Electron Spin Resonance Study of 10-(3-Dimethylamino-2-methylpropyl)-phenothiazine Cation Radical in Solution

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The e.s.r. spectrum of the cation radical of 10-(3-dimethylamino-2-methylpropyl)phenothiazine in sulphuric solution has been studied in the temperature range 233—333 K, with the help of computer simulation. The analysis at 293 K indicates the non-equivalence of the β-protons of the side-chain. Furthermore, the changes observed with the temperature affect both the linewidth and the hyperfine splittings of the β-protons. These results are interpreted on the basis of the branching of the side-chain at the γ-position and the resulting unsymmetrical character of the γ-carbon. The hyperfine splitting constant values $a_{(\beta\text{-H}_1)}$ and $a_{(\beta\text{-H}_2)}$ measured at 233 K have allowed us to calculate the dihedral angles between the $2p(\pi)$ orbital of the heterocyclic nitrogen and the β-H–C bonds.

The special electronic properties of phenothiazine (PTAZ) and its derivatives have stimulated interest in those compounds from very different points of view, as spin labels, as agents for the treatment of mental disorders, or as organic semiconductors. The use as spin labels of the cation radicals of these compounds has been restricted because of the complexity of their e.s.r. spectra. In contrast to the simplicity of the spectra of other spin labels such as the nitroxides, which can be analysed directly in many cases, the spectra of the cations of the phenothiazine derivatives require detailed analysis by means of computer simulation in order to obtain the information these compounds can offer. However, some of their applications, mainly in the pharmacological field, have aroused much interest, with the aim of elucidating how the presence of different side-chains in phenothiazine derivatives influences their properties.

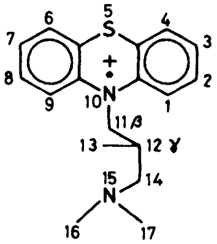
In this direction, several theoretical studies have appeared dealing with conformational aspects and electronic distribution in some phenothiazine derivatives with therapeutic interest.[1-3] The crystalline structures of some of them have also been determined using *X*-ray diffraction methods.[4-6] However, data on their behaviour in solution are relatively rare, particularly in relation to their conformational characteristics.[7,8]

One of the *N*-substituted phenothiazine derivatives with pharmacological properties as an antihistamine and neuroleptic agent is 10-(3-dimethylamino-2-methylpropyl)phenothiazine (alimemazine) (ALMZ). An e.s.r. study of this compound has been made by Fenner,[9] but his results contain some apparent differences from those obtained by other authors for similar compounds.[10,11] The e.s.r. spectra reported by them for several phenothiazine derivatives with a side-chain at nitrogen branched at the γ-position, although with low resolution, clearly present a four-component overall pattern. This structure seems to be incompatible with the hyperfine splitting (h.f.s.) constant values given by Fenner in his analysis, which considers the two β-protons in the side-chain to be equivalent.

Because of this discrepancy and in order to obtain more detailed information on the cation radical of ALMZ in solution we have studied, with the help of computer simulation, the e.s.r. spectrum of ALMZ$^+$ at room temperature, and have also followed the changes at temperatures between 233 and 333 K.

Experimental

The sample of ALMZ in neutral form has been supplied by Specia (Rhone-Poulenc Pharma), and the cation radical was obtained following Merkle's method.[12] For the e.s.r. study, the solid sample was dissolved in dilute sulphuric acid (25% v/v) using a concentration of 1 mg ml^{-1}. With the aim of improving dissolution, the mixture was heated gently while stirring.



The e.s.r. spectra were obtained in the X band with a JEOL PE-3X spectrometer and a JES-VT-3A variable-temperature controller. On-line with the spectrometer, a Digital Computer Controls D-116E minicomputer could digitize the spectra and use them for the simulation procedures. A Mn–MgO standard was used to calibrate the magnetic field.

Two different computer programs, elaborated in our laboratory for the analysis of e.s.r. spectra of these types of compounds, were used. One enables us to determine h.f.s. values through a trial-and-error process, by means of removing from the spectrum, in several steps, the contributions of the most extreme hyperfine components, and obtaining in this way a ' difference ' spectrum (' reduced ' spectrum) where the exact cancellation of the extreme peaks serves as indication of good fit. At the same time, the simulated spectrum of each contribution which is removed is also obtained. The other program simulates the full spectrum, and offers the possibility of reproducing an ' alternating linewidth effect ' between the groups arising from β-proton splittings.

