is higher than that of pyrimidine. In other words, the hydrophobic interactions between the substrate and the polynucleotide in the present reaction system are predominant and the hydrogen-bonding interactions are of the secondary importance. This feature was earlier proposed by Ts'o, *et al.*,[26] from the thermodynamic measurements of various nucleic acid bases and nucleosides in aqueous solution. They showed that the strength of the interactions of the free bases and nucleosides could be arranged in the series: purine–purine > purine–pyrimidine > pyrimidine–pyrimidine.

It would be useful to sum up the present investigation as follows. Model compounds of coenzyme I, nicotinamide derivatives containing nucleic acid bases, were synthesized. In the CN^- addition reaction of these compounds, polyelectrolytes were found to decelerate the reaction, as was the case for interionic reactions between oppositely charged ionic species. Taking advantage of the equilibrium nature of the CN^- adduct formation, we were able to find polyelectrolyte influence on the forward and backward reaction rates; the forward rate constant was decreased by the polyelectrolyte addition whereas the backward rate constant was not influenced at all. Thus, it is clear that the polyelectrolytes cannot be regarded as catalysts, if we follow the earlier definition by Ostwald.[27] It was pointed out that, like the polyelectrolytes, micelles affect the two elementary processes in a different proportion. The "catalytic" influences by polyelectrolytes and micelles are mainly due to the electrostatic interactions. The hydrophobic interactions are also important, especially so in micelle-containing systems.

Acknowledgment. The authors are pleased to acknowledge the support of this work by a research grant from the Ministry of Education. Thanks are due to Professor N. J. Leonard for kind communication and also to Professors T. Saegusa and Y. Itoh, Kyoto University, for their assistance in the nmr measurements.

(26) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Amer. Chem. Soc.*, **85**, 1289 (1963).

(27) W. Ostwald, *Phys. Z.*, **3**, 313 (1902).

The Structure of Dihydroisohistrionicotoxin, a Unique Unsaturated Alkaloid and Anticholinergic Agent

Isabella L. Karle

Contribution from the Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D. C. 20390. *Received December 5, 1972*

Abstract: Dihydroisohistrionicotoxin, (2-*pro*-*R*,6*S*,7-*pro*-*S*,8a*S*)-7-(*cis*-1-buten-3-ynyl)-8-hydroxy-2-(3,4-pentadienyl)-1-azaspiro[5.5]undecane, crystallizes as the hydrochloride in space group $P2_12_12_1$ with $a = 11.438 \pm 0.005$ Å, $b = 14.598 \pm 0.004$ Å, and $c = 11.379 \pm 0.004$ Å. The material, derived from the Colombian frog *Dendrobates histrionicus*, is a potent and selective inhibitor of cholinergic mechanisms. Its configuration is compared with the established configurations of other cholinergic agonists and antagonists. Structural and conformational parameters have been determined for the allene and vinylacetylene substituents in the solid state.

Dihydroisohistrionicotoxin, a venom isolated from the skin of the brightly colored Colombian frog *Dendrobates histrionicus*, has an unique chemical constitution (I) consisting of a spiro alkaloid moiety and two unsaturated side chains, one containing an allene moiety and the other a vinylacetylene moiety.[1] This compound and its congener, histrionicotoxin (II), appear to be the first examples of acetylenic and allenic bonds occurring in natural products from the animal kingdom.[1] The molecular formulas of both congeners had been elucidated by crystal structure analyses using X-ray diffraction;[1] however, the only crystal of I available for the initial experiment was disordered. Although the molecular formula could be established, the data could not be refined to a reasonable agreement factor. Since that time, more material has become available, new crystals have been grown, and the structure determination has been repeated. The agreement factor between the observed and calculated structure factors is now 4.8%.

The histrionicotoxins, of which I and II are examples, are a third class of alkaloids isolated from the skin secretions of tropical American frogs. Batrachotoxin (III), from the Colombian *Phyllobates aurotania*, is a steroidal alkaloid and the most toxic nonprotein substance known.[2-4] Pumiliotoxin C (IV), from the Panamian *Dendrobates pumilio*, is a *cis*-decahydroquinoline.[5] Although these toxins occur in frogs which all belong to the family *Dendrobatidae*, their molecular formulas are quite diverse.

