THE CRYSTAL STRUCTURE OF ACETYLCHOLINE CHLORIDE: A NEW CONFORMATION FOR ACETYLCHOLINE

John K. Herdklotz and Ronald L. Sass
Department of Chemistry
Rice University
Houston, Texas 77001

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The crystal structure of acetylcholine chloride has been determined from three-dimensional diffractometer data. The molecular geometry of this compound differs significantly from that observed in acetylcholine bromide but does agree with the results of molecular orbital calculations and NMR data on acetylcholine in solution.

INTRODUCTION

Structural properties of numerous molecules active in cholinergic systems have been studied in recent years. Our interest has been in the molecular properties of local anesthetics such as procaine (Beall, Herdklotz, and Sass, 1970) and procaine analogues. A primary consideration in these studies is the relationship between the conformational geometry of these molecules with that of acetylcholine, the natural intercellular effector in many nervous transmission systems. It is thus important that the structural features of acetylcholine be accurately established. The crystal structure of acetylcholine bromide was first reported by Sörum (1959). This analysis was questioned by Dunitz (1963) and Canepa (1964). A reinvestigation of the structure of acetylcholine bromide, using original X-ray diffraction data of Sörum, has been published by Canepa, Pauling and Sörum (1966).

Because of the relatively large estimated standard deviations of the bond distances (about 0.06Å) of acetylcholine bromide and because of the more fundamental question of the effects of crystal packing on structural geometry of molecules, we have undertaken the diffraction study of crystalline acetylcholine chloride.

EXPERIMENTAL

Crystals of acetylcholine chloride suitable for X-ray analysis were obtained by diffusion of anhydrous ether into an ethanol solution of the compound. Because acetylcholine chloride is highly deliquescent, the crystals were kept in a dry nitrogen atmosphere and were sealed in .2 mm diameter glass capillaries before being subjected to the X-ray beam. From preliminary examination of several crystals, one crystal was chosen and used throughout the analysis. The crystals are orthorhombic (space group $2_1 2_1 2_1$) with cell dimensions a = 10.071, b = 15.511, c = 6.380Å. Three dimensional intensity data were collected on a Phillips Pailred diffractometer employing equi-inclination geometry. All data were collected using silicon monochromatized molybdenum Kα radiation. The fixed counter moving crystal method was used. All reflections for which the counting statistical error exceeded 50% were rejected (Ladell, 1965). A total of 730 independent reflections were included in the analysis.

The positional parameters for the chlorine atom were obtained from a three-dimensional Patterson synthesis. The remaining non-hydrogen atoms were located in the structure using the heavy atom method. All hydrogen atom positional parameters were obtained from a three-dimensional Fourier difference synthesis. The structure was refined by the method of full-matrix least squares assigning weights based on counting statistics (Evans, 1961) to each reflection. Positional parameters and anisotropic temperature factors of 6.0 were assigned to the hydrogen atoms. The structure was refined to a final R value of 7.1% while the final weighted R was 2.3%. Final positional parameters for all atoms are shown in table 1.

RESULTS AND DISCUSSION

The measured bond distances and angles of acetylcholine chloride

Table 1. Positional Parameters
(Estimated Standard Deviations)

Atom	x	y	z
Cl	0.1602(2)	0.1643(1)	0.2397(5)
N(1)	-0.1006(5)	0.3562(4)	0.2673(11)
O(1)	0.0411(5)	0.4893(3)	0.5922(7)
O(2)	0.2230(6)	0.5632(4)	0.4937(8)
C(1)	-0.1905(8)	0.4331(6)	0.2848(15)
C(2)	-0.1798(8)	0.2748(5)	0.2592(17)
C(3)	-0.0212(13)	0.3625(8)	0.0652(12)
C(4)	-0.0110(10)	0.3487(6)	0.4532(12)
C(5)	0.0930(8)	0.4140(6)	0.4839(13)
C(6)	0.1224(9)	0.5607(6)	0.5891(12)
C(7)	0.0612(10)	0.6289(6)	0.7229(14)
H(1,1)	-0.243 (7)	0.432 (6)	0.195 (10)
H(1,2)	-0.241 (7)	0.425 (5)	0.415 (9)
H(1,3)	-0.138 (6)	0.486 (3)	0.226 (11)
H(2,1)	-0.234 (8)	0.274 (6)	0.182 (12)
H(2,2)	-0.117 (6)	0.217 (3)	0.294 (11)
H(2,3)	-0.240 (7)	0.273 (5)	0.406 (11)
H(3,1)	-0.068 (9)	0.354 (7)	-0.033 (9)
H(3,2)	0.031 (7)	0.432 (4)	0.073 (10)
H(3,3)	0.034 (9)	0.308 (5)	0.050 (14)
H(4,1)	-0.068 (7)	0.342 (6)	0.598 (9)
H(4,2)	0.042 (8)	0.294 (5)	0.450 (13)
H(5,1)	0.164 (7)	0.394 (3)	0.604 (9)
H(5,2)	0.146 (7)	0.445 (4)	0.335 (7)

