The Proton Magnetic Resonance Spectrum and Conformation of Acetylcholine

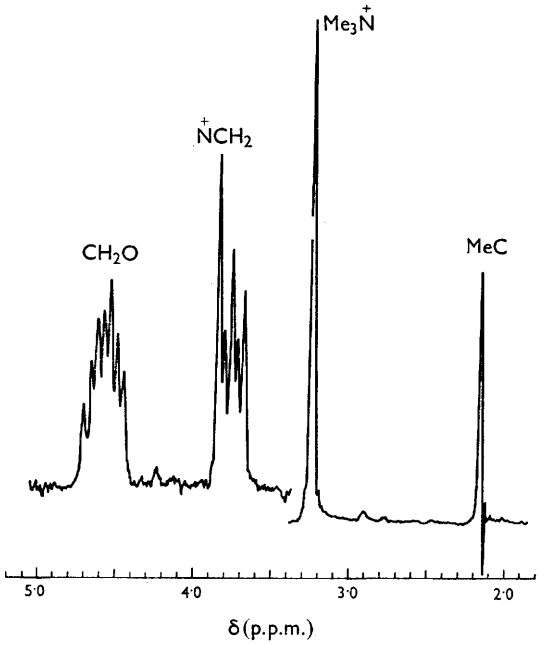
By C. C. J. CULVENOR and NORMAN S. HAM

[*Division of Organic Chemistry, C.S.I.R.O. Chemical Research Laboratories, Melbourne, Australia* (*C.C.J.C.*) *and Division of Chemical Physics, C.S.I.R.O. Chemical Research Laboratories, Melbourne, Australia* (*N.S.H.*)]

THE importance of acetylcholine in the transmission of nerve impulses has led to numerous attempts to define its conformation in aqueous solution.[1–3] We report a study of the proton magnetic resonance spectrum of acetylcholine in deuterium oxide which leads to the conclusion that the $^{+}$N–C–C–O system is in a *gauche*-arrangement and that the CH_2–O–CO–Me grouping has the normal conformer populations of a primary ester; on this basis the mean conformation of the molecule in aqueous solution is probably best depicted as in (I).

Canepa, Pauling, and Sorum[4] have just announced a crystal structure of acetylcholine bromide (II) based on a recalculation of the earlier results of Sorum.[1] This has a *gauche* $^{+}$N–C–C–O system but has an ester conformation markedly different from (I). We comment on this difference below.

The 60 Mc./sec. spectrum of acetylcholine chloride in D_2O solution is shown in the Figure. The four methylene protons show an unsymmetrical pattern with the low-field multiplet somewhat broader. Choline chloride shows similar methylene and $^{+}NMe_3$ signals apart from an overall high-field shift. This is largest for the low-field multiplet, which is thus assigned to the CH_2O group. Both molecules have two groups of equivalent methylene protons since each spectrum may be analysed as an A_2B_2 system in which the asymmetry arises because of a tripling of the low-field multiplet by an additional coupling of about 2c./sec. between the CH_2O group and the $^{+}$N atom (nuclear spin 1). In quaternary nitrogen compounds, the three-bond *NCCH* coupling is known to be larger (1·5—2·0 c./sec.) than the 2-bond *NCH* coupling (0—0·6 c./sec.).[5–8] This supports the assignment of the low-field multiplet to the CH_2O grouping. Taking N- and L-values ($N, L = J + J'$ where J, J' are the vicinal coupling constants) from appropriate line separations and refining to a best fit with the observed spectrum by means of an iterative calculation, the following parameters for acetylcholine are obtained: N, 9·49 c./sec., L, 4·43 c./sec. Assuming that the vicinal couplings are positive, the values are 7·0 and 2·5 c./sec.

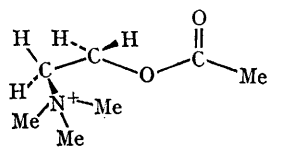


FIGURE

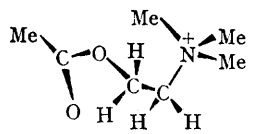
Varian A60 *proton magnetic resonance spectrum of acetylcholine chloride in* D_2O *with* δ *the downfield chemical shift in p.p.m. from the sodium* 3-*trimethylsilylpropane*-1-*sulphonate as internal reference.*