Histrionicotoxins have specific affinities as inhibitors of cholinergic receptor mechanisms in the neuromuscular system. In these molecules there are geometrical features which are comparable to portions of acetylcholine and other cholinergic materials. These

(1) J. W. Daly, I. L. Karle, C. W. Myers, T. Tokuyama, J. A. Waters, and B. Witkop, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1870 (1971).

(2) T. Tokuyama, J. W. Daly, B. Witkop, I. L. Karle, and J. Karle, *J. Amer. Chem. Soc.*, **90**, 1917 (1968).

(3) T. Tokuyama, J. W. Daly, and B. Witkop, *J. Amer. Chem. Soc.*, **91**, 3931 (1969).

(4) I. L. Karle and J. Karle, *Acta Crystallogr., Sect. B*, **25**, 428 (1969).

(5) J. W. Daly, T. Tokuyama, G. Habermehl, I. L. Karle, and B. Witkop, *Justus Liebigs Ann. Chem.*, **729**, 198 (1969).

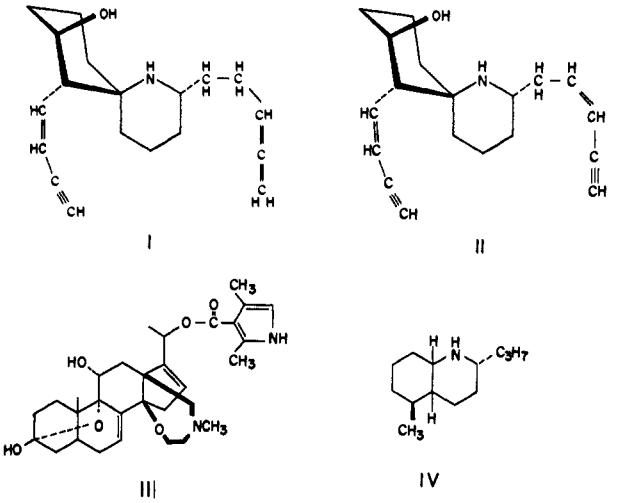
similarities in structure and conformation will be illustrated.

Experimental Section

Crystals of the hydrochloride of dihydroisohistrionicotoxin were prepared by Dr. T. Tokuyama of Osaka City University. Intensity data were collected on a four-circle automatic diffractometer using the θ–2θ scan technique with a $2.1° + 2\theta(\alpha_1)° - 2\theta(\alpha_2)°$ scan over 2θ. Background counts were measured for 10 sec at either end of the scan. The intensities were corrected for Lorentz and polarization factors and normalized structure factors $|E|$ were derived. Cell parameters and other physical data are listed in Table I.

Table I. Cell Parameters and Other Data

Formula	$C_{19}H_{27}NO \cdot HCl$
Mol wt	321.9
Color	Pale yellow
Habit	Prismatic *a*
Cross-section	0.4 × 0.5 mm
Space group	$P2_12_12_1$
a	11.438 ± 0.005 Å
b	14.598 ± 0.004 Å
c	11.379 ± 0.004 Å
Volume	1900.0 cm³
Z	4
Density (calcd)	1.125 g/cm³
Radiation	Cu Kα, 1.5418 Å
No. of independent reflections	1765



I II III IV

Fractional coordinates and thermal parameters for the heavy atoms are listed in Table II, and the approximate fractional coordinates for the hydrogen atoms as derived from the difference map are listed in Table III. Bond lengths and angles are shown in Figure 1 and torsional angles are shown in Figure 2.

