Self-association of Benzylpenicillin in Aqueous Solution: ^{1}H Nuclear Magnetic Resonance Study

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Summary Concentration dependence of ^{1}H n.m.r. spectra suggests that benzylpenicillin ions aggregate in aqueous solution primarily through hydrophobic interactions of the benzyl side chains.

ALTHOUGH n.m.r. spectra of aqueous solutions of penicillins are known to be concentration dependent,[1] no attempt appears to have been made to correlate this concentration dependence with association properties of penicillins. Controversial reports have been made regarding the state of penicillin salts in aqueous solutions; on the one hand they are said to behave as simple 1 : 1 electrolytes;[2] and on the other, to behave as colloidal electrolytes and to form micellar aggregates.[3,4] We report a ^{1}H n.m.r. study that

sheds some light on the self-association property of benzylpenicillin (penicillin G) in aqueous solution.

The high resolution 100 MHz ^{1}H n.m.r. spectrum of a 0·1 M-D_2O solution of potassium benzylpenicillin is given in Figure 1. Signal assignments[1,5] are also shown. Assignments of the signals of β-lactam protons (III and IV) are based upon the recent n.m.r. observations on partially deuteriated penicillins by Carlstedt *et al.*[6]

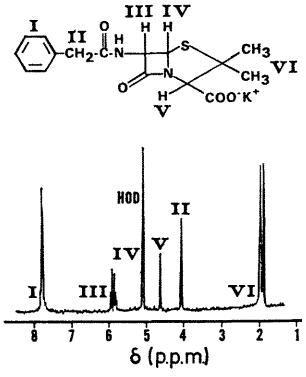

FIGURE 1. ^{1}H *n.m.r. spectrum* (100 *MHz*) *of potassium benzylpenicillin.*

The ^{1}H n.m.r. spectrum of potassium benzylpenicillin was examined over a concentration range 0·01—1·0 M. Figure 2 shows the concentration dependence of chemical shifts of the various protons.† The following features are apparent: (i) signals of the aromatic (I) and the adjacent methylene protons (II) undergo the most pronounced upfield shifts, (ii) signal of the proton (V) on the carbon bearing the ionized carboxyl group remains unaltered in its chemical shift, (iii) signals due to the two methyl groups (VI) on the thiazolidine ring undergo small upfield shifts, and (iv) signals due to the β-lactam protons experience small upfield shifts at first and then with a further increase in concentration, move downfield.

These results suggest that benzylpenicillin ions aggregate primarily by hydrophobic interactions involving the benzyl side chains. The aromatic hydrophobic cores constitute what can be described as the nonpolar core of the aggregate.‡ Overlap or stacking in this region would place benzyl groups in the shielding regions of the aromatic moieties of the neighbouring ions. Such an arrangement would explain why signals of protons (I) and (II) undergo the marked upfield shifts. The ionized carboxyl groups on the thiazolidine ring form the periphery of the aggregate in intimate contact with the surrounding water. The polarity and the magnetic character of the environment of proton (V), which is attached to the same carbon that bears the carboxyl group, remain unaltered as indicated by the unchanging chemical shift for this proton. The small upfield shifts of the methyl protons (VI) reflect the increasingly ordered solute structure as the aggregates are formed. Chemical shift behaviour of the β-lactam protons is not

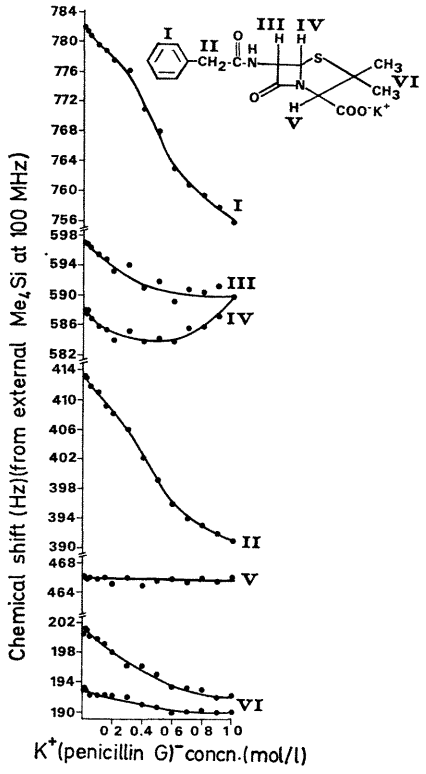

FIGURE 2. *Concentration dependence of proton chemical shifts of potassium benzylpenicillin in* D_2O *at* 30 °C.

readily understood, but the non-uniform trend suggests an involvement of the amide groups of adjacent penicillin ions in interionic hydrogen bonding which might be an additional factor reinforcing the aggregate structure.

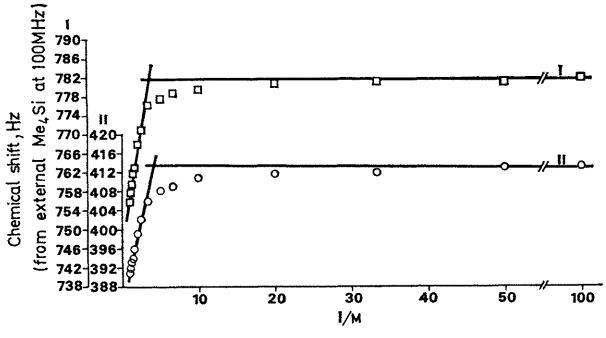

FIGURE 3. *Chemical shifts of aromatic* (I) *and methylene* (II) *protons of potassium benzylpenicillin* versus *reciprocal of molar concentration.*

Chemical shift data for the aromatic (I) and the methylene (II) protons are amenable to the mathematical treatment suggested by Muller and Birkhahn.[7] Plots of observed chemical shift against reciprocal of molar concentration,

† Bulk diamagnetic susceptibility corrections have not been made. These corrections would be significant near the upper limit of the concentrations used; however, neglecting them should not alter materially the conclusions in this communication.

‡ An analogy, helpful in visualizing the aggregate, is that of the classical spherical micelle of a typical anionic surface active agent; the nonpolar micellar core is formed by the hydrocarbon chains whereas the periphery is formed by the ionized polar groups and the associated counterions. However, the present n.m.r. data do not permit ruling out of the lamellar micelle in favour of the spherical micelle.

1/M, yield two linear regions which intersect at 1/M = 1/critical micelle concentration.§ Figure 3 shows such plots for both the aromatic (I) as well as the methylene (II) protons. Critical micelle concentration values obtained from these plots are 0·275 and 0·251 M, respectively, which are in good agreement with the value of 0·25 M, determined by McBain *et al.*[3] using cryoscopic and dye-solubilization methods. The curvature which the data points display in the region of intersection (Figure 3) indicates that the number of penicillin ions which come together to form the aggregates is probably not very large; if it were large, the data points would display an abrupt change.[8]

We have observed similar concentration dependence of chemical shift in aqueous solutions of potassium salt of phenoxymethylpenicillin (penicillin V). For both these penicillins, we have found that addition of 4 M-urea does not completely disrupt the aggregate structure which suggests that factors other than just hydrophobic interactions may be responsible for aggregation.

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§ According to Muller and Birkhahn,[7] critical micelle concentration is defined as that value of the solute concentration at which just half the total solute is present in the monomeric form.

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