At 293 K, the e.s.r. spectrum is not symmetrical (Figure 1a) and the hyperfine structure is less resolved at the high-field side, because of progressive line broadening. Because of that, in most cases only the low-field halves of the spectra have been simulated, and no attempt has been made of studying quantitatively the dependence of the linewidth on the quantum number $M_I(N)$. Thus, the given linewidth values apply strictly only to the $M_I(N) = +1$ component (low-field), where the agreement between simulated and experimental results can be most closely monitored.

The scheme that best reproduces the experimental spectrum

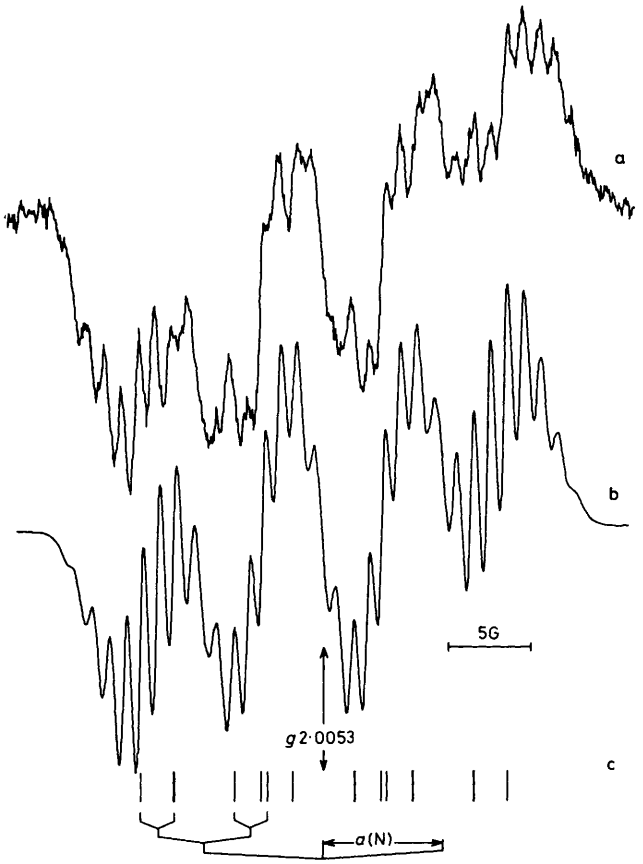


Figure 1. E.s.r. spectrum of ALMZ$^+$, at 293 K, in sulphuric solution: a, experimental; b, simulated; c, main hyperfine splitting diagram

of ALMZ$^+$ at 293 K (Figure 1) considers three types of hyperfine coupling, the N(10) nucleus (type 1), the two non-equivalent β-protons (type 2), and the four proton pairs in the benzene rings (type 3). In contrast with the interpretation of Fenner,[9] the spectrum cannot be simulated by considering the β-protons to be equivalent.

For each set of four groups of lines associated with type 2 h.f.s., two different linewidth values were used, one for the two central components (ΔH_C) and the other for the two lateral ones (ΔH_L). This 'alternating linewidth effect'[13,14] was found to be necessary for reproducing the overall slope appearing in the full experimental spectrum (Figures 1a and b). The Table shows the values of the e.s.r. parameters obtained by the analysis of the spectrum and optimized by computer simulation. The assigment of the h.f.s. constants corresponding to the protons in the lateral rings has been made on the basis of results obtained for 10-methylphenothiazine cation radical by MO calculations.[15]

