

## The Conformation of Acetylthiocholine in Solution

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

FROM structural data, largely measured in the crystalline state, Chothia and Pauling<sup>1,2</sup> have recently concluded that the conformation of cholinergic molecules effective at both muscarinic and nicotinic receptors has a *gauche* arrangement of the  $\text{N}-\text{C}-\text{C}-\text{O}$  system, or its equivalent. The *trans*- $\text{N}-\text{C}-\text{C}-\text{S}$  system for acetylthiocholine iodide, found in an X-ray diffraction study,<sup>3</sup> is at variance with this conclusion, since this compound has a weak muscarinic action but a

strong nicotinic action at ganglionic synapses.<sup>4</sup> 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  $^1\text{H}$  n.m.r. spectrum of acetylthiocholine in  $\text{D}_2\text{O}$  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  $\text{Me}_3\text{N}^+$  peak occurs at 3.2 p.p.m., while the methyl peak of the  $\text{CH}_3\text{CO}\cdot\text{S}$  fragment appears at 2.4 p.p.m. The  $-\text{CH}_2-\text{CH}_2-$  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  $\text{Me}_3\text{N}^+$  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  $^{14}\text{N}$  (spin 1) and the  $\beta$ -protons attached to sulphur.<sup>5</sup>

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 ( $K = J_{\text{AA}'} + J_{\text{BB}'}$ ), 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  $J_{\text{AA}'} - J_{\text{BB}'}$ ) 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  $^{14}\text{N}$ - $^1\text{H}$  coupling constant. These were 0.3 Hz for  $M$  and 0.75 Hz for  $J(\text{NCCH})$ .

For a 1,2-disubstituted ethane, Abraham and Pachler<sup>6</sup> 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,  $18.0 - 0.8 \Sigma E$ , gives a value of 6.6 Hz, which is to be compared with the experimental quantity  $\frac{1}{2}N + \frac{1}{2}L$ ; 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.<sup>6</sup> 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  $60^\circ$  or  $180^\circ$ , and (b) that the coupling constants will be the same for any two vicinal protons in a *gauche*-relationship.<sup>6,7</sup> More recent work<sup>7,8</sup> 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.<sup>7</sup> 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.<sup>7</sup>

There is a general paucity of data for proton chemical shifts in thiols and thioesters and the conformation in solution of the  $\text{S-CO-CH}_3$  grouping relative to the NCCS chain cannot be defined from n.m.r. data at this time.

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