

## Structure of Acetylcholine Tetraphenylborate

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(Received 6 November 1979; accepted 10 December 1979)

### Abstract

Crystals of acetylcholine tetraphenylborate,  $C_7H_{16}NO_2 \cdot C_{24}H_{20}B$ ,  $M_r = 465.45$ , are monoclinic, space group  $P2_1/c$ ,  $a = 9.228(3)$ ,  $b = 16.329(7)$ ,  $c = 22.159(10)$  Å,  $\beta = 124.71(5)^\circ$ ,  $Z = 4$ ,  $D_m = 1.129$ ,  $D_x = 1.126$  Mg m<sup>-3</sup>,  $V = 2744.9$  Å<sup>3</sup>. The structure was solved by direct methods and refined by full-matrix least squares to  $R = 0.064$ . The molecular geometry of acetylcholine in this salt differs from those observed in the bromide and iodide, but agrees with those of the chloride, perchlorate and  $\beta$ -resorcyate. The N–C–C–O torsion angle is observed to be synclinal ( $66.7^\circ$ ) and the C–C–O–C torsion angle is anticlinal–antiplanar ( $154.6^\circ$ ).

### Introduction

Acetylcholine is the natural intercellular transmitter substance in most intercellular peripheral nervous junctions. The structure of the acetylcholine ion in different environments, such as in crystals of the chloride, bromide, iodide, perchlorate and  $\beta$ -resorcyate, has been studied by a number of investigators, and many theoretical calculations have been published. The conformations of acetylcholine chloride, perchlorate and  $\beta$ -resorcyate are synclinal–antiplanar at N–C–C–O– and C–C–O–C= and those of acetylcholine bromide and iodide are synclinal–synclinal. This shows that the conformation of acetylcholine is labile and influenced by its environment. No definite rule can yet be established about the conformational changes of acetylcholine as a chemical substance. The structure of the tetraphenylborate salt has been analysed to study the conformation of the acetylcholine ion in the presence of a large ion. This investigation shows a synclinal–antiplanar conformation. On the basis of NMR data in solution, the observed conformation of acetylcholine in solution is also synclinal–antiplanar (Culvenor & Ham, 1966).

† Deceased 17 June 1976.

### Experimental

Crystals of acetylcholine tetraphenylborate, provided by Professor D. J. Jenden, are colourless plates, usually rectangular with elongation in the  $c$  direction, and show the forms  $\{010\}$  and  $\{100\}$ . Space group and preliminary cell parameters were determined from Weissenberg and precession photographs. The refined cell parameters and associated standard deviations were obtained by a least-squares refinement of three angular parameters of twelve reflections accurately aligned on a computer-controlled four-circle Stoe diffractometer (Busing, Ellison, Levy, King & Rosebury, 1968).

### Data collection and structure analysis

Three-dimensional X-ray intensity data from a single crystal ( $0.23 \times 0.35 \times 0.61$  mm) were collected on a Stoe computer-controlled four-circle diffractometer using Mo  $K\alpha$  radiation with a graphite monochromator (002), a coupled  $\theta$ – $2\theta$  step scan with  $\Delta 2\theta = 0.04^\circ$ , a counting time of 8 s per step and a peak width of  $1.6^\circ$ . In the range  $2\theta = 5$ – $45^\circ$ , 2051 intensities out of a possible 4009 in the quadrant of reciprocal space ( $hk \pm l$ ) were considered observed,  $I \geq 3\sigma(I)$ . Data were corrected for Lorentz and polarization effects but not for absorption or extinction.

The structure was solved by direct methods with the program *MULTAN* (Germain, Main & Woolfson, 1971). An  $E$  map calculated for the set with the absolute figure of merit (1.13), combined figure of merit (3.00) and  $\varphi_o$  (0.203) showed all the 35 non-hydrogen atoms.

