Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine II [1]
X-ray Structure Analysis of Trimethyl(4-oxopentyl)ammonium-chloride

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Dedicated to Prof. Walter Hoppe *on the Occasion of his 65th Birthday on March 21st, 1982*

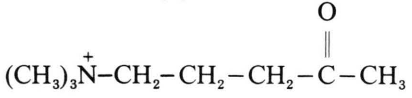
X-ray, Neurotransmitter, Cation-Anion Interactions, Structure-Activity Relationships

The crystal structure of Trimethyl(4-oxopentyl)ammonium-chloride ($[(CH_3)_3\overset{+}{N}{-}(CH_2)_3COCH_3]Cl^-$) (**1**) was determined by an X-ray structure analysis. **1** crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 10.440$ (3), $b = 14.600$ (9), $c = 6.804$ (9) Å and with four formula units per unit cell. The structure was solved by a Patterson and a successive Fourier synthesis. The least squares refinement yielded an R-value of 0.064 for 1077 observed reflections. The cation of **1** is derived from acetylcholine by replacement of the ester oxygen with a CH_2 group. It shows a potent nicotinic activity and a significant difference in conformation as compared with acetylcholine. In the crystal structure the anions are oriented stereospecifically with respect to the tetrahedron of the quaternary ammonium group. The geometry of two triangles formed by the quaternary nitrogen atom, the oxygen atom of the carbonyl group, and by either of the two anions nearest to the quaternary ammonium group is characteristic for the nicotinic activity of **1**.

Introduction

We have carried out a single-crystal X-ray structure analysis of the title compound (**1**) as a part of a systematic study of agonists of the natural neurotransmitter acetylcholine (**2**). In this paper primarily the stereochemical and crystallographic results are to be discussed. The general conclusions drawn from this and a series of X-ray structure analyses of cholinergic agonists [2] with respect to the structure-activity relationships in preliminary form are published separately [1, 3] and will be published in detail in the near future [4].

1



$$(CH_3)_3\overset{+}{N}{-}CH_2{-}CH_2{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}CH_3$$

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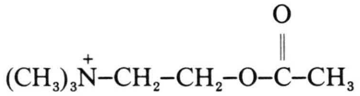

$$(CH_3)_3\overset{+}{N}{-}CH_2{-}CH_2{-}O{-}\overset{\overset{O}{\|}}{C}{-}CH_3$$

The cation of **1** is a derivative of acetylcholine, in which the ester oxygen is substituted by a methylene group. This chemical modification results in the exhibition of almost exclusively nicotinic activity in **1**. Its muscarinic activity is only very weak [5, 6].

Experimental

1 was synthesized following specifications given in the literature [5, 6]. Colourless, hygroscopic crystals were obtained by recrystallization from absolute ethanol. For the X-ray investigation a crystal of dimensions 0.18 × 0.21 × 0.71 mm was sealed under dry argon atmosphere in a Debye-Scherrer capillary. Rotation, Weissenberg and precession photographs yielded an unambiguous determination of the space group and preliminary lattice constants. The final lattice constants were determined by the exact angle positions of specifically chosen reflections by aid of an automatic single-crystal diffractometer (Siemens AED, off-line). The crystallographic data are summarized in Table I. With the diffractometer mentioned above 1119 independent reflections were recorded using Cu K_α radiation (Ni filter) up to a maximum scattering angle of $\Theta = 70°$ (5-point measurement, $\Theta/2\Theta$ scan mode, maximum of measuring time: 1.2 sec/10^{-2} ° (Θ), minimum: 0.06 sec/10^{-2} ° (Θ). 42 of the recorded reflections were

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Table I. Crystallographic data of **1**.

