photodestruction of photoaffinity reagents were (compound/time): 23/45 s, 10/>120 s (inefficient photolysis), 13/40 s, 17/45 s.

Experiments with chemically reactive affinity labels followed a similar pattern. Homogenate was bound with affinity label, spun at 20000g for 15 min, and then resuspended in the original volume. The bound membranes were incubated for various periods of time (up to 60 min) at 37 °C. Samples were then diluted and washed in the same manner as indicated for photolyzed samples. Control samples, which were processed in the same manner, consisted of membranes treated with no drug and samples bound with naloxone or fentanyl citrate. After resuspension in the original volume, samples were bound with tritiated drug.

Stereospecific binding of [^{3}H]naltrexone or [^{3}H]etorphine on samples treated and resuspended in the original volume was assayed on duplicate 2-mL samples as previously described.[20] Binding was assayed in the presence or absence of 1 μM naltrexone to obtain specific binding. Samples were incubated for 15 min at 37 °C, cooled for 10 min in an ice bath, and then filtered through Whatman GF/B filters. Filters were rinsed twice with 4 mL of buffer, dried, and counted in a toluene-based scintillation cocktail. Protein content was determined by the Lowry method.[27]

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(±)-2-Depentylperhydrohistrionicotoxin: A New Probe for a Regulatory Site on the Nicotinic Acetylcholine Receptor–Channel

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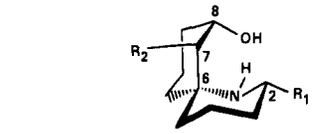
(±)-2-Depentylperhydrohistrionicotoxin (4), several of its analogues, and N- and O-substituted derivatives were prepared and tested for their effects on the neuromuscular transmission of the frog sartorius muscle. Compound 4, its *N*-methyl derivative 5, the *O*-acetyl derivative 9, and the quaternary methiodides 19 and 20 blocked the indirectly elicited twitch. The oxidation of 4 and 5 to ketones 12 and 14 and their reduction to the epimeric alcohols 17 and 18 afforded materials with substantially reduced activity. N-Acetylation of 4 to 11 changed the course of the activity to a transient potentiation of muscle twitch. Both 4 and 5 were not very toxic to mice after subcutaneous administration. (±)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-one (12) epimerized readily at room temperature to afford the epimer 13, and preparation of the hydrochloride of its N-methylated derivative 14 was accompanied by a retro-Michael reaction, affording the 2-*n*-butyl-3-[4-(methylamino)butyl]cyclohexene-2-one (22). The strongly hydrogen-bonded alcohol 4 was analyzed as the hydrobromide by a single-crystal X-ray analysis, confirming its structure.

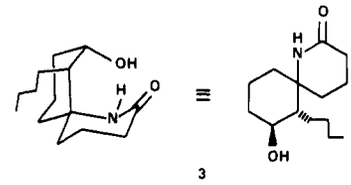
Natural histrionicotoxin (–)-1[1] and its fully hydrogenated, naturally derived congener perhydrohistrionicotoxin [(–)-H_{12}-HTX, (–)-2] are important biochemical tools for studying the mechanism of action of cholinergic agonists in the neuromuscular system (Chart I).[2a] Histrionicotoxin does not block interaction of acetylcholine with the nicotinic receptor, but instead interacts with a site on the associated ion channel. The interaction with histrionicotoxin appears to cause the channel to assume an inactive and nonconducting state. Binding of radioactive perhydrohistrionicotoxin is very slow unless a nicotinic agonist is present, indicating that binding of histrionicotoxin occurs to the channel primarily in the open configuration.[2b] Affinity of nicotinic agonists for its receptor and the extent of desensitization of the receptor are increased in the presence of isodihydrohistrionicotoxin.[2c] Histrionicotoxin enhances the conversion of acetylcholine receptor–channel complex to an inactive (desensitized) state. The receptor–channel is blocked in adrenal medulla cells where it serves to control catecholamine secretion.[2d] The unavailability of the natural toxin and congeners derived from it has been a serious handicap in exploring the biological potential of these spiroamines and performing a structure–activity relationship study. This problem can now be considered to have been solved with the several syntheses of (±)-2[3] and particularly with the recently

Chart I



1 $R_1 = CH_2CH=CH-C\equiv CH$, $R_2 = CH=CH-C\equiv CH$

2 $R_1 = n\text{-}C_5H_{11}$, $R_2 = n\text{-}C_4H_9$



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† NIADDK.
‡ University of Maryland.
§ National Heart, Blood, and Lung Institute.

(1) (a) B. Witkop, *Experientia*, **27**, 1121 (1971). (b) J. W. Daly, I. L. Karle, W. Myers, T. Tokuyama, J. A. Waters, and B. Witkop, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1870 (1971). (c) T. Tokuyama, K. Uenoyama, G. Brown, J. W. Daly, and B. Witkop, *Helv. Chim. Acta*, **57**, 2597 (1974). (d) J. W. Daly, T. Tokuyama, T. Fujiwara, R. J. Highet, and I. L. Karle, *J. Am. Chem. Soc.*, **102**, 830 (1980).

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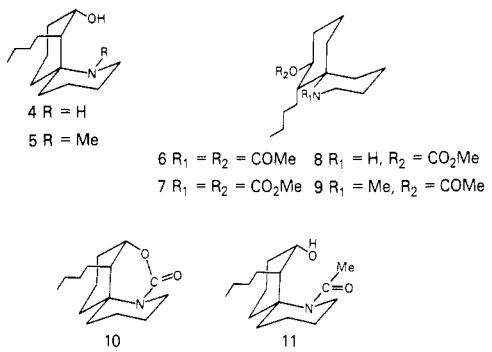
Chart II



completed synthesis of naturally derived (−)-**2**.[4] Nevertheless, information regarding the importance of the two hetero functionalities and the relationship of the relative stereochemistry of these molecules to their biological effects is scanty, and thus we have made a serious effort to investigate these particular aspects. We now report on the results of this investigation, carried out in the more easily accessible (±)-2-depentyl series.[5] The use of the depentyl compounds seemed justified, since (±)-**4** proved to have activity in the frog muscle preparation similar to (−)-**1**, (−)-**2**, or (±)-**2**. The depentyl analogue (**4**) appears to have about one-third the potency of (−)-**2** in this assay.

Chemistry. Lactam **3**, possessing the same relative configuration as the natural toxins **1** and **2**, seemed a logical starting material for our investigation and was prepared by modified procedures of Speckamp[3c,8] and Evans.[3d] Starting from (*E*)-1-bromo-4-nonene and glutarimide, lactam **3** was repeatedly obtained in an overall yield of 20% (see Experimental Section). Reduction of **3** with $LiAlH_4$ gave the (±)-2-depentyl analogue **4**, which crystallized as the free base and was also obtained as the crystalline hydrobromide. This material seemed identical with a compound reported by Corey et al.;[3a] however, no experimental details for its preparation or physical data for its characterization were given. Reductive N-methylation of the amino alcohol **4** afforded the crystalline amine **5** (Chart II).

The latter could also be obtained by $LiAlH_4$ reduction of carbamate **7**, obtained from **4**, by treatment with methyl chloroformate. Reaction of **4** with phosgene, in the presence of sodium bicarbonate, gave the cyclic carbamate **10**.[9] The carbamate **10** could not be cleaved with refluxing 3 N HCl and decomposed when refluxed with 64% aqueous hydrazine. The *N*-acetyl derivative **11** was obtained by treating the diacetyl derivative **6** with potassium carbonate in methanol.

