Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine, IV [1]

X-Ray Structure Analyses of Trimethylpentylammonium-chloride and (4-Acetoxybutyl)trimethylammonium-iodide

Alfred Gieren* and Michail Kokkinidis

Max-Planck-Institut für Biochemie, Arbeitsgruppe für Chemische Kristallographie,
Am Klopferspitz, D-8033 Martinsried/München, Bundesrepublik Deutschland

Z. Naturforsch. **41c**, 618–626 (1986); received April 12, 1984/November 27, 1985

X-Ray Structure Analyses, Neurotransmitter, Disorder, Structure-Activity Relationships

The crystal structures of the title compounds which display cholinergic activity at the ganglionic receptor have been determined by X-ray structure analysis. $[(CH_3)_3N^+C_5H_{11}]Cl^-$ (**1**) crystallizes in the orthorhombic space group Pbnm with half a formula unit per asymmetric unit, $a = 11.381(14)$, $b = 12.871(17)$, $c = 7.316(4)$ Å. The intensities of 1106 independent reflections were collected with an automatic diffractometer. The structure refinement converged at $R = 0.133$ for the 355 observed reflections. The cation of **1** is disordered. $[(CH_3)_3N^+(CH_2)_4-O-C(O)-CH_3]I^-$ (**2**) crystallizes in the orthorhombic space group $P2_12_12_1$ with four formula units per unit cell, $a = 16.783(8)$, $b = 10.276(6)$, $c = 7.427(10)$ Å. The intensities of 1469 independent reflections were collected. The structure refinement converged at $R = 0.071$ for 1383 observed reflections. In both compounds the trimethylammonio methyl groups are coordinated nearly tetrahedrally by four anions in the first coordination sphere. Anions which occupy a special face type (B) of the tetrahedron of the $(CH_3)_3N^+-CH_2$-group may be treated as a "model binding site" of the receptor. In the crystal structure of **2** the anions occupying B-type faces form together with the ammonium nitrogen and the carbonyl oxygen so called "Activity triangles". The almost equal geometries of these activity triangles are correlated with the mode of pharmacological action.

Introduction

A central problem in neurochemistry is the manner in which neurotransmitters interact with their receptor proteins to initiate a series of events that give rise to the final physiological response. In this context we carried out systematic investigations of the crystal structures of cholinergic compounds with the purpose to elucidate the structure-activity relationships of agonists of the natural neurotransmitter acetylcholine.

From these studies a model emerged which incorporates correlations between the X-ray structures and the pharmacological activity of cholinergic agonists. Several aspects of this model have been published earlier [1–5]; a detailed presentation is given in the following paper [6]. The basic concepts of the model are twofold: The preferred directionality of the pairing interaction between the cationic ammonium group of cholinergic stimulants and the complementary anionic binding site of the receptor may be obtained in the crystal structures from the direction of the vector connecting the ammonium nitrogen (N^+) with a monoatomic anion (A^-) which is specifically positioned with respect to the ammonium group. This anion is treated as a "model binding site" of the cholinoreceptor [2–6]. The second concept of the model concerns the differentiation of the cholinergic activity into the nicotinic and muscarinic mode. So called "activity triangles" defined by the $N^+ \rightarrow A^-$ vector and the vector connecting N^+ with a further polar binding site of the neurotransmitter cation exhibit geometrical features which are associated with the appearance of the nicotinic or muscarinic mode of action.

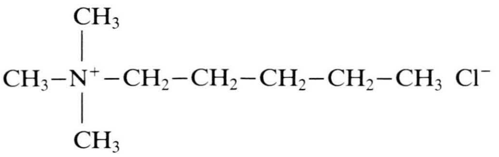
The present paper contains the crystallographic investigations of the cholinergic agonists **1** and **2** and a brief discussion of their possible interactions with the cholinoreceptor according to the concepts of the model described above. Both **1** and **2** are closely related to acetylcholine (**3**). In the case of **1** the carboxyl group of **3** is exchanged by an apolar CH_2-CH_2 group. In **2** the aliphatic part of the chain originating from the nitrogen atom is elongated by an ethylene group. These chemical modifications of the title compounds in comparison with acetylcholine

---

Reprint requests to Prof. A. Gieren.

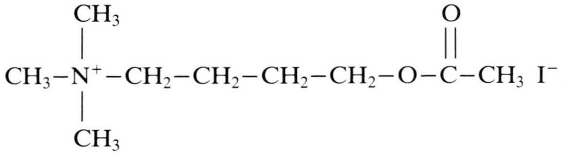
Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0500–0618 $ 01.30/0

give rise to striking differences in their cholinergic activity patterns. Whereas acetylcholine exhibits high activity with all modifications of the cholinoreceptor, **1** and **2** exhibit high nicotinic activities only at the ganglia; their actions at the receptor of the skeletal muscles and at the muscarinic receptor are very weak [7–11].