Formula	$(C_8H_{18}NO)^+Cl^-$
Crystal system	orthorhombic
Systematic absences	h00, $h = 2n + 1$
	0k0, $k = 2n + 1$
	00l, $l = 2n + 1$
Space group	$P2_12_12_1$
Lattice parameters [Å]	$a = 10.440$ (3)
	$b = 14.600$ (9)
	$c = 6.804$ (9)
Volume of the unit cell [Å^3]	1037.1
Molecular weight	179.7
Content of formula units:	
in the unit cell	4
in the asymmetric unit	1
Calculated density [gcm^{-3}]	$D_c = 1.15$
Measured density [gcm^{-3}]	$D_m = 1.14$

classified as unobserved ($I < 2\sigma_I$). To those reflections an intensity of $I_0 = 2\sigma_I$ was attributed. The linear absorption coefficient μ was calculated to be 29.2 cm^{-1}.

Solution of the structure and refinement

The reflection intensities were corrected for Lorentz and polarization effects in the usual way. No absorption correction was applied. *E* value statistics confirmed the existence of a noncentrosymmetric space group. For most of the computer calculations a special version [7] of the X-RAY 67 program system [8] was used with a Siemens 4004 computer, but also an IBM 360/91 and an Amdahl 470 computer were employed. The atomic scattering factors for C, N, O and Cl$^-$ were taken from International Tables for X-ray Crystallography [9] and those for H are given by Stewart, Davidson and Simpson [10].

By way of a Patterson synthesis the position of the chlorine atom was determined. A Fourier synthesis which was phased by the chlorine atoms yielded the positions of all non-hydrogen atoms. The least squares refinement (unit weights, full matrix), with anisotropic temperature parameters for all non-hydrogen atoms and isotropic ones for the hydrogen atoms, converged to an *R*-value of 0.064 ($R = \sum \left| |F_0| - |F_c| \right| / \sum |F_0|$) for the observed reflections. The unobserved reflections were incorporated into the computation of parameter shifts only under the condition that $|F_c| > |F_0|$. In the last steps of refinement reflections with $|\Delta F| > 5$ ($|\Delta F| = \left| |F_0| - |F_c| \right|$) were omitted from the computation of parameter shifts. In the last cycle of refinement the number of those reflections was only 17. The positions of the hydrogen atoms with the exception of H(18) were determined in the course of refinement with anisotropic temperature parameters by way of difference Fourier syntheses. The positional parameters of H(18) were calculated and not varied in the course of refinement. The atomic coordinates and temperature parameters are summarized in Table II. A list of the calculated and observed structure factors can be obtained by request from the authors.

*The structure of the cation of **1***

The molecular structure of the cation of **1** is represented in Fig. 1. The C–N- and C–C-bond lengths of type sp^3-sp^3 (average C–N(sp^3-sp^3): 1.50 Å, average C–C(sp^3-sp^3): 1.54 Å are within the limits of the expected values. While the C(6)-C(7) bond distance of bonding type sp^3-sp^2 with a length of 1.52 Å is also quite normal, it seems that the C(7)-C(8) length of the same type is somewhat shortened (1.47 Å). The C(7)-O(1) distance of the carbonyl group (1.21 Å) falls within the lower limits of the expected value. The atoms O(1) and C(8) indicate with 6.0 and 6.2 Å^2, respectively, a relatively high temperature factor in comparison to those of the other non-hydrogen atoms, which range from 2.7 to 4.2 Å^2. This phenomenon could mean either a greater thermal motion of O(1) and C(8), which can be described as a rotation around the C(7)-C(8) bond, or it could be interpreted as a partial disorder. In any case the shortening of the bond lengths therefore cannot be regarded as real. On the other hand it expresses a certain conformational flexibility of the cation. The conformational flexibility of neurotransmitter cations is regarded in the literature as

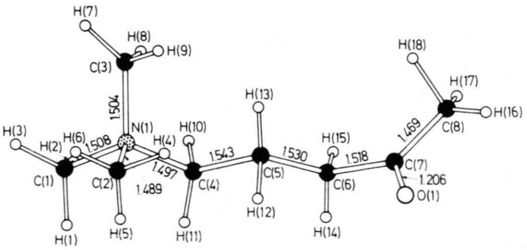

Fig. 1. Structure of the cation of **1**. The standard deviations of the given bond lengths are within the range of 0.013–0.023 Å.

