Aromatic Ring Dynamics in Crystalline Penicillins from Variable Temperature ^{13}C Cross-polarisation Magic-angle-spinning N.M.R.

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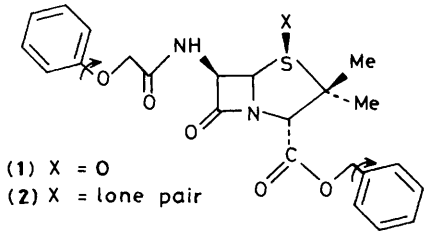
A combination of lineshape analysis and magnetisation transfer experiments in variable temperature ^{13}C cross-polarisation magic-angle-spinning n.m.r. spectroscopy has enabled the determination of rate and activation parameters for aromatic ring rotations over a motional frequency range of 10^{-2}—10^{6} Hz in crystalline penicillin derivatives.

In a recent ^{13}C cross-polarisation magic-angle-spinning (c.p.–m.a.s.) n.m.r. study of a series of crystalline penicillins we observed marked differences in the resonance lineshapes of the aromatic carbons.[1] We suggested that this was caused by differing dynamic properties of the aromatic rings in the various compounds, presumably arising from different molecular packing arrangements in the crystals. Here we report variable temperature (v.t.) studies of phenoxymethyl penicillin (penicillin v) benzyl ester β-sulphoxide (**1**) and its sulphide (**2**), which has permitted the dynamic properties of the phenoxy and benzyl rings in both molecules to be characterised in some detail.



(**1**) X = O
(**2**) X = lone pair

The upfield aromatic regions of the v.t. ^{13}C c.p.–m.a.s. n.m.r. spectra of (**1**) are shown in Figure 1. As the temperature is increased between 200 and 240 K, the two resonances of the *ortho* carbons in the phenoxy ring broaden, coalesce, and sharpen. These changes in lineshape are characteristic of site exchange effects which depend on the relative magnitudes of the rate constant and the frequency difference between isotropic chemical shifts describing the individual sites; such phenomena are well known in solution n.m.r.[2] As the temperature increases above 260 K, however, the site-averaged *meta* and *ortho* isotropic resonances broaden to the limits of observability, then sharpen to narrow resonances of 16 Hz half-height width at the highest temperature. The substantial line broadening observed here can be associated with the situation when the motional frequency becomes comparable to the ^{13}C–^{1}H dipolar decoupling frequency.[3] This dipolar broadening is at a maximum when the motional and decoupling frequencies are equal.

These observations provide an opportunity to obtain quantitative kinetic and activation parameters associated with the ring dynamic processes in crystalline (**1**). The lineshapes of the *ortho* phenoxy resonances of (**1**) in the 200—240 K interval

