Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine VI [1]

X-Ray Structure Analysis of Trimethyl[2-(propionyloxy)ethyl]-ammonium-iodide (O-Propionylcholine-iodide)

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The title compound (**1**) crystallizes in the orthorhombic, noncentrosymmetric space group $Pna2_1$ with $a = 10.241(11)$, $b = 12.903(12)$, $c = 9.312(9)$ Å and with one formula unit per asymmetric unit. The stereochemically comparable torsion angles of the cation of **1** and of acetylcholine chloride are analogous. In the crystal structure the trimethylammonio methyl group is surrounded by three anions in the first coordination sphere. The geometry of a triangle formed by one of these counterions which occupies a special face of the $\overset{+}{N}C_4$ tetrahedron of the $(CH_3)_3\overset{+}{N}-CH_2-R$ moiety, the nitrogen atom of the ammonium group and the oxygen atom of the carbonyl group is typical for nicotinic agonists.

Introduction

The title compound **1** ($[C_8H_{18}NO_2]^+ I^-$) which is derived from acetylcholine by substitution of the acetyl residue by propionyl, was investigated in the course of several X-ray structure investigations [1–9] of cholinergic agonists, carried out in order to elucidate structure-activity correlations.

$$(CH_3)_3\overset{+}{N}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_3 \quad I^- \qquad \mathbf{1}$$

The relatively small chemical modification of **1** when compared to acetylcholine, leads to a remarkable change of the pharmacological activity: The muscarinic activity of compound **1** is very weak, while its nicotinic activity at skeletal muscles is even higher than that of acetylcholine [10, 11].

Experimental

Colourless, hygroscopic and light-sensitive crystals of **1** were obtained by recrystallization of a commercially available sample from a methanol/n-hexane mixture (10:1). For the X-ray structure investigation a crystal with the dimensions $0.80 \times 0.61 \times 0.92$ mm was sealed under dry argon atmosphere in a Debye-Scherrer capillary. This crystal was handled as far as possible under exclusion of light.

Preliminary Weissenberg and precession photographs (CuK_α-radiation) yielded an orthorhombic crystal system and the systematic absences $0kl: k+l = 2n+1$, $h0l: h = 2n+1$ which are consistent with the space groups $Pna2_1$ (noncentrosymmetric, No. 33) or Pnam (centrosymmetric, No. 62). The final lattice constants were determined on an automatic single crystal diffractometer (Siemens AED I): $a = 10.241(11)$, $b = 12.903(12)$, $c = 9.312(9)$ Å; $V = 1230.5$ Å^3, $Z = 4$, $D_x = 1.55$ gcm^{-3}, $D_m = 1.53$ gcm^{-3}. 1266 independent reflections were collected using MoK_α radiation up to a maximum scattering angle of $\theta = 26°$ (5-point measurement, $\theta/2\theta$ scan mode). 101 of these reflections were classified as unobserved ($I < 2\sigma_I$). No absorption correction was applied ($\mu(MoK_\alpha) = 26.05$ cm^{-1}).

Structure solution and refinement

E value statistics indicated the presence of a noncentrosymmetric space group ($Pna2_1$) with a distinct centrosymmetric portion (iodide positions). The space group was confirmed by the result of the structure solution. The atomic scattering factors for C, N, O and I were taken from the International Tables for X-ray Crystallography [12, 13] and those for H are given by Stewart, Davidson and Simpson [14]. For most computer calculations the X-RAY-67 program system [15] was used.

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The I atomic position was determined by way of Patterson synthesis. A subsequent Fourier synthesis yielded the positions of all non-hydrogen atoms. Because the I atomic positions alone give rise to the symmetry of the centrosymmetric space group Pnam, this Fourier synthesis yielded a superposition of the structure and its mirror image. However on the basis of the known chemical constitution and with the knowledge of approximate bond lengths and angles, both the enantiomorphous structures were easily separated. The least squares refinement, carried out first with isotropic and then with anisotropic temperature parameters, converged to an R-value of 0.063 ($R = \Sigma \left| |F_o| - |F_c| \right| / \Sigma |F_o|$). The positions of the hydrogen atoms were partially determined by way of difference Fourier syntheses and partially calculated. Their positional and temperature parameters were not varied in the course of the refinement.