Table II. Fractional atomic coordinates and thermal parameters. Standard deviations in the least significant digits are given in parentheses. Isotropic and anisotropic thermal parameters are defined as $T = \exp(-B \sin^2\theta/\lambda^2)$ and $T = \exp[-1/4 (B_{11} h^2 a^{*2} + B_{22} k^2 b^{*2} + B_{33} l^2 c^{*2} + 2B_{12} h k a^* b^* + 2B_{13} h l a^* c^* + 2B_{23} k l b^* c^*)]$.

Atom	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl	0.3570 (2)	0.1633 (2)	0.2675 (4)	3.3 (1)	3.2 (1)	4.3 (1)	−0.4 (1)	0.1 (1)	−0.4 (1)
O (1)	−0.2507 (9)	−0.0583 (7)	0.4812 (16)	4.9 (4)	7.0 (6)	6.1 (5)	−2.2 (4)	1.4 (5)	−1.2 (5)
N (1)	0.1394 (7)	0.1521 (5)	0.7595 (13)	2.6 (3)	2.7 (3)	2.9 (3)	−0.3 (3)	−0.2 (4)	0.0 (3)
C (1)	0.2001 (12)	0.2457 (7)	0.7494 (25)	4.3 (5)	2.6 (4)	5.2 (6)	−1.1 (4)	−0.3 (6)	−0.3 (6)
C (2)	0.0599 (15)	0.1472 (12)	0.9409 (19)	3.5 (6)	5.9 (8)	3.0 (5)	−0.6 (6)	0.2 (5)	−0.1 (5)
C (3)	0.2463 (10)	0.0831 (8)	0.7694 (23)	3.0 (4)	3.6 (5)	5.3 (7)	0.5 (4)	−1.0 (6)	−0.2 (6)
C (4)	0.0604 (12)	0.1394 (8)	0.5779 (16)	2.7 (5)	3.3 (5)	2.9 (4)	−0.3 (4)	−0.4 (4)	0.1 (4)
C (5)	−0.0179 (11)	0.0497 (9)	0.5801 (18)	3.0 (5)	3.5 (5)	3.5 (5)	−0.9 (4)	−0.4 (4)	0.0 (4)
C (6)	−0.0744 (14)	0.0332 (9)	0.3755 (20)	5.1 (6)	3.9 (6)	3.5 (5)	−1.5 (5)	−0.7 (5)	0.4 (4)
C (7)	−0.1582 (11)	−0.0518 (8)	0.3761 (18)	3.1 (4)	4.5 (5)	4.1 (5)	−1.1 (4)	−0.9 (5)	0.0 (5)
C (8)	−0.1214 (14)	−0.1259 (10)	0.2414 (36)	5.6 (7)	4.1 (6)	8.9 (11)	−0.9 (5)	1.8 (10)	−1.3 (8)