Structure

The configuration of dihydroisohistrionicotoxin (I) is shown in the stereodiagram in Figure 3. The absolute configuration is assumed to be the same as that established for the congener histrionicotoxin (II) by the anomalous dispersion of the Br^- ion.[1] The bond lengths, bond angles, and conformations are very nearly the same in both congeners for atoms N(1) to C(16). Each contains an intramolecular NH···O hydrogen bond. In the piperidine rings in I and II, the CNC angles are 117.5 and 116.0°, respectively. The magni-

Table II. Fractional Coordinates, Standard Deviations, and Thermal Parameters for Dihydroisohistrionicotoxin[a]

Atom	*x*	*y*	*z*	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl^-	−0.1214 (1)	0.0536 (1)	0.0547 (1)	4.06	4.99	4.96	−0.32	−0.26	0.26
N(1)	0.1146 (3)	0.3933 (2)	−0.0247 (2)	4.30	4.37	3.70	−0.45	−0.09	−0.10
C(2)	0.0485 (3)	0.4441 (3)	−0.1227 (3)	4.42	7.09	3.84	−0.12	−0.42	0.01
C(3)	0.0580 (4)	0.5469 (3)	−0.1034 (3)	6.48	5.39	6.08	0.15	−0.70	1.12
C(4)	0.0234 (4)	0.5753 (3)	0.0203 (3)	7.06	5.97	5.91	1.16	0.63	0.16
C(5)	0.0993 (3)	0.5247 (2)	0.1072 (3)	5.83	4.75	5.04	−0.36	−0.10	−0.51
C(6)	0.0868 (3)	0.4204 (2)	0.1018 (3)	3.80	4.36	3.83	−0.50	0.06	−0.28
C(7)	−0.0384 (3)	0.3873 (2)	0.1318 (3)	4.31	6.21	4.05	−0.88	0.04	−0.12
C(8)	−0.0448 (4)	0.2825 (3)	0.1382 (3)	7.05	6.26	4.98	−2.57	0.31	0.26
C(9)	0.0454 (4)	0.2411 (3)	0.2197 (4)	8.48	6.34	5.78	−1.81	0.25	1.35
C(10)	0.1680 (4)	0.2717 (3)	0.1857 (4)	7.57	6.32	5.77	0.36	−0.65	0.95
C(11)	0.1781 (3)	0.3760 (3)	0.1801 (3)	5.11	5.98	3.96	−0.14	−0.54	0.46
C(12)	−0.0817 (3)	0.4310 (3)	0.2435 (3)	4.98	7.66	4.54	−1.12	0.28	−0.25
C(13)	−0.1892 (4)	0.4616 (3)	0.2602 (3)	5.30	9.47	5.40	−0.51	0.79	−1.24
C(14)	−0.2788 (4)	0.4574 (4)	0.1725 (4)	4.68	9.31	7.03	−0.44	1.26	−0.38
C(15)	−0.3548 (4)	0.4552 (4)	0.1009 (4)	5.13	12.11	8.05	0.13	−0.25	−0.76
C(16)	0.0979 (4)	0.4167 (3)	−0.2395 (3)	5.90	7.47	4.45	−1.07	−0.43	−0.38
C(17)	0.0728 (5)	0.3219 (3)	−0.2810 (4)	8.93	7.21	7.16	−1.11	1.54	−1.04
C(18)	0.1094 (5)	0.3139 (3)	−0.4116 (4)	8.58	8.17	6.12	−0.75	−1.05	−1.88
C(19)	0.1900 (5)	0.2574 (3)	−0.4488 (4)	8.50	6.61	5.24	−1.47	−0.20	−1.09
C(20)	0.2678 (5)	0.2029 (4)	−0.4896 (5)	9.13	9.50	8.91	−1.07	0.59	−1.51
O	−0.0306 (3)	0.2502 (2)	0.0194 (2)	7.80	5.54	5.47	−2.47	0.68	−0.58

[a] Thermal parameters are expressed in the form $T = \exp[-1/4(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$.

Phases based on the coordinates of the Cl^- ion, derived from a Patterson map computed with $|E_h|^2 - 1$ as coefficients, were used as input for a recycling procedure[6] employing the tangent formula.[7] The *E* map computed with the phases derived from the tangent formula clearly indicated all the C, N, and O atoms of the molecule. The 28 hydrogen atoms were located in a difference map computed after full-matrix least-squares anisotropic refinement of the 22 heavy atoms. Thermal parameters for the hydrogen atoms were assumed to be the same as for the heavy atom to which they are attached. The weighting function was based on counting statistics. Refinement of the heavy atoms with the parameters for the 28 hydrogen atoms held constant resulted in an *R* factor of 4.8% for the 1765 data.[8]

(6) J. Karle, *Acta Crystallogr., Sect. B*, **24**, 182 (1968).
(7) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956).