Refinement of the scale factor and overall temperature factor on these atoms gave  $R = 0.273$ . Five cycles of full-matrix least-squares refinement of positional and isotropic thermal parameters and an overall scale factor for the non-hydrogen atoms reduced  $R$  to 0.147. A difference Fourier map clearly indicated all H atom positions. Final cycles of full-matrix anisotropic refinement including the H atom positional parameters were continued until all parameter shifts were  $\leq 0.1\sigma$ .

Table 1. *Final positional parameters* ( $\times 10^4$ ) *for non-hydrogen atoms, with estimated standard deviations*

	<i>x</i>	<i>y</i>	<i>z</i>
O(1)	5022 (6)	4033 (3)	1764 (2)
O(2)	5202 (9)	5316 (3)	2069 (4)
N(1)	6803 (6)	2347 (3)	2197 (3)
C(1)	5100 (9)	2304 (4)	1444 (4)
C(2)	8329 (9)	2468 (4)	2145 (4)
C(3)	7077 (11)	1543 (4)	2574 (4)
C(4)	6774 (8)	2995 (4)	2685 (3)
C(5)	6708 (9)	3876 (4)	2459 (3)
C(6)	4413 (10)	4794 (4)	1609 (4)
C(7)	2793 (9)	4896 (4)	874 (4)
B(1)	7912 (8)	2753 (4)	4961 (3)
C(8)	6053 (7)	2877 (4)	4153 (3)
C(9)	5443 (8)	3651 (4)	3825 (3)
C(10)	3886 (9)	3756 (5)	3131 (4)
C(11)	2897 (8)	3087 (5)	2726 (3)
C(12)	3470 (8)	2313 (5)	3013 (3)
C(13)	5037 (7)	2204 (4)	3709 (3)
C(14)	9464 (7)	2788 (4)	4808 (3)
C(15)	10047 (7)	2105 (4)	4625 (3)
C(16)	11203 (8)	2151 (5)	4421 (4)
C(17)	11804 (8)	2893 (6)	4367 (4)
C(18)	11272 (9)	3581 (5)	4539 (4)
C(19)	10134 (8)	3530 (4)	4749 (3)
C(20)	8201 (7)	3487 (3)	5531 (3)
C(21)	9881 (8)	3699 (3)	6147 (3)
C(22)	10115 (8)	4290 (4)	6635 (3)
C(23)	8715 (9)	4698 (4)	6569 (3)
C(24)	7051 (8)	4497 (4)	5975 (3)
C(25)	6798 (7)	3903 (4)	5485 (3)
C(26)	7908 (7)	1893 (3)	5344 (3)
C(27)	9438 (8)	1434 (4)	5836 (3)
C(28)	9406 (9)	720 (4)	6181 (3)
C(29)	7848 (10)	436 (4)	6057 (4)
C(30)	6333 (9)	876 (4)	5589 (4)
C(31)	6344 (8)	1596 (4)	5250 (3)

Isotropic thermal parameters ( $U_{iso}$ ) for all H atoms were fixed at  $0.0770 \text{ \AA}^2$ . The final  $R$  is  $0.064$ . The function minimized was  $\sum w(|F_o| - |F_c|)^2$  with  $w = 1$  for all reflections. Atomic scattering factors for neutral C, N, O, B atoms were those of Cromer & Waber (1965) and for H atoms from *International Tables for X-ray Crystallography* (1962). The final positional parameters for non-hydrogen atoms are listed in Table 1 and those of H atoms in Table 2.\*

### Results and discussion

Fig. 1 shows the atomic numbering. Bond distances and angles for a unique molecule are listed in Table 3.

