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Inhibitors of Acetylcholinesterase. Crystal Structure of Neostigmine Bromide

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The conformation of the potent competitive inhibitor of acetylcholinesterase neostigmine has been determined by X-ray diffraction analysis of crystals of the bromide and is consistent with the conformation proposed by Chothia and Pauling for substrates of the enzyme.

Neostigmine is a potent competitive inhibitor of the enzyme acetylcholinesterase[1] which plays a necessary role in cholinergic nervous transmission processes by hydrolyzing the nervous transmitter substance acetylcholine. We have analyzed the crystal structure of neostigmine bromide. The observed conformation of the molecule closely resembles the conformation of substrates of AChE proposed by Chothia and Pauling.[2]

Crystals of neostigmine bromide from BDH Ltd., recrystallized from 1:1:1 MeOH–Me_2CO–EtOAc, are monoclinic clear tabular plates, space group $P2_1/c$, a = 1605.3 (6), b = 778.1 (3), c = 1083.0 (5) pm, β = 92.85 (1)°, Z = 4 formula units per unit cell. A unique quadrant of data measured using ω–2θ step scans and Zr-filtered Mo Kα radiation in the range $2\theta \leqslant 45°$ together with unfiltered Mo K radiation in the range $40° \leqslant 2\theta \leqslant 60°$ on a computer-controlled[3] Stoe 4-circle diffractometer provided 5324 measurements which gave a total of 4133 unique observations and yielded 2619 unique observed intensities with $I \geqslant 3\sigma(I)$. The two data sets were placed on a common scale by least-squares fit of multiply observed reflections. The empirical absorption correction of Phillips,[4] modified for a 4-circle instrument, was applied and the data were corrected for long-term variation in the intensity of the incident radiation by standardization to the normalized value of a periodically measured standard diffraction maximum. The structure was solved by Patterson and Fourier methods and refined by the full-matrix least-squares technique with anisotropic thermal parameters for all atoms other than H and an overall thermal parameter for the H atoms to a present value of the residual R = 0.077. Full details of the structure analysis will be published elsewhere (T.J.P. in preparation). Some intramolecular distances and torsion angles and their associated esd's are presented in Table I.

A perspective drawing of the neostigmine molecule as observed in crystals of the bromide is presented in Figure 1, showing 50% probability ellipsoids of thermal vibration for the non-H atoms. The conformation is at first sight a 2-parameter problem. Only the torsion angles τ C(3)–N(1)–C(4)–C(5) and τ C(5)–C(6)–O(1)–C(10) may vary because the group C(11)–C(12)–N(2)–C(10)–O(2)–O(1)–C(6) is constrained to be planar by the amide bond N(2)–C(10) and the partial double-bond character of C(10)–O(1). Examination of a space-filling CPK model of the molecule, however, shows that τ C(3)–N(1)–C(4)–C(5) is constrained to be 0 or 180° by H–H repulsions and the only remaining variable parameter is τ C(5)–C(6)–O(1)–C(10). We find that the benzene ring is planar to within 0.9 pm and that the dimethyl carbamate group is planar to within 6 pm. The angle between the plane of the carbamate group and the plane of the benzene ring is 31.8°. This angle between the two planes corresponds to a torsion angle τ C(5)–C(6)–O(1)–C(10) of 148.2°. There is evidence of some form of intramolecular interaction between the C(7) H atom and the carboxyl oxygen atom O(2). The C(7)–H distance is long (116 pm), the O(2)–H distance is short (223 pm) for a van der Waals contact and, more significantly, the center of the electron-density peak for this H atom is displaced 35 pm out of the plane of the benzene ring towards O(2) which is 99 pm distant from this plane. The other three H atoms bonded to C(5), C(8), and C(9) are all within 5 pm of the plane. One of the methyl C atoms [C(3)] of the ammonium group lies in the plane of the benzene ring and τ C(3)–N(1)–C(4)–C(5) is 177°. The other two methyl C atoms of this ammonium group are symmetrically related to the benzene ring.

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(1) R. B. Barlow, "Introduction to Chemical Pharmacology," 2nd ed, Methuen, London, 1968.

(2) C. H. Chothia and P. J. Pauling, *Nature (London)*, **223**, 919 (1969).

(3) W. R. Busing, R. B. Ellison, H. A. Levy, S. P. King, and R. T. Roseberry, The Oak Ridge Computer-Controlled X-Ray Diffractometer, ORNL-4143 (1968).

(4) D. C. Phillips, A. C. T. North, and F. S. Mathews, *Acta Crystallogr.*, **A24**, 351 (1968).

