Observation of Discrete Thiazolidine Ring Conformations in Frozen Aqueous Solutions of Penicillins by ^{13}C CP–MAS NMR Spectroscopy

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Resonances from two distinct thiazolidine ring conformers have been resolved in the ^{13}C CP-MAS spectra of frozen aqueous solutions of penicillins; the relative intensities of the resonances correlate well with previous estimates of the populations of the different conformers in liquid aqueous solutions.

Single crystal X-ray diffraction studies have revealed that penicillins crystallize with one of two distinct conformations of the five-membered thiazolidine ring.[1,2] These conformations are denoted C3′ and S1′, based on the ring torsion angles and generally correlating with which atom of the five membered ring lies furthest from a plane defined by the remaining four atoms.[3,4] ^{13}C cross polarization magic angle spinning (CP-MAS) NMR studies of a series of crystalline penicillins have shown that the 2βMe ^{13}C isotropic chemical shift is a sensitive probe of these solid state thiazolidine ring conformations with 2βMe chemical shifts for the rings in the C3′ and S1′ conformations differing by approximately 6 ppm.[3] By contrast, ^{13}C 2βMe chemical shifts of penicillins in aqueous solution[5] are intermediate between these values, suggesting that the thiazolidine ring conformers may be rapidly interconverting in solution.[3] Indeed, theoretical studies have suggested that the energy difference between the thiazolidine conformers is only 2 kJ mol^{-1}.[6]

In order to explore further the populations of the different conformers we have recorded spectra of penicillins in frozen aqueous solution at low temperatures. The samples were prepared by dissolution of the penicillins in water at various concentrations, and transferring the solutions to rotors which were introduced into a pre-cooled NMR probe. The spectra were found to be independent of solution concentration in the range 1.00 to 0.03 mol dm^{-3}.

