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# NMR STUDY OF GANGLION-BLOCKING AND CURARE-LIKE DIMETHONIUMS CONFORMATION IN AQUEOUS SOLUTIONS

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## 1. Introduction

At present the isolation of the Acetylcholine (Ach) receptors are the subject of many investigations [1, 2] and several studies concerning the conformation of cholinergic compounds related to Ach are undertaken [3, 4]. We report here on the conformation, in water solution, of dimethoniums with general formula:

$$(CH_3)_3\,\overset{+}{N}{-}(CH_2)_n{-}\overset{+}{N}\,(CH_3)_3,\ 2\,X^-$$

It is a well known fact that the biological activity of these bications varies with "n" [5]. From these findings several authors have attempted to relate the activity to the cation structure [6, 7]. The only known results are those obtained from crystal datas by X-ray diffraction where all methoniums have a *trans* conformation [4, 8, 9]. It is interesting and perhaps biologically more significant to study the molecular conformation in water solutions. We used high resolution NMR spectroscopy and studied methoniums from the trimethonium (n = 3) to the decamethonium (n = 10). We shown that the $\overset{+}{N}$-$CH_2{-}CH_2$-moieties are blocked in *trans* conformation in every case. However, compounds which have blocking activity exist in solution in many conformation states.

## 2. Materials and methods

Methoniums with n = 3 and 4 are a gift from Mrs. Y. Barrans who synthesised them. Compounds n = 5, 6, 10 are Fluka products. NMR spectra were obtained on a HA-100 Varian spectrometer in $D_2O$ solution at concentrations varying from 0.25 to 1 mole/1. We used either external TMS lock or a water soluble silane as internal reference.

## 3. Results

Until n = 6 we observed one individual multiplet for each chemically different proton, as can be seen in figs. 2–5. When n > 6 all remaining protons give an additional line at the same position as the $\gamma$ methylene protons. Chemical shifts are summarized in table 1.

The $\alpha$ methylene protons are coupled to those of the $\beta$ methylene and to the nitrogen nucleus, however, J $^{14}$NCH is small [12], so we have neglected it, in a first approximation. The $\alpha$ signal is a quintuplet and its shape does not vary from trimethonium (n = 3) to the decamethonium (n = 10). This structure indicates that free rotation between the $\alpha$–$\beta$ bond does not occur. On the other hand, the spectrum is not sensitive to a temperature increase from 25 to 90°, nor to a pH or solvent change. So, there is not an equilibrium between rotamers but $\alpha$ and $\beta$ methylenes groups are blocked either in *trans* or *gauche* conformations.

The multiplet was then analysed as the AA′ part of an AA′BB′ spectrum. Using classical parameters [13] we obtained directly on the spectrum $|L| = |J - J'| = 6.9$ Hz and $N = J + J' = 17.5$ Hz. To determine the $L$ sign we used the well known Abraham and Pachler [14] law connecting $\bar{J} = \frac{1}{3}(J_{trans} + 2\,J_{gauche})$ to the sum of the $C_\alpha{-}C_\beta$ substituent electronegativities $\Sigma E_i$. For dimethoniums $\Sigma E_i = 14.02$, so the expected value is $\bar{J} = 6.8$ Hz, which is to be compared to the ex-

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Table 1

| ppm/TMS | $^{+}N(CH_3)_3$ | $CH_{2\alpha}$ | $CH_{2\beta}$ | $CH_{2\gamma}$ |
|---|---|---|---|---|
| n = 3 | 3.29 | 3.54 | 2.43 | |
| n = 4 | 3.25 | 3.53 | 1.98 | |
| n = 5 | 3.23 | 3.45 | 1.97 | 1.53 |
| n = 6 | 3.10 | 3.30 | 1.80 | 1.38 |
| n = 10 | 3.07 | 3.23 | 1.67 | 1.25 |

perimental ones: $\overline{J}$ = 9.97 Hz if $L$ is positive, $\overline{J}$ = 7.6 Hz if $L$ is negative. This comparison proves that $L$ must be negative and consequently the *trans* conformer predominates. The difference between measured 7.6 Hz, and calculated value, 6.8 Hz, is similar to deviations usually observed, every time the *trans* rotamer exists.

Rigorous calculations of AA′ part of the spectrum with: $J_t$ = 12.2 Hz, $J_g$ = 5.3 Hz and $J_{gem}$ = –12.0 Hz conduce to a good agreement with the experimental spectrum as can be seen on fig. 1.

To find out the conformational state of methylenes further than the β group, we made a priori calculations of the whole spectrum with a modified Laocoon 3 program adapted to a 360/44 IBM computer. We did not attempt to use iterative processes, nor to assign the lines because the experimental multiplets are not sufficiently well resolved compared to the expected ones. Likewise we have not measured splittings less than 1 Hz and we have neglected the $^{14}$NCCH coupling for β protons. For comparison, we calculated the whole six spins system spectrum (fig. 2) for the trimethonium which is all *trans* from previous results. The calculated spectrum with only previously determined coupling constants agrees fairly well with the experimental one.