Hydrogen Atoms

Atom	x/a	y/b	z/b	B	Atom	x/a	y/b	z/c	B
H (1)	0.125 (11)	0.294 (7)	0.735 (20)	4.8 (27)	H (10)	0.138 (15)	0.140 (9)	0.480 (21)	5.7 (34)
H (2)	0.235 (15)	0.257 (10)	0.621 (23)	5.6 (38)	H (11)	0.004 (11)	0.192 (9)	0.582 (18)	3.2 (27)
H (3)	0.260 (13)	0.251 (9)	0.889 (20)	4.2 (30)	H (12)	−0.101 (12)	0.069 (8)	0.675 (19)	5.2 (32)
H (4)	0.023 (13)	0.085 (10)	0.914 (24)	5.4 (37)	H (13)	0.035 (11)	0.001 (7)	0.629 (17)	3.7 (26)
H (5)	−0.007 (14)	0.186 (10)	0.934 (22)	4.8 (37)	H (14)	−0.121 (15)	0.079 (10)	0.340 (24)	7.2 (46)
H (6)	0.115 (12)	0.156 (8)	1.046 (17)	3.6 (27)	H (15)	−0.020 (13)	0.033 (9)	0.262 (25)	6.3 (36)
H (7)	0.299 (20)	0.060 (14)	0.946 (36)	12.8 (73)	H (16)	−0.186 (12)	−0.169 (9)	0.250 (25)	6.3 (34)
H (8)	0.288 (12)	0.092 (9)	0.603 (20)	4.2 (29)	H (17)	−0.079 (14)	−0.120 (10)	0.112 (25)	6.3 (38)
H (9)	0.200 (11)	0.028 (9)	0.810 (20)	5.1 (32)	H (18)	−0.034 (—)	−0.151 (—)	0.338 (—)	11.7 (66)

one of the conditions for its physiological function [11].

The bond angles defined by non-hydrogen atoms are given in Table III. The conformation of the cation is described by the torsion angles $\tau 1 - \tau 4$ (Fig. 2), which are compared with those found from acetylcholine chloride (AcCh Cl) [12], acetylcholine bromide (AcCh Br) [13] and acetylcholine iodide (AcCh I) [14] (Table IV). Each of the acetylcholine salts exhibits for $\tau 2$ a *gauche*- and for $\tau 4$ a *trans*-conformation. In contrast to these findings, **1** exhibits a *trans*-conformation for $\tau 2$ and a skew-conformation for $\tau 4$. A *trans*-conformation of $\tau 2$ is also displayed by the cations of acetylthiocholine bromide and acetylselenocholine iodide [15]. In this cations in comparison with acetylcholine the ester oxygen was replaced by its higher homologs sulfur and selenium.

In Fig. 3 the conformation of the cation of **1** is compared with a conformation (**1a**) in which the torsion angle $\tau 2$ is the same as that of acetylcholine ($\tau 2 = 80°$). As can be seen in Fig. 3 in conformation

Table III. Bond angles defined by the non hydrogen atoms of the cation of **1**. Standard deviations in the least significant digits.

C(1)−N(1)−C(2)	108.4 (10)
C(1)−N(1)−C(3)	107.3 (8)
C(1)−N(1)−C(4)	107.9 (9)
C(2)−N(1)−C(3)	110.1 (10)
C(2)−N(1)−C(4)	111.8 (9)
C(3)−N(1)−C(4)	111.3 (9)
N(1)−C(4)−C(5)	112.9 (9)
C(4)−C(5)−C(6)	109.3 (10)
C(5)−C(6)−C(7)	110.4 (11)
C(6)−C(7)−C(8)	116.7 (11)
C(6)−C(7)−O(1)	121.9 (12)
C(8)−C(7)−O(1)	121.4 (12)

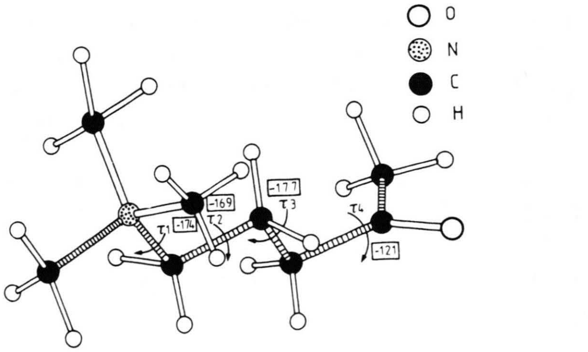

Fig. 2. Torsion angles $\tau 1 - \tau 4$ of the cation of **1**.

Table IV. Comparison of torsion angles $\tau 1 - \tau 4$ in **1** and acetylcholine.