(8) Tables of the structure factors will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D. C. 20036, by referring to code number JACS-73-4036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

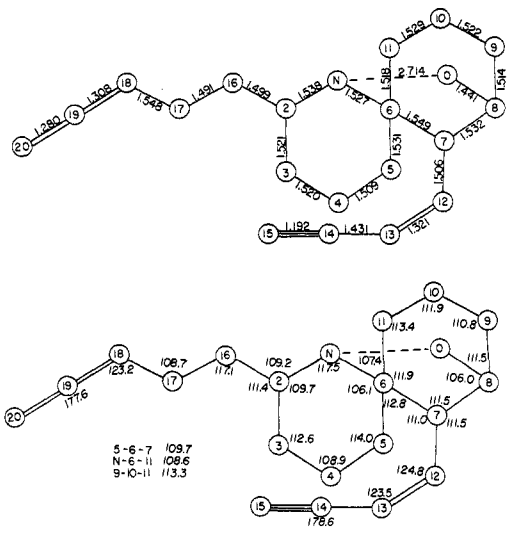


Figure 1. Bond lengths and angles in dihydroisohistrionicotoxin. The standard deviations range from 0.0040 Å for the C–N bonds to 0.0075 Å for C(19)–C(20). For the angles associated with the two rings, the standard deviations are of the order of 0.3° and they increase to 0.5° for the angles near the ends of the two chains.

Table III. Approximate Coordinates for Hydrogen Atoms Derived from a Difference Map

Atom	x	y	z
H(N-1)	0.1022	0.3292	−0.0332
H(N-2)	0.2047	0.4173	−0.0266
H(2)	−0.0372	0.4254	−0.1107
H(3-1)	0.1344	0.5713	−0.1204
H(3-2)	−0.0114	0.5683	−0.1621
H(4-1)	−0.0643	0.5679	0.0460
H(4-2)	0.0276	0.6476	−0.0187
H(5-1)	0.1877	0.5453	0.0863
H(5-2)	0.0838	0.5435	0.1892
H(7)	−0.0996	0.4070	0.0681
H(8)	−0.1331	0.2557	0.1665
H(9-1)	0.0190	0.1658	0.2143
H(9-2)	0.0354	0.2658	0.3063
H(10-1)	0.2112	0.2529	0.2499
H(10-2)	0.1923	0.2429	0.1148
H(11-1)	0.2651	0.3956	0.1475
H(11-2)	0.1686	0.3813	0.2683
H(12)	−0.0138	0.4339	0.3123
H(13)	−0.2251	0.4864	0.3385
H(15)	−0.4229	0.4404	0.0480
H(16-1)	0.1889	0.4213	−0.2283
H(16-2)	0.0650	0.4550	−0.3240
H(17-1)	0.1381	0.2647	−0.2358
H(17-2)	−0.0132	0.3162	−0.2732
H(18)	0.0537	0.3470	−0.4673
H(20-1)	0.3733	0.2253	−0.5145
H(20-2)	0.2448	0.1462	−0.5402
H(0)	−0.0416	0.1822	0.0367

tudes of the angles alternate within the piperidine rings where the average values of the angles at N(1), C(3), and C(5) are 114.7° as compared with 108.2 and 109.3° for the average of the angles at C(2), C(4), and C(6) in I and II. A similar, though smaller, alternation in angular values is found in the piperidyl ion in the crystal of piperidine hydrochloride.[9]