In the last steps of the refinement 18 structure factors with $|\Delta F| > 8$ ($\Delta F = |F_o| - |F_c|$) were omitted from computation of the parameter shifts. The unobserved reflections were incorporated with $|F_o| = 4\sigma_F$ into the computation of the parameter shifts only under the condition that $|F_c| > 4\sigma_F$. The atomic coordinates and temperature parameters are summarized in Table I. A list of observed and calculated structure factors can be obtained by request from the authors. The high temperature parameters of the ethyl group atoms C(7) and C(8) indicate a statistical disorder of this part of the molecule, also confirmed by a final difference Fourier synthesis which yielded an additional maximum of electron density that must be interpreted as an alternative position of C(8).

Description and discussion of the molecular structure

The molecular structure of the cation of **1** is shown in Fig. 1. Bond lengths and angles of the cation are summarized in Table II. The atoms C(2), N(1), C(4), C(5) on one hand and C(4), C(5), O(1), C(6), O(2), C(7), C(8), respectively, on the other hand are approximately co-planar. The angle between the least squares planes is 68°. The torsion angles $\tau_1 - \tau_4$ which have been discussed in connection with structure-ac-

Table I. Fractional atomic coordinates and thermal parameters. Standard deviations in form of the last significant figure(s) 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	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
I (1)	0.2132 (1)	0.0541 (1)	0.0	4.4 (1)	6.3 (1)	8.1 (1)	0.4 (1)	−0.1 (1)	−0.1 (3)
O(1)	0.0960 (20)	0.3334 (15)	0.2855 (26)	5.7 (8)	4.4 (8)	5.5 (10)	1.5 (7)	0.8 (8)	−0.6 (8)
O(2)	−0.0587 (23)	0.4007 (23)	0.4211 (32)	5.5 (9)	9.9 (17)	7.0 (14)	2.6 (10)	1.3 (10)	−1.0 (12)
N(1)	0.2903 (19)	0.3980 (15)	0.0421 (28)	4.3 (7)	3.1 (7)	5.4 (15)	0.1 (6)	−1.1 (7)	2.0 (8)
C(1)	0.3581 (26)	0.3384 (28)	0.1549 (46)	3.7 (10)	5.4 (15)	6.8 (19)	0.5 (9)	−0.3 (12)	0.8 (15)
C(2)	0.3332 (42)	0.3588 (36)	−0.1003 (40)	7.3 (18)	6.6 (21)	4.1 (14)	−0.6 (17)	1.3 (14)	−0.9 (14)
C(3)	0.3205 (25)	0.5060 (19)	0.0673 (43)	5.0 (10)	3.7 (10)	8.8 (20)	−1.3 (8)	−2.3 (12)	−2.9 (13)
C(4)	0.1425 (23)	0.3800 (21)	0.0468 (35)	4.0 (8)	4.7 (10)	6.5 (17)	−0.7 (8)	−0.9 (10)	1.1 (11)
C(5)	0.0748 (23)	0.4094 (23)	0.1769 (39)	3.7 (9)	5.6 (13)	6.3 (17)	1.0 (8)	−0.1 (10)	0.5 (12)
C(6)	0.0282 (33)	0.3384 (21)	0.4079 (41)	6.8 (14)	3.7 (10)	6.3 (16)	1.4 (11)	0.7 (14)	0.6 (12)
C(7)	0.0794 (51)	0.2603 (41)	0.5076 (97)	15.9 (35)	11.3 (27)	5.8 (20)	5.6 (27)	1.3 (47)	−0.2 (36)
C(8)	0.0279 (68)	0.2641 (87)	0.6634 (86)	13.2 (46)	30 (11)	8.5 (36)	2.6 (68)	2.3 (34)	−4.5 (58)

Hydrogen Atoms

Atom	x	y	z	B[Å^2]	Atom	x	y	z	B[Å^2]
H(11)	0.320	0.258	0.104	7	H(41)	0.126	0.307	0.021	7
H(12)	0.325	0.352	0.261	7	H(42)	0.100	0.423	−0.040	7
H(13)	0.463	0.345	0.149	7	H(51)	−0.008	0.419	0.161	7
H(21)	0.430	0.398	−0.133	7	H(52)	0.110	0.484	0.213	7
H(22)	0.260	0.381	−0.177	7	H(71)	0.055	0.184	0.472	7
H(23)	0.345	0.279	−0.095	7	H(72)	0.185	0.267	0.516	7
H(31)	0.420	0.521	−0.002	7	H(81)	0.023	0.333	0.676	7
H(32)	0.336	0.525	0.174	7	H(82)	0.095	0.235	0.737	7
H(33)	0.245	0.555	0.020	7	H(83)	−0.065	0.234	0.671	7

