

The Crystal and Molecular Structure and Absolute Configuration of L-(+)-(S)-Acetyl- β -methylcholine Iodide

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Crystals of L-(+)-acetyl- β -methylcholine iodide, $C_8H_{18}NO_2I$, are orthorhombic, space group $P2_12_1(D_2^4)$, $a = 28.16(3)$, $b = 7.60(1)$, $c = 5.94(1)$ Å, $Z = 4$. The absolute configuration of the molecule was determined as *S*. The conformation of the molecule described by the torsion angles N–C–C–O ($+87^\circ$) and C–C–O–C (-144°) is similar to that of other muscarinic agonists.

Introduction

In cholinergic nervous systems, acetyl- β -methylcholine [(CH₃)₃NCH₂CH(CH₃)OCOCH₃] retains the muscarinic activity of acetylcholine [(CH₃)₃NCH₂CH₂OCOCH₃] at the post-ganglionic parasympathetic nervous junction but not the nicotinic activity at the ganglion and motor end plate. Ellenbrock & van Rossum (1960) and Beckett, Harper & Clitherow (1963) have synthesized the two enantiomers of the compound, determined their absolute configuration and measured their muscarinic activity using guinea pig ileum. The L-(+)-(S) enantiomer is about as active as acetylcholine as a muscarinic agonist, while the D-(–)-(R) enantiomer is only $\frac{1}{240}$ th as active. We describe here the structure analysis of L-(+)-(S)-acetyl- β -methylcholine iodide.

Experimental results

Crystals of L-(+)-(S)-acetyl- β -methylcholine iodide, provided by Professor A. H. Beckett, are colourless plates with principal faces (100). These plates are usually rectangular with elongation in the *c* direction. Optical extinction occurs parallel to the long edges of the rectangular plates.

Crystal data

L-(+)-(S)-Acetyl- β -methylcholine iodide, $C_8H_{18}NO_2I$, $M_r = 287.15$, $a = 28.156(27)$, $b = 7.601(6)$, $c = 5.941(5)$ Å, $Z = 4$, $d_c = 1.50$ g cm⁻³. Laue symmetry *mmm*; systematic absences: $h00$: $h = 2n + 1$; $0k0$: $k = 2n + 1$; $00l$: $l = 2n + 1$; space group $P2_12_1(D_2^4)$.

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X-ray diffraction data of a crystal $0.04 \times 0.16 \times 0.36$ mm, coated in Formvar, were measured on a home-built computer-controlled (PDP-8/S) Stoe four-circle diffractometer. The control programs used were those of Busing, Ellison, Levy, King & Roseberry (1968). Cell parameters and their standard deviations and the orientation of the crystal in the laboratory system were determined by a least-squares analysis of the observed maxima in three parameters of 12 diffraction maxima. Data were measured for one quadrant of the sphere of reflexion within the range $2\theta = 0.5\text{--}45.0^\circ$; the diffraction data were measured in three sets (Busing *et al.*, 1968). Within the range $2\theta = 0.5\text{--}10^\circ$, Zr-filtered Mo radiation was used with the moving-crystal stationary-detector method (ω scan) in which the crystal is oscillated three times around the reflecting position (once for the measurement with the detector in the appropriate position and twice with the detector offset to measure the background). Data for the range $2\theta = 5\text{--}25^\circ$ were measured using Zr-filtered Mo radiation and the moving-crystal moving-detector (2θ scan) method. Data in the range $2\theta = 20\text{--}45^\circ$ were measured using the 2θ scan with unfiltered Mo radiation. A standard diffraction maximum was measured after every 40 data measurements. A constant-time-measuring technique was used in which the time for the measurement of the diffraction maximum was approximately 80 s and the total time for the measurement of the two background measurements was approximately 40 s. The *c* axis of the crystal was mounted parallel to the diffractometer ϕ axis. 3028 diffraction maxima were measured. The effect of the absorption of X-rays by the crystal was determined as a function of ϕ by measuring the intensity of the 006 diffraction maximum with $\chi = 90^\circ$ at 5° intervals over the range $\phi = 0\text{--}360^\circ$.