	$\tau 1$ (°)	$\tau 2$ (°)	$\tau 3$ (°)	$\tau 4$ (°)
1	− 174 (*trans*)	− 169 (*trans*)	− 177 (*trans*)	− 121 (skew)
AcCh Cl	171 (*trans*)	85 (*gauche*)	− 167 (*trans*)	− 175 (*trans*)
AcCh Br	− 175 (*trans*)	78 (*gauche*)	79 (*gauche*)	− 174 (*trans*)
AcCh I	− 174 (*trans*)	89 (*gauche*)	83 (*gauche*)	180 (*trans*)

1a there is an additional short H --- H contact distance between a methyl group of the quaternary ammonium group and the methylene group positioned in the same position as the ester oxygen in acetylcholine. The convolution of conformation **1a** into **1** is obviously caused by the replacement of the ester oxygen of acetylcholine by the bulkier CH_2 group. As mentioned, the substitution of the ester oxygen in acetylcholine by the bulkier sulfur or selenium results in the same change in conformation. Also in this case a too small contact distance between a CH_3 group of the quaternary ammonium group and the atoms which replace the ester oxygen might be the reason for the conformational change. As it can be shown by CNDO calculation [16] the conformation **1a** is by about 7.3 kcal/mol higher in energy compared with **1**. One can conclude from this that **1** also does not attack the receptor in form of the $\tau 2$-gauche-conformation to a notable extent.

Furthermore the conformational change of the cation in **1** compared with acetylcholine concerning $\tau 4$ is correlated with the replacement of the ester oxygen by a methylene group (Fig. 4). The π-interaction in the ester group of acetylcholine forces a

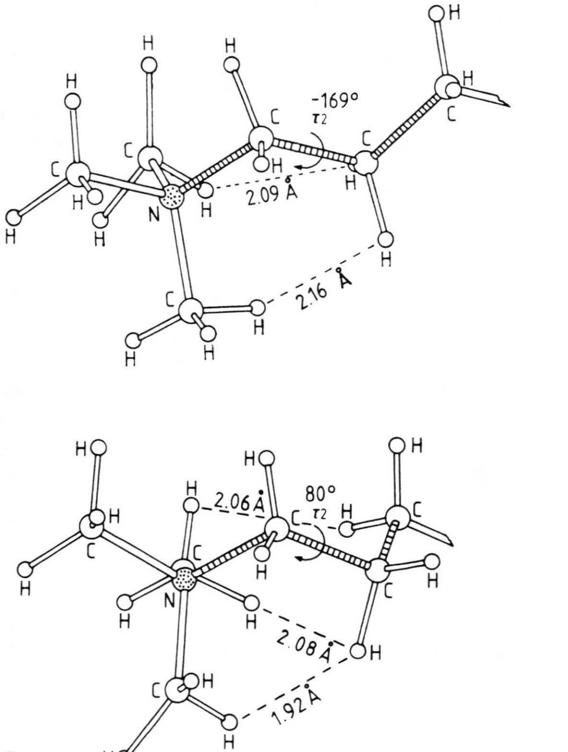

Fig. 3. Conformation of **1** with respect to the torsion angle $\tau 2$ compared with a conformation **1a** which corresponds to acetylcholine.