For tetramethonium neither the eight spins system spectrum calculated for all *trans* conformation (fig. 3a), nor that corresponding to the free rotation around the ββ′ methylene groups (fig. 3b) are in good agreement with the experimental one. However, the fitting was found to be somewhat better in the former case.

Concerning pentamethonium (fig. 4), the eight spins spectrum corresponding to αβγβ′ groups was calculated with αβ groups *trans* blocked and free rotation for the others. It is in good agreement with the experimental spectrum.

For hexamethonium (fig. 5) the spectrum looks like that of pentamethonium. Moreover, the calculated

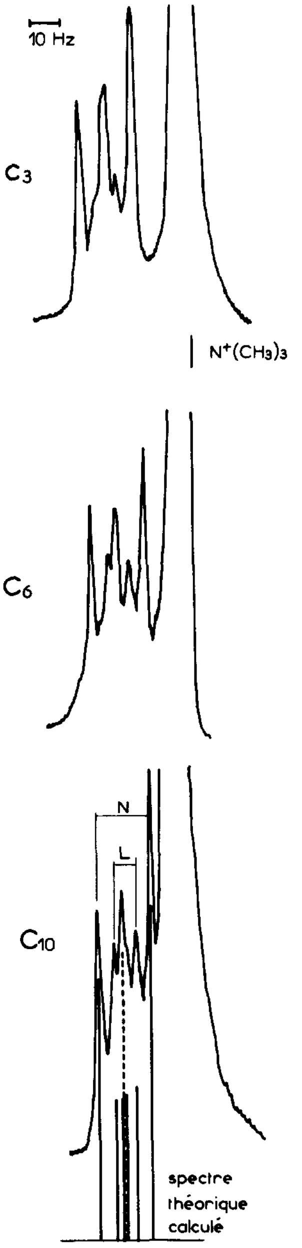

Fig. 1. Experimental and calculated spectra for α methylene protons.

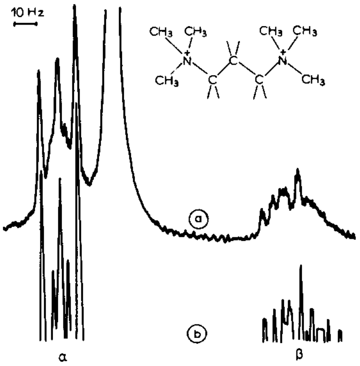

Fig. 2. Trimethonium: a) experimental; b) calculated.

line for γ protons in the case of free rotation agrees quite well with the experimental one.

## 4. Discussion

The above finding strongly suggests that the α and β methylene groups of dimethoniums in aqueous solution are blocked in *trans* conformation, oppositely to the cholinergic compounds of the Ach serie which are in *gauche* conformation. Moreover all the methylenes of the corresponding tertiary amines, like the NN dimethyl-1, 6 diamino hexane (cf. fig. 5c) are freely rotating. So a $(CH_3)_3X$ voluminous moiety is necessary to block αβ groups. Notice that when there are no interactions between $(CH_3)_3X$ and γ substituent, the *trans* conformation exists either with a charged $(CH_3)_3\overset{+}{N}$ group, or with non polar groups like in $(CH_3)_3C{-}CH_2{-}CH_2Cl$ [14] and $(CH_3)_3SiCH_2{-}CH_2{-}CH_2SO_3^-$.

Since the α and β groups are blocked, the trimethonium and possibly the tetramethonium are in solution, as in the solid state, only in the all *trans* conformation. When $n \geqslant 5$ only α and β groups remain blocked, so they exist in solution in various conformations, oppositely to the solid state. Nevertheless the number of rotamers is smaller than expected; indeed for pentamethonium only 9 rotamers are likely (with only 4 different) and 27 for hexamethonium, instead of 81 and 243, respectively, in the homologous alkane chain. Nevertheless this leads to $3^7$ conformations for decamethonium, a really very large number.

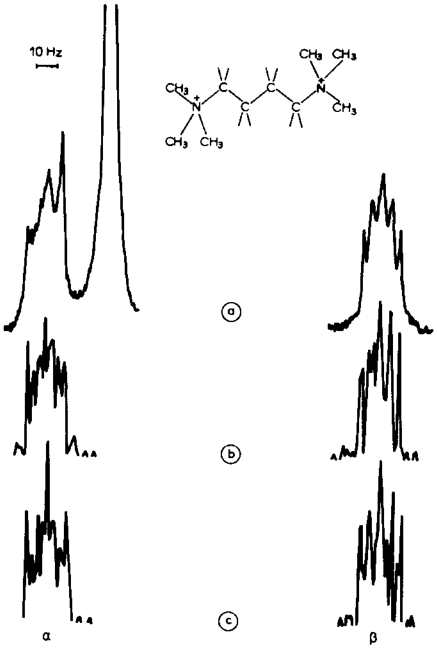

Fig. 3. Tetramethonium: a) experimental ; b) calculated TTT; c) calculated TLT.