Table II. Bond lengths [Å] and angles [°] defined by the non hydrogen atoms. Standard deviations are given in form of the last significant figure(s) in parentheses.

a) Bond lengths		b) Bond angles	
O(1)–C(5)	1.43 (4)	C(5)–O(1)–C(6)	120 (2)
O(1)–C(6)	1.34 (4)	C(1)–N(1)–C(2)	109 (3)
O(2)–C(6)	1.21 (4)	C(1)–N(1)–C(3)	107 (2)
N(1)–C(1)	1.48 (5)	C(1)–N(1)–C(4)	111 (2)
N(1)–C(2)	1.49 (5)	C(2)–N(1)–C(3)	114 (3)
N(1)–C(3)	1.45 (3)	C(2)–N(1)–C(4)	105 (2)
N(1)–C(4)	1.53 (3)	C(3)–N(1)–C(4)	111 (2)
C(4)–C(5)	1.45 (5)	N(1)–C(4)–C(5)	117 (2)
C(6)–C(7)	1.47 (8)	O(1)–C(5)–C(4)	110 (2)
C(7)–C(8)	1.54 (12)	O(1)–C(6)–O(2)	120 (3)
		O(1)–C(6)–C(7)	109 (4)
		O(2)–C(6)–C(7)	131 (4)
		C(6)–C(7)–C(8)	117 (5)

tivity relationships of cholinergic agents [16–19] are shown in Fig. 2. They are comparable to those of acetylcholine chloride [20] that also exhibits a *trans, gauche, trans, trans* conformation, while the conformations of acetylcholine bromide [21] and iodide [22] are *trans, gauche, gauche, trans*. The torsion angles $\tau_1-\tau_4$ of the cation of **1** are in very good agreement with values which have been considered to be typical for a lot of muscarinic agonists [19]. However, compound **1** is a potent nicotinic agonist, while its muscarinic activity is negligibly low. As a consequence it must be necessarily concluded that the conformational angles – found in crystal structures – alone, are not adequate for the differentiation between nicotinic and muscarinic activity modes [1].

Due to the analogous conformational angles τ_1 and τ_2, similar contact distances between a methyl group (C(1)), which is *gauche* relative to N(1)–C(4), and the ester oxygen atom, appear both in the crystal structure of **1** and in the crystal structures of the acetylcholine halides. The relevant distances for this interaction are shown in Fig. 3. They are of special interest due to the relatively short contact distances indicating the formation of a weak intramolecular H-bridge between the CH_3 group and the ester oxygen

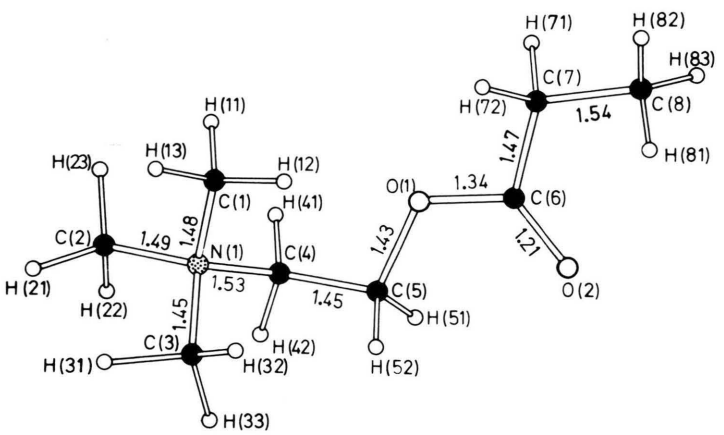

Fig. 1. Structure of the cation of **1**.