The three sets of diffraction data were processed using a Fortran program for an IBM 360/65 computer written by Miss Margaret Dellow and Dr T. J.

Petcher. Absorption of X-rays by the crystal was corrected by the method of North, Phillips & Mathews (1968), modified for a four-circle instrument. Variations in the properties of the diffractometer, as a function of temperature and other unknown variables, were corrected by multiplying all maxima by a factor equal to the mean of all standard maxima divided by the mean of the two standard maxima measured at the beginning and end of each set of 40 data measurements. As a quadrant of the sphere of diffraction maxima was collected, each independent diffraction maximum was measured at least twice. Common reflexions were averaged.

Structure analysis

A three-dimensional Patterson synthesis was calculated. Large peaks on the Harker sections $x = \frac{1}{2}$, $y = \frac{1}{2}$ and $z = \frac{1}{2}$ were interpreted as vectors between I

Table 1. Final positional ($\times 10^4$, for H $\times 10^3$) and thermal parameters

(a) Nonhydrogen atoms	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> (Å ²)
I	715 (1)	1525 (2)	1210 (3)	*
N	4274 (6)	3515 (21)	2321 (21)	3.6
C(1)	4300 (8)	1878 (23)	901 (35)	4.5
C(2)	4348 (8)	5175 (26)	741 (37)	4.6
C(3)	4676 (6)	3453 (29)	4034 (38)	3.9
C(4)	3811 (6)	3593 (27)	3545 (38)	3.6
C(5)	3388 (8)	3504 (36)	2167 (36)	5.5
C(8)	2965 (9)	4310 (31)	3443 (60)	7.9
O(1)	3258 (5)	1652 (24)	1788 (24)	5.4
C(6)	3047 (14)	995 (46)	-319 (64)	11.3
O(2)	3032 (9)	2154 (31)	-1810 (45)	12.2
C(7)	2998 (10)	-921 (34)	-264 (47)	8.3

(b) Hydrogen atoms (as determined from the final difference Fourier syntheses). Hydrogen atoms are numbered H(*nm*), where *n* is the number of the bonded carbon atom.

	<i>x</i>	<i>y</i>	<i>z</i>
H(11)	481	455	472
H(12)	481	227	472
H(13)	500	318	278
H(21)	430	55	133
H(22)	411	214	-56
H(23)	463	182	0
H(31)	435	636	194
H(32)	411	486	0
H(33)	467	514	-17
H(41)	370	245	483
H(42)	370	455	500
H(51)	333	386	56
H(81)	306	536	389
H(82)	272	364	472
H(83)	259	477	222

* Anisotropic thermal parameters ($\times 10^4$) for the iodide ion:

b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
22	127	303	4	-33	0

atoms related by the screw axes parallel to the *a*, *b* and *c* axes respectively. The coordinates of the I atom determined from the Patterson synthesis are $x = 0.0711$, $y = 0.1488$ and $z = 0.1248$. The positions of all non-hydrogen atoms were determined from an observed Fourier synthesis phased on the I atom. The structure was refined by full-matrix least-squares analysis, with anisotropic thermal parameters for the I atom and isotropic thermal parameters for all other atoms, to a value of the conventional residual $R = 0.069$. The scattering factors used were those of Cromer & Waber (1965). The scattering factor of the I atom was reduced by the real part of the anomalous dispersion. Each structure amplitude $F(hkl)$ was weighted in the least-squares analysis by the statistical weight $4I(hkl)/\sigma(I)$, where $\sigma(I)$ is the statistical standard deviation of *I*.

Observed and difference Fourier syntheses were calculated using atomic positions and thermal parameters derived from the least-squares analysis. Nine H atoms were clearly indicated in the difference synthesis with peak heights of about 1 e. It was possible to find six other H atoms whose peaks were somewhat more diffuse. There was no indication in the difference synthesis of the H atoms attached to the acetoxy methyl group [C(7)].

