# Conformations in solution of some cholinergic and anticholinergic compounds

Interest in the structure-activity relations of cholinergic compounds has prompted detailed X-ray analyses of acetylcholine, *S*(+)-acetyl-β-methylcholine, *R*(+)-acetyl-α-methylcholine, L-(+)-muscarine and L-(+)-2(*S*)-methyl-4(*R*)-trimethylammonium-methyl-1,3-dioxolan iodide (for relevant references see Chothia, 1970). As a result of these studies the conformational similarity of these compounds in the crystal phase is now well established. Additionally it has been suggested, from a consideration of the magnitude of stereodependent vicinal proton–proton coupling constants from nmr studies, that acetylcholine has a similar preferred conformation in $D_2O$ solution (Culvenor & Ham, 1966). However, since the generality and accuracy of this type of approach for determining the conformation in solution of cholinergic (and anti-cholinergic) compounds remains to be established and since it is not known whether all the above cholinergic compounds are conformationally similar in crystal form and in solution we have attempted to use two other methods to assess the solution conformation of the O–C–C–N chain of acetyl α- and β-methylcholines. The first method depends on reports that $^{14}$N-H vicinal coupling constants are stereodependent (Mooney & Winson, 1969) and the second method utilizes reports that the molecular rotations of chiral molecules may be calculated and are conformation dependent (Lemieux & Martin, 1970).

*Method* 1. The quadrupole moment associated with the $^{14}$N nucleus (spin I = 1) precludes observation of spin–spin coupling to nitrogen in all but a few compounds. However, when there is a highly symmetrical field gradient about the nitrogen nucleus, such as in quaternary salts, appreciable vicinal coupling constants may be observed particularly at high temperatures with $D_2O$ and DMSO as solvent. Such couplings are stereodependent; for example, for vicinal $^{14}$N–H dihedral angles of 60°, 80°, 120° and 0° $J_{^{14}N\text{-}H}$ is <1, <1, <1 and 1–3 Hz respectively (Terui, Aono & Tori, 1968), and more recently it has been shown that for compounds in which the vicinal dihedral angle is 180° $J_{^{14}N\text{-}H}$ was 6·9–9·7 Hz (Bothner-By & Cox, 1969).

The nmr spectrum of acetyl α-methylcholine (I) has been measured in $D_2O$ at 90° and found to be first order.* The β-protons at δ 4·48 ppm were equivalent and appeared as a pair of superimposed triplets as a consequence of coupling with the α-proton ($J$ = 4 Hz) and with $^{14}$N ($J$ = 2 Hz). Depending on values assigned to $J_{gauche}$ and $J_{trans}$ the value of 4 Hz for $J_{H\alpha,\,H\beta}$ is consistent with a rapidly equilibrating mixture of conformers A and B or of a mixture of A, B and C (Fig. 1). However the vicinal $J_{^{14}N\text{-}H}$ value of 2 Hz is similar to that shown by acetylcholine where an antiperiplanar N–C–C–O arrangement was thought unlikely (Culvenor & Ham, 1966), whereas in acetylthiocholine and acetylselenocholine, where an antiperiplanar N–C–C–S(Se) arrangement was favoured, the vicinal $J_{^{14}N\text{-}H}$ value was < 0·7 Hz (Cushley & Mautner, 1970). Thus it is unlikely that conformer C of acetyl α-methylcholine (I) is present to any appreciable extent in solution. This result indicates a difference between the solution and crystal structures of I since crystallographic data indicated that both conformers B and C were important (Chothia & Pauling, 1969).


I R = $CH_3$ III R = $CH_3$

II R = $Ph_2C(OH)$- IV R = $Ph_2C(OH)$-

* It was necessary to measure $^{14}$N–H coupling constants at 90° in order to sufficiently reduce the relaxation time of the N nucleus to allow the appearance of well resolved $^{14}$N–H couplings.

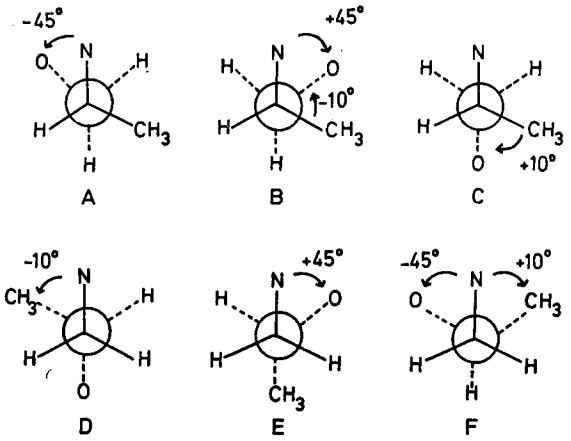



FIG. 1. The conformers of *S*(—)acetyl-α-methylcholine (A, B and C), and *S*(+)acetyl-β-methylcholine (D, E and F) are shown, together with the contributions (e.g. −45°⟋) of various gauche contributions to molecular rotation.