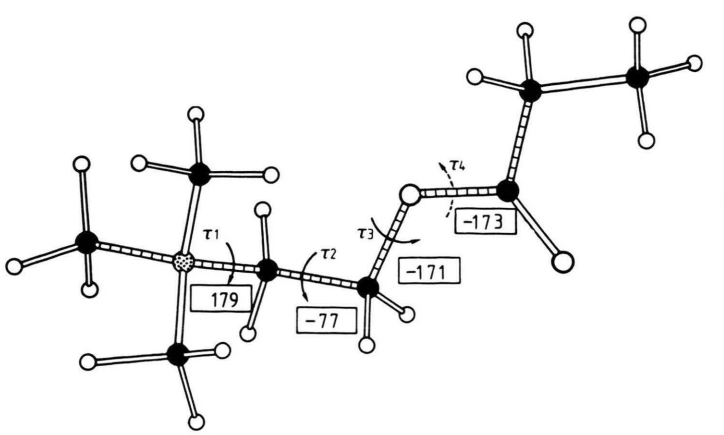

Fig. 2. Torsion angles of the cation of **1**.

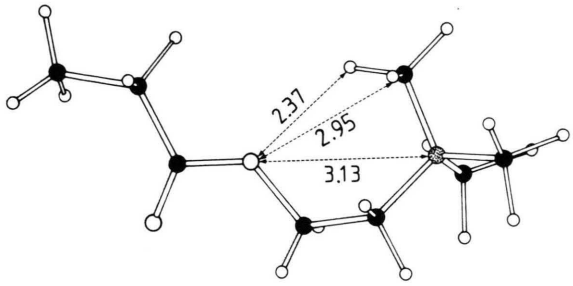

Fig. 3. Intramolecular contact distances between the ester oxygen and the trimethylammonium group.

atom, which effects the stabilization of a conformation (τ_2 = *gauche*) that has been regarded as biologically active [23, 24].

The intramolecular contact distance between the positively charged nitrogen atom of the quaternary ammonium group and the oxygen atom of the carbonyl group which carries a partial negative charge, is important for structure-activity considerations. In **1** it is 5.02 Å, being considerably longer than the equivalent distance in acetylcholine chloride (4.80 Å) [20], acetylcholine bromide (4.41 Å) [21] and acetylcholine iodide (4.54 Å) [22].

Crystal structure and biological activity

As we previously reported, the anions in the crystal structures of halides of cholinergic neurotransmitters can be used as models for the anionic adsorption site of the receptor [1–5]. In the crystal structure of **1** (Fig. 4), the quaternary ammonium group is coordinated by three anions close to it (Table III a), while all other $I^- - N^+$ distances are longer than 5 Å. Each of these anions occupies a tetrahedral face of the trimethylammonio alkyl group, specified as type A, B, or C. The definition of these face types has been given earlier [1, 5]. The strongest Coulombic interaction occurs between N^+ and the anions occupying faces of type A and B. Both tetrahedral faces of the trimethylammonio methyl group of type B are formed by two methyl groups and the methylene group of the carbon chain, with the restricting condition, that the anion occupying a face of this type "sees" three H-atoms [1]. In the structure of **1** only one of the two B-faces is occupied by an anion within the first coordination sphere. We have shown [1–5], that the adsorption face of the trimethylammonio

Table III. a) Short $N^+ - - - I^-$ distances [Å] in the crystal structure of **1**. The face types of the tetrahedron formed by the $(CH_3)_3N^+ - CH_2$-group are given by A, B and C [1, 5]. The index of the I anions is defined by the symmetry operation in parentheses which must be applied to the atomic position of I given in Table I.

(A) N(1)–I_1 4.39(2) $(x + 1/2, -y + 1/2, z)$
(B) N(1)–I_2 4.52(2) (x, y, z)
(C) N(1)–I_3 4.72(3) $(-x + 1/2, y + 1/2, z + 1/2)$.

b) Angles [°] between the $N^+ \rightarrow I^-$ vectors and the $N^+ - C$ bonds.