These 15 H atoms were included in further least-squares analysis with a fixed overall *B* of 3.2 Å², but the H atom positions were not refined. The structure was refined to give a final residual $R = 0.052$, and resulted in the atomic coordinates and thermal parameters listed in Table 1.*

Discussion

Molecular structure

The derived interatomic bond distances and angles are given in Fig. 1. The high standard deviations of

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32963 (4 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 2. Torsion angles (°)

C(1)-N(1)-C(4)-C(5)	-57
C(2)-N(1)-C(4)-C(5)	+63
C(3)-N(1)-C(4)-C(5)	-175
N(1)-C(4)-C(5)-O(1)	+87
N(1)-C(4)-C(5)-C(8)	-158
C(4)-C(5)-O(1)-C(6)	-144
C(8)-C(5)-O(1)-C(6)	+98
C(5)-O(1)-C(6)-C(7)	+177
C(5)-O(1)-C(6)-O(2)	+5

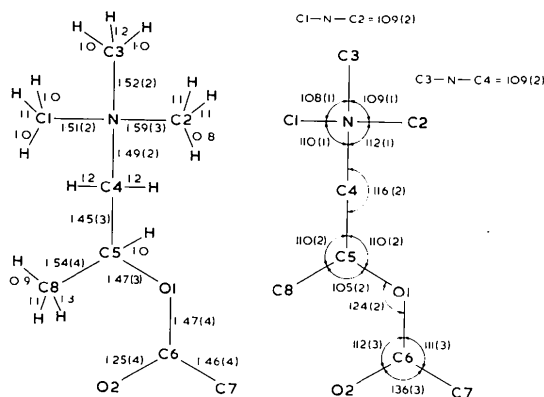


Fig. 1. L-(+)-Acetyl- β -methylcholine cation: bond lengths (Å) and angles ($^{\circ}$).

the atomic coordinates mean that the exact observed values of the bond lengths and angles do not have significance. Most values are within less than two standard deviations of expected values and the remainder are within three.

Atoms C(8), C(6), O(2) and C(7) have high temperature factors. In the packing of the molecules in the crystal, the acetoxy group has few contacts with neighbouring molecules and some freedom of movement (see below). It is not clear from this structure analysis whether the large thermal parameters are due to disorder in the atomic positions or to large vibrational displacements.

The torsion angles of the molecule are listed in Table 2. Steric repulsion causes the trimethylammonium group to be staggered relative to the rest of the molecule $\{\tau[C(3)-N(1)-C(4)-C(5)]$ is -175° . Electrostatic attraction between the quaternary group and O(1) makes $\tau[N-C(4)-C(5)-O(1)]$ synclinal ($+87^{\circ}$) (Coggon, McPhail & Roe, 1969; Brennan, Ross, Hamilton & Shefter, 1970; and Baker, Chothia, Pauling & Petcher, 1971). It also fixes the value of $\tau[C(4)C(8)-C(5)-O(1)-C(6)]$ as the synclinal conformation brings the acetoxy group close to the quaternary ammonium group [$C(1)-O(2) = 3.36(3)$ and $C(1)-C(6) = 3.92(4)$ Å] and so any appreciable decrease in the observed value of $\tau[C(4)-C(5)-O(1)-C(6)]$ is prevented by steric hindrance. Any increase in the value of $\tau[C(4)-C(5)-O(1)-C(6)]$ is prevented by the contacts between the acetoxy group and the β -methyl group C(8) [$C(8)-C(6) = 3.37(5)$ and $C(8)-O(2) = 3.53(4)$ Å]. The observed planarity of the ester group is that normally observed.

Absolute configuration of L-(+)-acetyl- β -methylcholine

The data collected for this structure were all values of hkl and $\bar{h}kl$ in the sphere $2\theta = 1-45^{\circ}$. These two sets of diffraction maxima are related by Friedel's law (Laue

