A neutron diffraction study of an acetylcholine analogue-*erythro*(±)-α,β-dimethylacetylcholine iodide

Nuclear magnetic resonance (Culvenor & Ham, 1966) and x-ray diffraction studies (Canepa, Pauling & Sörum, 1966) of acetylcholine have established that the conformation of the choline residue (i.e., N^+-C-C-O atoms) is synclinal (*gauche*) both in deuterium oxide and in the solid state. It has been suggested (Canepa & others, 1966; Martin-Smith, Smail & Stenlake, 1967a) that this spatial arrangement owes at least part of its stability to N^+-C-H . . . O hydrogen bonding of the type proposed by Sutor (1963). Infrared data (Martin-Smith & others, 1967b) on this molecule have been interpreted to be consistent with the presence of C-H . . . O hydrogen bonds. The nmr spectra does not, however, substantiate the existence of such an interaction. X-ray studies of acetylcholine and many of its analogues have never been of sufficient accuracy to locate the protons and thus clarify this question. A neutron diffraction study of an acetylcholine analogue (*erythro*-(±) α,β-dimethylacetylcholine iodide) was undertaken to establish whether the synclinal conformation of the N^+-C-C-O system is in fact stabilized by C-H . . . O hydrogen bonding. The non-hydrogen atom structure of this molecule has been studied by x-ray methods (Shefter, Sackman & others, 1970) and shown to have a similar N^+-C-C-O conformation to that of acetylcholine.

Crystals of sufficient size for a neutron diffraction study (approximately 4 × 2 × 2 mm) were grown from an ethanol–ether solution. The crystallographic parameters obtained for these monoclinic crystals by x-ray diffraction (Shefter & others, 1970) were used in the neutron study. Intensity data were collected on an automated four-circle diffractometer with a neutron wavelength of 1·038 Å and to a maximum *two theta* value of 85°. These data were processed in the usual manner correcting for the Lorentz factor and absorption to obtain structure amplitudes. An extinction correction was applied during the structure refinement. All of the hydrogen positions were located from a difference Fourier synthesis using the non-hydrogen positions determined by x-ray diffraction as the phasing model. Positional and thermal parameters have been refined by a full matrix least squares method to a current R factor (usual reliability index) of 0·080 for 2084 observed reflections. The standard errors obtained for the bond lengths are on the average 0·008 Å for those involving non-hydrogen atoms and about 0·02 Å for the non-hydrogen to hydrogen distances. A detailed structural report will be published.

The upper limit for the distance between a hydrogen and an oxygen atom at which the two atomic species might be considered as forming a hydrogen bond is 2·4 Å (Hamilton, 1968). This value is 0·2 Å less than the sum of the van der Waals radii of

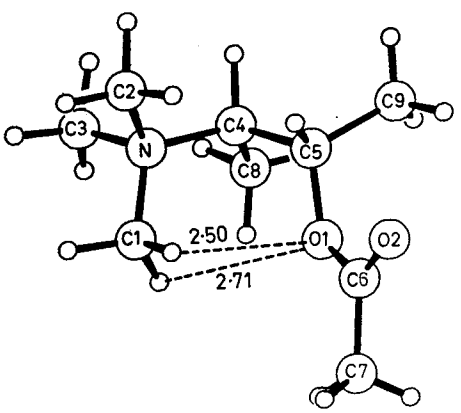

FIG. 1

the respective atoms. The neutron diffraction study (Coppens, 1964) of *o*-nitrobenzaldehyde provides experimental justification for the use of this limiting distance.

The hydrogen atoms which are closest to the acetoxy oxygen [O(1)] are clearly denoted in Fig. 1. The H . . . O distances indicate that hydrogen bonding between the cationic portion of the molecule and the ester linkage is not involved in the stabilization of the conformation of the choline moiety. This is further supported by the C(1)-H . . . O(1) angles which deviate substantially from linearity (100° and 88°). The stabilization of the *synclinal* arrangement of the N^+-C-C-O grouping is in all probability greatly influenced by the electrostatic interaction between the basic portion of the molecule (ester linkage) and the acidic quarternary nitrogen (Shefter & Mautner, 1969).

Mathieson (1965) has shown that the preferred conformation for secondary esters has a synperiplanat (0° ± 30°) distribution of H-C-O-C angles [H-C (5)-O(1)-C(6) in this structure]. The possibility has been raised that this conformation might be stabilized by a C(5)-H . . .O(2) hydrogen bond. This is based on the fact that a torsion angle of 0° would make the H to O(2) distance approximately 2·2 Å (well within the correct range for such an interaction). In the *erythro*-acetylcholine analogue the O(2) . . . H distance is 2·46 Å and the torsion angle [H-C (5)-O(1)-C(6)] is −35° for the $\alpha(R)$ $\beta(S)$ isomer. Again the possibility of C-H . . . O hydrogen bonding as a mode of conformational stabilization has been eliminated. In fact, the hydrogen has a repulsive effect on the conformation of the C(5)-O(2) ester bond.

The relevance of the conformational parameters to cholinergic activity of this molecule has been discussed at some length (Shefter & others, 1970; Shefter, 1970) and requires no further comment here.

This work was supported in part by a Public Health Service Grant (CA-10104) to Eli Shefter, and by the United States Atomic Energy Commission.

We are also indebted to Prof. E. E. Smissman of the University of Kansas for supplying the material.

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June 18, 1970

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