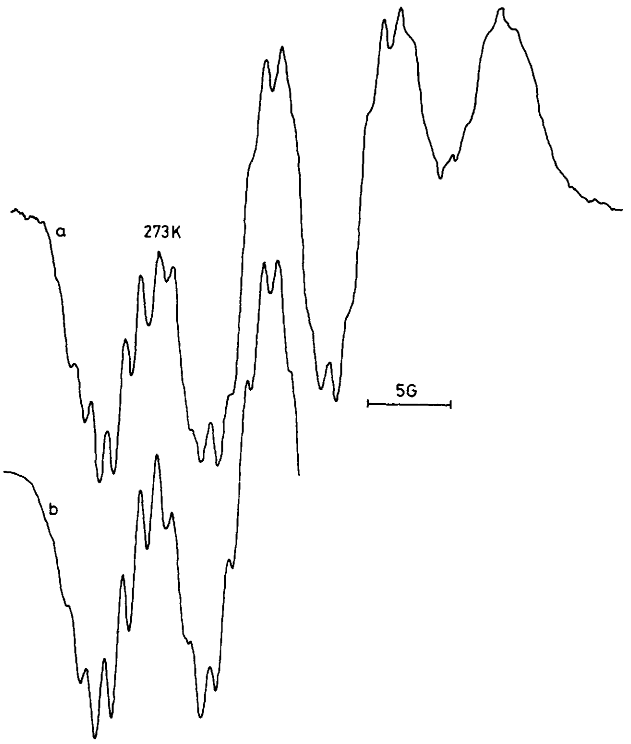


Figure 2. a, Experimental and b, simulated e.s.r. spectra at 273 K

E.s.r. spectra were also recorded at different temperatures between 183 and 333 K. Particular attention was paid to the range between 293 K and the freezing point of the solution (219—233 K [16]) in order to determine the effect of slowing down the tumbling of ALMZ$^+$ molecules on the e.s.r. spectra. In a similar way as in the case of the results at 293 K, the e.s.r. parameters obtained at 273, 253, and 233 K, given in the Table, were optimized using computer simulation by a trial-and-error process (Figures 2 and 3).

E.s.r. data for ALMZ$^+$

T/K	a(N)	a(β-H$_1$)	a(β-H$_2$)	a(3-H)	a(1-H)	a(2-H)	a(4-H)	$\Delta H_L(G)$ [a]	$\Delta H_C(G)$ [b]	g Value
293	7.08	1.96	5.48	1.96	1.04	0.96	0.28	0.60	0.76	2.0053
273	7.08	1.92	5.48	1.96	1.04	0.96	0.28	0.80	0.96	
253	7.08	1.88	5.52	1.96	1.04	0.96	0.28	1.00	1.08	
233	7.08	1.76	5.64	1.96	1.04	0.96	0.28	1.40	1.40	
Room temperature [c]	6.9	3.5	3.5	2.1	(1.0)					

[a] Peak-to-peak linewidth of the lateral groups of lines of the quartet corresponding to β-protons. [b] Peak-to-peak linewidth of the central ones. [c] Values given by Fenner.[9] Hyperfine splittings and ΔH_{pp} values are expressed in 10^{-4} T units; the resolution of the simulation method is 0.04×10^{-4} T.

The results of the simulation process indicate that the non-equivalence of the β-protons is kept along this temperature range, but some significant changes in the corresponding h.f.s. constants have been observed. The value of $a_{(\beta\text{-H}_1)}$ decreases with temperature and $a_{(\beta\text{-H}_2)}$ increases, the sum of both values being practically constant for all temperatures.

The linewidths increase at lower temperature, showing that the molecular tumbling rate becomes lower.[17] The variation of both linewidth values of ΔH_L and ΔH_C with T^{-1} is plotted in Figure 4; it can be seen that the moderate alternating linewidth effect, observed at 293 K, decreases with decreasing temperature and disappears at 233 K, where both lines merge.

