Conformational Behaviour of Cromakalim and Related Potassium Channel Activators

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A re-investigation by NMR spectroscopy of the dynamic behaviour of cromakalim and related compounds has shown that previous evidence for a single rigid conformation in solution was incorrect.

In a recent communciation, Cassidy *et al.*[1] reported that the potassium channel activating agent cromakalim (**1**) adopts a single rigid conformation in solution, *i.e.*, rotation about the C–N bond joining the pyrrolidone ring to the benzopyran is prevented. Evidence for this was based on lack of temperature dependence of the spectrum, NOE difference experiments and similar ^{13}C relaxation times in both rings. We find these results surprising in view of barriers to rotation measured in similar compounds,[2] and our own work on cromakalim and a series of related compounds, including the piperidinone (**2**), the methyl pyrrolidine (**3**) and the extremely potent antihypertensive and bronchopulmonary agent Ro 31-6930 (**4**) synthesised in our laboratories.

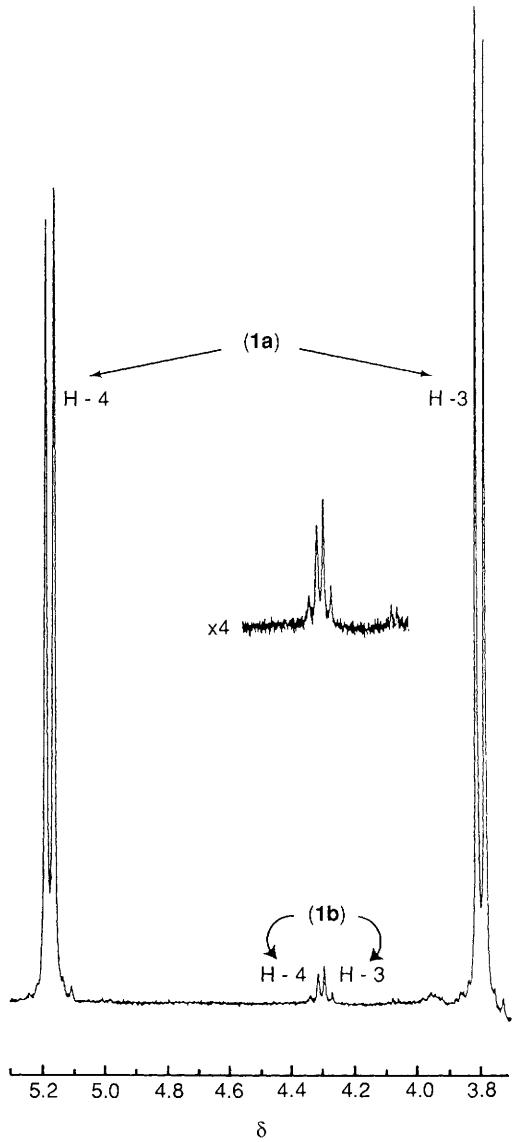


Figure 1. Part of the 400 MHz ^1H NMR spectrum of cromakalim (**1**) in deuteriomethanol solution at −60 °C. Doublets for the protons H-3 and H-4 can be seen for the two forms (**1a**) and (**1b**) in a ratio 97.5 : 2.5. The anisotropy of the carbonyl group causes a low field shift of H-4 in (**1a**) and a smaller low field shift of H-3 in (**1b**). The spectrum was determined on a Bruker AM-400 spectrometer fitted with an Aspect 3000 computer.

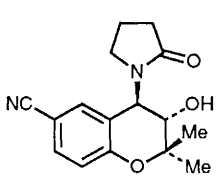
The following three reasons can be put forward for lack of temperature dependence of the NMR spectrum of (**1**) in deuteriochloroform solution. (i) Rotation is stopped at ambient temperatures in a single conformation, and no other forms are present; (ii) rotation is fast but one conformation is overwhelmingly predominant in the solvent used; (iii) chemical shifts for different conformations are accidentally equivalent. The second of these possibilities seemed most likely, and confirmed by our own experiments on cromakalim in deuteriomethanol solution. Unlike the situation in deuteriochloroform,† the NMR spectrum of (**1**) in CD_3OD is considerably broad between 20 and −40 °C, sharpening up again at −60 °C to show two forms in the ratio 97.5 : 2.5 (Figure 1). The chemical shifts of the methine proton H-4 (major form δ 5.19 and minor form δ 4.30) clearly show that the major form is (**1a**) and the minor (**1b**). The energy difference between the two forms is 2.2 kcal mol^{-1} (1 cal = 4.185 J), very close to the value calculated previously.[1] The barrier to rotation is conservatively estimated to lie in the range 11.5—13.5 kcal mol^{-1} indicating that rotation is still very fast at ambient temperatures.