Chart III

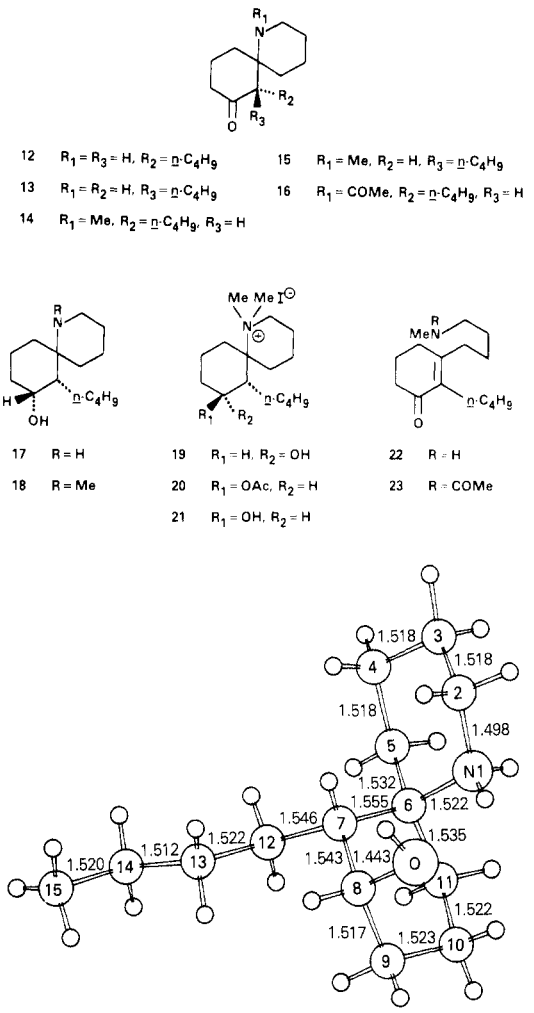


Figure 1. X-ray structure and bond lengths for (±)-2-depentylperhydrohistrionicotoxin hydrobromide (**4**·HBr). ESDS of bond length is <0.006 Å.

(2) (a) E. X. Albuquerque, E. A. Barnard, T. H. Chiu, A. J. Lapa, J. O. Dolly, S. Jansson, J. Daly, and B. Witkop, *Proc. Natl. Acad. Sci.*, **70**, 949 (1973); E. X. Albuquerque, K. Kuba, and J. W. Daly, *J. Pharmacol. Exp. Ther.*, **189**, 513 (1974); A. J. Lapa, E. X. Albuquerque, J. W. Daly, and B. Witkop *Pharmacologist*, **15**, 171 (1973); J. Elliott and M. A. Raftery, *Biochemistry*, **18**, 1968 (1979); E. X. Albuquerque, M. Adler, C. E. Spivak, and L. Aguayo, *Ann. N.Y. Acad. Sci.*, **358**, 204 (1980). (b) M. E. Eldefrawi, R. S. Aronstam, N. M. Bakry, and E. X. Albuquerque, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2309 (1980); R. S. Aronstam, A. T. Eldefrawi, I. N. Pessah, J. W. Daly, E. X. Albuquerque, and M. E. Eldefrawi, *J. Biol. Chem.*, **256**, 2843 (1981). (c) W. Burgermeister, W. A. Catterall, and B. Witkop, *Proc. Natl. Acad. Sci.*, **74**, 5754 (1977). (d) D. L. Kilpatrick, R. Slepteis, and N. Kirshner, *J. Neurochem.*, **37**, 125 (1981). (e) T. Tokuyama, J. W. Daly, and B. Witkop, *J. Am. Chem. Soc.*, **91**, 3931 (1969).

(3) (a) E. J. Corey, J. F. Arnett, and G. N. Widiger, *J. Am. Chem. Soc.*, **97**, 430 (1975); E. J. Corey, M. Petrzilka, and Y. Ueda, *Helv. Chim. Acta*, **60**, 2294 (1977). (b) M. Aratani, L. V. Dunkerton, T. Fukuyama, Y. Kishi, H. Kakoi, S. Sugiura, and S. Inoue, *J. Org. Chem.*, **40**, 2009 (1975); T. Fukuyama, L. V. Dunkerton, M. Aratani, and Y. Kishi, *ibid.*, **40**, 2011 (1975). (c) H. E. Schoemaker and W. N. Speckamp, *Tetrahedron Lett.*, 1515 and 4841 (1978); H. E. Schoemaker and W. N. Speckamp, *Tetrahedron*, **36**, 951 (1980). (d) D. A. Evans and E. W. Thomas, *Tetrahedron Lett.*, 411 (1979). (e) T. Ibuka, H. Minakata, Y. Mitsui, and Y. Inubushi, "Symposium Papers", Symposium on the Chemistry of Natural Products, 22nd, Nagoya, Japan, 1979, pp 351–358; T. Ibuka, Y. Mitsui, K. Hayashi, H. Minakawa, and Y. Inubushi, *Tetrahedron Lett.*, **22**, 4425 (1981). (f) R. J. Cvetovich, Ph.D. Thesis, Columbia University, 1978.

(4) K. Takahashi, B. Witkop, A. Brossi, M. A. Maleque, and E. Albuquerque, *Helv. Chim. Acta*, **65**, 252 (1982).

(5) The IUPAC nomenclature for (±)-**4** is [(6*R*,7*S*,8*S*) + (6*S*,7*R*,8*R*)]-7-*n*-butyl-1-azaspiro[5.5]undecan-8-ol. Because of the complexity of this name, we have chosen to use, throughout this paper, the informal nomenclature derived from the name histrionicotoxin.

(6) I. L. Karle, *J. Am. Chem. Soc.*, **95**, 4036 (1973).

(7) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).

(8) We thank Professor W. N. Speckamp, University of Amsterdam, The Netherlands, for having provided us, very early and prior to publication, with detailed procedures for making lactam **3**. We also thank Professor Y. Kishi, Harvard University, for having provided us the authentic sample of lactam **3**.

(9) We thank Professor E. Winterfeldt, University of Hannover, Federal Republic of Germany, for having provided us with spectral data of the cyclic carbamate **8**.

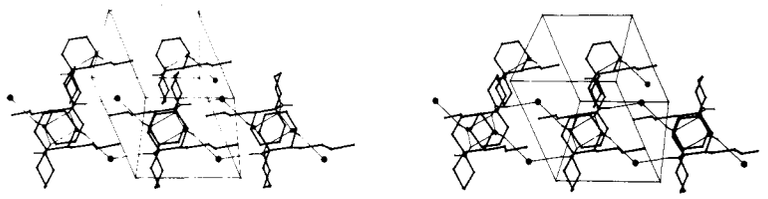


Figure 2. Packing and hydrogen bonds in crystals of (±)-2-depentylperhydrohistrionicotoxin. The vertical axis is *c*.

Oxidation of the amino alcohols **4** and **5** and the *N*-acetyl derivative **11** with pyridinium chlorochromate in methylene chloride afforded ketones **12**, **14**, and **16**, obtained after filtration through a silica gel column (Chart III).

Ketones **12** and **14** were unstable, and **12** epimerized readily at room temperature in methanolic HCl. TLC analysis of the equilibrated mixture showed the presence of almost equal amounts of starting material and the newly formed epimer **13**. Structure **13** was confirmed, after column chromatography on the basis of spectral properties. Preparation of the hydrochloride salt from **14** with methanolic HCl gave a mother liquor that contained a cyclohexenone derivative (**22**) which is the result of a retro-Michael reaction.[10a,b] The amine **22** was converted into its *N*-acetyl derivative **23** with acetic anhydride and pyridine for characterization.