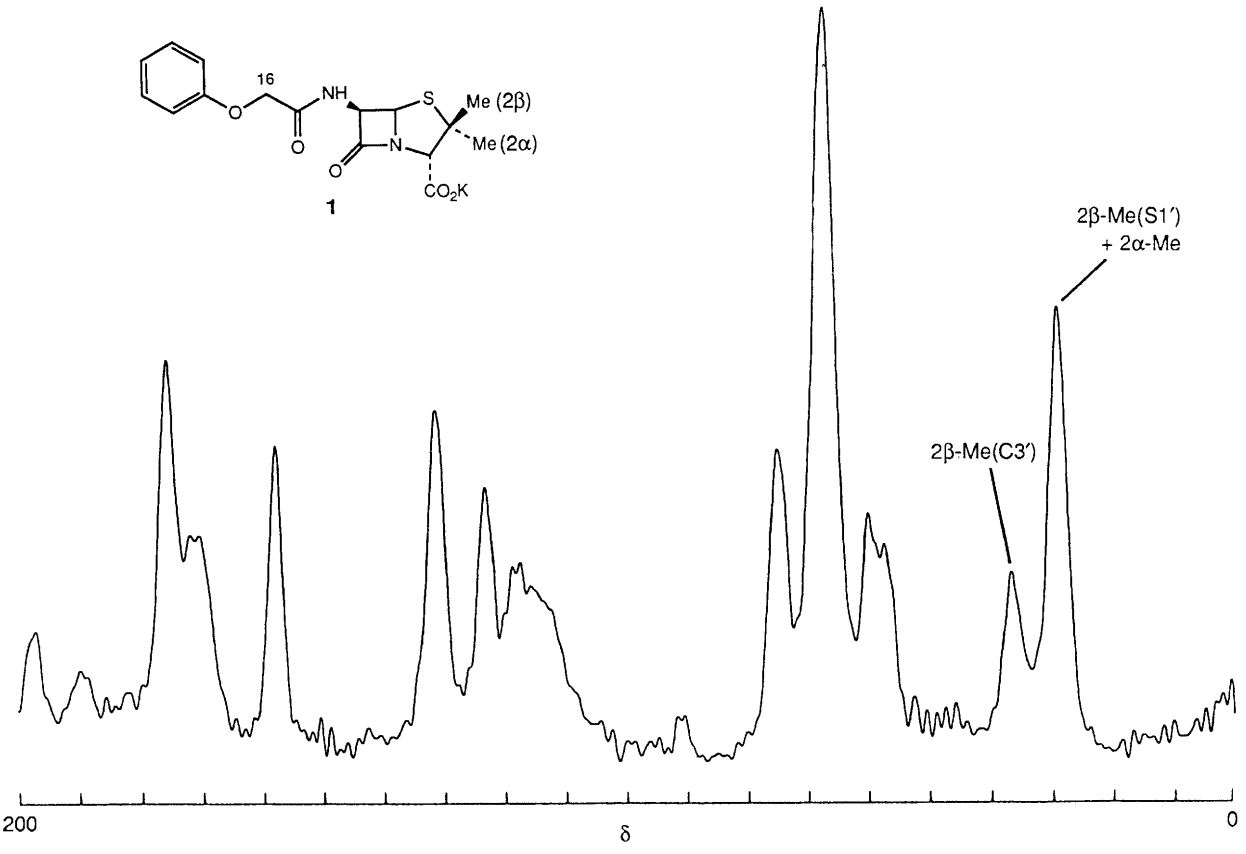

Fig. 1 Low temperature ^{13}C CP-MAS spectrum of a frozen aqueous solution of potassium penicillin V at *T ca.* 200 K. ^{13}C CP-MAS spectra were collected on Bruker CXP-200 and MSL-200 instruments operating at ^{13}C and ^{1}H frequencies of 50.3 and 200.1 MHz, respectively. Liquid solutions in 7 mm (o.d.) zirconia rotors were introduced into a pre-cooled NMR probe (at 210 or 200 K). The samples were spun slowly at ν_r *ca.* 500 Hz for *ca.* 5 min in order to produce a rotationally stable frozen solution. The MAS frequency was then increased to ν_r *ca.* 3 kHz and the probe allowed to equilibriate for 15 min. ^{13}C CP-MAS spectra were then collected using the standard single contact cross polarisation sequence[11,12] with contact times of t_{cp} = 0.75 ms, ^{13}C–^{1}H dipolar decoupling fields of ν *ca.* 75 kHz and recycle delays of 3 s. Chemical shifts were referenced externally to the upfield resonance of adamantane at δ 29.23 with respect to Me_4Si. The indicated temperature of the Bruker double-bearing MAS probe was calibrated against the well known temperature dependent chemical shifts of samarium ethanoate tetrahydrate[13,14] and the well defined phase transitions of a number of compounds[13–15]. Dry boil-off nitrogen gas was used throughout as the pneumatic gas.

At temperatures below 220 K, CP-MAS spectra were obtained with good signal-to-noise ratios, showing resolved resonances for many of the carbon atoms. The resonances are, however, considerably broader than those of crystalline penicillin samples, with $\Delta\nu_{1/2}$ typically 200 rather than 25 Hz, but are comparable to resonances obtained for penicillins in the amorphous state.[3] We presume that the increased linewidths are caused by the existence of extensive disorder in the samples, hence there are a range of different environments for individual molecules in the frozen solutions and amorphous solids when compared with the regular packing in the crystalline samples. Such a distribution of chemical environments will give rise to resonances which are inhomogeneously broadened as a consequence of the resulting distribution of chemical shifts.[7] Similar conclusions have been drawn from spectra of carbohydrates in frozen solutions.[8]

At temperatures above 220 K, the signal-to-noise ratio in the CP-MAS spectra was observed to deteriorate substantially; we have found, however, that the sensitivity of ^{13}C single pulse excitation experiments employing recycle delays of 3 s increases at these higher temperatures. Both these observations suggest that significant motion remains in the solid matrix under these conditions. Preliminary ^{2}H NMR studies, similar to those described for frozen $^{2}H_2O$ by Wittebort *et al.*,[9] provide additional evidence for this proposition.

Two resonances can be resolved from methyl group carbons in the frozen solution spectra of both potassium penicillin G and potassium penicillin V **1**, the penicillins studied in this work. Fig. 1 shows a low temperature ^{13}C CP-MAS spectrum of a frozen aqueous solution of potassium penicillin V at $T \approx$ 200 K; in Fig. 2, the interpretation of such spectra is shown by comparison with spectra of the liquid aqueous solution and with spectra of crystalline penicillins. In each spectrum of the frozen solution one resonance, at approximately δ 27, is close to the position where the resonance of the 2α-Me carbon occurs in both C3′ and S1′ puckers in the crystalline state, (δ 27.0 ± 2.0) and close to where the resonance of the 2β-Me group carbon occurs in the S1′ conformation (δ 29.7 ± 0.8). Spectral simulations (Fig. 2) show that with the linewidths characteristic of the frozen solutions these individual signals would not be resolved. The other resonance, at approximately δ 35, is of much lower intensity and is at a shift corresponding closely to that found for the 2β-Me carbon resonance in molecules with the C3′ pucker, δ 35.6 ± 0.9. These observations are consistent with the situation in which both the C3′ and S1′ ring puckers are present in the penicillin molecules contained within the frozen solution and in contrast to the situation found for the liquid aqueous solution, (Fig. 2), distinct resonances are observed from molecules in the two states. From the frequency separation of the 2β-Me resonances observed in the spectra of crystalline samples in the two