	N(1)–C(1)	N(1)–C(2)	N(1)–C(3)	N(1)–C(4)
N(1)–I_1	71	71	71	176
N(1)–I_2	68	69	175	71
N(1)–I_3	65	163	56	92

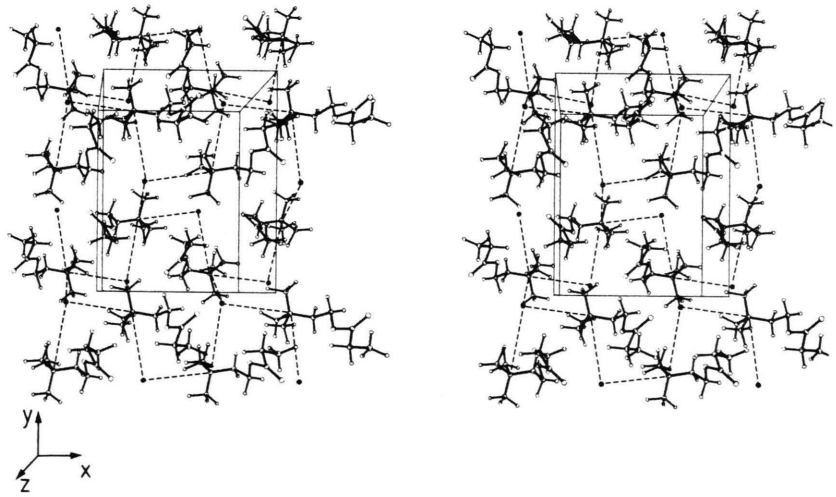

Fig. 4. Crystal structure of **1**.

methyl group at the active site of the receptor very probably is a tetrahedral face of type B.

The orientational parameters of the N^+-I^- vectors relative to the quaternary trimethylammonio methyl group are summarized in Table IIIb. The N^+-I^- vectors usually are directed perpendicularly to a tetrahedral face in its center of gravity and are co-linear with the $C-N^+$ bond of the methyl or methylene group, respectively, opposite to the tetrahedral face. In an ideal case, being very well realized in **1**, if the faces of type A and B are occupied by I^-, the angles between the N^+-I^- vector and the N^+-C bonds are 180° or 70.5°, respectively. Significant deviations from these angles are found, if a face of type C is occupied. The characteristic arrangement of monoatomic anions relative to the trimethylammonio methyl group was confirmed recently [25, 26] by a systematic search on the basis of the Cambridge Crystallographic Data File [27].

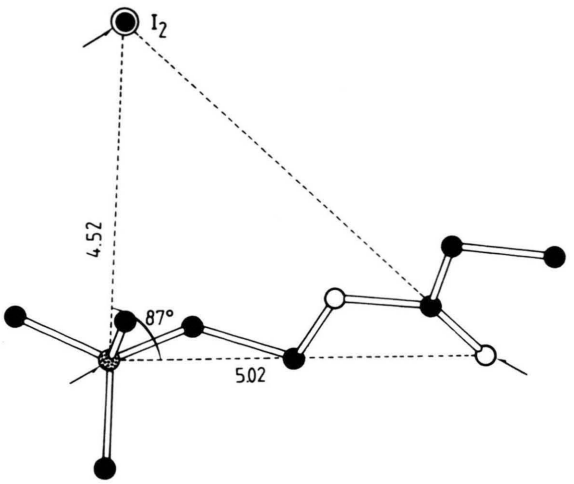

Fig. 5. Nicotinic "activity triangle" in the crystal structure of **1**.

In Fig. 5 a triangle is shown, formed by polar centers of the crystal structure, namely the nitrogen of the ammonium group, the I^- anion occupying one face of type B and the oxygen of the carbonyl group. A pronounced correlation has been found between the geometries of such, so called activity triangles and the activity mode (muscarinic or nicotinic) of a compound. The angle of 87° between the N^+-I^- and the N^+-O (carbonyl) vectors is typical for a nicotinic agonist at skeletal muscles like **1** [1–5]. Another triangle is formed between the same N^+-I^- vector and the N^+-O (ester oxygen) vector with an angle between these vectors of 72° and a $N^+-O(1)$ distance of 3.13 Å. Although this triangle could be interpreted as a muscarinic activity triangle of second type [1], its geometry deviates from that considered as typical for such activity triangles. In principle, an additional muscarinic triangle of this type may be formed by occupation of the second – in this structure non-occupied – B face by an anion. However, although a muscarinic activity triangle of second type with nearly normal geometry is thus possible, with respect to the activity, the presence of a single triangle of the above type is in general associated with weak muscarinic actions [1, 7].

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