The *cis*-butenynyl chain, C(7),C(12)–C(15), is planar to within ±0.003 Å. The C—C≡C angle is 178.6° with atom C(15) trans to the double bond. A direct comparison can be made with the parameters in CH_2=CH—C≡CH where the structure was determined in the vapor phase by electron diffraction.[10] The angles are almost exactly the same and the bond lengths, (C=C) = 1.344 Å, (C—C) = 1.434 Å, and (C≡C) = 1.215 Å, are within 0.02 Å of the values in the present study. An even closer agreement is found with crystalline *p,p'*-HC≡C—C_6H_4—C≡CH where the single bond (1.444 Å) is between a triple bond (1.188 Å) and an aromatic bond in the benzene ring and the C—C≡C angle is 178.6°.[11] The length of a single C—C bond appears to be greatly affected by the number of adjacent multiple bonds. The value of 1.431 Å for C(13)–C(14) found in dihydroisohistrionicotoxin is quite consistent with the empirical curve derived for the C–C bond length as a function of the number of adjacent atoms.[12]

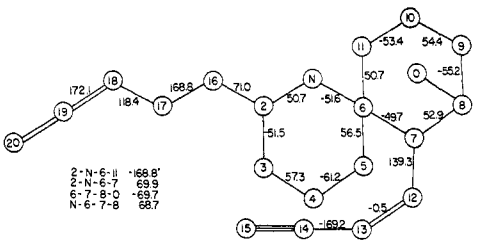


Figure 2. Torsional angles.

The differences in molecules I and II occur between atoms C(16) and C(20) both in chemistry and conformation. Whereas the *cis*-pentenynyl chain in II is planar, the allenic pentadienyl chain in I has atoms C(17)–C(20) in a plane to within ±0.002 Å while atom C(16) is rotated 62° from the trans conformation into an anti-gauche conformation (see Figure 2). Atom C(17) is gauche with respect to the N atom in I whereas it is trans in II, thus causing the dissimilar side chains to project away from the molecules in different directions in I and II. The allene moiety is not quite linear with a C=C=C angle of 177.6°. Atom C(20) is trans with respect to C(17). The bond lengths of 1.280 and 1.308 Å for the allene moiety can be compared with 1.283 Å for the central bond and 1.318 Å for the outer C=C bonds observed for H_2C=C=C=CH_2 in the vapor state[13] and 1.289 Å for the C=C bonds in O=C=C=C=O in the vapor state.[14]

Unusually low values of 1.49 and 1.50 Å have been observed for the single C(2)–C(16) and C(16)–C(17) bonds with a correspondingly large value of 117.1° for the C(2)C(16)C(17) angle. To eliminate the possibility of some undetected systematic error, the data collection was repeated on another diffractometer using Mo radiation instead of Cu radiation. The least-squares refinement resulted in an agreement

(9) C. Rérat, *Acta Crystallogr.*, **13**, 72 (1960).

(10) T. Fukuyama, K. Kuchitsu, and Y. Morino, *Bull. Chem. Soc. Jap.*, **42**, 379 (1969).

(11) N. A. Ahmed, A. I. Kitaigorodsky, and M. I. Sirota, *Acta Crystallogr., Sect. B*, **28**, 2875 (1972).

(12) See, *e.g.*, (a) B. P. Stoicheff, *Tetrahedron*, **17**, 135 (1962); (b) J. Karle, "Electron Diffraction" from "Determination of Organic Structures by Physical Methods," Vol. 5, F. C. Nachod and J. J. Zuckerman, Ed., Academic Press, New York, N. Y., 1973, p 21; and (c) K. Kuchitsu in "Gas Electron Diffraction" from "MTP International Review of Science," G. Allen, Ed., Medical and Technical Publishing Co., Ltd., Oxford, in press.

(13) A. Almenningen, O. Bastiansen, and M. Traetteberg, *Acta Chem. Scand.*, **15**, 1557 (1961).

(14) M. Tanimoto, K. Kuchitsu, and Y. Morino, *Bull. Chem. Soc. Jap.*, **43**, 2776 (1970).