Reduction of ketones **12** and **14** with $NaBH_4$ in methanol afforded stereoselectively the epimeric alcohols **17** and **18**. Both showed strong absorption in their IR spectra at 3640 cm^{-1}, attributable to the free OH group. In contrast, the IR spectra of the amino alcohols **4** and **5** indicated strong intramolecular hydrogen bonding when measured at different concentrations in CCl_4. Inspection of models suggested that in **4** and **5** a six-membered ring could be formed by hydrogen bonding between the amino and alcohol functionalities, introducing steric hindrance and necessitating the breaking of an N···H–O bond before alkylation of the nitrogen could occur. Quaternization of the tertiary amine **5** was unusually difficult and was only possible after reacting **5** with MeI in acetone solution at 40–50 °C for almost 2 weeks. This conclusion has now been further strengthened by the finding that the epimeric alcohol **18** readily afforded a methiodide upon treatment with MeI in acetone solution at room temperature. The O-acetylated amine **9**, differing in its conformation from that of the hydrogen-bonded free alcohol **5**,[11] needed milder conditions for quarternization. Above observations were further supported by single-crystal X-ray analysis described below.

Single Crystal X-ray Analysis of (±)-2-Depentylperhydrohistrionicotoxin Hydrobromide. As in other cholinergic inhibitors reported by Karle,[1b,6] the hydroxy oxygen atom is axial, and the bond lengths and angles are also very similar to those reported.[1b,6] The crystal conformation and bond lengths are shown in Figure 1. There is an intramolecular N–H···O (hydroxy) hydrogen bond with an N···O length of 2.774 Å, an H···O distance of 2.165 Å, and an N–H···O angle of 132°. (For discussion of hydrogen bonds, we will adopt the van der Waals radii of Bondi[7] which lead to van der Waals distances for C···Br, O···N, N···Br, O···Br, H···Br, and H···O of 3.55, 3.07, 3.40, 3.37, 3.05, and 2.72 Å, respectively.) Very similar conformations of the N and O atoms and hydrogen bonds have been reported.[1b,6] Torsion angles of the piperidine ring correspond to an almost ideal chair conformation while the other ring is slightly flatter, probably because of the presence of the axial substituents. Packing in the crystal (Figure 2) is strongly influenced by hydrogen bonding and electrostatic attractions. The second amino H atom forms a hydrogen bond to the Br^-, one *a* translation from the refined position: N···Br, 3.320 Å; H···Br, 2.491 Å; N–H···Br, 172°. The N···Br hydrogen bond is doubtless reinforced by electrostatic interaction between the positively charged part of the molecule and the Br^-, although only the N atom lies within a van der Waal distance of the Br. The H atom involved in the intramolecular bond to the hydroxy atom actually forms a bifurcated bond, since it also interacts with a second O (hydroxy) atom in a molecule related by a center of symmetry on the *ab* plane: N···O, 2.975 Å; H···O, 2.313 Å; N–H···O, 138°. The hydroxy group is also hydrogen bonded to the Br– ion with coordinates as refined: O···Br, 3.258 Å; H···Br, 2.596 Å; O–H···Br, 161°. There are, thus, centrosymmetric pairs of cations linked by two hydrogen bonds, and the pairs are linked into infinite chains along the *a* axis by the Br^- ions. All other nonbonded contacts are greater than or equal to van der Waals distances.

The results of the X-ray analysis of **4**·HBr are consistent with the spectroscopic data of **4**·HBr, which is expected to have the same conformation in solution. Similar hydrogen bonds and conformations could be supposed in the free bases of **4** and **5** on the basis of their ^1H NMR spectra.[11] Similarity of the result of the X-ray analysis of histrionicotoxin hydrochloride (**1**·HCl)[1b,6] to that of **4**·HBr, lacking the C-5 side chain, suggested that conformations and hydrogen bonds in these molecules may play an important role in their biological activities.

Supplementary material available includes three tables which contain atomic parameters, bond angles, and the listing of the observed and calculated structure factors.

Biological Results and Discussion

Effects of Synthetic (±)-Depentylperhydrohistrionicotoxin and Its Analogues on the Neuromuscular Transmission of the Frog Sartorius Muscle. (±)-2-Depentylperhydrohistrionicotoxin (**4**) and its analogues **5**–**12**, **14**, and **16**–**21** were tested on the neuromuscular transmission of three to five frog sciatic nerve sartorius muscle preparations (Table I). Compound **2**, chosen as a standard, is naturally derived (–)-H_{12}-HTX, which was found equiactive with its (+) enantiomer and its racemate.[4] All but **6** and **7** partially blocked the twitch of the frog muscle, but to different degrees. Thus, 50 μM (±)-2-depentylperhydrohistrionicotoxin (**4**) and its ana-

(10) (a) E. J. Corey and R. D. Balanson, *Heterocycles*, **5**, 445 (1976). (b) Magnus et al. reported that secondary amines of type **12** underwent a retro–Mannich reaction and converted into hexahydroquinoline derivatives. J. J. Venit and P. Magnus, *Tetrahedron Lett.*, **21**, 4815 (1980).

(11) The structures shown in the charts represent one of the possible conformers. The conformational analysis of spiroamines **4**–**11** was guided by a careful interpretation of their ^1H NMR spectra. The results are in good agreement with the findings reported by Speckamp [H. E. Schoemaker and W. N. Speckamp, *Tetrahedron Lett.*, 4841 (1978)]. That is, in NMR spectra, the $W_{1/2}$ of the signals of the C_8 proton of **4** and **5** is 8 Hz. On the other hand, the signals of the C_8 protons of O-protected compounds are observed as a triplet of doublets ($J_1 = J_2 = 10.5$ Hz, $J_3 = 4.5$ Hz). Since prefered conformations of **12**–**21** are not confirmed by their spectroscopic data, their structures are shown as Chart III.

Table I. Effects of (±)-Depentylperhydrohistrionicotoxin and Its Analogues on the Sciatic Nerve Sartorius Muscle Preparation of the Frog[a]

	% block of muscle twitch at time shown[b]				% max transient potentiation of muscle[b]	
	indirect		direct			
no.	30 min	60 min	30 min	60 min	indirect	direct
2	100	100	47 ± 3	45 ± 5		
4	96 ± 1	100 ± 5	52 ± 8	52 ± 8		
5	78 ± 1	92 ± 5	24 ± 4	30 ± 8		
6	0	0	0	0	125 ± 37	118 ± 6
7	0	0	0	0		
8	67 ± 14	82 ± 5	14 ± 1	25 ± 2		
9	88 ± 7	94 ± 8	22 ± 2	25 ± 2		
10	35 ± 1	66 ± 1	22 ± 9	31 ± 10		
11	10 ± 5	26 ± 2	20 ± 2	24 ± 7	116 ± 2	103 ± 5
12	46 ± 4	58 ± 4	8 ± 5	25 ± 5		
14	49 ± 8	56 ± 1	26 ± 8	33 ± 5		
16	7 ± 1	20 ± 4	0	6 ± 3	117 ± 5	102 ± 2
17	55 ± 9	61 ± 9	19 ± 5	30 ± 8		
18	62 ± 9	65 ± 8	5 ± 3	5 ± 3		
19	74 ± 8	83 ± 9	48 ± 2	49 ± 3		
20	85 ± 5	91 ± 4	34 ± 8	47 ± 1		
21	39 ± 6	49 ± 7	29 ± 5	43 ± 5		