\* Lists of structure factors, anisotropic thermal parameters and bond distances involving H atoms have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35036 (17 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

The observed bond distances and angles are in good agreement with those found in related structures. The calculations of mean-plane equations and deviations of atoms from those planes for certain groups of atoms

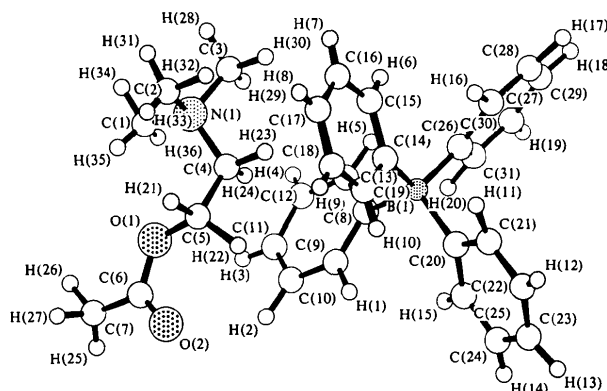


Fig. 1. View of acetylcholine in crystals of the tetraphenylborate with observed hydrogen positions.

Table 2. *Fractional coordinates* ( $\times 10^3$ ) *for hydrogen atoms*

	<i>x</i>	<i>y</i>	<i>z</i>
H(1)	612 (8)	421 (4)	414 (3)
H(2)	354 (9)	435 (4)	287 (4)
H(3)	185 (8)	316 (4)	224 (4)
H(4)	277 (9)	186 (4)	277 (4)
H(5)	530 (8)	160 (4)	396 (4)
H(6)	962 (7)	159 (3)	463 (3)
H(7)	1144 (8)	167 (4)	430 (3)
H(8)	1244 (7)	287 (3)	423 (3)
H(9)	1157 (10)	403 (4)	450 (4)
H(10)	986 (8)	401 (4)	491 (3)
H(11)	1092 (9)	345 (4)	611 (4)
H(12)	1106 (9)	435 (4)	693 (4)
H(13)	892 (7)	511 (4)	697 (3)
H(14)	613 (7)	476 (4)	596 (3)
H(15)	567 (7)	381 (3)	511 (3)
H(16)	1056 (8)	163 (4)	583 (3)
H(17)	1061 (11)	39 (5)	667 (5)
H(18)	786 (7)	-10 (4)	634 (3)
H(19)	521 (8)	73 (4)	555 (3)
H(20)	505 (10)	189 (5)	483 (4)
H(21)	771 (10)	401 (5)	230 (4)
H(22)	701 (10)	421 (5)	299 (5)
H(23)	797 (10)	288 (5)	326 (4)
H(24)	575 (9)	280 (4)	273 (4)
H(25)	189 (15)	519 (7)	83 (6)
H(26)	209 (16)	438 (8)	46 (7)
H(27)	297 (11)	515 (6)	52 (5)
H(28)	730 (9)	111 (5)	229 (4)
H(29)	626 (10)	147 (5)	262 (4)
H(30)	841 (13)	151 (6)	316 (6)
H(31)	840 (8)	199 (4)	187 (3)
H(32)	964 (12)	236 (6)	275 (5)
H(33)	816 (9)	284 (4)	194 (4)
H(34)	514 (10)	185 (5)	114 (5)
H(35)	491 (11)	274 (5)	117 (5)
H(36)	401 (11)	219 (5)	144 (4)

show that the ester group of acetylcholine and the phenyl rings of tetraphenylborate are planar within experimental error. Some torsion angles in this structure are compared in Table 4 with the corresponding angles of other acetylcholine ions in crystals of the

chloride (Herdklotz & Sass, 1970), bromide (Svinning & Sørum, 1975; N. Datta, P. Mondal & P. J. Pauling, unpublished), iodide (Jagner & Jensen, 1977), perchlorate (Mahajan & Sass, 1974) and  $\beta$ -resorcyate (Jensen, 1975). The conformation of the trimethylammonium as measured around the C(4)–N(1) bond is almost staggered [ $C(3)–N(1)–C(4)–C(5) \approx 174^\circ$ ]. The torsion angles  $N(1)–C(4)–C(5)–O(1)$  and  $C(4)–C(5)–O(1)–C(6)$  are the most important in the definition of the overall conformation of the acetylcholine ion. In acetylcholine tetraphenylborate, the torsion angle  $N(1)–C(4)–C(5)–O(1)$  is  $66.7^\circ$  (the smallest yet observed) and  $C(4)–C(5)–O(1)–C(6)$  is  $154.6^\circ$ , similar to one conformation of lactoylcholine observed in crystals of the iodide (Chothia & Pauling, 1968).