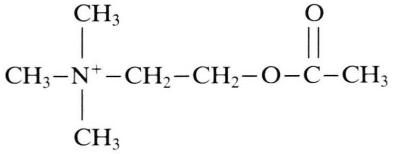
$$CH_3-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_2-CH_2-CH_2-CH_2-CH_3\ Cl^-$$

1



$$CH_3-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_2-CH_2-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3\ I^-$$

2



$$CH_3-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3$$

3

Experimental

Compound **1** was synthesized [8] following a procedure given in ref. [9]. Colourless, hygroscopic crystals were obtained by recrystallization from absolute ethanol. For the crystallographic study a crystal with the dimensions 0.14 × 0.11 × 0.72 mm was selected. The synthesis of **2** is described by Barrass *et al.* [10]. A suitable crystal for X-ray structure analysis with the dimensions 0.62 × 0.36 × 0.74 mm was isolated by recrystallizing a sample of **2** [12] from a methanol/acetone mixture (2:1).

Due to their moisture sensitivity the crystals of **1** and **2** were sealed prior to further investigations under dry Ar gas in Debye-Scherrer capillaries. In both cases preliminary Weißenberg and precession photographs were taken to establish the approximate unit cell dimensions and the space groups. The photographs showed for **1** the Laue group mmm, and the systematic absences 0kl : k = 2n + 1; h0l : h + 1 = 2n+1. These are consistent with the orthorhombic space groups Pbnm (centrosymmetric) or $Pbn2_1$ (non-centrosymmetric). For **2** the space group is orthorhombic, $P2_12_12_1$. In both cases accurate unit-cell dimensions were obtained on a punch tape controlled single crystal diffractometer (Siemens-AED I, MoK_α radiation). This instrument was also used for the measurement of the reflection intensities in the $\theta/2\theta$ scan mode (5-point measurement) up to a maximum θ angle of 26°. Reflections with $I < 2\sigma_I$ were classified as unobserved. Their intensities were set to $I = 2\sigma_I$. No absorption corrections were applied. The reflection intensities were corrected for Lorentz- and polarization effects.

The lattice constants and other crystallographic data are: **1** a = 11.381(14), b = 12.871(17), c = 7.316(4) Å; V = 1071.7 Å^3; D_x = 1.03, D_m = 1.04 g·cm^{-3}; Z = 4; $\mu(MoK_\alpha)$ = 0.30 mm^{-1}; 1106 independent reflections, 751 reflections unobserved. **2** a = 16.783(8), b = 10.276(6), c = 7.427(10) Å; V = 1280.9 Å^3; D_x = 1.54 g·cm^{-3}, D_m = 1.56 g·cm^{-3}; $\mu(MoK_\alpha)$ = 2.5 mm^{-1}; 1469 independent reflections, 86 reflections unobserved.

Solution and refinement of the structures

The atomic scattering factors for O,N,C,Cl$^-$ [13] and I [14] were taken from International Tables for X-ray Crystallography, those for H are given in [15]. Computer calculations were carried out using a special version [16] of the X-Ray-67 program system [17] on a Siemens-4004 computer.

For compound **1** *E*-value statistics indicated a centrosymmetric space group. Assuming space group Pbnm the structure was solved by direct methods using the program PHASDT [16, 18]. The cations are disordered via the crystallographic mirror plane. The atomic positions of C(4), C(5) and C(7) of the pentyl residue are into two positions which are symmetric with respect to this plane. Two methyl carbon atoms of the quaternary ammonium group are related to each other by this mirror plane. At this stage all other atoms occupy special positions on the crystallographic mirror plane. The least squares refinement of this model (full matrix, anisotropic temperature parameters for Cl$^-$, isotropic ones for the rest of the nonhydrogen atoms) yielded extremely high temperature parameters for atoms C(6) and C(8) on the mirror plane. Therefore the refinement was continued by splitting C(6) and C(8) into double positions and yielded reasonable temperature factors for both atoms.

Due to the small number of observed reflections no anisotropic temperature parameters were introduced for the C- and N-atoms and furthermore the

Table I. Fractional atomic coordinates, thermal parameters and occupation densities (M) of compound **1**. Standard deviations of 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 $T = \exp[-0.25(B_{11} h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$.

ATOM	x	y	z	M	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl(1)	0.8760(3)	0.1660(3)	0.2500		8.8(2)	7.5(2)	7.9(2)	1.2(2)	0.0	0.0
					B					
N(1)	0.5781(7)	0.3449(7)	0.2500		6.6(2)					
C(1)	0.6476(8)	0.3688(7)	0.0835(14)		9.3(3)					
C(3)	0.4677(10)	0.4098(9)	0.2500		6.7(3)					
C(4)	0.5492(12)	0.2329(10)	0.3231(18)	0.5	6.1(4)					
C(5)	0.4536(10)	0.1792(10)	0.2074(24)	0.5	6.1(4)					
C(6)	0.4490(10)	0.0671(9)	0.2889(25)	0.5	5.6(4)					
C(7)	0.3570(12)	0.0048(11)	0.1902(19)	0.5	6.9(4)					
C(8)	0.3507(11)	−0.1077(9)	0.2775(40)	0.5	7.3(4)					

H-atoms were not incorporated into the refinement. Under these limiting conditions the refinement converged to an R value ($R = \sum \big| |F_o| - |F_c| \big| / \sum |F_o|$) of 0.133. In the last cycles of refinement 4 reflections with $|\Delta F| = \big| |F_o| - |F_c| \big| > 6$ were omitted from the calculation of parameter shifts. The unobserved reflections were only included in the calculation of parameter shifts, when $|F_c| > |F_o|$. The hydrogen atoms shown in the following figures are calculated under consideration of standard bond lengths and angles. Table I contains the result of the refinement in the space group Pbnm.

