# The Conformation of Acetylthiocholine in Solution

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*Summary* It is concluded from $^1H$ n.m.r. spectra that acetylthiocholine has a *trans* $^+N$–C–C–S conformation in solution.

FROM structural data, largely measured in the crystalline state, Chothia and Pauling[1,2] have recently concluded that the conformation of cholinergic molecules effective at both muscarinic and nicotinic receptors has a *gauche* arrangement of the N–C–C–O system, or its equivalent. The *trans*-N–C–C–S system for acetylthiocholine iodide, found in an *X*-ray diffraction study,[3] is at variance with this conclusion, since this compound has a weak muscarinic action but a strong nicotinic action at ganglionic synapses.[4] However, with such flexible molecules there is a need for conformational data relating directly to aqueous solutions. From an analysis of the n.m.r. spectrum we show that acetylthiocholine has the *trans*-conformation also in deuterium oxide.

The $^1H$ n.m.r. spectrum of acetylthiocholine in $D_2O$ at 100 MHz shows two multiplets centred about 3·3 and 3·5 p.p.m. downfield from the reference, sodium 3-trimethylsilylpropane 1-sulphonate. The $Me_3N^+$ peak occurs at 3·2 p.p.m., while the methyl peak of the $CH_3CO{\cdot}S$ fragment appears at 2·4 p.p.m. The $-CH_2-CH_2-$ multiplets are not symmetrical; the low-field one shows eight lines, whereas the higher-field one is more complex. Part of the high-field multiplet is masked by the edge of the $Me_3N^+$ peak and one band shows a distinct tripling. One expects the protons adjacent to the positively charged nitrogen to resonate at a lower field than those attached to the less electronegative S atom. The spectral asymmetry could then arise from a tripling within the high-field multiplet due to coupling between $^{14}N$ (spin 1) and the $\beta$-protons attached to sulphur.[5]

The multiplets were analysed as an AA′BB′ system. The chemical-shift difference between the two sets of protons, of about 20 Hz at 100 MHz, is less than the probable sum of the geminal coupling constants ($K = J_{AA'} + J_{BB'}$), so that the spectrum should not be particularly sensitive to the value of $K$. The two central lines of the $K$ (symmetric) quartet were identified by finding that pair of lines which has the same average frequency as the pair split by N, the sum of the vicinal coupling constants ($J,J'$). The spectrum superficially looks as if $M$ (defined as $J_{AA'} - J_{BB'}$) could be zero. Trial calculations showed that if the two outside members of the $M$ (antisymmetric) quartet were assumed to be masked by any of the other bands, the relative intensity relationships were wrong unless $M$ was less than 0·5 Hz. An iterative calculation on the eight lower-field lines refined the parameters $N$ and $L$ (equal to $J - J'$) to the values 16·4 and 6·45 Hz, respectively. The high-field multiplet was then used to obtain refined values for $M$ and the $^{14}N$–$^1H$ coupling constant. These were 0·3 Hz for $M$ and 0·75 Hz for $J(NCCH)$.

For a 1,2-disubstituted ethane, Abraham and Pachler[6] have indicated two methods by which the sign of $L$, and hence the preferred conformer, may be determined. One method involves spectra run in a series of solvents and the other uses a correlation between spectral parameters and the electronegativities of the substituents. For acetylthiocholine the sum of the CC-substituent electronegativities, $\Sigma E$, is about 14·3, so that their relationship, $18{\cdot}0 - 0{\cdot}8\,\Sigma E$, gives a value of 6·6 Hz, which is to be compared with the experimental quantity $\frac{1}{2}N + \frac{1}{6}L$; this is 9·3 Hz for $L$ positive and 7·1 Hz for $L$ negative. This comparison indicates therefore that $L$ is negative and that the *trans*-conformer predominates.[6] Similar positive deviations from the relationship are also observed for other *trans*-1,2-disubstituted ethanes.

This interpretation of the sign of $L$ involves the simplifying assumptions (a) that all dihedral angles have the ideal values 60° or 180°, and (b) that the coupling constants will be the same for any two vicinal protons in a *gauche*-relationship.[6,7] More recent work[7,8] shows that the *gauche*-vicinal coupling constants in the *trans*- and *gauche*-conformers are different, and that in the *trans*-conformer the *gauche*-vicinal coupling $J_G^T$ is rather insensitive to the nature of the substituents.[7] In the present instance the two vicinal coupling constants, $J$ and $J'$, have values of 5·0 and 11·4 Hz, respectively. Consideration of the algebra involved shows that this can only be so if the *trans*-conformer is strongly preferred. The smaller coupling constant, 5·0 Hz, then approaches $J_G^T$ and the value here is very similar to those reported by Abraham *et al.* for *trans*-conformers.[7]

There is a general paucity of data for proton chemical shifts in thiols and thioesters and the conformation in solution of the $S{\cdot}CO{\cdot}CH_3$ grouping relative to the NCCS chain cannot be defined from n.m.r. data at this time.

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