Table 3. Absolute configuration of L-(+)-acetyl- β -methylcholine

hkl	F_{obs}	F_{calc}^*	$F_{\text{obs}}(hkl) - F_{\text{obs}}(\bar{h}kl)$	$F_{\text{calc}}(hkl) - F_{\text{calc}}(\bar{h}kl)$
1 1 1	97.4	86.3	-3.2	-1.2
$\bar{1}$ 1 1	100.6	87.5		
1 1 2	118.8	105.4	-1.6	-2.7
$\bar{1}$ 1 2	120.4	108.1		
1 2 1	78.2	71.8	-1.6	-2.6
$\bar{1}$ 2 1	79.8	74.4		
1 3 1	66.3	56.5	+3.9	+1.8
$\bar{1}$ 3 1	62.4	54.7		
1 4 1	51.2	44.3	+2.6	+0.9
$\bar{1}$ 4 1	48.6	43.4		
2 1 1	114.8	99.1	+2.7	+0.6
$\bar{2}$ 1 1	112.1	98.5		
2 1 2	63.2	52.4	+2.3	-1.3
$\bar{2}$ 1 2	60.9	53.7		
2 1 3	51.4	43.7	-1.5	-2.4
$\bar{2}$ 1 3	52.9	46.1		
2 2 3	52.6	44.8	+1.6	+1.2
$\bar{2}$ 2 3	51.0	43.6		
3 1 1	152.6	133.3	+1.8	-0.9
$\bar{3}$ 1 1	150.8	134.2		
4 1 2	28.6	25.8	+1.6	+2.7
$\bar{4}$ 1 2	27.0	23.1		
5 1 1	112.7	100.3	-2.9	-3.3
$\bar{5}$ 1 1	115.6	103.6		
5 2 2	29.8	25.2	+2.4	+2.2
$\bar{5}$ 2 2	27.4	23.0		
6 1 1	101.9	87.4	-1.6	-4.0
$\bar{6}$ 1 1	103.5	91.4		
6 1 3	47.0	39.3	+1.8	+1.5
$\bar{6}$ 1 3	45.2	37.8		
7 1 1	58.0	47.2	+1.6	+0.6
$\bar{7}$ 1 1	56.4	46.6		
9 1 2	33.9	28.7	-2.5	-1.4
$\bar{9}$ 1 2	36.4	30.1		
12, 1, 3	36.8	30.7	-1.6	-3.3
$\bar{12}$, 1, 3	38.4	34.0		
12, 3, 3	24.1	20.7	+1.8	-0.5
$\bar{12}$, 3, 3	22.3	21.2		
13, 1, 1	64.1	55.8	-1.6	-1.9
$\bar{13}$, 1, 1	65.7	57.7		

* F_{calc} was calculated for the S enantiomer.

symmetry mmm) but not by the crystal point-group symmetry 222. Iodine diffracts Mo $K\alpha$ radiation anomalously with a real part $\Delta f' = -0.64$ and an imaginary part $\Delta f'' = +2.15$ (Cromer, 1965). The contribution of the imaginary part of the anomalous iodine scattering factor to $F(hkl)$ and $F(\bar{h}kl)$ will be in opposite phase.

The structure factors used in the refinement were the average of $F_{\text{obs}}(hkl)$ and $F_{\text{obs}}(\bar{h}kl)$ so the contribution of the imaginary component of the anomalous-scattering factor for iodine was cancelled out. After the refinement of the structure was completed, the imaginary component was included in a calculation of the structure factors $F_{\text{calc}}(hkl)$ and $F_{\text{calc}}(\bar{h}kl)$, and the results were compared with the quadrant of un-averaged observed data. All pairs of $F_{\text{calc}}(hkl)$ and