Table I shows, that the temperature factor of C(1) is significantly higher than those of the rest of the atoms of the cation. It can be concluded that C(1) represents a superposition of two nearby atomic positions. Furthermore the electron density distribution in a difference Fourier map (omitting C(1)) allows the interpretation of an overlap of two atomic positions for C(1). Atom Cl(1) also displays a relatively high temperature factor (B = 8.1 Å^2) which points to a slight disorder. In the structure of O-ethylcholine-chloride, where the cations are disordered, we also found high temperature factors for the Cl anions [1].

In Fig. 1 the numbering scheme is shown. The atoms N(1) and C(3), which are located on the mirror plane and also the mirror imaged atomic positions C(1) and C(1′) – the primed atomic positions are mirror-equivalent – are common to both disordered cations. All other atomic positions are split into two.

Because of the disorder also alternative space groups were tested, namely the noncentrosymmetric Pbn2_1, the monoclinic subgroups of Pbnm and P$\bar{1}$. In the first case the least squares refinement resulted in an R-value not significantly [19] different from the previous one; in the other space groups structure factor calculations resulted in higher R-values.

The cation of **1** exhibits mirror symmetry *via* a noncrystallographic molecular mirror plane formed by the C atoms of the pentyl residue, N$^+$ and a *trans* positioned C-atom of the $(CH_3)_3N^+CH_2$-moiety. Both *gauche* CH_3 groups are mirror equivalent via this plane, while via the crystallographic mirror plane a *gauche* CH_3 group is related to the *trans* one (Fig. 1). This type of arrangement of the disordered $(CH_3)_3N^+-CH_2$-group, the crystallographic symmetry neglecting the molecular one, has been also observed in some other cholinergic agents investigated by us [2].

For compound **2** E-value statistics indicated a noncentrosymmetric space group. The I$^-$ position was detected by a Patterson synthesis, a following Fourier synthesis yielded the remaining positions of nonhydrogen atoms. The least squares refinement converged to an R-value of 0.071 with anisotropic temperature parameters for the nonhydrogen atoms. The positions of the H-atoms were partially found by

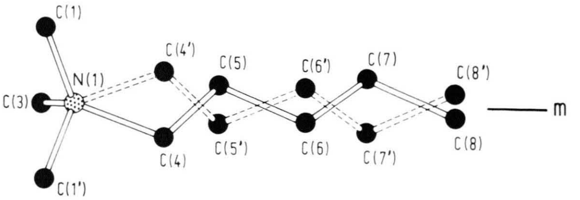


Fig. 1. Assignment of the atomic positions to the disordered cations. Atomic positions marked by ′ result from the coordinates summarized in Table I by the crystallographic mirror plane at $z = 0.25$.

Table II. Fractional atomic coordinates and thermal parameters of compound **2**. Standard deviations in the least significant digits are given in parentheses.

ATOM	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
I (1)	0.1050(1)	0.1685(2)	−0.0160(3)	3.4(1)	4.2(1)	4.9(1)	0.7(1)	−0.3(1)	−0.1(1)
O(1)	0.1387(19)	0.5358(25)	0.3085(71)	5.1(13)	4.4(10)	10.6(31)	0.9(9)	0.9(19)	1.8(15)
O(2)	0.2121(15)	0.3822(21)	0.4265(43)	3.8(9)	4.7(9)	4.4(12)	0.3(7)	0.1(10)	0.6(10)
N(1)	0.3885(13)	0.0748(20)	−0.0118(40)	3.1(8)	3.3(7)	4.2(13)	0.0(7)	0.6(14)	0.8(10)
C(1)	0.3821(32)	0.1554(40)	−0.1885(67)	6.4(24)	4.6(16)	5.4(20)	1.9(18)	2.3(20)	1.3(17)
C(2)	0.3216(18)	−0.0173(27)	−0.0002(78)	2.8(10)	4.1(10)	8.7(32)	−0.7(9)	−2.0(22)	−0.6(22)
C(3)	0.4652(19)	−0.0019(29)	−0.0176(78)	3.2(10)	4.6(11)	6.3(23)	0.4(9)	−0.5(18)	−0.1(21)
C(4)	0.3960(23)	0.1593(37)	0.1502(52)	2.8(10)	4.0(12)	4.8(15)	0.1(13)	0.4(13)	−0.2(13)
C(5)	0.3266(20)	0.2509(29)	0.1835(57)	2.7(11)	4.4(12)	4.8(16)	0.3(10)	−1.1(13)	−0.7(13)
C(6)	0.3464(20)	0.3469(40)	0.3317(63)	2.7(11)	6.6(18)	5.4(19)	0.1(12)	−0.2(12)	−1.5(19)
C(7)	0.2812(25)	0.4471(35)	0.3617(78)	4.6(16)	4.4(14)	6.9(25)	−0.6(12)	2.3(18)	0.1(17)
C(8)	0.1441(23)	0.4418(29)	0.3943(53)	5.2(16)	3.0(11)	4.0(16)	0.7(10)	0.0(14)	−1.3(11)
C(9)	0.0757(23)	0.3696(33)	0.4764(83)	4.2(13)	5.3(14)	6.6(26)	0.7(11)	0.8(22)	0.1(17)