[a] All compounds were used at 50 μM concentrations. [b] Muscles were exposed to toxins (50 μM) for 60 min. The values shown are means ± SD from at least three muscles. [c] Transient potentiation developed following addition of the toxin into the bath and lasted for approximately 5–15 min. There was no contracture at this concentration. There was only partial recovery (40–70%) following washout with normal physiological Ringer's solution for 60 min.

logues **5**, **8–12**, **14**, and **16–21** blocked the indirectly elicited twitch by 100, 92, 82, 94, 66, 26, 58, 56, 20, 61, 65, 83, 91, and 49% of control in 60 min, respectively (Table I). Equimolar concentrations of analogues **6**, **11**, and **16** did not appreciably block the direct or indirect twitches during this period but rather potentiated transiently both direct and indirect twitches within 5–15 min of exposure. The transient potentiation was more pronounced at high concentrations and was followed by depression of the indirect twitch. There was no potentiation of either direct or indirect twitches in the presence of (±)-depentylperhydrohistrionicotoxin or other analogues. On the basis of the twitch tension results, **4**, **5**, **8**, **9**, **19**, and **20** can be described as moderately potent blockers of neuromuscular transmission, and analogues **10–14**, **16–18**, and **21** can be described as weak blockers. Analogue **7** had no effect on either twitch. The rank order of potencies of these compounds was as follows: **4 > 9 > 5 > 20 > 19 > 8 > 10 > 21 > 18 > 17 > 12 > 14 > 11 > 16 > 6 = 7**.

According to these results, N-methylation of **4** did not particularly effect its activity (92%). Although chirality is not considered to be essential in this assay,[4] the relative stereochemistry of C_8-O and C_6-N seems important. The rank order of potency clearly shows that all the compounds having an axial OH substituent which can interact with the nitrogen functionality were more potent than their epimers. Oxidation of the hydroxy groups to ketones or epimerization of the hydroxy group reduced the activity to a range of 56–65%, while protection of the hydroxy group did not affect the activity very much (82–92%). N-Acetylation changed the course of the activity to transient potentiation of muscle twitch.

These data are entirely based on the evaluation with frog sartorius muscle preparations. Since the practical importance of this test is unknown, it would seem prudent to evaluate these compounds using other screening methods. This would greatly aid in obtaining a better understanding of the meaning of the observed biological changes resulting from the structural modification of histrionicotoxin. With the contemporary availability of depentylperhydrohistrionicotoxins, further research in this area should be stimulated and, hopefully, help to clarify whether these or structurally related compounds have medicinal utility.

Assays for Antinociceptive Activity and Toxicity. In the hot-plate assay for antinociceptive activity,[12-14] depentylperhydrohistrionicotoxin (**4**) did not show a dose–response relationship (e.g., 20% of the mice were effected at 100 mg/kg, but 50% were effected at 10 and 20 mg/kg). *N*-Methyl-2-depentylperhydrohistrionicotoxin (**5**) was essentially inactive in that assay (10% of the mice were effected at 50 mg/kg). The assay used NIH general-purpose mice. The drug was introduced sc, **4**·HBr in H_2O and **5** in dilute HCl. When the same route of administration, type of mouse, and solvent were used, acute (24 h) toxicities were run. Compound **5** was somewhat more toxic than **4** (30% of the mice died at 100 mg/kg with **5**; none out of ten mice were effected at 100 mg/kg with **4**). Insufficient compound was available to determine exact LD_{50} values, but both **4** and **5** obviously had LD_{50} values greater than 100 mg/kg.

Nothing is known about the toxicity of the histrionicotoxins present in the defensive skin secretions of frogs. In these frog skin secretions, batrachotoxins were found to be highly toxic, and pumiliotoxin C^{2e} was found to be relatively nontoxic. The data reported above suggest that (±)-2-depentylperhydrohistrionicotoxin (**4**) is a relatively nontoxic material, at least to mice.

Experimental Section

X-ray Crystallography. Crystals of (±)-depentylperhydrohistrionicotoxin hydrobromide were grown from a solution of ethyl acetate. They crystallized in triclinic space group $P\bar{1}$: a = 7.902 (1), b = 8.271 (1), c = 13.299 (1) Å, α = 103.03 (1)°, β = 94.12 (1)°, γ = 113.81 (1)°, V = 760.84 Å^3, Z = 2, Dx = 1.337 cm^{-3}; 3133 (596 < 1s) reflections were collected in the 20 range 2–150° using Cu Kα X-radiation (graphite monochromator) with a CAD-4 Nonius diffractometer.

The structure was solved using Patterson and direct Fourier methods. Evidence was found in a difference map for all the

(12) N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953).
(13) A. E. Jacobson and E. L. May, *J. Med. Chem.*, 8, 563 (1965).
(14) L. Atwell and A. E. Jacobson, *Lab Anim.*, 7, 42 (1978).

hydrogen atoms. The structure was refined by full matrix least squares using anisotropic thermal parameters for C, N, O, and Br. Isotropic thermal parameters were assigned to H atoms but were not refined. Thermal parameters for hydrogen atoms were assumed to be the same as the isotropic values for the heavy atoms to which they are attached. The final R value was 3.7%. Programs used were mainly from the XRAY72 system.[15]

Chemical Method. Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Serivices and Instrumentation of this laboratory. IR spectra were obtained on a Beckman 4230 instrument. NMR spectra were determined by using a Varian HR-220 spectrometer or a JEOL FX-100 spectrometer with Me_4Si as an interval reference. Chemical-ionization (CI) mass spectra were obtained by using a Finnigan 1015 D spectrometer, electron-impact (EI) mass spectra 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 an 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 liquid chromatography 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 or 230–400 mesh) were from EM Laboratories. Uniformity of the oily compounds **6**, **7**, **10**, **16**, and **23** was confirmed by TLC analysis.

(6*S*,7*S*,8*S*)- and (6*R*,7*R*,8*R*)-7-*n*-Butyl-8-hydroxy-1-azaspiro[5.5]undecan-2-one (3). The modified procedures of Speckamp[3c,8] and Evans[3d] were carried out. Thus, the bromomagnesium salt of glutarimide [prepared from glutarimide (56.5 g, 0.5 mol) with an equivalent amount of MeMgBr] was reacted with an equivalent amount of (*E*)-1-nonen-4-ylmagnesium bromide [prepared from (*E*)-1-bromonon-4-ene (103 g, 0.5 mol) and Mg (12.15 g, 0.5 mol) in THF (300 mL)] in THF at 25 °C for 15 h. After workup, the crude ketoamide [(96 g, isolated as a solid and partially purified by recrystallization from Et_2O-*n*-hexane, mp 90–91 °C (lit.[3d] 90–91 °C); IR (KBr) 3400, 3200, 1715, 1655 cm^{-1}; NMR ($CDCl_3$) δ 6.00 and 5.80 (each br s, each 1 H, 2 NH), 5.56–5.22 (m, 2 H, vinyl H), 2.70–1.00 (m, 18 H), 0.92 (br s, 3 H, CH_2CH_3); CIMS (NH_3), m/e 240 ($M^+ + 1$); EIMS, m/e 239 (M^+)] was dissolved in HCO_2H (2 L) and allowed to stir at 25 °C for 72 h. Workup and recrystallization from *i*-Pr_2O yielded the formate of **3**, mp 148–149 °C (lit.[3c] 144–146 °C, lit.[3d] 148–149 °C). Analytical HPLC (*n*-hexane/*i*-PrOH, 95:5; 2 mL/min) of the crude mixture showed the ratio of the formate of **3** (t_R = 11.6 min) and its isomers (t_R = 5.6 and 7.6 min) was 55:45. Purification of the mother liquor with preparative HPLC (*n*-hexane/*i*-PrOH/EtOH, 89:8:3, as an eluent) afforded an additional amount of the formate of **3**. The formate was hydrolyzed with NaOMe in MeOH to give **3** (23 g, 20%): mp 135–136 °C (lit.[3b,d] 133–134 °C, lit.[3c] 135–136 °C), which was identical in every respect with the authentic sample.[8]