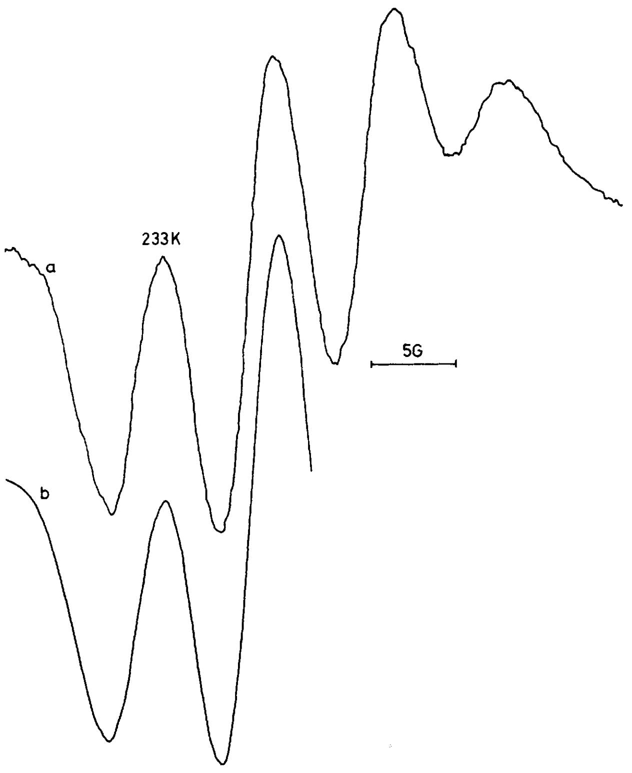


Figure 3. a, Experimental and b, simulated e.s.r. spectra at 233 K

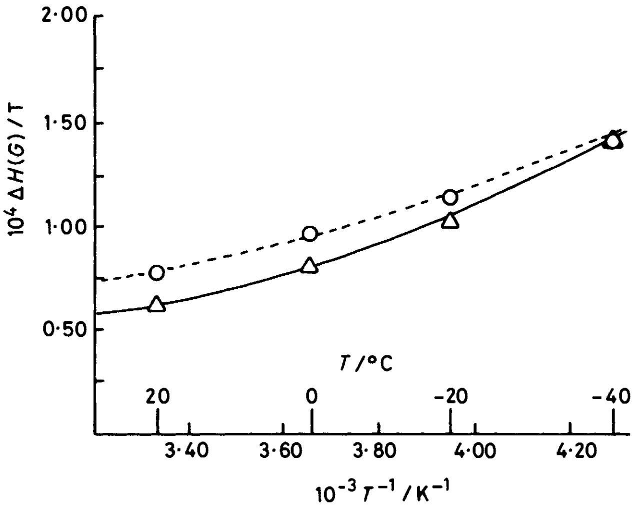


Figure 4. Linewidth values $\Delta H(G)$ *versus* T^{-1}, for the lateral (solid line) and central (broken line) components of the quartet associated with type 2 hyperfine splitting

Moreover, with decreasing temperature, the lineshape becomes more unsymmetrical; this effect is related to the larger variation of the linewidth with T^{-1} for the lines at higher magnetic field.

Below 233 K more important changes appear in the spectrum: the second and third lines tend to approach while the fourth moves away from the centre; a substantial increment in the linewidth is also observed. These effects are indicative of a considerable decrease of the molecular tumbling rate corresponding to freezing of the solution.

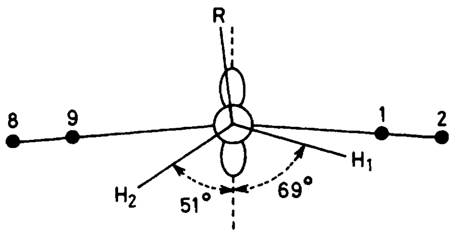


Figure 5. Preferred conformation of ALMZ$^+$at 233 K

On the other hand, on increasing the temperature above 293 K, certain modifications are observed in the spectra, but they do not affect the h.f.s. constants of types 1 and 2, whose evolution is central to our study. The linewidth alternating effect seems to be smaller in the spectrum taken at 333 K.

Discussion

The e.s.r. spectra of PTAZ$^+$ and some of its N-substituted derivatives, obtained for the same solvent and similar experimental conditions, show important differences in the linewidth of its components. For ALMZ$^+$, the lines are broader than for many other PTAZ cation derivatives, and its spectrum is less resolved.

The substituent on nitrogen in phenothiazine derivatives is expected to adopt particular conformations trying to minimize its steric interactions with the aromatic ring system, particularly with the 1- and 9-hydrogens.[7] The non-equivalence of the β-protons induced by these conformational effects has been indicated for neutral molecules by *X*-ray studies [18] and quantum chemical calculations [3] even in the case of symmetrical substituents.