The N atom has been observed to be synclinal to the ether O atom in almost all acetylcholine-type structures. The torsion angle  $C(4)–C(5)–O(1)–C(6)$  is variable between synclinal and antiplanar, although the antiplanar conformation is almost universal in other esters (G. P. Jones & P. J. Pauling, unpublished).

It appears that acetylcholine adopts the synclinal–antiplanar conformation in the presence of light cations, and synclinal–synclinal in the presence of heavy cations.

Table 3. *Interatomic distances (Å) and angles (°) involving non-hydrogen atoms with estimated standard deviations in parentheses*

C(1)–N(1)	1.51 (1)	C(17)–C(16)	1.37 (1)
C(2)–N(1)	1.49 (1)	C(18)–C(17)	1.36 (1)
C(3)–N(1)	1.50 (1)	C(19)–C(14)	1.40 (1)
C(4)–N(1)	1.53 (1)	C(19)–C(18)	1.37 (1)
C(5)–O(1)	1.46 (1)	C(20)–B(1)	1.65 (1)
C(5)–C(4)	1.51 (1)	C(21)–C(20)	1.41 (1)
C(6)–O(1)	1.33 (1)	C(22)–C(21)	1.37 (1)
C(6)–O(2)	1.20 (1)	C(23)–C(22)	1.39 (1)
C(7)–C(6)	1.46 (1)	C(24)–C(23)	1.38 (1)
C(8)–B(1)	1.64 (1)	C(25)–C(20)	1.41 (1)
C(9)–C(8)	1.41 (1)	C(25)–C(24)	1.37 (1)
C(10)–C(9)	1.40 (1)	C(26)–B(1)	1.64 (1)
C(11)–C(10)	1.38 (1)	C(27)–C(26)	1.41 (1)
C(12)–C(11)	1.38 (1)	C(28)–C(27)	1.40 (1)
C(13)–C(8)	1.42 (1)	C(29)–C(28)	1.38 (1)
C(13)–C(12)	1.40 (1)	C(30)–C(29)	1.38 (1)
C(14)–B(1)	1.65 (1)	C(31)–C(26)	1.42 (1)
C(15)–C(14)	1.40 (1)	C(31)–C(30)	1.40 (1)
C(16)–C(15)	1.38 (1)		