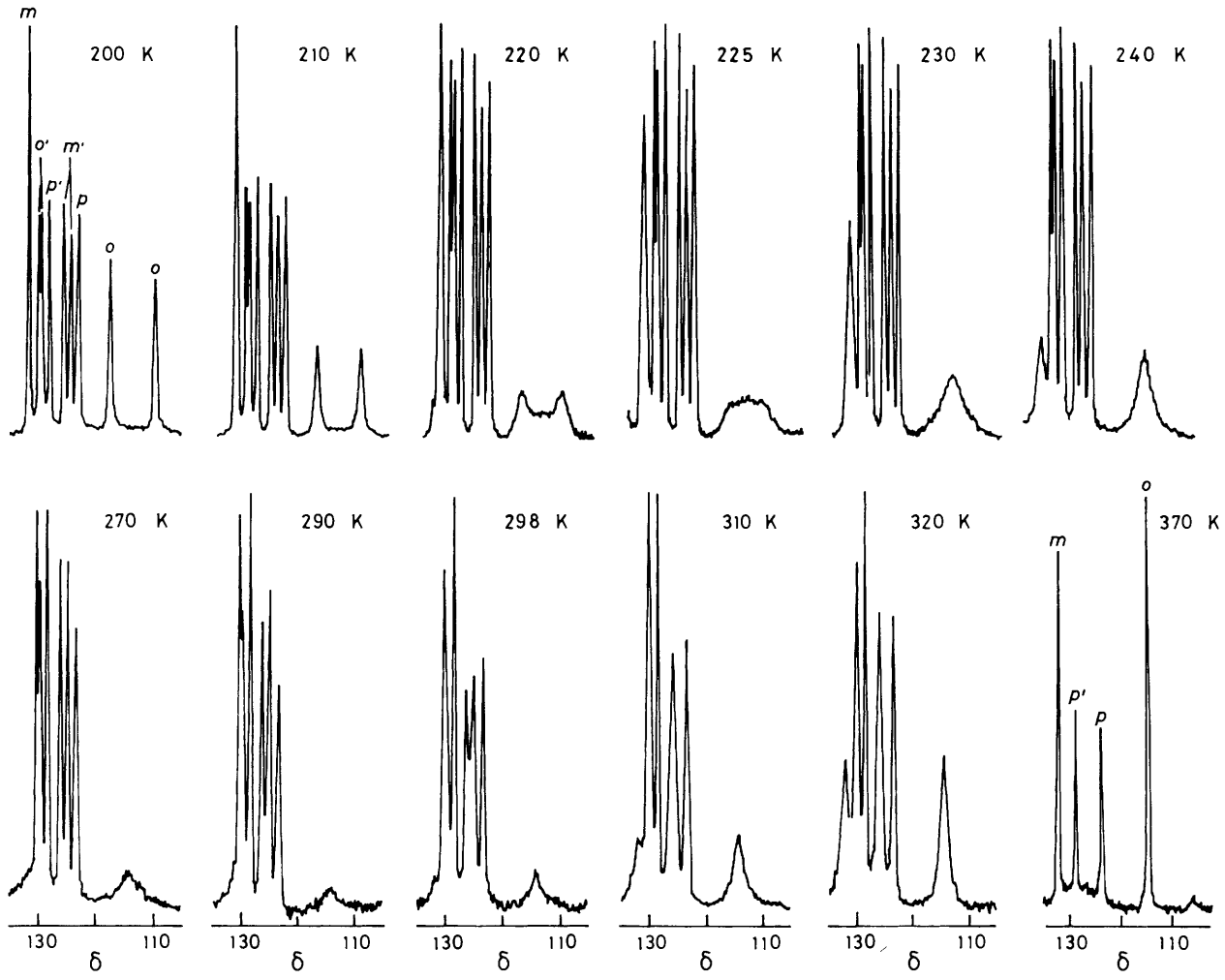


Figure 1. Variable temperature ^{13}C c.p.–m.a.s. n.m.r. spectra of (**1**). Phenoxy *ortho* (*o*), degenerate *meta* (*m*), and *para* (*p*); benzyl *ortho* (*o'*), *meta* (*m'*), and *para* (*p'*) carbon resonances as labelled. M.a.s. between 4.0 and 4.2 kHz. Larmor frequencies at our field are ν_0^C 50.32, ν_0^H 200.13 MHz; ^{13}C–^1H dipolar decoupling frequency $\nu_1^H \approx 75$ kHz.

could be closely simulated by a single correlation time two-site exchange model arising from discrete flips of the phenoxy ring about its local C_2 axis; this enabled the rate constants for the ring flips to be determined over this temperature range. Maximum dipolar broadening occurs experimentally at 285 K and the flip rate at this temperature could therefore be obtained from this experiment. The consistency between rates derived from these independent measurements is evident in the Arrhenius plot, Figure 2, which indicates that the energy barriers defining the phenoxy ring flips in (**1**) remain unchanged over flip rates of 10^1—10^6 Hz. The activation parameters for (**1**) within 95% confidence limits are E_a 43 ± 3 kJ mol^{-1} and A_0 2×10^{12}—5×10^{13} s^{-1}. Additional line broadening which follows from subtle interactions of the full anisotropic chemical shift, m.a.s. frequency, and motional rate[4] is not significant at our field and m.a.s. frequency within either the site exchange or dipolar broadening rate regimes.

In the variable temperature spectra of (**2**), the *ortho* phenoxy resonances undergo site exchange lineshape changes qualitatively similar to those observed for (**1**) but in the higher temperature range of 290—330 K. The site exchange lineshapes for the *ortho* phenoxy resonances of (**2**) were also well simulated by a single correlation time two-site exchange model. Although maximum dipolar broadened *ortho* phenoxy resonances corresponding to a rapid phenoxy flip rate of ~75 kHz was not observed in the temperature range 240—330 K, very slow kinetic behaviour could be probed between 240 and 260 K by a series of variable mixing time, rotationally synchronised, magnetisation transfer (m.t.) experiments.[5] The independently derived but complementary m.t. and site exchange rate data are again found to be consistent, Figure 2. The activation parameters for the phenoxy ring flip of (**2**) within 95% confidence limits are E_a 89 ± 1 kJ mol^{-1} and A_0 7×10^{17}—2×10^{18} s^{-1}.

The n.m.r. data also provide information on the reorientations of the more hindered benzyl rings of (**1**) and (**2**). The *ortho* benzyl resonances of (**1**) coalesce at 298 K, Figure 1. Similarly, the *meta* benzyl resonances of (**1**), which have a greater 'slow' site exchange frequency separation, coalesce at 310 K. As the temperature increases, the motional frequency moves from the site exchange regime to the dipolar broadening regime with a concomitant increase in linewidth. An estimate of $50 < E_a < 80$ kJ mol^{-1} can be made from the coalescence temperatures. The benzyl resonances of (**2**) remain in the slow site exchange limit over the temperature range 240—330 K and the benzyl ring flip activation energy is therefore much greater than that of the motional phenoxy ring, $E_a > 100$ kJ mol^{-1}.