For several phenothiazine derivatives having symmetrical 10-substituents the e.s.r. spectrum of the radical cation in solution at 293 K shows seven components,[10,11] which can be explained by assuming the equivalence of the β-protons together with the approximate relation $a_N \simeq 2a_{(\beta\text{-H})}$. A seven-line pattern with relative intensities 1 : 2 : 2 : 2 : 2 : 2 : 1 is then predicted for those derivatives, in accord with the experimental results. The magnetic equivalence of both β-hydrogens in this case may be due to the existence of a single symmetrical stable conformation for which the dihedral angles θ_1 and θ_2, formed by the $2p(\pi)$ orbital of the nitrogen heterocyclic atom and the β-H–C bonds, are equal, but may result also from motional averaging between two interconvertible, symmetrically related conformations each having non-equivalent β-protons, a circumstance which is known for nitroxide radicals.[19-21]

In the case of ALMZ$^+$, a different behaviour is found. In the temperature range studied, the spectra show four main groups of lines, which can only be simulated on the assumption of non-equivalent β-protons (Figure 5). The equivalence or non-equivalence of these protons can then explain the differences in the number of main groups of lines which can be observed in the spectra of different PTAZ-type cation radicals having nitrogen substituents with two β-protons.[10,11] In our case, varying the temperature of the solution does not change the splitting pattern, but both the observed variations in $a_{(\beta\text{-H})}$ and the presence of a linewidth alternating effect indicate the presence of conformational changes; again, the existence of more than one relevant conformation can be assumed. Probably, no more than two of them are of practical importance in determining the e.s.r. spectrum; because of the chirality of the γ-carbon, they will no longer be mutually

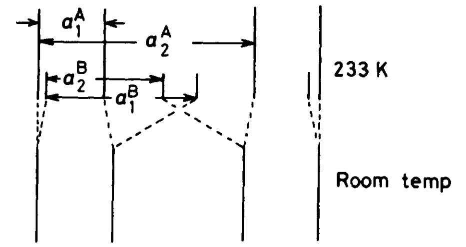


Figure 6. Splitting pattern corresponding to h.f.s. of β-protons for the two conformers A and B, assuming unequal weights

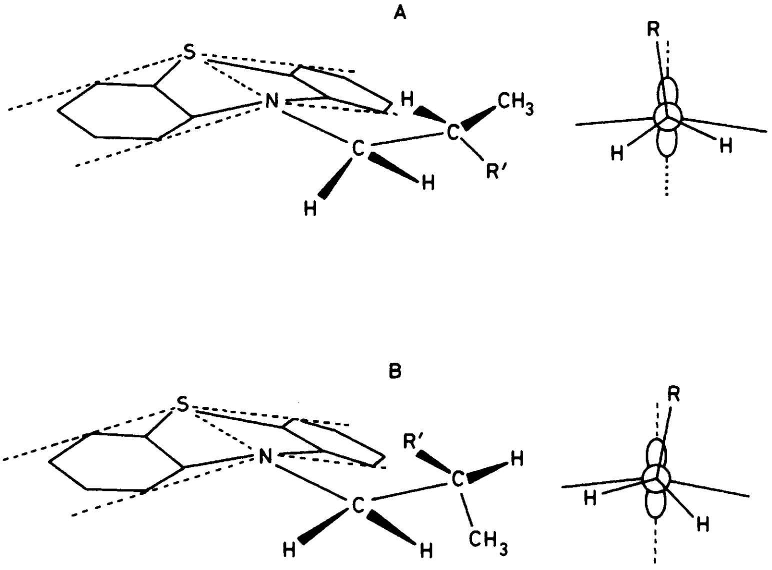


Figure 7. Possible preferred conformations of the side-chain in ALMZ$^+$

symmetrical, and furthermore their respective populations will be different.

The experimental results can be accounted for in the following way. The existence of linewidth alternation indicates that the interconversion between two conformations is not fast, or at least remains below the fast exchange limit over most of the temperature range studied. It can be explained by accepting that the rotations around the N(10)–C(11) or C(11)–C(12) bonds are restricted due to the conformational barriers between the two preferred conformations A and B. At 233 K, the interconversion process between the A and B conformers is probably slow enough and thus the ' linewidth alternating effect ' disappears. In this situation we should expect to observe for the second type of splitting eight components (four for each conformation). The presence of only four at this temperature can be explained by considering very different populations for both conformations (Figure 6), so that at this temperature we observe mainly one of them. At higher temperatures the coalescence process will give four components, whose h.f.s. constants will be the weighted average of those of the parent conformations (Figure 6). In this scheme, the sum of both constants $a_{(\beta\text{-H}_1)} + a_{(\beta\text{-H}_2)}$ is taken as nearly equal in both conformations, bearing in mind that the outermost lines are much less broadened by the coalescence process than the central lines, as shown experimentally. Exact equality, however, is not required by symmetry, due to the chiral character of the substituent. The fact that a finite change in the values of the h.f.s. constants takes place indicates that the second conformation has a non-negligible weight, although its spectral lines at low temperature (223 K) cannot be resolved. In the high temperature range (above 233 K) the small changes in h.f.s. constants can be explained on the basis of the dependence with temperature of the population ratio, which affects the weighted average of the h.f.s. constants.