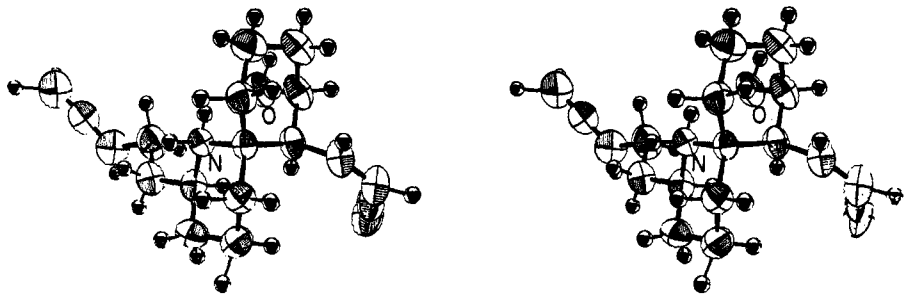


Figure 3. Stereodiagram of dihydroisohistrionicotoxin. The thermal ellipsoids for the C, N, and O atoms are drawn at the 50% probability level. The stereo figures in this paper were prepared with a computer program (ref 22).

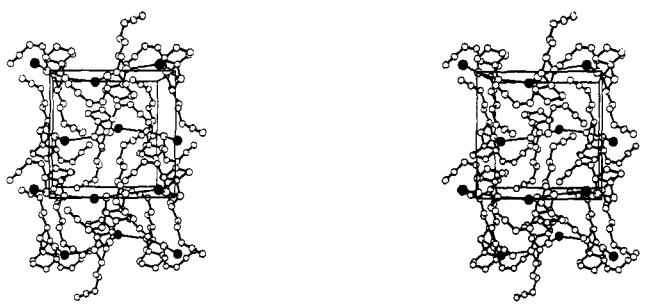


Figure 4. Stereodiagram of the packing of the molecules. The Cl^- ions are shaded and the NH···Cl^-, OH···Cl^- and NH···O hydrogen bonds are indicated by light lines. The axial directions are: $a\rightarrow$, $c\uparrow$, and b down into the plane of the page.

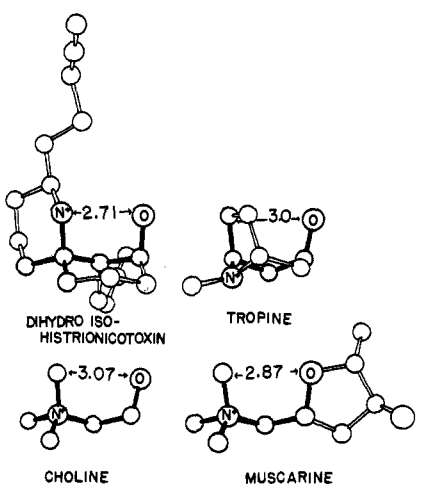


Figure 5. A comparison of the conformations of four materials exhibiting cholinergic activity (only the choline moiety of acetylcholine and the tropine moiety of scopolamine are shown). The drawings are to scale and were prepared from the atomic coordinates of their respective crystal structures.

factor of 5.5% for 1892 data and the values for the bond lengths and angles were within the standard deviations quoted for the results from the Cu data. There have not been any previous studies of a moiety similar to the pentadienyl chain for direct comparison.

Dihydroisohistrionicotoxin has an intramolecular NH···O hydrogen bond of 2.714 Å, creating a third ring around the spiro carbon atom. Two other hydrogen bonds involve the Cl^- ions, NH···Cl^- of length 3.136 Å and OH···Cl^{-1} of length 3.078 Å. These three hydrogen bonds link molecules into continuous chains around the screw axes parallel to the a direction. The stereodiagram in Figure 4 illustrates the mode of hydrogen bonding and the packing in the crystal. The closest intermolecular approaches in the crystal involve the Cl^- ions. Each Cl^- ion is surrounded by five molecules. Aside from the NH···Cl^- and OH···Cl^- bonds, the nearest distances are: $Cl^-\cdots C(15)'$ at 3.53 Å $(-x, -1/2 + y, 1/2 - z)$, $Cl^-\cdots C(8)'$ at 3.58 Å (x, y, z), $Cl^-\cdots C(11)'$ at 3.67 Å $(1 - x, -1/2 + y, 1/2 - z)$, and $Cl^-\cdots C(12)'$ at 3.73 Å $(1/2 + x, -1/2 - y, 1 - z)$. The primed atoms are in molecules related to the x, y, z coordinates by the symmetry operations shown in parentheses. The only other intermolecular distances of less than 3.8 Å are $C(13)\cdots C(20)'$ at 3.58 Å $(1 - x, -1/2 + y, 1/2 - z)$ and $C(4)\cdots C(19)'$ at 3.70 Å $(1/2 + x, -1/2 - y, -z)$. The two hydrocarbon side chains do not pack efficiently in the crystal. The relatively few close contacts between molecules account for the low density of 1.125 g/cm^3 for the crystal.