Hydrogen Atoms

ATOM	x	y	z	B	ATOM	x	y	z	B
H(11)	0.399	0.112	−0.313	5	H(42)	0.449	0.218	0.137	5
H(12)	0.419	0.239	−0.167	5	H(51)	0.315	0.305	0.062	5
H(13)	0.321	0.184	−0.196	5	H(52)	0.275	0.195	0.220	5
H(21)	0.276	0.037	0.000	5	H(61)	0.355	0.291	0.456	5
H(22)	0.325	−0.071	0.126	5	H(62)	0.401	0.395	0.300	5
H(23)	0.322	−0.085	−0.109	5	H(71)	0.300	0.518	0.458	5
H(31)	0.500	0.048	0.038	5	H(72)	0.268	0.493	0.236	5
H(32)	0.482	−0.017	−0.159	5	H(91)	0.067	0.292	0.397	5
H(33)	0.457	−0.095	0.043	5	H(92)	0.024	0.429	0.489	5
H(41)	0.401	0.098	0.266	5	H(93)	0.092	0.336	0.614	5

way of difference Fourier syntheses and partially calculated at expected positions. The positional parameters of the H-atoms and their isotropic temperature factors, which were set to 5 Å^2, were not varied in the course of refinement.

Reflections (25) with $|\Delta F| > 11$ were not used for calculation of parameter shifts during the last cycles of refinement. The unobserved reflections were set to $|F_o| = 4\sigma_F$ ($I_o = 2\sigma_I$). They were incorporated in the calculation of the parameter shifts, only if $|F_c| > |F_o|$. The final positional and temperature parameters are summarized in Table II.

Table III. Bond angles of the cations of compounds **1** and **2**. Standard deviations in terms of the last significant digit are given in parentheses.

1

C(1) −N(1)−C(1′)	110(1)
C(1) −N(1)−C(3)	109(1)
C(1) −N(1)−C(4)	126(1)
C(1′)−N(1)−C(3)	109(1)
C(1′)−N(1)−C(4)	91(2)
C(3) −N(1)−C(4)	109(1)
N(1) −C(4)−C(5)	112(1)
C(4) −C(5)−C(6)	103(1)
C(5) −C(6)−C(7)	109(1)
C(6) −C(7)−C(8)	109(1)

2

C(1)−N(1)−C(2)	110(3)	C(4)−C(5)−C(6)	111(3)
C(1)−N(1)−C(3)	108(3)	C(5)−C(6)−C(7)	113(3)
C(1)−N(1)−C(4)	112(3)	C(6)−C(7)−O(2)	109(3)
C(2)−N(1)−C(3)	108(2)	C(7)−O(2)−C(8)	115(3)
C(2)−N(1)−C(4)	113(3)	O(1)−C(8)−O(2)	124(4)
C(3)−N(1)−C(4)	105(3)	C(9)−C(8)−O(1)	125(4)
C(5)−C(4)−N(1)	115(3)	C(9)−C(8)−O(2)	111(3)

Description and discussion of the structures of the cations of 1 and 2

The structure of the cation of compound **1** is shown in Fig. 2a. The bond lengths and the bond angles summarized in Table III are mainly within the region of the expected values under consideration of the high standard deviations. Only the bond angles

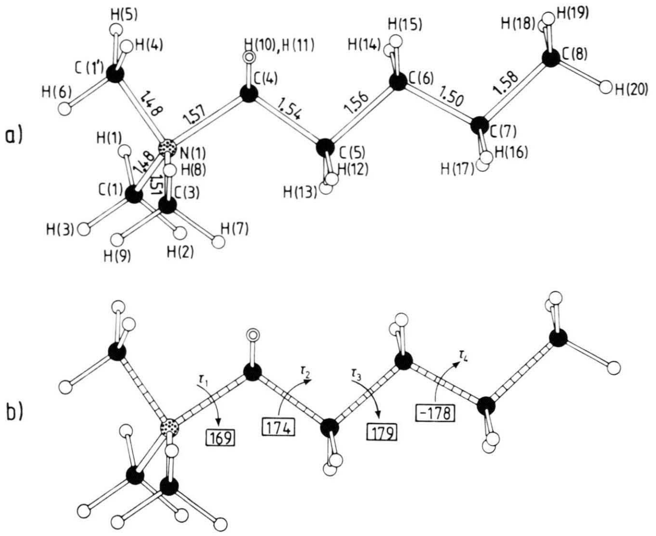


Fig. 2. a) Structure of the trimethylpentylammonium cation in the crystal structure of **1**. The atomic position C(1′) is the mirror image of C(1) (mirror plane at $z = 0.25$). The standard deviations of the given bond lengths are in the range of 0.01–0.03 Å.
b) Torsion angles of the cation of **1**.

