 (3 br. *s*, each 1H, HN, HO, and HCl); 4.30 (br. *s*, 1H, H–C(8)); 3.64–3.10 (*m*, 1H, H–C(2)); 2.50–1.00 (*m*, 27H); 0.92 and 0.89 (2 br. *s*, each 3H, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$).

$C_{19}H_{37}NO \cdot HCl$ (331.97) Calc. C 68.74 H 11.54 N 4.22% Found C 68.53 H 11.60 N 3.98%

[10]) Since natural HTX and congeners (**1**–**4**) are not soluble in $CHCl_3$ as hydrochlorides, the optical rotation of **6a** · HCl was also measured in EtOH.

Synthesis of (+)-perhydrohistrionicotoxin (**6b**) *and (+)-2-epi-perhydrohistrionicotoxin* (**15b**). These compounds were prepared from **14b** and were identical with **6a** and **15a**, respectively, except for optical rotation. **15b** · HCl: m.p. 223–225° (MeOH/Et_2O), mixed m.p. with **15a** · HCl 198–200°, $[\alpha]_D^{25} = +46.2°$ ($c = 1.06$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +48.4°$ ($c = 1.06$, $CHCl_3$).

$C_{19}H_{37}NO \cdot HCl$ (331.97) Calc. C 68.74 H 11.54 N 4.22% Found C 68.58 H 11.61 N 3.91%

6b · HCl: m.p. 184–186° (MeOH/Et_2O), mixed m.p. with **6a** · HCl 159–161°, $[\alpha]_D^{25} = +35.8°$ ($c = 1.00$, $CHCl_3$), $[\alpha]_{Hg\,578}^{25} = +37.4°$ ($c = 1.00$, $CHCl_3$).

$C_{19}H_{38}NO \cdot HCl$ (331.97) Calc. C 68.74 H 11.54 N 4.22% Found C 68.52 H 11.61 N 4.04%

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