From these remarks, it seems to be possible to calculate the probability distribution versus the $N^+{-}N^+$ distance for each compound, and to correlate this function to the blocking activity. This work has been done by E.W. Gill [7], under several assumptions and it is interesting to discuss now two of them. Firstly, the assumption that there is the same energy difference between *trans* and *gauche* rotamers as in alkane compounds is true for central methylenes groups, but not for α and β groups. This leads to distributions curves very different from that computed by E.W. Gill. Secondly, it seems clear now that conformational equilibrium can be drastically changed, when going from solute to adsorbed state [15]. So when the biological bication is fixed to the receptor, interaction energies are able to alter conformational equilibriums, especially if the energy difference between conformers is small compared to interaction energy.

From the above results, we can suggest that:

i) The *trans* conformation for α and β methylenes is preserved, the energy necessary to change it being higher than thermal energy.

ii) For central methylenes, the energy difference

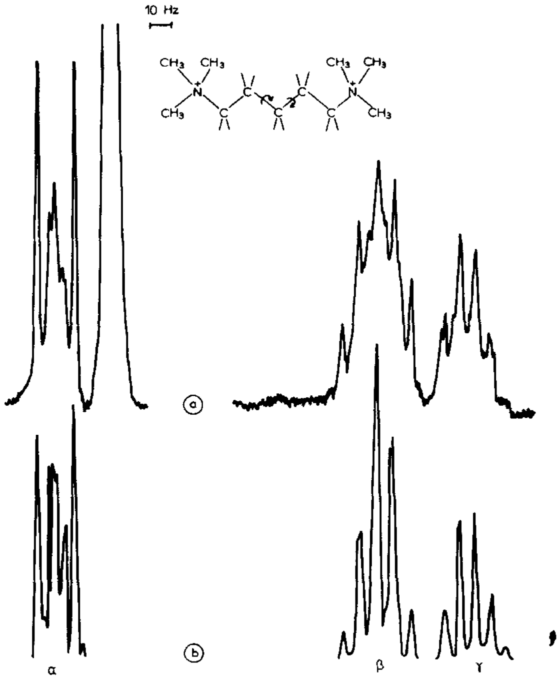

Fig. 4. Pentamethonium: a) experimental; b) calculated TLLT.

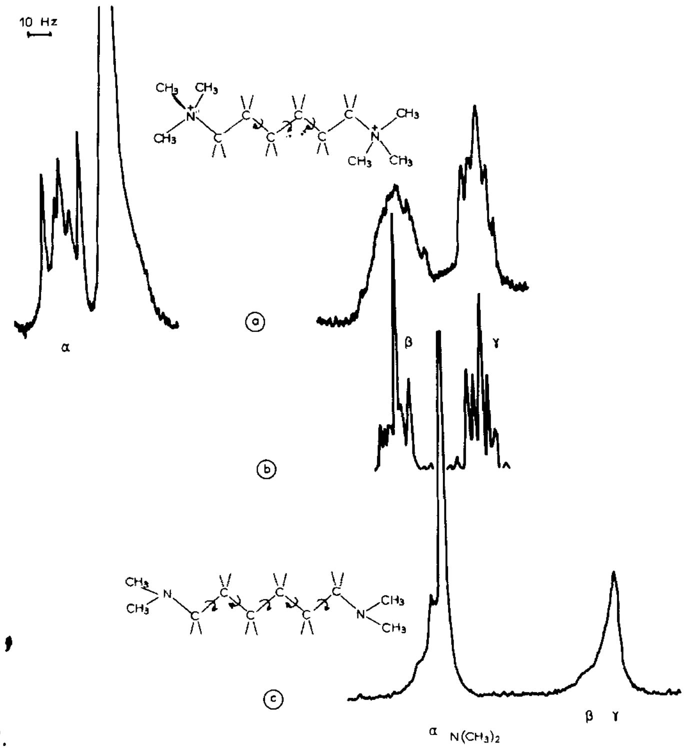

Fig. 5. Hexamethonium: a) experimental; b) calculated γ multiplet for LLL; c) spectrum of the corresponding tertiary amine.

between rotamers is small, so conformational equilibriums can be modified and this leaves open the possibility that the whole all *trans* conformation exists on acting.

Following these restrictions, it is not just now possible to give a significant $N^+$–$N^+$ distance corresponding to curare-like activity. So we are studying homologue products in which the number of conformational possibilities is smaller. From this point of view *p*-phenethyl dimethonium, which is 3 times more potent than hexamethonium [16], can only exist in three conformations. If the α–β groups are, like in dimethoniums studied previously, blocked in *trans* conformation it would correspond to a $N^+$–$N^+$ distance of 7.85 Å. This value is certainly close to the best distance corresponding to maximum activity for compounds with ganglion-blocking activity.

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