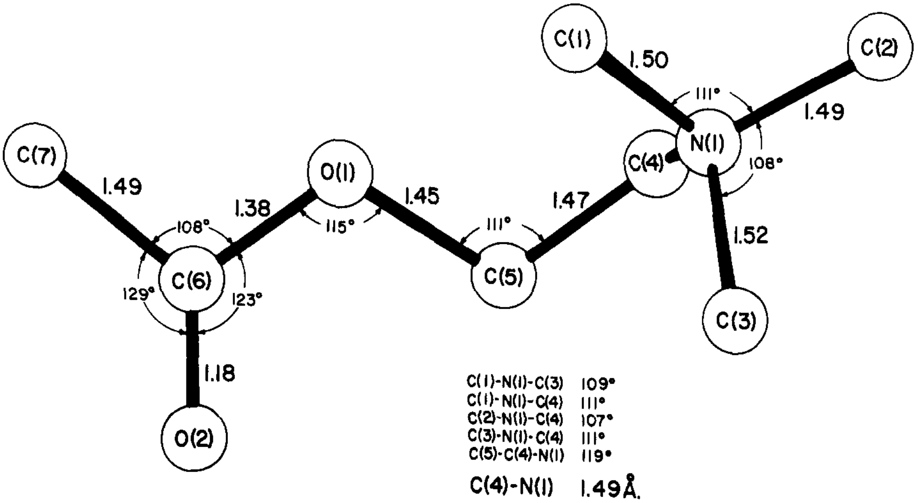
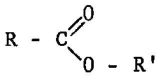


Figure 1: Molecular Dimensions of
Acetylcholine Chloride

(excluding hydrogen atoms) are shown in figure 1. The estimated standard deviations of the bond distances are all approximately ± 0.01Å and of the bond angles are all less the ± 1°. The atom numbering scheme is equivalent to that of Canepa, Pauling and Sörum for acetylcholine bromide.

The C(4)-N(1) bond distance of 1.49Å agrees well with the average value of C-N$^+$H$_3$ single bond distances observed in a variety of compounds. For example in noradrenaline hydrochloride (Carlström and Bergin, 1967), the observed C-N bond length is 1.493Å. The various carbon-carbon bonds are all normal and comparable to equivalent bond distances found in procaine hydrochloride. In the carboxylate group the C(6)-O(2) observed distance of 1.18Å is shorter while the C(6)-O(1) distance of 1.38Å is considerably longer than the related values of 1.20 ± .01Å and 1.33Å reported for methyl formate from microwave data (Curl, 1959). The two principle resonance structures expected for the carboxylate group are



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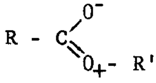

A. B.

The C-O bond lengths observed in acetylcholine indicate that structure A is the predominant contributor (over 90%) while structure B is relatively unimportant.

The conformation of the acetylcholine molecule has been of major concern for several years. The fundamental biological importance of this molecule has prompted investigation on many cholinergic effectors in an attempt to define the stereochemistry of the interactions of these molecules with membrane receptor and with acetylcholinesterase.

In the crystal structure of acetylcholine bromide (Canepa, Pauling and Sörum, 1966), the N$^+$-C(4)-C(5)-O(1) group was observed in the gauche configuration with a torsion angle of 77°. These authors also report a C(4)-C(5)-O(1)-C(6) torsion angle of 79°, resulting in an overall gauche-gauche arrangement extending from N$^+$ to C(6). In all systems containing the N$^+$-C-C-O group for which the crystal structures are known, the oxygen atom is gauche to the nitrogen atom (Sundaralingam, 1968). However, it has

Table 2. Selected Torsional Angles in Crystalline Acetylcholine Chloride and Acetylcholine Bromide

Angle	Chloride	Bromide
C(2)-N(1)-C(4)-C(5)	171.4°	~180°
N(1)-C(4)-C(5)-O(1)	84.7°	77°
C(4)-C(5)-O(1)-C(6)	166.9°	79°
C(5)-O(1)-C(6)-O(2)	5.2°	0°

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been pointed out by Mathieson (1965) that the trans C-O-C-C conformation is normally found in primary esters. The gauche-trans conformation is indeed observed on a number of related structures: Procaine (Beall, Herdklotz, and Sass, 1970), dl-lactoylcholine (Chothia and Pauling, 1968), muscarine iodide (Jellinek, 1957) are examples. Culvenor and Ham (1966), on the basis of NMR data, claim the predominant solution conformer of acetyl choline to be gauche-trans. We have also found this configuration to exist in crystalline acetylcholine chloride. The observed pertinent torsional angles are presented in table 2.

These results are consistent with the theoretical conformational analysis of acetylcholine by Kier (1967). Kier, using extended Hückel calculations, predicted only one stable configuration, namely the gauche-trans. Liquori, Damiani and de Coen (1968) calculated minimum energy conformations of acetylcholine on the basis of van der Waals pairwise interactions between non-bonded atoms and 3-fold torsional potentials. They found four stable conformations separated in energy be less than 1 kcal/mole. Their lowest energy conformation was the fully extended trans chain. Two of the other stable conformations are those observed in crystalline acetylcholine chloride and bromide. Some questions remain about these calculations, however, since no choline derivatives have been observed in the fully trans configuration.

The relative position of the quaternary ammonium group to the ester oxygen appears to be an important feature in the interaction of acetylcholine with nerve receptors. We have observed an N···O(1) distance of

3.26Å and a C(1)···O(1) distance of 3.17Å. These are comparable to the values of 3.29Å and 3.02Å observed in acetylcholine bromide. In spite of the conformational differences existing between these two crystalline structures, these interaction distances remain nearly constant.

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