It is of interest that an anticholinergic analogue of I, benziloyl α-methylcholine (II), shows a similar spectrum ($J_{H\alpha H\beta}$ = 4Hz, $J_{^{14}N-H}$ = 2Hz) to I and can be confidently assigned a similar conformation in solution.

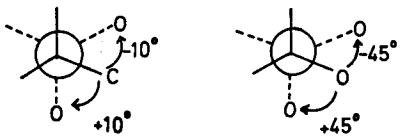
The spectrum of acetyl β-methylcholine (III) in $D_2O$ at 90° was also first order with non-equivalent α-protons at δ 3·58 and 3·82 ppm with vicinal coupling constants of 2·8 and 8·5 Hz respectively. These coupling constants are consistent with conformers D and E but not with F (Fig. 1). Conformer F was also precluded because no vicinal $^{14}N$–H coupling was detected and only F should show such a coupling. Distinction between conformers D and E may be made from a consideration of the magnitude of the $J_{gauche}$ coupling constant. It is well known that gauche coupling constants are smaller when one proton is antiperiplanar to an electronegative substituent, such as in E, than otherwise, such as in D where the gauche coupling would be expected to be nearer 5Hz than the observed 2·8Hz (Booth, 1965). Thus it appears that for acetyl β-methylcholine conformer E is favoured in solution and in crystal form (Chothia & Pauling, 1969a).

The anticholinergic analogue of III, benziloyl β-methylcholine (IV) did not give a first order spectrum and no vicinal $^{14}N$–H coupling could be detected. Thus as for III, conformation F is unlikely to be present to any significant extent in solution.

It is encouraging that with the choline-like compounds examined, for which H–H couplings have been measured, there is good agreement between results based on these values and those based on $^{14}N$–H couplings. Additionally the conformational assignments to I and III in solution by the nmr method were in agreement with those obtained by molecular rotational considerations. It is recognized that the preferred conformation of acylic molecules in solution can depend on the temperature of the solution but since for the choline like molecules described the vicinal H-H couplings showed no significant variation with temperature increase there is no reason to suppose that the solution conformations at room temperature differed from those at 90°.

*Method* 2. Molecular rotations ($[M]_D$) of chiral molecules may be calculated using the empirical treatment of Lemieux & Martin (1970). In their approach only relations between *gauche* situated groups are considered to contribute significantly to the overall molecular rotation, and for example a *gauche* C/O relation is assigned a value of 10° and a gauche O/O relation a value of 45°. The signs of these *gauche* contributions to

molecular rotation are illustrated:




Since the molecular rotations of methyl 4,6-*O*-benzylidene-α-D-altropyranoside ($[M]_D$ + 350°) and its 3-deoxytrimethylammonium derivative ($[M]_D$) + 370°) show only a small difference it has been assumed for the purposes of the present discussion that $\overset{+}{N}$/O and $\overset{+}{N}$/C contributions to molecular rotation differ little from O/O and O/C contributions. Thus the measured molecular rotation* of +70° for acetyl S(+)-β-methylcholine suggests that in solution conformer E preponderates (calculated $[M]_D = +45°$, Fig. 1) since the occurrence of conformers D and F or a mixture of conformers would cause a marked reduction in the magnitude or change in the sign of the calculated rotation, or both. Similarly, the measured molecular rotation* of *S*(−)-acetyl-α-methylcholine (−18°) is most nearly consistent with a mixture of conformers A and B in approximately equal proportions (calculated $[M]_D = -5°$, Fig. 1) since no other conformer or mixture of conformer gives a calculated $[M]_D$ of similar sign and magnitude. These results therefore suggest that if more realistic values can be obtained for $\overset{+}{N}$/C and $\overset{+}{N}$/O *gauche* contributions toward molecular rotation, the method of comparing observed and calculated molecular rotations may provide accurate information about the relative proportions of conformers in chiral cholinergic and anticholinergic compounds.

In summary, both the nmr and molecular rotational data indicate that acetyl β-β-methylcholine has a similar conformation in solution to that found in the crystal lattice. For acetyl α-methylcholine the nmr, molecular rotation and X-ray data indicate that two or more conformations are equally favoured and that the preferred conformations in solution and in the crystal lattice may differ.

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* Molecular rotations are calculated from the specific rotations quoted by INCH, T. D. & LEWIS, G. J. (1970), *Carbohydrate Res.* in press.