C(1)–N(1)–C(4) and C(1′)–N(1)–C(4) show significant deviations due to the superposition of two atoms in the positions C(1) and C(1′), respectively. The assumption that C(1) is an averaged position is supported by the fact, that the average of the angles C(1)–N(1)–C(4) and C(1′)–N(1)–C(4) is equal to a normal tetrahedral angle. The correct positions of C(1) and C(1′) for the two cations disordered via the crystallographic mirror plane can be obtained by rotating the group C(1)–N(1)–C(1′) by ca. 20° around N(1)–C(3) clockwise for the cation shown in Fig. 2 and counterclockwise for the mirror image, respectively.

The conformation of the cation of **1** is described by the torsion angles $\tau_1 - \tau_4$ which are given in Fig. 2b. The unusual deviation of the torsion angle τ_1 from 180° is an artefact caused by the disorder of C(1) and C(1′) respectively, as discussed above. At τ_2 the conformation of **1** *(trans)* deviates significantly from the conformation of acetylcholine salts *(gauche)* [20–24]. On the other hand at τ_3 the conformation of **1** *(trans)* is similar to the conformations of acetylcholine chloride [20], perchlorate [23] and β-resorcyclate [24], whereas acetylcholine bromide [21] and iodide [22] exhibit *gauche* conformations. The disorder of the cation in the crystal structure of **1** indicates a conformational flexibility which is regarded relevant for the biological activity [25].

The structure of the cation of compound **2** is shown in Fig. 3. The bond lengths and angles (Table III) are as expected within the standard deviations. The conformational angles are given in Table IV. The chain C(3)–N(1)–C(4)–C(5)–C(6)–C(7) is *all-trans*-planar and forms an angle of 82° with

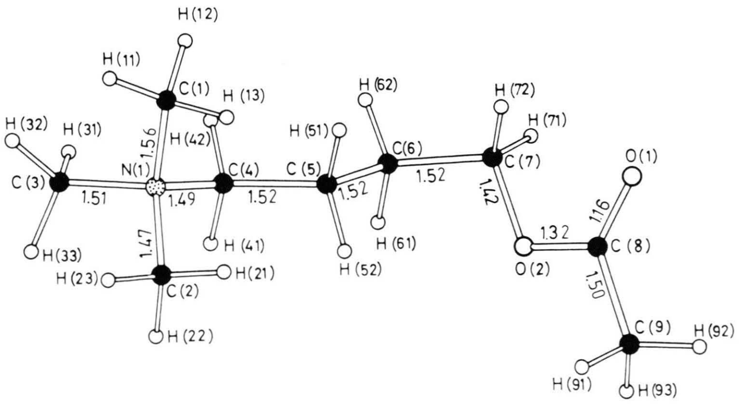


Fig. 3. The structure of the cation in **2**. The standard deviations of the bond lengths given are in the region 0.04–0.06 Å.

Table IV. Torsion angles (°) of the cation of compound **2** with standard deviations in parentheses.

C(1)−N(1)−C(4)−C(5)	61(4)
C(2)−N(1)−C(4)−C(5)	−64(4)
C(3)−N(1)−C(4)−C(5)	178(3)
N(1)−C(4)−C(5)−C(6)	−171(3)
C(4)−C(5)−C(6)−C(7)	176(3)
C(5)−C(6)−C(7)−O(2)	65(5)
O(1)−C(8)−O(2)−C(7)	5(6)
C(9)−C(8)−O(2)−C(7)	−177(4)
C(6)−C(7)−O(2)−C(8)	−155(4)

the plane of the atoms C(7), O(1), O(2), C(8), and C(9) which are coplanar due to mesomeric effects. The intramolecular contact distances N^+−ester oxygen (O(2)) and N^+−carbonyl oxygen (O(1)), which are of interest with respect to biological activity, are 5.42 and 6.76 Å, respectively.

The crystal packing as a model for the interactions with the cholinoreceptor

The crystal packings of compounds **1** (Fig. 4) and **2** (Fig. 5) are characterized by Coulombic interactions between the quaternary ammonium groups and the anions. In compound **1** the anions and cations are arranged in layers which coincide with the crystallographic mirror plane. The two shortest N^+-Cl^- contact distances occur within the layers, two longer ones between the layers (Table V).