These results are emphasised further by the piperidone derivative (**2**), examined in three solvents. In deuteriochloroform, as with (**1**), a single form with sharp peaks is observed, whereas in deuteriomethanol and [$^2H_6$]dimethylsulphoxide two forms are observed in the ratio 88 : 12 at room temperature, again favouring the configuration (**2a**). The energy difference between (**2a**) and (**2b**) for these more polar solvents is 1.2 kcal mol^{-1}, and the barrier to rotation $\Delta G^{\ddagger}$ 16 kcal mol^{-1} (±0.5 kcal mol^{-1}). In ^2H_2O on the other hand, a more physiologically relevant solvent, the ratio of isomers is *ca.* 91 : 9, suggesting that factors other than dielectric constant are affecting the rotamer preferences. Such small energy differences do not preclude the higher energy conformer from being that bound by the receptor. An attempt to raise the barrier to rotation by increasing the steric hindrance as in the methyl substituted derivative (**3**), was successful but not to the extent of 'freezing out' rotamers about the C–N bond,‡ since peaks were still broad at 20 °C, indicating that slow rotation was still occurring.

The pyridine *N*-oxide derivative Ro 31-6930 (**4**) is a highly potent antihypertensive and bronchopulmonary agent now in development in our laboratories. It provides an interesting

† We estimate from low temperature NMR studies of cromakalim in deuteriochloroform that if a minor conformer is present, the concentration will be ≤0.5%.

‡ A barrier to rotation of ~23 kcal mol^{-1} is required for stable rotamers at 20 °C.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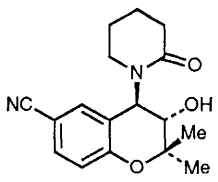
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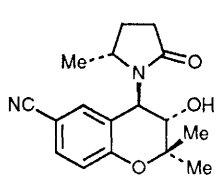
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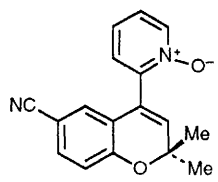
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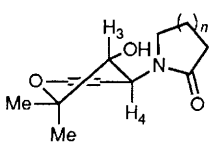
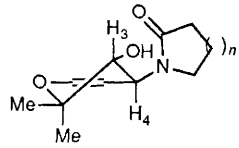


(**1a**); $n = 1$
(**2a**); $n = 2$



(**1b**); $n = 1$
(**2b**); $n = 2$

case of enantiomorphic behaviour, since in principle, at low temperatures the molecule is chiral for the same reason as biphenyl derivatives though racemised by rotation through 180°. The prochiral methyl groups provide a good probe for studying the barrier to rotation. In deuteriomethanol solution they appear in the NMR spectrum as a sharp singlet (δ 1.57) at 20 °C, and as two singlets at δ 1.51 and 1.61 at −60 °C. Coalescence of the two singlets at −10 °C indicates a barrier $\Delta G^{\ddagger}$ of 13.0 ± 0.5 kcal mol^{-1}.§

The behaviour of these and a large number of similar compounds clearly shows that these molecules are by no means rigid, and the behaviour in a relatively non-polar solvent ($CDCl_3$) is misleading. NOE difference experiments are not good criteria for defining conformational dynamics in small molecules, and ^{13}C relaxation times are even more prone to misinterpretation. Variable temperature behaviour in solvents of varying polarity is more definitive and usually reliable.

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§ The normal approximation for exchange between two equally populated sites was used to calculate $\Delta G^{\ddagger}$ *i.e.*, $K = \pi\Delta\gamma/\sqrt{2}$ at coalescence, where K is the rate of exchange, and $\Delta\gamma$ the maximum shift difference in Hz between the exchanging sites at low temperature.

References

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