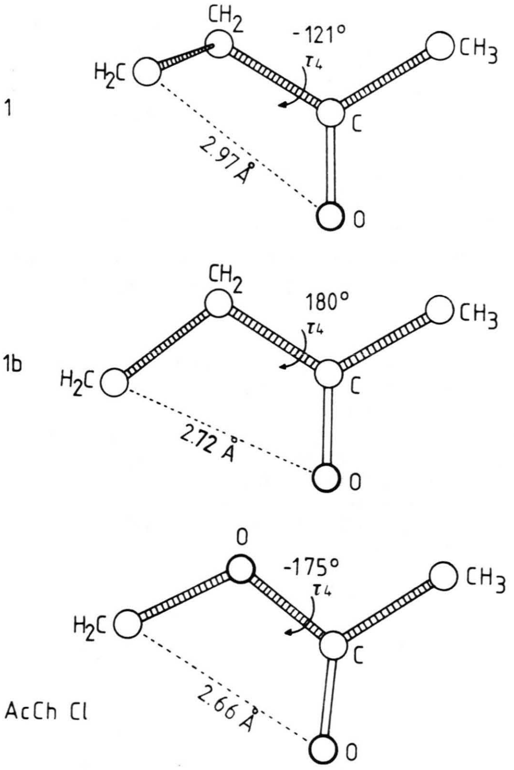

Fig. 4. Conformation of **1** concerning $\tau 4$ compared with acetylcholine chloride (AcCh Cl) and an acetylcholine-corresponding conformation of **1** (**1b**).

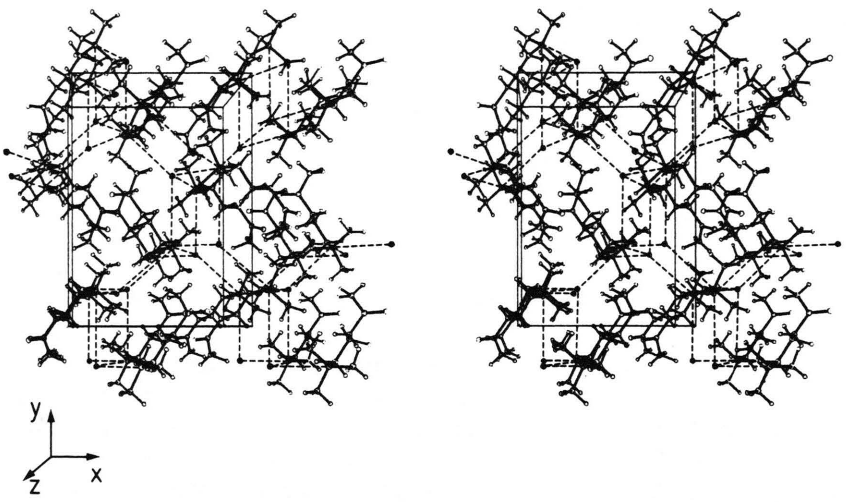

Fig. 5. Coulomb interaction between the quaternary ammonium group and Cl^- anions in the crystal structure of **1**.

planar arrangement of this group inclusively the carbon of the methylene group bound to the ester oxygen. The loss of the ester function in **1** allows this carbon to deviate from a planar arrangement, resulting in a diminuation of the non-bonding interaction between the carbonyl oxygen and the vicinal CH_2 group. In acetylcholine itself the repulsion of these groups is over-compensated by the gain of mesomeric energy of the carboxylate group.

Crystal structure

The crystal packing is essentially fixed through the Coulomb-interaction of the trimethylammonium group with its neighbouring anions (Fig. 5). Each ammonium group is approximately tetrahedrally coordinated through four nearest Cl-anions, each anion by four cationic groups. In Table V the shortest $N^+ \cdots Cl^-$ distances are summarized. The neighbouring anions of the quaternary ammonium group arrange themselves stereospecifically with respect to this group in such a way that each anion-nitrogen vector is positioned perpendicularly to a tetrahedral face of the N^+C_4 group in the opposite direction of an N → C vector of a *trans* positioned CH_3 or CH_2 group. The angle between the *trans* positioned

Table V. a) Short $N^+ - Cl^-$ distances [Å] in the crystal structure of **1**. A, B and C describe the face types of the tetrahedron of the $(CH_3)_3N^+CH_2$-group, introduced in Fig. 6. The index of the Cl anions is defined in respect to the symmetry operation (in parentheses), which is to be applied on the Cl^- position given in Table II.