The anions in **1** and **2** exhibit the same arrangement pattern relative to the quaternary ammonium

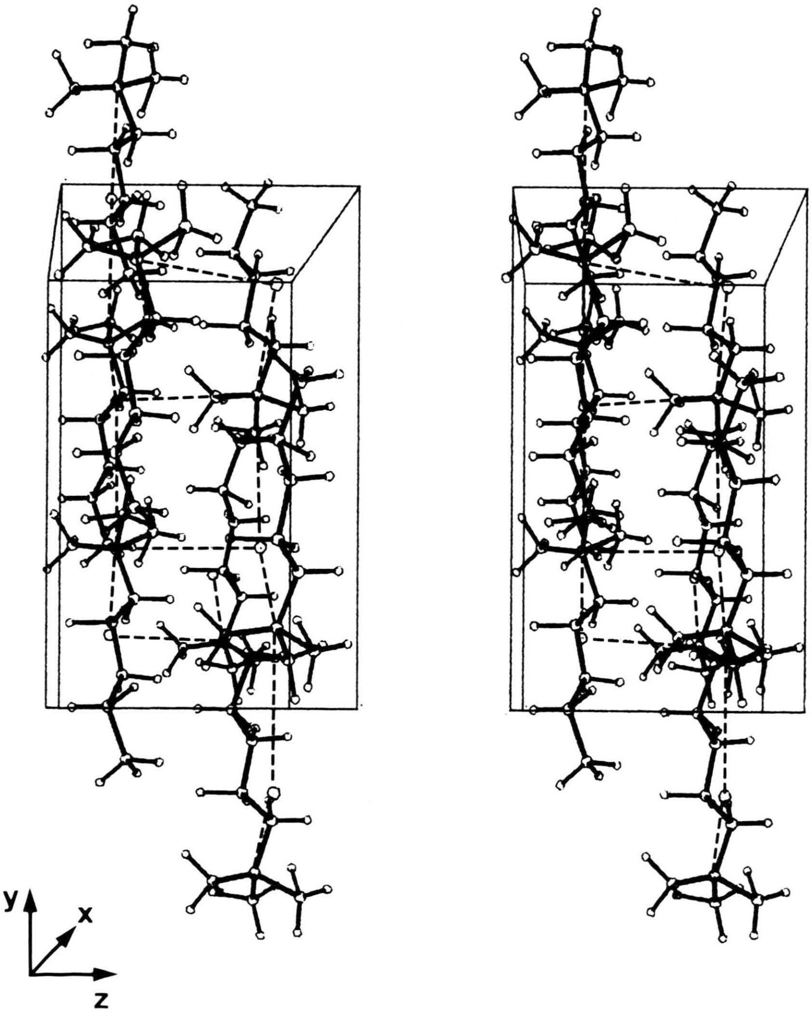


Fig. 4. The crystal structure of **1**. For reasons of better clarity, only one of the disordered cations is shown (space group $Pbn2_1$). The actual crystal packing can be obtained by letting the cations being mirror-imaged through the mirror planes of the space group Pbnm at $z = 0.25$ and $z = 0.75$. Short N^+--Cl^- contacts are indicated by the broken lines.

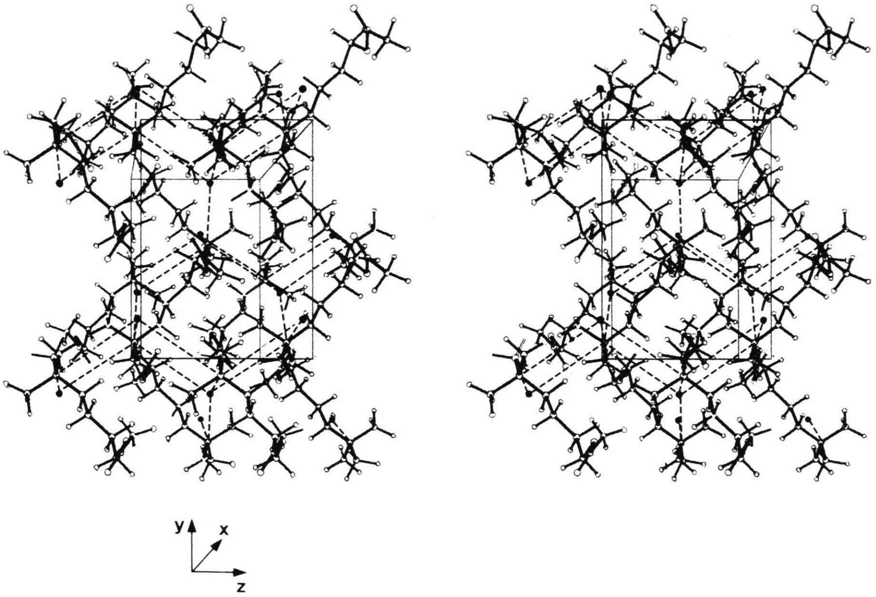


Fig. 5. Crystal packing of **2**. Short $N^+ - - I^-$ distances are indicated by broken lines.

group which we also encountered in the crystal structures of other halides of cholinergic compounds regardless of their crystallographic differences [1–6]: The quaternary ammonium group is surrounded almost tetrahedrally by four anions in the first coordination sphere. The vectors $N^+-X^-(X=Cl,I)$ are oriented nearly perpendicularly with respect to the faces of the tetrahedron of the quaternary ammonium group. They cut these faces in their center of gravity and lie therefore in the same direction as the N–C bond of the CH_3 or CH_2 group, which is opposite to the anion with respect to the tetrahedral face. In case of an ideal geometry one out of the four anion–N^+–C angles formed between the N^+–anion vector and the N^+–C bonds has the value of 180°, the other three of 70.5°.