Discussion

The histrionicotoxins are inhibitors which selectively interact with cholinergic mechanisms[15] in the nervous system and thereby prevent transynaptic transmission of nerve impulses. The molecules I and II have a polar side and two aliphatic side chains to which much more rigidity is imparted by the presence of double and triple bonds than in saturated chains of comparable length. It is conceivable that these rigid chains may play a role in breaking up the phospholipid membrane in order for the molecule to gain access to the receptor sites, although the perhydro derivative with two saturated side chains is still active.

Crystal structure analyses have been reported for many diverse materials which are active at cholinergic sites. Conformational analyses relating activity with structural parameters have been published, *e.g.*, by Sundaralingam,[16] Beers and Reich,[17] Baker, *et al.*,[18] and Martin-Smith.[19] It has been found that active derivatives of choline and muscarine assume the gauche conformation in the N+CCO moiety (see Figure 5) with a N+CCO torsional angle in the range +73 to

(15) E. X. Albuquerque, J. W, Daly, and B. Witkop, *Proc. Nat. Acad. Aci. U. S.*, in press; E. X. Albuquerque, A. J. Lapa, J. W. Daly, and B. Witkop, "Histrionicotoxin on Skeletal Muscle. I. Cholinergic Effects," Fifth International Congress on Pharmacology, San Francisco, Calif., April 1972.

(16) M. Sundaralingam, *Nature (London)*, 217, 35 (1968).

(17) W. H. Beers and E. Reich, *Nature (London)*, 228, 917 (1970).

(18) R. W. Baker, C. H. Chothia, P. Pauling, and T. J. Petcher, *Nature (London)*, 230, 439 (1971).

(19) M. Martin-Smith in "Drug Design," Vol. II, A. Arien, Ed., Academic Press, New York, N. Y., 1971, p 503.

+137°. Compounds active at the muscarinic receptor sites appear to require a $(CH_3)_3N^+$ group and, in addition, an alkyl or NH_2 group at the position of the acetoxy methyl in acetylcholine.[18] For the nicotinic receptor sites, the groupings which appear to be essential for activity are a quaternary N^+ moiety and a hydrogen bond receptor located approximately 5.9 Å from the center of the positive charge.[17]

It may be instructive to examine whether there are geometrically similar groupings in the histrionicotoxins and cholinergic agonists and antagonists. In Figure 5, the geometry of isohistrionicotoxin is compared with muscarine, tropine, and the choline moiety in acetyl- or succinylcholine. Coordinates determined from crystal structure analyses[20,21] were used to execute precise drawings of the configurations by computer.[22] Similar geometric, although not necessarily chemical, moieties are outlined with heavy lines. Although there is a group of atoms in the histrionicotoxins with a geometry resembling that of choline, the histrionicotoxin structure does not possess the requirements as mentioned above either for muscarinic or nicotinic activity. For this potent inhibitor, the occurrence of a N^+ atom with a hydrogen bond receptor only 2.71 Å from the N^+ apparently is sufficient for activity.