(B)	$N(1)-Cl_1$	3.999 (8)	$(x-1/2,\ -y+1/2,\ -z+1)$
(B)	$N(1)-Cl_2$	4.048 (9)	$(x,\ y,\ z)$
(A)	$N(1)-Cl_3$	4.140 (9)	$(x,\ y,\ z+1)$
(C)	$N(1)-Cl_4$	4.605 (8)	$(-x+1/2,\ -y,\ z+1/2)$

b) Angles (°) between the $N^+ \rightarrow Cl^-$ vectors and the $N^+ \rightarrow C$ vectors.

	N(1)-C(1)	N(1)-C(2)	N(1)-C(3)	N(1)-C(4)
$N(1)-Cl_1$	72(1)	70(1)	180(1)	69(1)
$N(1)-Cl_2$	72(1)	179(2)	69(1)	68(1)
$N(1)-Cl_3$	77(1)	67(1)	65(1)	175(1)
$N(1)-Cl_4$	155(1)	87(1)	47(1)	84(1)

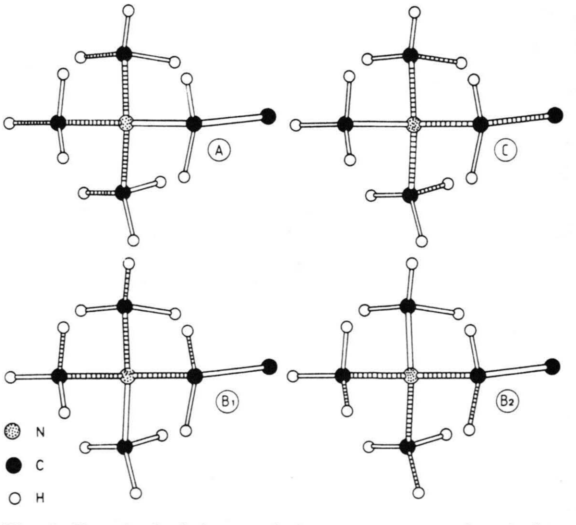

Fig. 6. Tetrahedral faces of the quaternary trimethylammonium group in **1**.

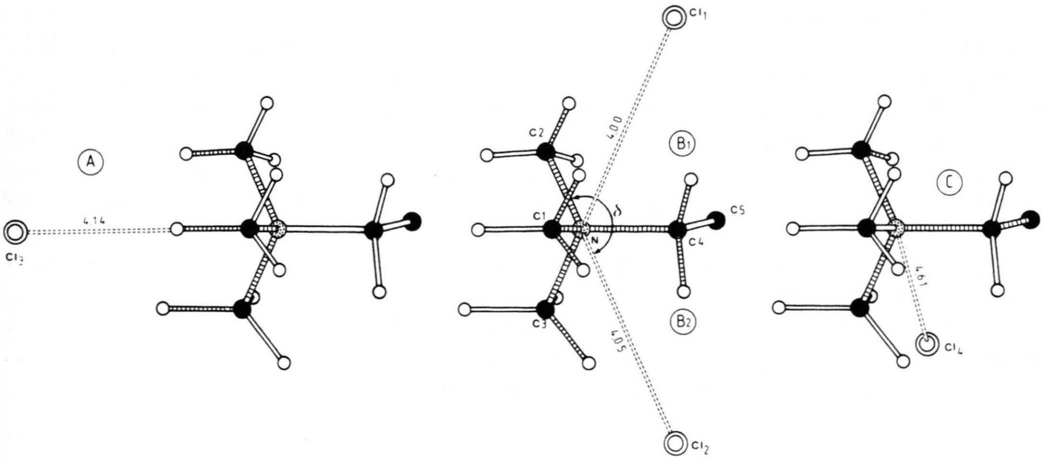

Fig. 7. Arrangement of anions in **1** relative to the quaternary ammonium group.