(±)-Depentylperhydrohistrionicotoxin [(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-ol] (4). To a stirred suspension of $LiAlH_4$ (1.14 g, 30 mmol) in THF (40 mL) was added dropwise a solution of the lactam (**3**; 2.39 g, 10 mmol) in THF (40 mL). The resulting mixture was refluxed for 15 h in a current of argon and then cooled with an ice bath, and a saturated aqueous solution of Na_2SO_4 was added slowly. The mixture was filtered through Celite, and the filter cake was washed with CH_2Cl_2. The combined organic layer was washed with brine, dried ($MgSO_4$), and evaporated to leave a solid, which was chromatographed on silica gel with CH_2Cl_2/MeOH/NH_4OH (85:14:1) as the eluent. The appropriate fractions after evaporation afforded **4** as a white solid (1.82 g, 81%). Recrystallization from petroleum ether (bp 37.8–56.9 °C) gave an analytical sample of **4**: mp 63–64 °C; IR (KBr) 3310, 3100 cm^{-1}; IR (CCl_4) 3225 cm^{-1}; NMR ($CDCl_3$) δ 3.86 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 3.10–2.60 (m, 2 H, C_2 H_2), 2.10–1.00 (m, 19 H), 0.90 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4) m/e 226 ($M^+ + 1$); EIMS, m/e 225 (M^+). Anal. ($C_{14}H_{27}NO$) C, H, N. The 2,4,6-trinitrobenzenesulfonate of **4** gave mp 180–182 °C (EtOH). Anal. ($C_{14}H_{27}NO{\cdot}C_6H_3N_3O_9S$) C, H, N, S. A saturated solution of HBr in Et_2O was added to a solution of the free base **4** (1.8 g) in MeOH and evaporated to leave a syrup, which was dissolved in AcOEt and cooled. Crystalline **4** was collected by filtration, and the filtrate was concentrated and cooled to give another crop of **4**·HBr. The same operation was repeated, and 1.87 g (61%) of **4**·HBr was obtained: mp 110–111 °C (EtOAc); IR (KBr) 3240, 3150 cm^{-1}; NMR ($CDCl_3$) δ 8.80, 8.56, 5.16 (each br s, each 1 H, D_2O exchangeable, OH, NH, HBr), 4.28 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 3.60–2.88 (m, 2 H, C_2 H_2), 2.44–1.00 (m, 19 H), 0.88 (br t, J = 6 Hz, 3 H, C-CH_3). Anal. ($C_{14}H_{27}NO{\cdot}HBr$) C, H, N, Br.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-7-*n*-Butyl-1-methyl-1-azaspiro[5.5]undecan-8-ol (5). Method A. A mixture of the amino alcohol **4** (225 mg, 1 mmol), formaldehyde (37%, 2 mL), and MeOH (10 mL) was stirred for 5 h at 25 °C. To the solution was added $NaCNBH_3$ (100 mg, 1.59 mmol), and the mixture was stirred for 1 h at 25 °C. The mixture was diluted with H_2O, extracted with Et_2O, dried ($MgSO_4$), and evaporated to leave a solid, which was chromatographed on silica gel with CH_2Cl_2/MeOH/NH_4OH (90:9:1) as the eluent. The appropriate fraction afforded **5** (220 mg, 92%): mp 69–70 °C [from petroleum ether (bp 37.8–56.9 °C)]; IR (KBr) 3400, 3350 cm^{-1}; IR (CCl_4) 3160 cm^{-1}; NMR ($CDCl_3$) δ 8.10 (br s, 1 H, OH), 3.88 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8H), 3.62–3.40 (m, 1 H), 2.62–2.46 (m, 1 H), 2.40 (s, 3 H, NCH_3), 2.05–1.00 (m, 19 H), 0.83 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4) m/e 240 ($M^+ + 1$); EIMS, m/e 239 (M^+). Anal. ($C_{15}H_{29}NO$) C, H, N. 2,4,6-Trinitrobenzenesulfonate of **5**: mp 174–175 °C (EtOH). Anal. ($C_{15}H_{29}NO{\cdot}C_6H_3N_3O_9S$) C, H, N, S.

Method B. To a stirred suspension of $LiAlH_4$ (20 mg, 0.53 mmol) in THF (3 mL) was added a solution of the carbamate **7** (20 mg, 0.06 mmol) in THF (2 mL). The mixture was refluxed for 15 h under argon. The mixture was cooled with an ice bath, and H_2O was added cautiously. It was filtered through Celite, and the filter cake was washed with CH_2Cl_2. The combined filtrate was washed with H_2O, dried ($MgSO_4$), and evaporated to give **5** (9 mg, 63%), identical with the sample described in method A.

Method C. Similar treatment of the cyclic carbamate **10** (25 mg, 0.1 mmol) with $LiAlH_4$ (20 mg, 0.53 mmol) in THF (5 mL) afforded **5** (16 mg, 67%), which was identical with the sample described in method A.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-8-Acetoxy-1-acetyl-1-azaspiro[5.5]undecane (6). A mixture of the amino alcohol **4** (225 mg, 1 mmol), Ac_2O (3 mL), and pyridine (3 mL) was set aside at 25 °C for 15 h. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in Et_2O and washed with 10% HCl and H_2O, followed by saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated to leave **6** as an oil (299 mg, 97%): bp 130 °C (0.25 mmHg); IR (film) 1730, 1640 cm^{-1}; NMR ($CDCl_3$) δ 4.75 (t of d, $J_1 = J_2$ = 10.5 Hz, J_3 = 4.5 Hz, 1 H, C_8 H), 3.55–3.18 (m, 3 H), 2.95–2.50 (m, 1 H), 2.06 and 2.00 (each s, each 3 H, 2 Ac), 1.96–1.00 (m, 17 H), 0.84 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4), m/e 310 ($M^+ + 1$); EIMS, m/e 309 (M^+). Exact mass calcd for $C_{18}H_{31}NO_3$, 309.2302; found, 309.2304. Anal. ($C_{18}H_{31}NO_3$) C, H, N.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-1-Carbomethoxy-8-[(carbomethoxy)oxy]-1-azaspiro[5.5]undecane (7). Method A. To a mixture of the amino alcohol **4** (225 mg, 1 mmol) and K_2CO_3 (600 mg, 4.35 mmol) in CH_2Cl_2 (10 mL) was added dropwise methyl chloroformate (500 mg, 5.29 mmol), and the mixture was stirred at 25 °C for 15 h. The mixture was washed with H_2O, dried ($MgSO_4$), and evaporated to leave an oil, which was chromatographed on silica gel with CH_2Cl_2/MeOH (99:1) as the eluent to afford **7** (200 mg, 59%): bp 140 °C (0.1 mmHg); IR (film) 1748, 1695 cm^{-1}; NMR ($CDCl_3$) δ 4.60 (t of d, $J_1 = J_2$ = 10.5 Hz, J_3 = 4.5 Hz, 1 H, C_8 H), 3.76 and 3.64 (each s, each 3 H, 2 OMe), 3.40–2.80 (m, 2 H), 2.60–2.20 (m, 1 H), 2.12–1.90 (m, 1 H), 1.90–1.00 (m, 17 H), 0.84 (br t, J = 6 Hz, 3 H, CH_2CH_3); EIMS, m/e 341 (M^+). Exact mass calcd for $C_{18}H_{31}NO_5$, 341.2200; found, 341.2202. Anal. ($C_{18}H_{31}NO_5$) C, H, N.