Nearly ideal geometrical conditions are observed when anions occupy the sterically unhindered faces of type A and B. (The classification in face types A, B and C is given in refs. [2–4]). Furthermore, face types A and B are also associated with the shortest N^+–anion distances, *i.e.* the strongest Coulombic interactions (Tables V, VI). The analysis of the anionic environment of the quaternary ammonium group [1–6, 26] and quantum mechanical calculations [27] lead to the conclusion that the nearly ideal geometry associated with A or B faces is energetically the most preferred one. This result is probably also valid in solution [27]. The sterically hindered C-type faces [2–4] are normally less favourably occupied (Tables V, VI).

The combination of the structural framework of the N^+–anion interaction with pharmacological observations [2, 3, 5, 6] provides strong evidence that cholinergic agonists predominantly attack the anionic binding site of the receptor via faces of type B. Therefore we suggested that monoatomic anions occupying B faces in the crystal structures of

Table V. Shortest N^+–anion contact distances in the crystal structures of **1** and **2**. The letters A, B and C give the type of the faces of the tetrahedron of the quaternary trimethylammonio methyl group which are occupied by anions. (For definition compare [2–4].) In parentheses are given the symmetry operations which are applied to the Cl^- and I^- positions in Tables I and II, respectively.

1

(B)	N(1)–Cl(1)	4.10(1)	(x , y , z)
(A)	N(1)–Cl(1)*	4.17(1)	(−x+1.5, y+0.5, z)
(B)	N(1)–Cl(1)**	4.32(3)	(x−0.5, −y+0.5, z+0.5)
(C)	N(1)–Cl(1)***	4.32(3)	(x−0.5, −y+0.5, z−0.5)

2

(B)	N(1)–I(1)*	4.45(3)	(−x+0.5, −y, z+0.5)
(B)	N(1)–I(1)**	4.49(2)	(x+0.5, −y+0.5, −z)
(A)	N(1)–I(1)***	4.50(3)	(−x+0.5, −y, z−0.5)
(C)	N(1)–I(1)****	4.86(2)	(x, y, z)

Table VI. Angles (°) between the $N^+ \rightarrow$anion vectors and the $N^+ \rightarrow C$ vectors of the $(H_3C)_3N^+-CH_2$-group in **1** and **2**. The symmetry operations which have to be applied in the case of the anions are given in Table V. Face type definition same as in Table V.

1

Face type	N→C N→Cl	N(1)−C(1)	N(1)−C(1′)	N(1)−C(3)	N(1)−C(4)
B	N(1)−Cl(1)	71	71	179	70
A	N(1)−Cl(1)*	74	74	64	159
B	N(1)−Cl(1)**	170	66	65	65
C	N(1)−Cl(1)***	66	170	65	98

2

Face type	N→C N→I	N(1)−C(1)	N(1)−C(2)	N(1)−C(3)	N(1)−C(4)
B	N(1)−I(1)*	177	67	73	70
B	N(1)−I(1)**	77	173	68	63
A	N(1)−I(1)***	66	73	70	173
C	N(1)−I(1)****	80	52	160	88

cholinergic compounds may be treated as a crude model for the anionic binding site of the receptor. The vectors connecting N^+ with these anions give in a first approximation the favourable directionality of the interaction between the $N^+(CH_3)_3$-group and the anionic binding site of the receptor.

In the case of compound **2**, besides the quaternary ammonium group, a further pharmacologically active centre, the carbonyl oxygen [28], is present. According to our previous work [1–5], "activity triangles" can be formed by the nitrogen of the quaternary ammonium group, an anion occupying a B-type face and a second active centre of the cation. With respect to cholinergic interactions it was suggested [2], that the capability of both pharmacologically active groups (*i.e.* the quaternary ammonium group and the carbonyl oxygen in the case of **2**) to interact with their corresponding binding sites of the receptor, requires a degree of geometrical complementarity between the activity triangles and the active centre(s) of the receptor. This hypothesis was confirmed by the fact that the geometries of the activity triangles are unambiguously associated with the nicotinic or muscarinic mode of action of the compounds [1–6].

In the crystal structure of **2**, two activity triangles are formed, both of them having the $N^+ \rightarrow O$ (carbonyl) vector in common, whereas the $N^+ \rightarrow$anion vector is formed with the anions occupying the B-type faces (I(1)* and I(1)**, Table V). One of these triangles is shown in Fig. 6. The other triangle differs only slightly in the angle σ (97° and 94°, respectively). The angle σ, which has been found to be the relevant parameter for the differentiation between the nicotinic and muscarinic activity mode, is in both cases in very good agreement with the typical values for nicotinic activity.