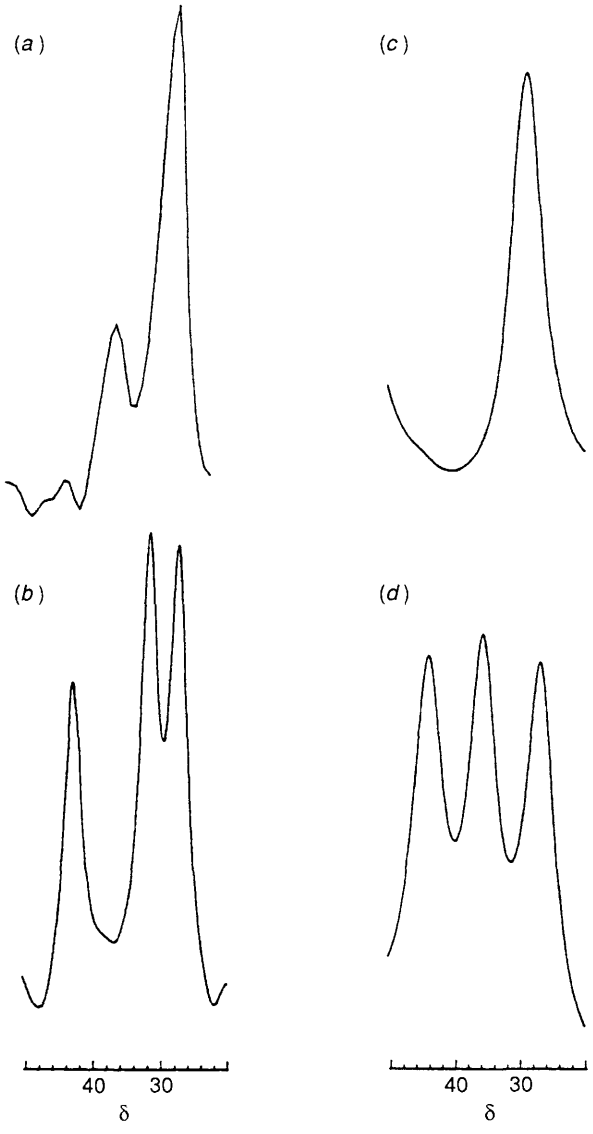

Fig. 2 Part of the ^{13}C CP-MAS spectra of penicillins showing resonances of the 2α- and 2β-Me carbons: (*a*) frozen aqueous solution of *ca.* 0.5 mol dm^{-3} potassium penicillin V at *T ca.* 190 K with MAS ν_r *ca.* 3.1 kHz. Resonances at δ *ca.* 35 and 27 were assigned to 2β-Me (C3′ conformation) and to 2β-Me (S1′ conformation) and 2α-Me (C3′ and S1′ conformations), respectively; (*b*) *ca.* 1 mol dm^{-3} liquid solution of potassium penicillin G in 25% D_2O–H_2O at *T ca.* 298 K; (*c*) crystalline ampicillin anhydrate (S1′ thiazolidine conformation); (*d*) crystalline potassium penicillin G (C3′ thiazolidine conformation). Spectra (*b*)–(*d*) are convoluted with a linebroadening of 230 Hz to enable comparison with (*a*). The peak at δ *ca.* 43 in (*b*) and (*d*) is from the C-16 carbon which resonates downfield of δ 50 in the other samples.

conformational states, we can estimate that such a situation would arise if the rate of interconversion between the conformers were to be less than approximately 100 s^{-1}. It appears, therefore, that at low temperatures in the frozen matrix the rapid conformational interconversion characteristic of the liquid solution has been slowed substantially.

From the integrated intensities of the methyl group resonances in the frozen solution spectra it is possible to estimate the populations of the molecules in the different ring puckers. This requires the assumption that the resonance lineshapes and the cross-polarization dynamics are comparable in the different methyl sites, and that we therefore observe 2α-Me : 2β-Me integrated intensities of 1 : 1 for each thiazolidine conformation present in the frozen solution. This assumption is valid for a large number of crystalline penicillins, whose ^{13}C CP-MAS spectra show 2β-Me : 2α-Me integrated intensities of 1 : 1 over a large temperature range.[3] The results of this analysis give approximate population ratios for potassium penicillin G of 0.45 : 0.55 and for potassium penicillin V of 0.40 : 0.60, for the C3′ : S1′ conformations. These ratios can be compared with those obtained in liquid aqueous solution at 298 K from the chemical shift data; for a series of penicillin solutions average populations of the C3′ and S1′ conformers were estimated to be 0.32 : 0.68.[3] The two sets of data are sufficiently in agreement with each other to give confidence that the populations present in the frozen matrix reflect to a good approximation those present in the solution prior to freezing. The differences between the values may reflect experimental error in the methods of estimating the populations in the two states, or may reflect some change in population generated by the freezing process. Further experiments will be needed to distinguish between these possibilities.

The ability to obtain spectra of frozen solutions offers the opportunity to explore the influence of solvent interactions on conformations and dynamical properties of a wide range of molecules. Furthermore, previous studies[10] have shown the value of frozen samples as a means of stabilising intermediates which are otherwise short-lived. The present results suggest that studies of frozen solutions will provide a means of exploring equilibrium processes which in liquid solution are too rapid to probe by NMR techniques.

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