Considering that the spectrum at 233 K is due to only one conformation instead of an averaging of two, the values obtained for the h.f.s. constants corresponding to the β-protons can be used to calculate the dihedral angles θ^A_1 and θ^A_2 of the preferred conformation, using relation (1) proposed initially by Heller and McConnell.[22]

In applying equation (1) to the case of ALMZ$^+$ we can assume that the constant B is not changed significantly by the

$$a(\beta\text{-H}) = \rho_N B\cos^2\theta + B_0 \qquad (1)$$

nature of the 10-substituent, and that B_0 is small enough to be neglected.[7] With this assumption, the values $\rho_N \simeq 0.29$ and $B = 49$ G obtained by Sullivan and Bolton [15] for 10-methylphenothiazine cation radical have been used; this results in $\theta^A_1 \simeq 69$ and $\theta^A_2 \simeq 51°$ for ALMZ$^+$ in solution.

The fact that $\theta^A_1 + \theta^A_2 \simeq 120°$ indicates that both β-protons are located at the same side of the folded plane containing the aromatic rings, contrary to the *X*-ray and molecular orbital results obtained for several phenothiazine derivatives in the neutral (non-radical) form.[2,3,18] Both β-protons will thus occupy the same type of position in relation to the folded plane, either both *endo*, or both *exo*.

The experimental results do not allow us to distinguish between these two possibilities. Because of the steric interaction of the protons with 1- and 9-H in the aromatic rings, the *exo*-conformation is energetically more favourable. In this case the repulsions between the $\beta\text{-H}_1\text{–H}(1)$ and $\beta\text{-H}_2\text{–H}(9)$ would induce a higher folding of the ring plane than for phenothiazine. However, the folding could be significantly different for cation radicals. In the neutral molecules, both sulphur and nitrogen atoms have a hybridization with important sp^3 character, and the lone electron pairs force non-planar character on the nitrogen substituents. The radical has one lone pair less, and thus an increased sp^2 character at nitrogen, which will result in a smaller deviation from planarity for the aromatic system. If the folding were small enough, both β-protons could be accommodated in the *endo*-position. In the following discussion we will consider the β-protons to be in the *endo*-position (Figure 5); the same arguments are valid for the case of *exo*-position.

Assuming that both protons are in the *endo*-position, a conformational barrier between two possible conformations is due to rotations about the C(11)–C(12) bond as indicated in Figure 7. While conformation A is probably the more stable, conformation B may be significantly populated. It is of interest that, because of the different steric repulsions of the substituents at the γ-carbon (Figure 7), the R group will move to different sides of the plane of symmetry of the aromatic system, and thus the roles of both β-protons in determining the h.f.s. constants are changed on passing from A to B, *i.e.*, $\theta^{A}_{1} > \theta^{A}_{2}$; $\theta^{B}_{1} < \theta^{B}_{2}$ and thus $a^{A}_{1} < a^{A}_{2}$; $a^{B}_{1} > a^{B}_{2}$.

The considerable difference between both conformations for each β-H h.f.s. constant thus explains that conformation B, in spite of its relatively low weight, can significantly affect the h.f.s. constants in the high-temperature region (Figure 6). This would not happen in a situation where $a^{A}_{1} \simeq a^{B}_{1}$ and $a^{A}_{2} \simeq a^{B}_{2}$.