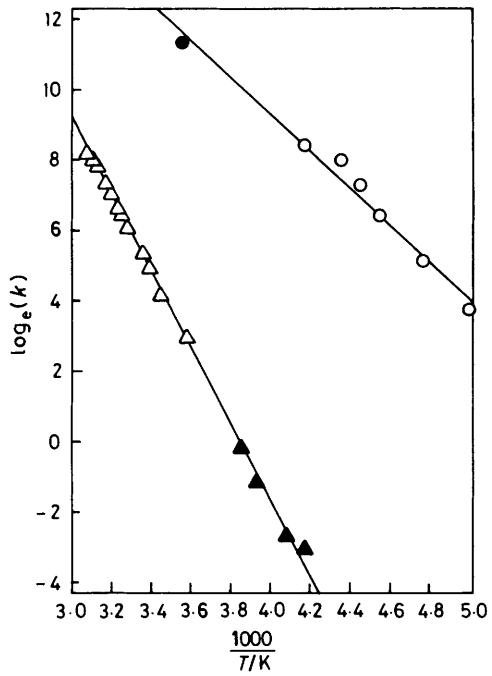


Figure 2. Arrhenius plot of phenoxy ring two-site exchange rates in (**2**) (△ = site exchange; ▲ = magnetisation transfer) and (**1**) (○ = site exchange; ● = maximum dipolar broadening). For the site exchange lineshape analysis, an estimate of 19 Hz for the *ortho* phenoxy linewidths of (**1**) in the slow exchange limit was made from measurements of the motionally unmodulated aromatic resonances at 200 K; the static *ortho* phenoxy linewidths of (**2**) were determined directly at 240 K to be 23 Hz. The frequency separation of the two *ortho* phenoxy resonances of (**2**) is 283 Hz; the evolution time and m.a.s. frequency in the magnetisation transfer experiments were 883 μs and 3395 ± 3 Hz, respectively, with mixing times ranging from 0.5 to 12.0 s.

Aromatic ring rotations have previously been observed by ^{13}C c.p.–m.a.s. n.m.r. in glassy polymers[6] and their precursors[7] although in contrast with the results described above, only site exchange phenomena were observed. The transition from slow to fast site exchange was, however, over a larger temperature range of approximately 150 K. These dynamic processes were best described by distributions of correlation times,[8] presumably reflecting a distribution of aromatic environments in the materials. Our observation of single correlation time ring flips raises questions about the mechanism of such large amplitude motions in densely packed and well ordered molecular solids. A recent simulation of ring motions in an idealised array of aromatic rings has suggested that ring flips may be co-operative events.[9] It is interesting that the activation parameters of the phenoxy and benzyl ring flips are different in both (**1**) and (**2**) and consequently the phenoxy and benzyl ring flip events apparently occur with no direct correlation between these two motions within each crystal.

Our correlation time model and activation energies are comparable to results obtained from wide line ^{2}H n.m.r. spectra of crystalline amino acids and polypeptides selectively deuteriated at motional aromatic sites.[10] The techniques described here offer a complementary method for studying dynamic processes in solids which is applicable in a direct manner over a wide range of motional frequencies.

One of us (J. M. T.) thanks the E. P. Abraham Education Trust, the E.P.A. Cephalosporin Trust, Beecham Pharmaceuticals, and Pembroke College, Oxford, for generous financial support. This work was also supported by the S.E.R.C. We thank S. J. Heyes for useful discussions.

Received, 11th January 1988; Com. 8/00081F

References

1 N. J. Clayden, C. M. Dobson, L-Y. Lian, and J. M. Twyman, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1933.
2 I. D. Campbell, C. M. Dobson, G. R. Moore, and R. J. P. Williams, *FEBS Lett.*, 1976, **70**, 96.
3 W. P. Rothwell and J. S. Waugh, *J. Chem. Phys.*, 1981, **74**, 2721.
4 A. Schmidt, S. O. Smith, D. P. Raleigh, J. E. Roberts, R. G. Griffin, and S. Vega, *J. Chem. Phys.*, 1986, **85**, 4248; D. Suwelack, W. P., Rothwell, and J. S. Waugh, *ibid.*, 1980, **73**, 2559.
5 C. Connor, A. Naito, K. Takegoshi and C. A. McDowell, *Chem. Phys. Lett.*, 1985, **113**, 123; K. Takegoshi and C. A. McDowell, *J. Am. Chem. Soc.*, 1986, **108**, 6852.
6 A. N. Garroway, W. M. Ritchey, and W. B. Moniz, *Macromolecules*, 1982, **15**, 1051.
7 B. Everatt, R. K. Harris, A. M. Kenwright, K. J. Packer, and B. P. Stark, *Magn. Reson. Chem.*, 2986, **25**, 1986.
8 J. I. Kaplan and A. N. Garroway, *J. Magn. Reson.*, 1982, **49**, 464.
9 D. Perchak, J. Skolnick, and R. Yaris, *Macromolecules*, 1987, **20**, 121.
10 Y. Hiyama, J. V. Silverton, D. A. Torchia, J. T. Gerig, and S. J. Hammond, *J. Am. Chem. Soc.*, 1986, **108**, 2715; D. M. Rice, Y. C. Meinwald, H. A. Scheraga, and R. G. Griffin, *ibid.*, 1987, **109**, 1636; R. J. Wittebort, E. T. Olejniczak, and R. G. Griffin, *J. Chem. Phys.*, 1987, **86**, 5411.