Method B. A mixture of the amine **8** (142 mg, 0.5 mmol), K_2CO_3 (276 mg, 2.0 mmol), and methyl chloroformate (200 mg, 2.1 mmol) in CH_2Cl_2 (3 mL) was stirred at 25 °C for 15 h. Workup

(15) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, XRAY72, Technical Report TR-192, Computer Center, University of Maryland 1972.

as above afforded 7 (152 mg, 89%), which was identical with the sample described in method A.

Cyclic Carbamate of the Amino Alcohol (10). A mixture of the amino alcohol 4 (225 mg, 1 mmol), phosgene (12.5% in toluene, 2 mL), a saturated aqueous solution of $NaHCO_3$ (10 mL), and $CHCl_3$ (5 mL) was stirred at 25 °C for 3 h. The mixture was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel with CH_2Cl_2/MeOH (99:1) as the eluent to afford **10** (196 mg, 78%): bp 145 °C (0.1 mmHg); IR (film) 1690 cm^{-1}; NMR ($CDCl_3$) δ 4.50–4.20 (m, 2 H, C_8 H and C_2 H), 3.84–2.40 (m, 1 H, C_2 H), 2.20–1.00 (m, 19 H), 0.92 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4), m/e 252 ($M^+ + 1$); EIMS, m/e 251 (M^+). Exact mass calcd for $C_{15}H_{25}NO_2$, 251.1884; found, 251.1885. Anal. ($C_{15}H_{25}NO_2$) H, N; C: calcd, 71.67; found, 71.01.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-1-Acetyl-7-*n*-butyl-1-azaspiro[5.5]undecan-8-ol (11). A mixture of the diacetate (150 mg, 0.49 mmol), K_2CO_3 (276 mg, 2 mmol), and MeOH (10 mL) was stirred at 25 °C for 15 h. The mixture was diluted with CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), and evaporated to leave a solid, which was recrystallized from Et_2O (117 mg, 90%): mp 138–139 °C; IR (KBr) 3300, 1610 cm^{-1}; NMR ($CDCl_3$) δ 3.55–3.00 (m, 3 H), 3.24–3.05 (m, 1 H), 2.88–2.70 (m, 1 H), 2.50 (br s, 1 H, OH, D_2O exchanged), 2.04 (s, 3 H, Ac), 2.02–1.20 (m, 17 H), 0.84 (br t, J = 6 Hz, 3 H, CH_2CH_3); EIMS, m/e 267 (M^+). Anal. ($C_{16}H_{29}NO_2$) C, H, N.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-7-*n*-Butyl-8-[(carbomethoxy)oxy]-1-azaspiro[5.5]undecane (8). To a stirred solution of the amino alcohol 4 (225 mg, 1 mmol) and Et_3N (0.5 mL, 3.6 mmol) in CH_2Cl_2 (5 mL) was added slowly methyl chloroformate (0.5 mL, 6.5 mmol), and stirring was continued for 1 h. The mixture was washed with brine, dried ($MgSO_4$), and evaporated to leave an oil, which was chromatographed on silica gel with CH_2Cl_2/MeOH/NH_4OH (90:9:1) as the eluent. The appropriate fraction was evaporated, and the residue was distilled to give 8 (220 mg, 78%): bp 120 °C (0.25 mmHg); IR ($CHCl_3$) 1740 cm^{-1}; NMR ($CDCl_3$) δ 4.78 (t of d, $J_1 = J_2$ = 10.5 Hz, J_3 = 4.5 Hz, 1 H, C_8 H), 3.76 (s, 3 H, OMe), 2.90–2.60 (m, 2 H, C_2 H_2), 2.16–1.00 (m, 19 H), 0.88 (br t, J = 6 Hz, 3 H, CH_2CH_3). Exact mass calcd for $C_{16}H_{29}NO_3$, 283.2146; found, 283.2147. Hydrochloride of 8: mp 204–206 °C (MeOH–Et_2O); IR (KBr) 1740, 1580 cm^{-1}; NMR ($CDCl_3$) δ 10.10 and 7.76 (each br s, each 1 H, NH and HCl), 4.84 (1 H, br s, C_8 H), 3.78 (s, 3 H, OCH_3), 3.40–3.00 (m, 2 H, C_2 H_2), 2.34–1.00 (m, 19 H), 0.88 (br t, J = 6 Hz, 3 H, CH_2CH_3). Anal. ($C_{16}H_{29}NO_3$·HCl) C, H, N.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-8-Acetoxy-7-*n*-butyl-1-methyl-1-azaspiro[5.5]undecane (9). A solution of the alcohol 5 (100 mg, 0.42 mmol), Ac_2O (0.1 mL), and pyridine (0.1 mL) in CH_2Cl_2 (2 mL) was set aside at 25 °C for 15 h. The mixture was evaporated under reduced pressure, and the residue was distilled: bp 130 °C (0.25 mmHg); yield 80 mg (67%); IR (film) 1735 cm^{-1}; NMR ($CDCl_3$) δ 4.85 (t of d, $J_1 = J_2$ = 10.5 Hz, J_3 = 4.5 Hz, 1 H, C_8 H), 2.65–2.40 (m, 2 H), 2.16 and 2.02 (each s, each 3 H, Ac and NCH_3), 2.00–1.00 (m, 17 H), 0.86 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4), m/e 282 ($M^+ + 1$); EIMS, m/e 281 (M^+). Exact mass calcd for $C_{17}H_{31}NO_2$, 281.2353; found, 281.2355. Hydrochoride of 9: mp 233–235 °C (MeOH–Et_2O); IR (KBr) 1725 cm^{-1}; NMR ($CDCl_3$) δ 10.60 (br s, 1 H, HCl), 4.85 (t of d, $J_1 = J_2$ = 10.5 Hz, J_3 = 4.5 Hz, 1 H, C_8 H) 3.50–3.30 (m, 1 H), 3.12–2.95 (m, 1 H), 2.68 (d, J = 6 Hz, 3 H, NCH_3), 2.50–1.00 (m, 19 H), 2.04 (s, 3 H, Ac), 0.88 (br t, J = 6 Hz, 3 H, CH_2CH_3). Anal. ($C_{17}H_{31}NO_2$·HCl) C, H, N.