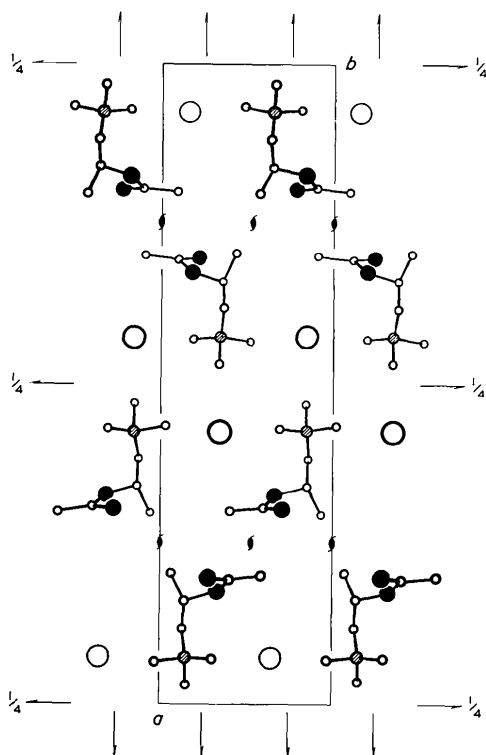


Fig. 2. L-(+)-Acetyl- β -methylcholine iodide: view of the unit cell in the c direction.

$F_{\text{calc}}(\bar{h}kl)$ which differed by more than 1.5 electrons are shown in Table 3 with the values of $F_{\text{obs}}(hkl)$ and $F_{\text{obs}}(\bar{h}kl)$. Examination of these results shows that the sign of the differences between $F_{\text{calc}}(hkl)$ and $F_{\text{calc}}(\bar{h}kl)$ is the same as the sign of the difference between $F_{\text{obs}}(hkl)$ and $F_{\text{obs}}(\bar{h}kl)$ in seventeen cases and differs in three. The absolute configuration of L-(+)-acetyl- β -methylcholine iodide is reliably determined to be S at C(5) confirming the chemical result of Ellenbrock & van Rossum (1960) and Beckett *et al.* (1963).

Molecular packing

The molecular packing in the crystal is illustrated in Fig. 2 and the intermolecular contacts are listed in Table 4. As can be seen from Fig. 2, the molecules form a bilayer structure whose plane is parallel to the crystal (100) plane. Adjacent bilayers are held together by ionic attraction between the iodide ions and the quaternary ammonium groups. Each I atom is in contact with four molecules in the same layer [two close contacts (I-CH₃ 3.68 and 3.80 Å) and two not so close (I-CH₃ 4.11 and 4.07 Å)], and two molecules in the adjacent layer (I-CH₃ 4.06 and 4.22 Å) (see Table 4).

Table 4. Intermolecular contacts (Å)

		Symmetry and translation of second atom		
I-C(2)	3.68	$\frac{1}{2} - x$,	$-y$,	$\frac{1}{2} + z$; $+b$
I-C(1)	3.80	$\frac{1}{2} - x$,	$-y$,	$\frac{1}{2} + z$
I-C(2)	4.11	$\frac{1}{2} - x$,	$-y$,	$\frac{1}{2} + z$; $+b, -a$
C(3)	4.18			
I-C(1)	4.07	$\frac{1}{2} - x$,	$-y$,	$\frac{1}{2} + z$; $-c$
C(3)	4.15			
I-C(3)	4.06	$\frac{1}{2} + x$,	$\frac{1}{2} - y$,	$-z$; $-a, +b$
I-C(2)	4.22	$\frac{1}{2} + x$,	$\frac{1}{2} - y$,	$-z$; $-a$
C(1)-C(3)	3.88	$-x$,	$\frac{1}{2} + y$,	$\frac{1}{2} - z$; $+a, -b$
C(2)-C(3)	3.71	$-x$,	$\frac{1}{2} + y$,	$\frac{1}{2} - z$; $+a$
C(4)-O(2)	3.69	x ,	y ,	z ; $+c$
C(8)-O(2)	3.26	x ,	y ,	z ; $+c$
C(8)-C(7)	3.81	$\frac{1}{2} - x$,	$-y$,	$\frac{1}{2} + z$
O(2)-C(7)	3.67	$\frac{1}{2} - x$,	$-y$,	$\frac{1}{2} + z$; $-b$

Close contacts occur between O(2) of one molecule and C(4) and C(8) of an adjacent molecule in the c direction. Across the 'interior' of the bilayer the only van der Waals contact is C(8)-C(7) (3.81 Å). As noted above, this loose packing of the acetoxy end of the molecule results in some disorder.

The implications of the molecular structure of acetyl- β -methylcholine observed here for its activity in cholinergic nervous systems is discussed by Baker, Chothia, Pauling & Petcher (1971).

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