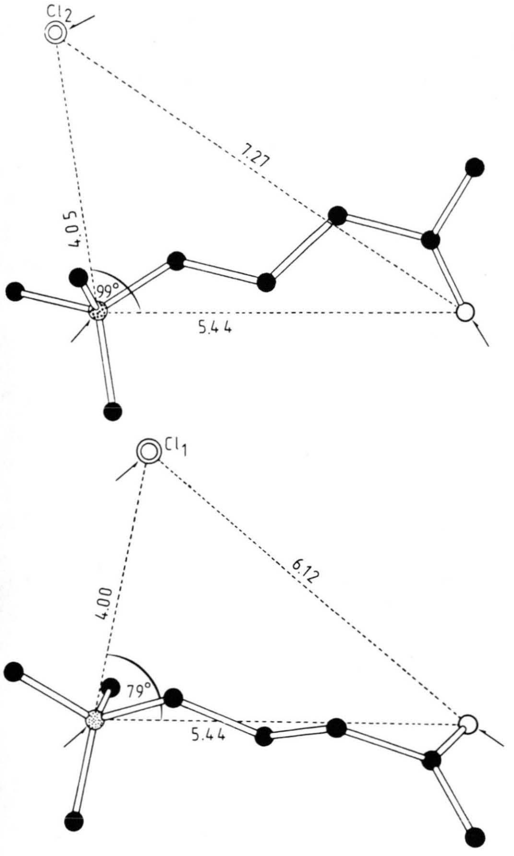

Fig. 8. Nicotinic "activity triangles" in **1**. The arrows mark the atoms which define the plane of projection.

$CH_3(CH_2)$ group and the vector cation-anion is in the ideal case 180 °.

The four tetrahedral faces of the trimethyl-alkyl-ammonium group of cholinergic neurotransmitters can be classified into three types (Fig. 6). The type of face A is formed by three methyl groups, the types B and C by two methyl groups and the methylene group of the carbon chain. Face types B and C are differentiated in such way that an anion, which is brought near face B, "sees" three H-atoms, but brought near face type C "sees" two H-atoms and the carbon (C5) of the carbon chain. Face B occurs doubled due to a local mirror symmetry.

In Fig. 7 the arrangement of the four anions nearest to the quaternary ammonium group with respect to this group is represented. As can be seen from Table Va and Fig. 7, the anions occupying face type B show the shortest distances to N^+. The Cl-anion occupying face A has an insignificantly longer (about 0.1 Å) N^+-Cl^--distance; in contrast the N^+-Cl^--distance of the anion occupying the C-face is significantly longer. In Table Vb the angles between the N^+-Cl^-- and N-C-vectors of the N^+C_4 group are summarized. The N^+-Cl_4^--vector is not only the longest but also the one wich differs mostly from the ideal direction. The ideal direction, assuming an undistorted tetrahedron for the NC_4-group, requires for three of these angles a value of 70.5° and for one angle a value of 180°.

As we could show by the comparison of the structure analyses of salts of cholinergic neurotransmitters performed by us or described in the literature, the anions which occupy faces of type B can be used as a model for the anionic adsorption site for the neurotransmitter on the receptor molecule. For the physiological activity of the cholinergic neurotransmitters it is generally necessary to have a second polar group in addition to the quaternary ammonium group. Consequently not only are the ammonium-anion vectors of interest, but also the vector triangles formed by the quaternary nitrogen, an anion occupying a face of type B of the N^+C_4 tetrahedron and the second-partially charged centre which is in the case of **1** the carbonyl oxygen. With these "activity triangles" the activity mode can be differentiated into nicotinic and muscarinic. Since in the crystal structure of **1** both faces of type B of the N^+C_4 group are occupied by an anion, two "activity triangles" can be found with a characteristic geometry of the nicotinic activity mode (Fig. 8).

Besides the cation-anion interaction, which leads to some Cl^- --- H contact distances shorter than the sum of the van-der-Waals-radii, there appear in the crystal structure no intermolecular interactions manifested by unusually short contact distances.

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