(20) (a) M. E. Senko and D. H. Templeton, *Acta Crystallogr.*, **13**, 281 (1960); (b) F. Jellinek, *ibid.*, **10**, 277 (1957).
(21) J. W. Visser, J. Manassen, and J. L. de Vries, *Acta Crystallogr.*, 7, 288 (1954).
(22) C. K. Johnson, ORTEP, ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

A General Method for the Determination of Precursor Configuration in Biosynthetic Precursor–Product Relationships. Derivation of Pipecolic Acid from D-Lysine, and of Piperidine Alkaloids from L-Lysine[1]

E. Leistner, R. N. Gupta, and Ian D. Spenser*

Contribution from the Department of Chemistry, McMaster University, Hamilton, Ontario, Canada. *Received December 14, 1972*

Abstract: The configuration of a metabolic precursor is determined experimentally by comparison of the $^3H/^{14}C$ ratio of the metabolic product with that of a doubly labeled substrate. This new method for the determination of precursor stereochemistry in intact systems does not depend on a comparison of incorporation efficiencies. It is shown by this method that the alkaloids sedamine, *N*-methylpelletierine, and *N*-methylallosedridine from two *Sedum* species and anabasine from *Nicotiana glauca* are derived from L-lysine, whereas pipecolic acid, from each of these plants, is derived from D-lysine.

In biosynthetic tracer studies in which specifically ^{14}C-labeled amino acids are used as substrates, the DL racemates are most frequently employed since they are more readily available and generally less expensive than correspondingly labeled samples of the L and D enantiomers. If nonrandom incorporation of label from a ^{14}C-labeled DL-amino acid into a biosynthetic product is observed, the assumption tends to be made (in the absence of evidence to the contrary) that it is the L enantiomer which represents the normal metabolic substrate and serves as the actual precursor (*e.g.*, ref 2 and 3).

Tracer experiments intended to establish, in intact systems, which one of the two enantiomers of an amino acid served as the actual precursor have, in general, led to results which were indicative rather than conclusive, and have on occasion yielded contradictory data. Inferences were based:

(i) on a comparison of the efficiencies,[4] observed in parallel experiments, of incorporation of radioactivity from the labeled DL racemate and the labeled L and D isomers of the substrate into the biosynthetic product,[6]

(1) Presented as part of a Symposium lecture at the 23rd International Congress of Pure and Applied Chemistry, IUPAC, Boston, Mass., July 1971, Symposium O-13, Abstract No. 91, p 36.
(2) E. Leete, *J. Amer. Chem. Soc.*, **84**, 55 (1962).
(3) A. J. Birch and K. R. Farrar, *J. Chem. Soc.*, 4277 (1963).
(4) Incorporation efficiencies have been expressed in several ways: *specific radiochemical yield* (100 × molar specific activity of product/molar specific activity of substrate) or its reciprocal, *dilution value* (molar specific activity of substrate/molar specific activity of product), are expressions of tracer concentration within the isolated product. Such values depend[5] not only on the rate of conversion of substrate to product, but also on the pool sizes, within the intact system, of the substrate and of the intermediates between substrate and product, as well as on the amount of product present in the system at the time of administration of tracer. Apart from demanding rigorous purification of end product, these values are independent of manipulative variables such as losses in the course of isolation of product. The *per cent incorporation* of activity into the product (100 × total activity recovered in product/total activity administered in substrate), on the other hand, depends on the chemical yield of product which, in turn, is a function of the skill of the experimenter.
(5) J. E. Watkin and A. C. Neish, *Can. J. Biochem. Physiol.*, **38**, 559 (1960).
(6) *E.g.*, incorporation of cystine into benzylpenicillin,[7] of tryptophan into echinulin,[8] actinomycin,[9] and pyrrolnitrin,[10] of valine and α-hydroxyvaleric acid into valinomycin,[11] of valine into benzylpenicillin,[12,13] into sporidesmolide 1,[14] and into penicillin N and cephalosporin C,[15] of α-aminoadipic acid into penicillin N and cephalosporin C,[16] of ornithine into bacitracin,[17] of lysine into pipecolic acid,[18,19] and of 5-hydroxylysine into 5-hydroxypipecolic acid.[20]
(7) H. R. V. Arnstein and P. T. Grant, *Biochem. J.*, **57**, 353 (1954).
(8) J. C. MacDonald and G. P. Slater, *Can. J. Microbiol.*, **12**, 455 (1966).
(9) A. Albertini, O. Tiboni, and O. Ciferri, *J. Label. Compounds*, **2**, 90 (1966).
(10) R. Hamill, R. Elander, J. Mabe, and M. Gorman, *Antimicrob. Ag. Chemother.*, 338 (1967).