For 1,2-disubstituted ethanes, n.m.r. data have been used in favourable instances by Abraham and Pachler to determine which conformer predominates.[9] For the $^+NCH_2$–CH_2–O system, conformers (III), (IV), and (V) are possible. Assuming 60° dihedral angles and only two vicinal coupling constants, J_t and J_g, and defining L as $J_{\alpha\beta} - J_{\alpha'\beta}$, then the spectral quantity $\frac{3}{2}N + \frac{1}{2}L$ is equal to $J_t + 2J_g$, and independent of conformer population. Which conformer predominates follows from determination of the sign of L. L is negative if the *trans*-form is more stable, positive if the *gauche*-form is more stable, and zero if the conformers are of equal energy. The expected constancy of $\frac{3}{2}N + \frac{1}{2}L$ for spectra run in different solvents was found to be of no value for the sign determination; no spectra could be obtained in non-polar solvents and spectra measured in D_2O, D_2O–pyridine, and trifluoroacetic acid have essentially the same N, L-values.

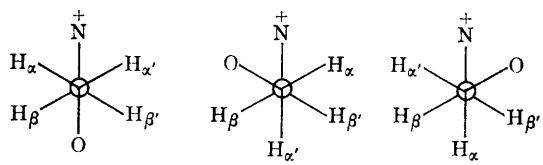
Abraham and Pachler have also demonstrated an approximate linear relationship between $J_{av} = \frac{1}{3}(J_t + 2J_g) = \frac{1}{2}N + \frac{1}{6}L$ and ΣE, the sum of the electronegativities of the substituents attached to the C–C fragment concerned. For acetylcholine in



(I)



(II)



(III) *trans* (IV) *gauche* (V) *gauche*

D_2O, $J_{av} = 4{\cdot}0$ if L is negative and 5·5 if L is positive. The corresponding ΣE-values from their relationship would be 17·5 or 15·5. Since acetylcholine has $\Sigma E \sim 15{\cdot}3$,[10] this argument strongly suggests that L is positive, and that the *gauche*-forms predominate.

It is noteworthy that the magnitude of L for acetylcholine is larger than in any of the nine XCH_2–CH_2Y compounds examined by Abraham and Pachler, despite a small value for N. This implies that acetylcholine exists largely in one conformation. The J,J'-values also support the *gauche*-conformation, for if (IV) and (V) predominate then $J' = 2{\cdot}5$ approaches J_g whereas $J = 7{\cdot}0$ approaches $\frac{1}{2}(J_t + J_g)$: on this basis J_t would be 11·5 c./sec. However, if the *trans*-form (III) predominates the small coupling is again J_g and the 7·0 c./sec. coupling becomes J_t, which is abnormally low. The electronegativity of substituents could not account for such a low value of J_t since, as already shown, this argues for a *gauche*-conformation.

Thus the n.m.r. evidence for acetylcholine favours a high proportion of a conformation which is *gauche* about the $\overset{+}{N}$–C–C–O bond. The *gauche*-conformation is apparently the preferred form of many 1,2-disubstituted ethanes of this type, both insolution, *e.g.*, $HOCH_2{\cdot}CH_2OH$, $MeOCH_2{\cdot}CH_2OH$,

and $MeOCH_2{\cdot}CH_2O{\cdot}CO{\cdot}Me$,[9] and in crystal structures, *e.g.*, choline chloride,[11] L-α-glycerophosphorylcholine.[12]

The CH_2O proton shifts for choline and acetylcholine are 4·02 and 4·53 p.p.m. respectively; the difference, 0·51 p.p.m., is a normal primary acylation shift and implies that the conformer populations of the ester system are normal.[13] Infrared evidence is also in agreement with acetylcholine having a normal ester group.[14] The conclusion drawn from n.m.r. evidence[13] was that in a primary ester the lowest-energy states have the hydrogen atoms on the α-carbon of the alcohol moiety essentially *cis* to the carbonyl group. They may be (i) symmetrically placed about the plane of the ester group; (ii) eclipsing in turn the carbonyl group as in secondary esters; or (iii) possibly occupying an in-between position. The *X*-ray structures of primary esters have been reported to favour the symmetrical arrangement[15] but this is not the case for the acetylcholine bromide structure (II) of Canepa *et al.*[4] In (II) the CH_2O protons apear to be in positions which are unusual in the light of the above evidence. Structure (II) is apparently not a major conformer of acetylcholine in aqueous solution; it would be consistent with an A_2B_2 spectrum if it were interconverting rapidly with its mirror image but it would be expected to give a smaller than normal acylation shift.

We thus conclude that in aqueous solution acetylcholine is best represented as in (I).

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