(6*R*,7*S*)- and (6*S*,7*R*)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-one (12) and (6*R*,7*R*)- and (6*S*,7*S*)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-one (13). Method A. A mixture of the amino alcohol 4 (225 mg, 1 mmol), PCC (450 mg, 2.09 mmol), and CH_2Cl_2 (5 mL) was stirred for 2 h at 25 °C. The mixture was diluted with Et_2O (10 mL), and stirring was continued for 0.5 h. It was then filtered through a silica gel column with CH_2Cl_2/MeOH/NH_4OH (90:9:1) as the eluent. Removal of the solvent afforded **12** as an oil (143 mg, 64%): bp 100 °C (0.25 mmHg); IR (film) 1705 cm^{-1}; NMR ($CDCl_3$) δ 2.96–2.64 (m, 2 H, C_2H_2), 2.46–2.20 (m, 3 H), 2.05–1.00 (m, 16 H), 0.86 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4), m/e 224 ($M^+ + 1$); EIMS, m/e 223 (M^+). Hydrochloride of 12: mp 175–176 °C (MeOH–Et_2O); IR (KBr) 1712 and 1570 cm^{-1}; NMR ($CDCl_3$) δ 9.80 and 8.80 (each br s, each 1 H, NH and HCl), 3.60–2.86 (m, 2 H), 2.55–2.00 (m, 3 H), 2.00–1.00 (m, 16 H), 0.88 (t, J = 6 Hz, CH_2CH_3). Anal. ($C_{14}H_{25}NO$·HCl) C, N; H: calcd, 10.09; found, 10.54. The mother liquor of **12**·HCl afforded, after basification and chromatography on silica gel with CH_2Cl_2/MeOH (9:1) as the eluent, the C_7 epimer (**13**): IR ($CHCl_3$) 1700 cm^{-1}; NMR ($CDCl_3$) δ 2.84–2.70 (m, 2 H), 2.70–2.50 (m, 1 H), 2.50–2.38 (m, 1 H), 2.38–2.12 (m, 1 H), 2.10–1.00 (m, 16 H), 0.90 (t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (CH_4), m/e 224 ($M^+ + 1$); EIMS, m/e 223 (M^+).

Method B. Similar treatment of the amino alcohol **17** (20 mg, 0.09 mmol) with PCC (50 mg, 0.23 mmol) afforded the ketone **12** (14 mg, 69%), which was identical with the sample described in method A.

(6*R*,7*S*)- and (6*S*,7*R*)-7-*n*-Butyl-1-methyl-1-azaspiro[5.5]undecan-8-one (14) and 2-*n*-Butyl-3-[4-(methylamino)butyl]cyclohexen-2-one (22). Method A. A mixture of the amino alcohol 5 (239 mg, 1 mmol), PCC (431 mg, 2 mmol), and CH_2Cl_2 (5 mL) was stirred at 25 °C for 4 h. The mixture was diluted with Et_2O (10 mL) and filtered through a silica gel column with CH_2Cl_2/MeOH/NH_4OH (90:9:1) as the eluent. Removal of the solvent afforded **14** as an oil (209 mg, 88%): bp 95 °C (0.25 mmHg); IR ($CHCl_3$) 1700 cm^{-1}; NMR ($CDCl_3$) δ 2.72–2.40 (m, 3 H), 2.40–2.20 (m, 2 H), 2.22 (s, 3 H, NCH_3), 2.10–1.00 (m, 16 H), 0.86 (t, J = 6 Hz, 3 H, CH_2CH_3); EIMS, m/e 237 (M^+). Exact mass calcd for $C_{15}H_{27}NO$, 237.2093; found, 237.2093. Hydrochloride of **14**: mp 166–168 °C (MeOH–Et_2O); IR (KBr) 1715 cm^{-1}; NMR ($CDCl_3$) δ 10.70 (br s, 1 H, HCl), 3.40 and 3.14 (each br s, each 1 H, C_2H_2), 2.76 (d, J = 6 Hz, 3 H, NCH_3), 2.60–2.30 (m, 3 H, C_7 H and C_9 H_2), 2.30–1.00 (m, 16 H), 0.88 (t, J = 6 Hz, 3 H, CH_2CH_3). Anal. ($C_{15}H_{27}NO$·HCl) C, H, N.

The mother liquor of the hydrochloride of **14** afforded the hydrochloride of **22**, after evaporation of the solvent: IR ($CHCl_3$) 1655 cm^{-1}; NMR ($CDCl_3$) δ 6.66 (br s, 2 H, NH and HCl), 3.00–2.85 (2 H, m), 2.65 (s, 3 H, NCH_3), 2.50–2.20 (m, 8 H), 2.10–1.70 (m, 4 H), 1.70–1.45 (m, 2 H), 1.45–1.15 (m, 4 H), 0.90 (br t, J = Hz, 3 H, CH_2CH_3). Basification with NH_4OH and extraction with Et_2O afforded the free base of **22**: IR ($CHCl_3$) 1655, 1615 cm^{-1}; NMR ($CDCl_3$) δ 2.73–2.52 (m, 2 H, NCH_2), 2.44 (s, 3 H, NCH_3), 2.59–2.19 (m, 8 H), 2.02–1.82 (m, 2 H), 1.66–1.42 (m, 4H), 1.42–1.16 (m, 4 H), 0.90 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (NH_3), m/e 238 ($M^+ + 1$). Acetylation of **22** with Ac_2O and pyridine yielded **23**: IR ($CHCl_3$) 1665, 1630 cm^{-1}; NMR ($CDCl_3$) δ 3.52–3.22 (m, 2 H, NCH_2), 3.01 and 2.94 (each s, each 1.5 H, NCH_3), 2.74–2.18 (m, 8 H), 2.02–1.76 (m, 2 H), 1.76–1.14 (m, 8 H), 0.90 (br t, J = 6 Hz, 3 H, CH_2CH_3). Exact mass calcd for $C_{17}H_{29}NO_2$, 279.2197; found, 279.2192.

Method B. Under the same conditions as above, the amino alcohol **18** (20 mg, 0.08 mmol) with PCC (40 mg, 0.19 mmol) yielded the ketone **14** (13 mg, 65%), which was identical with the sample described in method A.

(6*R*,7*S*)- and (6*S*,7*R*)-1-Acetyl-7-*n*-butyl-1-azaspiro[5.5]undecan-8-one (16). A mixture of the alcohol **9** (267 mg, 1 mmol) and PCC (500 mg, 2.33 mmol) was stirred in CH_2Cl_2 (10 mL) at 25 °C for 2 h. The mixture was diluted with Et_2O and filtered through silica gel with Et_2O as the eluent. Evaporation of the solvent afforded **16** (167 mg, 63%): bp 140 °C (0.2 mmHg); IR (film) 1708 and 1640 cm^{-1}; NMR ($CDCl_3$) δ 4.34 (d, J = 10 Hz, 1 H), 3.42–3.20 (m, 2 H), 3.20–2.80 (m, 1 H), 2.48–2.16 (m, 2 H), 2.08 (s, 3 H, Ac), 2.00–1.00 (m, 14 H), 0.84 (t, J = 6 Hz, 3 H, CH_2CH_2); CIMS (CH_4), m/e 266 ($M^+ + 1$); EIMS, m/e 265 (M^+). Exact mass calcd for $C_{16}H_{27}NO_2$, 265.2040; found, 265.2041. Anal. ($C_{16}H_{27}NO_2$) H, N; C: calcd, 72.41; found, 71.58.

(6*R*,7*S*,8*R*)- and (6*S*,7*R*,8*S*)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-ol (17). To a stirred solution of the ketone **12** (100 mg, 0.45 mmol) in MeOH (2 mL) was added $NaBH_4$ (20 mg, 0.52 mmol), and stirring was continued at 25 °C for 10 min. The mixture was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and evaporated to give **17** as an oil: IR (CCl_4) 3640 cm^{-1}; NMR ($CDCl_3$) δ 4.12 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 2.96–2.62 (m, 2 H, C_2 H_2), 1.80–1.00 (m, 18 H), 0.90 (br t, J = 6 Hz, 3 H, CH_2CH_3). Hydrochloride of **17** (97 mg, 82%): mp 250–252 °C (MeOH–Et_2O); IR (KBr) 3360, 1580 cm^{-1}; NMR ($CDCl_3$/CD_3OD, 9:1) δ 4.12 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 3.26–2.80 (m, 2 H, C_2H_2), 2.25–1.00 (m, 19 H), 0.90 (br t, J = 6 Hz, 3 H, CH_2CH_3). Anal. ($C_{14}H_{27}NO$·HCl) C, H, N.