TABLE I

SOME INTRAMOLECULAR DISTANCES[a] AND TORSION ANGLES[b] IN THE NEOSTIGMINE MOLECULE[c]

N(1)-O(1)	478.1 (8)
N(1)-C(10)	604.9 (9)
N(1)-O(2)	646.5 (8)
N(1)-N(2)	693.2 (9)
N(1)-C(11)	687.7 (12)
N(1)-C(12)	833.4 (14)
C(1)-N(1)-C(4)-C(5)	64.4 (7)
C(2)-N(1)-C(4)-C(5)	−55.3 (7)
C(3)-N(1)-C(4)-C(5)	177.5 (5)
C(5)-C(6)-O(1)-C(10)	153.6 (6)
C(6)-O(1)-C(10)-O(2)	5.5 (6)
C(6)-O(1)-C(10)-N(2)	−174.0 (6)

[a] Picometers. [b] Degrees. [c] Estimated standard deviations refer to the last significant digit quoted. The estimated standard deviations in parentheses are derived from the appropriate elements of the inverse matrix of the least-squares normal equations.

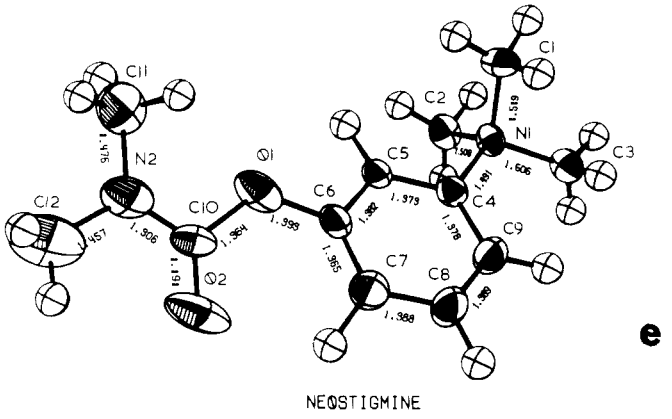

Figure 1.—A computer-drawn[5] perspective drawing of the molecule of neostigmine observed in crystals of the bromide. The angle between the planes of the dimethylcarbamate group and the benzene ring is 31.8°. Bond lengths are in angström units (1 Å = 100 pm).

This crystal structure analysis of a semirigid molecule which is a potent competitive inhibitor of acetylcholinesterase provides information about the conformation of both inhibitors and substrates of the enzyme. Although there are three carbon atoms between N(1) and O(1) in neostigmine and only two carbon atoms between the corresponding two atoms in ACh, the conformations of the two molecules are comparable because of the planarity of the intervening benzene ring in neostigmine. In particular, the observed conformation of neostigmine (Figure 2) is the same as that of substrates proposed by Chothia and Pauling[2] when they considered interaction of ACh (Figure 3) with the esterase.[6] The observed torsion angle τ C(10)-O(1)-C(6)-C(5) of neostigmine is the same as τ C(6)-O(1)-C(5)-C(4)

(5) C. K. Johnson, 'ORTEP, a Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, ORNL-3794, Revised (1965).

(6) This is not the conformation of ACh relevant to interaction with receptors. See C. H. Chothia and P. J. Pauling, *Proc. Nat. Acad. Sci. U. S.*, **65**, 477 (1970), C. H. Chothia, *Nature (London)*, **225**, 36 (1970), and C. H. Chothia, P. J. Pauling, and T. J. Petcher, in preparation.

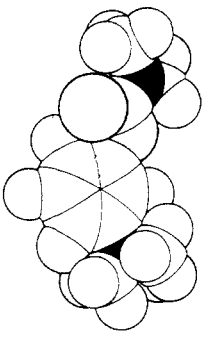

Figure 2.—A projection of the molecule of neostigmine in the orientation allowing comparison of the observed conformation of neostigmine with that conformation of ACh proposed as relevant to interaction with AChE.[2]

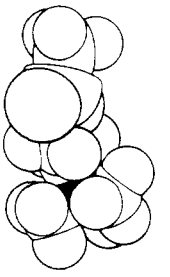

Figure 3.—A projection of the molecule of ACh in the conformation proposed as relevant to interaction with AChE.

of ACh (+150°). The carbamate group N(2)-C(10)-O(2)-O(1)-[C(6)-C(5)] and the Me_3N^+ group of neostigmine are in the same relative disposition as the AcO group C(7)-C(6)-O(2)-O(1)-[C(5)-C(4)] and the Me_3N^+ group of ACh. The carbamate group of neostigmine is about 133 pm further distant from the NMe_3^+ group than is the AcO group of ACh but AChE does not appear to be very specific to this distance.[1]

Since neostigmine is not an asymmetric molecule and both the conformation shown and its mirror image are present in crystals of the bromide, no information can be derived from this crystal structure analysis about the sign of the relevant torsion angles. It has been proposed,[2] however, that the arrangement of the AcO group with respect to the Me_3N^+ group involves a torsion angle τ C(6)-O(1)-C(5)-C(4) of +150 and not −150°. The conformation of neostigmine and of ACh consistent with this conclusion is that shown in Figures 2 and 3. Though the fit between the neostigmine molecule and the esterase must be good in order that neostigmine be a potent competitive inhibitor of the esterase, the compound is an inhibitor rather than a substrate because of the substitution of the Me_2N group for the Me group of substrates. No carbamates, carbamoylcholine for example, are substrates of AChE[1] and the enzyme appears to be very specific to the Me group.[7]

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(7) Personal communication from S. A. Bernhard.