O(2)–C(6)–O(1)	118.8 (7)	C(18)–C(19)–C(14)	123.2 (6)
C(2)–N(1)–C(1)	110.9 (5)	C(19)–C(14)–B(1)	122.0 (5)
C(3)–N(1)–C(1)	108.3 (5)	C(19)–C(14)–C(15)	113.6 (5)
C(3)–N(1)–C(2)	107.1 (5)	C(19)–C(18)–C(17)	120.8 (7)
C(4)–N(1)–C(1)	112.9 (5)	C(20)–B(1)–C(8)	111.0 (5)
C(4)–N(1)–C(2)	111.1 (5)	C(20)–B(1)–C(14)	110.6 (5)
C(4)–N(1)–C(3)	106.2 (4)	C(21)–C(20)–B(1)	122.1 (5)
C(4)–C(5)–O(1)	109.6 (5)	C(22)–C(21)–C(20)	122.0 (6)
C(5)–C(4)–N(1)	116.1 (5)	C(23)–C(22)–C(21)	122.4 (6)
C(6)–O(1)–C(5)	118.5 (5)	C(24)–C(23)–C(22)	116.9 (6)
C(7)–C(6)–O(1)	114.1 (6)	C(24)–C(25)–C(20)	123.1 (5)
C(7)–C(6)–O(2)	127.1 (7)	C(25)–C(20)–B(1)	123.5 (5)
C(9)–C(8)–B(1)	122.4 (5)	C(25)–C(20)–C(21)	114.2 (5)
C(10)–C(9)–C(8)	122.6 (6)	C(25)–C(24)–C(23)	121.3 (6)
C(11)–C(10)–C(9)	120.4 (6)	C(26)–B(1)–C(8)	111.1 (5)
C(12)–C(11)–C(10)	119.3 (6)	C(26)–B(1)–C(14)	113.1 (5)
C(12)–C(13)–C(8)	121.9 (6)	C(26)–B(1)–C(20)	105.8 (4)
C(13)–C(8)–B(1)	122.1 (5)	C(27)–C(26)–B(1)	123.9 (5)
C(13)–C(8)–C(9)	115.2 (5)	C(28)–C(27)–C(26)	122.7 (6)
C(13)–C(12)–C(11)	120.5 (6)	C(29)–C(28)–C(27)	120.8 (6)
C(14)–B(1)–C(8)	105.3 (4)	C(30)–C(29)–C(28)	118.1 (6)
C(15)–C(14)–B(1)	123.8 (5)	C(30)–C(31)–C(26)	121.7 (6)
C(16)–C(15)–C(14)	123.4 (6)	C(31)–C(26)–B(1)	121.1 (5)
C(17)–C(16)–C(15)	120.4 (6)	C(31)–C(26)–C(27)	114.7 (5)
C(18)–C(17)–C(16)	118.5 (6)	C(31)–C(30)–C(29)	121.9 (6)

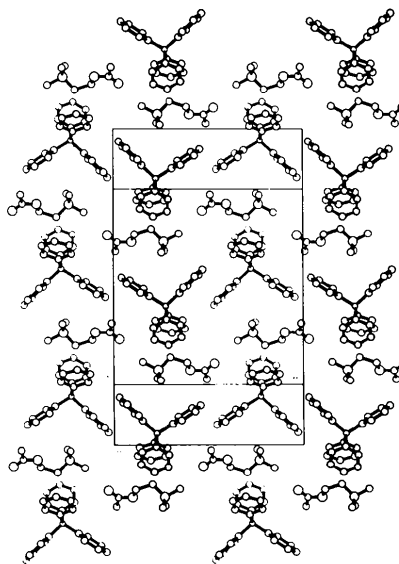


Fig. 2. Molecular packing of acetylcholine tetraphenylborate viewed down the  $-a^*$  axis.

Table 4. *Comparison of selected torsion angles (°)*

$\sigma(\tau) = 1.5^\circ$  for tetraphenylborate.

	Chloride	Bromide	Bromide*	Iodide	Perchlorate	Tetraphenyl- borate	$\beta$ -Resorcyate (a)	(b)
C(3)–N(1)–C(4)–C(5)	171.4	175.5	–174.8	–174.1	168.3	173.7	–179.9	–175.5
N(1)–C(4)–C(5)–O(1)	84.7	78.4	78.5	89	73.7	66.7	84.3	76.7
C(4)–C(5)–O(1)–C(6)	–166.9	78.9	78.5	83	179.8	154.6	158.8	163.4
C(5)–O(1)–C(6)–O(2)	5.2	4.1	4.7	–0.9	0.8	–5.4	1.9	0.06

\* N. Datta, P. Mondal & P. J. Pauling (unpublished).

Important intramolecular distances N(1)···O(1), C(1)···O(1), N(1)···C(6), N(1)···C(7) in this crystal are 3.07, 2.92, 4.38 and 5.22 Å respectively, in agreement with the results of Baker, Chothia, Pauling & Petcher (1971) for muscarinic agonists. There are no unusually short intermolecular distances in the structure. The molecular packing viewed down  $-a^*$  is shown in Fig. 2.

We thank Professor D. J. Jenden for the sample and John Cresswell for photographs. This work has been supported by the British Medical Research Council and the United States Institutes of Health under grant 2 ROI NS 12021 and the National Institute of Mental Health under grant 2 ROI MH 24284.

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