(6*R*,7*S*,8*R*)- and (6*S*,7*R*,8*S*)-7-*n*-Butyl-1-methyl-1-azaspiro[5.5]undecan-8-ol (18). To a stirred solution of the ketone

14 (100 mg, 0.42 mmol) in MeOH (2 mL) was added $NaBH_4$ (20 mg, 0.52 mmol), and stirring was continued for 30 min at 25 °C. The mixture was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and evaporated to afford 18 as an oil: IR (CCl_4) 3635, 3480 cm^{-1}; NMR ($CDCl_3$) δ 4.20 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 2.80–2.30 (m, 2 H, C_2 H_2), 2.20 (s, 3 H, NCH_3), 2.10–1.00 (m, 19 H), 0.92 (br t, J = 6 Hz, 3 H, CH_2CH_3). Hydrochloride of 18 (77 mg, 67%): mp 239–241 °C (MeOH–Et_2O); IR (KBr) 3330 cm^{-1}; NMR ($CDCl_3$) δ 10.02 (br s, 1 H, OH), 4.26 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 3.50–2.75 (m, 2 H, C_2 H_2), 2.60 (d, J = 6 Hz, 3 H, NCH_3), 2.50–1.00 (m, 19 H), 0.92 (br t, J = 6 Hz, 3 H, CH_2CH_3); EIMS, m/e 239 (M^+ – HCl). Anal. ($C_{15}H_{29}NO$·HCl) C, H, N, Cl.

(6*R*,7*S*,8*R*)- and (6*S*,7*R*,8*S*)-7-*n*-Butyl-8-hydroxy-1,1-dimethyl-1-azoniaspiro[5.5]undecane Iodide (19). A mixture of the amino alcohol 18 (120 mg, 0.5 mmol), methyl iodide (1 mL, 16 mmol), and acetone (3 mL) was set aside at 25 °C for 5 h. The precipitate was collected by filtration and recrystallized to give 19 (119 mg, 62%): mp 165–167 °C (MeOH–Et_2O); IR (KBr) 3370, 1480, 1455 cm^{-1}; NMR (CD_3OD) δ 4.10 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 3.86–3.70 (m, 1 H), 3.35–3.20 (m, 1 H), 3.20 and 3.05 (each s, each 3 H, 2 NCH_3), 2.40–1.20 (m, 19 H), 0.94 (br t, J = 6 Hz, CH_2CH_3); CIMS (NH_3), m/e 254 (M^+ + 1 – HI). Anal. ($C_{16}H_{32}NOI$) C, N; H: calcd, 8.46; found, 8.04.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-8-Acetoxy-7-*n*-butyl-1,1-dimethyl-1-azoniaspiro[5.5]undecane Iodide (20). A solution of the amine 11 (140 mg, 0.5 mmol) and MeI (1 mL, 16 mmol) in acetone (2 mL) was stirred at 40 °C for 3 days. Evaporation of the mixture afforded an oil, which was washed with Et_2O. The residue was crystallized from EtOAc to give 20 (90 mg, 43%): mp 153–154 °C (EtOAc); IR (KBr) 1725, 1480, 1450 cm^{-1}; NMR (CD_3OD) δ 5.02 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H), 3.95–3.80 (m, 1 H, C_2 H), 3.40–3.30 (m, 1 H, C_2 H), 3.24 and 3.10 (each s, each 3 H, 2 NCH_3), 2.15 (s, 3 H, Ac), 2.50–1.20 (m, 19 H), 0.94 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (NH_3), m/e 296 (M^+ + 1 – HI). Anal. ($C_{18}H_{34}NO_2I$) C, H, N.

(6*R*,7*S*,8*S*)- and (6*S*,7*R*,8*R*)-7-*n*-Butyl-8-hydroxy-1,1-dimethyl-1-azoniaspiro[5.5]undecane Iodide (21). A mixture of the amino alcohol 5 (100 mg, 0.42 mmol), MeI (1 mL, 16 mmol), and acetone (10 mL) was set aside at 40–50 °C for 11 days. (The reaction can be monitored by TLC and NMR in acetone-d_6.) The mixture was evaporated, and the residue was washed with EtOAc. Recrystallization from MeOH–Et_2O gave 21 (61 mg, 38%): mp 164–165 °C; IR (KBr) 3420, 1485 cm^{-1}; NMR (CD_3OD) δ 3.75 (br s, $W_{1/2}$ = 8 Hz, 1 H, C_8 H) 3.45–2.80 (m, 2 H, C_2H_2), 3.17 and 3.04 (each s, each 3 H, 2 NCH_3), 2.40–1.00 (m, 19 H), 0.95 (br t, J = 6 Hz, 3 H, CH_2CH_3); CIMS (NH_3), m/e 254 (M^+ – HI + 1). Anal. ($C_{16}H_{32}NOI$) C, H, N.

Electrophysiological Techniques. All experiments were performed at room temperature (20–22 °C) on sciatic sartorius muscle preparations of the frog, *Rana pipiens*. The physiological solutions used had the following composition (mM): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4, 1.3; NaH_2PO_4, 0.7. The solution was bubbled with 100% O_2 and 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 a Ag–AgCl salt bridge electrode connected to a wet electrode.[2a] 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 recorded by attaching the muscle to a Grass FT03 force displacement transducer. The twitches were also displayed on a Grass polygraph.

The stock solution (10–500 mM) were made in 95% ethanol and stored as refrigerated stock solutions. They were diluted with physiological solution immediately before use.

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Supplementary Material Available: Tables containing atomic parameters, bond angles, and observed and calculated structure factors (3 pages). Ordering information is given on any current masthead page.

Monophenolic Octahydrobenzo[*f*]quinolines: Central Dopamine- and Serotonin-Receptor Stimulating Activity

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Eight monophenolic *cis*- and *trans*-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolines have been synthesized and tested for central dopamine- and serotonin-receptor stimulating activity, using biochemical and behavioral tests in rats. The *trans*-7-, -8-, and -9-hydroxy isomers all elicited central pre- and postsynaptic dopaminergic receptor stimulation, while the *trans*-10-hydroxy isomer was devoid of dopaminergic activity but instead showed central serotoninergic activity. In all four isomeric pairs, the trans isomers were consistently much more potent than their corresponding cis analogues. The apparent presynaptic selectivity of the dopaminergic cis isomer *cis*-9-hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline could not be confirmed due to the toxic properties of this compound. Central dopamine receptors (autoreceptors and postsynaptic receptors) can accept dopaminergic compounds with one of possibly two N-substituents being larger than *n*-propyl, if this substituent is properly oriented in relation to the rest of the molecule.

Recently, we described the unique pharmacological profile of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (1, 3-PPP), indicating selective central dopamine (DA) autoreceptor stimulating activity.[1] In another study, we compared the four isomeric monohydroxylated 2-(di-*n*-propylamino)tetralins 2–5, with regard to their effects upon central monoaminergic transmission.[2] It was demon-

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