On the other hand however, the N^+-O(carbonyl) distance in the case of the ganglion-active compound

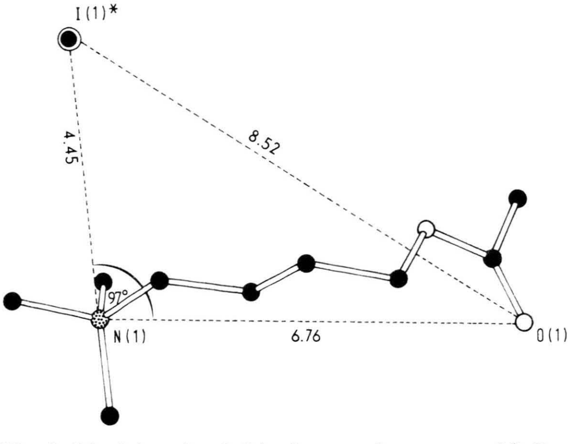


Fig. 6. "Activity triangle" in the crystal structure of **2**. Projection on the N,I,O(carbonyl) plane. σ = 97°.

2 is 1–2 Å longer than in the case of nicotinic agonists which are active at the skeleton muscles. A very similar activity triangle and distance have been found [2] in the crystal structure of (R,S)-lactoylcholine iodide [29], a compound which is also active at the ganglionic receptor. Thus we conclude that the ganglionic receptor exhibit a binding site which is further apart from the binding site of the ammonium group than the corresponding binding site at the nicotinic receptor of skeleton muscles. This result is consistent with several other experimental observations which suggest the existence of two modifications of the nicotinic receptor, *i.e.* the modification occurring at skeletal muscles and the modification occurring at ganglia [30]. In addition compound **1** shows that considerable activation of the ganglionic receptor may be achieved with just the interactions of the quaternary ammonium group.

[1] A. Gieren and M. Kokkinidis, Z. Naturforsch. **37c,** 977 (1982).

[2] M. Kokkinidis, Doctoral Thesis, TU München 1981.

[3] A. Gieren and M. Kokkinidis, Naturwissenschaften **68,** 482 (1981).

[4] A. Gieren and M. Kokkinidis, Z. Naturforsch. **37c,** 282 (1982).

[5] M. Kokkinidis and A. Gieren, Trends Pharm. Sci., **1984,** p. 369.

[6] A. Gieren and M. Kokkinidis, Z. Naturforsch. **41c,** 627 (1986).

[7] D. J. Triggle, in: Neurotransmitter-Receptor-Interactions, p. 251–252, Academic Press, London–New York 1971;
D. J. Triggle and C. R. Triggle, in: Chemical Pharmacology of the Synapse, p. 308–309, Academic Press, London–New York–San Francisco 1976.

[8] J. Bernhardt and E. Neumann, private communication. We thank the cited colleagues for kindly supplying us a sample of **1**.

[9] H. R. Ing, P. Kordik, and D. P. H. Tudor Williams, Brit. J. Pharmacol. **7,** 103 (1952).

[10] B. C. Barrass, R. W. Brimblecombe, P. Rich, and J. V. Taylor, Br. J. Pharmac. **39,** 40 (1970).

[11] A. M. Lands and C. J. Cavallito, J. Pharmacol. Exp. Ther. **110,** 369 (1954).

[12] We thank Prof. J. G. Cannon for kindly supplying to us a sample, from which we recrystallized **2**.

[13] International Tables for X-ray Crystallography, **Vol. III,** Table 3.3.1 A, Kynoch Press, Birmingham 1962.

[14] International Tables for X-ray Crystallography, **Vol. IV,** Table 2.2 A, Kynoch Press, Birmingham 1962.

[15] R. F. Stewart, E. R. Davidson, and W. T. Simpson, J. Chem. Phys. **42,** 3175 (1965).

[16] W. Hoppe, J. Gassmann, and K. Zechmeister, in Crystallographic Computing (F. R. Ahmed, ed.), p. 26, Munksgaard, Copenhagen 1970.

[17] J. M. Stewart, X-RAY-67 System. Tech. Rep. TR-67-58, Computer Science Center, Univ. of Maryland, College Park, Maryland 1967.

[18] K. Zechmeister, Doctoral Thesis, TU München 1969.

[19] W. C. Hamilton, Acta Cryst. **18,** 502 (1965).

[20] J. K. Herdklotz and R. L. Sass, Biochem. Biophys. Res. Commun. **40,** 583 (1970).

[21] T. Svinning and H. Sörum, Acta Cryst. **B31,** 1581 (1975).

[22] S. Jagner and B. Jensen, Acta Cryst. **B33,** 2757 (1977).

[23] V. Mahajan and R. L. Sass, Cryst. Mol. Struct. **4,** 15 (1974).

[24] B. Jensen, Acta Chem. Scand. **B29,** 531 (1975).

[25] F. W. Schueler, J. Amer. Pharm. Ass. **45,** 197 (1956).

[26] R. E. Rosenfield jr. and P. Murray-Rust, J. Am. Chem. Soc. **104,** 5427 (1982).

[27] F. Zuccarello, A. Raudino, and G. Buemi, Chem. Physics Letters **70,** 565 (1980).

[28] M. J. Michelson and E. V. Zeimal, in: Acetylcholine, an Approach on the Molecular Mechanism of Action, Pergamon Press, Chap. 4, Oxford 1973.

[29] C. Chothia and P. Pauling, Acta Cryst. **B33,** 1851 (1977).

[30] R. W. Brimblecombe, in: Drug Actions in Cholinergic Systems, p. 52, the Mac Millan Press Ltd., London 1974.