Other possible conformational changes deserve some comment. For example, a conversion between the conformations (both β-protons *endo*) and (both β-protons *exo*) can take place by an inversion of the folding of the aromatic part of the molecule around the S–N axis, and also by a rotation of *ca.* 180° around the N–C(11) bond. Inspection of the resulting steric repulsions shows that such conversion is not expected to interchange the relative deviations of β-H_1 and β-H_2, but leaves $\theta^{A}_{1} \simeq \theta^{B}_{1}$ and $\theta^{A}_{2} \simeq \theta^{B}_{2}$, and therefore cannot explain, alone, the observed changes of the h.f.s. with temperature. On the other hand, a conformational change leaving R and a β-proton in the *exo*-position and the other β-proton in the *endo*-position (as in the neutral molecule [2,3,18]) is not compatible with the sum $a_1 + a_2$ being essentially unchanged, as found experimentally.

In summary, the γ-branching of the 10-substituent in $ALMZ^+$ gives chiral character to the γ-carbon, which leads to non-equivalence of β-protons; it further induces a difference in energy in the preferred conformations whose populations depend therefore on temperature. At low temperature (233 K), the difference in population and the restriction in the movement of the side-chain are large enough to observe mainly the influence of the most favoured conformation in the e.s.r. spectrum. The dihedral angles θ^{A}_{1} and θ^{A}_{2} can thus be measured, and the values they take are probably conditioned by the degree of folding of the ring system and the steric hindrance of the side chain. Above 233 K the second conformation shows an influence on the e.s.r. spectrum, due to motional exchange between both conformations leading to a weighted average of the respective spectra; this effect is revealed in variations in the values of $a_{(\beta\text{-H})}$ and in a moderate linewidth alternating effect. At higher temperatures (*ca.* 333 K), this last effect seems to decrease, due probably to a faster interchange between both conformations. The constant heterocyclic nitrogen hyperfine splitting constant indicates that the unpaired electron spin density in this atom does not change significantly with temperature in the range explored.

References

1 J. J. Kaufman and E. Kerman, 'Quantum Chemical and Theoretical Techniques for the Understanding of the Action of Drugs which affect the C.N.S.,' 6th C.N.S. Jerusalem Symposium on Quantum Chemistry and Biochemistry, Jerusalem, 1974. p. 523.
2 J. J. Kaufman, *Int. J. Quant. Chem.*, 1979, **16**, 221.
3 J. L. Coubeils and B. Pullman, *Theor. Chim. Acta*, 1972, **24**, 35.
4 J. J. H. McDowell, *Acta Crystallogr.*, 1969, **B25**, 2175; 1970, **B26**, 954.
5 P. Marsau, *Acta Crystallogr.*, 1971, **B27**, 42.
6 M. C. Malmstrom and A. W. Cordes, *J. Heterocycl. Chem.*, 1972, **9**, 325.
7 D. Clarke, B. C. Gilbert, P. Hanson, and C. M. Kirk, *J. Chem. Soc., Perkin Trans. 2*, 1978, 1103.
8 P. Hanson, W. J. Isman, R. J. Lewis, and W. A. Stuckburn, *J. Chem. Soc., Perkin Trans. 2*, 1981, 1492.
9 H. Fenner, *Arch. Pharm.*, 1970, **303**, 919.
10 C. Thiery, J. Capette, J. Meunier, and F. Leterrier, *J. Chim. Phys.*, 1968, **66**, 234.
11 L. Levy, T. N. Tozer, L. D. Tuck, and D. B. Loveland, *J. Medi. Chem.*, 1972, **15**, 898.
12 F. H. Merkle and C. A. Disher, *J. Pharm. Soc.*, 1964, **53**, 965.
13 G. K. Fraenkel, *J. Phys. Chem.*, 1967, **71**, 139.
14 J. E. Wertz and J. R. Bolton, 'E.S.R. Elementary Theory and Practical Applications,' McGraw-Hill, New York, 1972, p. 208.
15 P. D. Sullivan and J. R. Bolton, *J. Magn. Reson.*, 1969, **1**, 356.
16 W. Biltz and O. Hulsmann, *Z. Anorg. Chem.*, 1934, **218**, 369.
17 Ref. 14, p. 215.
18 P. Marsau, *Acta Crystallogr.*, 1971, **B27**, 2058.
19 C. Lagercrantz and M. Setaka, *Acta Chem. Scand.*, 1974, **B28**, 619.
20 B. C. Gilbert, J. P. Larkin, and R. O. C. Norman, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1272.
21 B. C. Gilbert and M. Trenwith, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1934.
22 C. Heller and H. McConnell, *J. Chem. Phys.*, 1960, **32**, 1535.

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