A Solid-state ^{13}C Nuclear Magnetic Resonance Study of the Conformational States of Penicillins

Nigel J. Clayden, Christopher M. Dobson,* Lu-Yun Lian, and J. Mark Twyman
Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR

^{13}C Cross polarization–magic angle spinning spectra have been obtained for crystalline penicillins in which essentially all the resonances have been resolved and assigned. Comparison of the spectra of a series of penicillins of known structure has enabled separation of the effects of intermolecular and intramolecular interactions to be achieved. Two aspects of particular interest have emerged from the work. First, dynamic behaviour of the sidechain is evident in the spectra and this has been shown to depend critically on the crystalline environment of the penicillin molecules. Second, by correlating chemical shifts in the series of molecules, it has proved possible to predict features in the solid-state conformations of penicillins of unknown crystal structure. Further, on comparison with solution spectra, the existence of rapidly interconverting thiazolidine ring puckers in solution is suggested and their relative populations estimated for the different penicillins studied.

The penicillins are a family of antibiotics with a general structure of a variable sidechain attached to a fused β-lactam–thiazolidine ring system (Table 1). A variety of penicillins with different side-chains have been isolated or synthesized and shown to have varying pharmacological activities.[1] Although many detailed features of the biological action of the penicillins by inhibiting the final steps of bacterial cell wall synthesis are well known, the underlying reasons for this variation have remained unclear.[2,3]

Many penicillins have known solid-state structures as determined from single-crystal *X*-ray analyses (Table 1). Several distinct conformations of the thiazolidine ring and of the side-chain have been identified in these studies and attempts have been made to correlate these features with biological activity.[3,4] Solution n.m.r. studies have, however, suggested that the conformational states of the molecules are not necessarily the same in solution as in the solid state[5–7] although a detailed conformational analysis in solution has not yet been reported.

One approach to a direct comparison of the conformations of a molecule in a solid and in solution is to compare its n.m.r. spectra in the two states.[8] By combining rapid magic angle spinning (MAS) with high power proton decoupling it is now possible in favourable cases to obtain spectra of solids with a resolution approaching that of the solution state.[9,10] Although several examples of this comparative procedure have been reported,[11–13] the method has not yet been widely applied. Also, there have been uncertainties in the separation of intramolecular and intermolecular influences on the n.m.r. parameters.[8–10] In this paper we report the results of high-resolution ^{13}C n.m.r. studies of a series of crystalline penicillins. By correlating the spectral features with structural information available from *X*-ray diffraction studies it has been possible to identify the influence of different structural features on the n.m.r. parameters. It has proved possible to use these correlations to predict the conformational state of the thiazolidine ring for crystalline penicillins which have not been studied by single-crystal *X*-ray diffraction, and for all the penicillins in solution.

Experimental

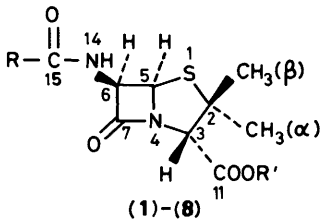
The penicillins studied here are listed in Table 1. Sodium salts of oxacillin, cloxacillin, dicloxacillin, methicillin, amoxycillin, and ampicillin were kindly donated by Beecham Pharmaceuticals, Brockham Park, Surrey. Sodium penicillin G, potassium penicillin G, procaine penicillin G, penicillin V, ampicillin anhydrate, ampicillin trihydrate, and further sodium ampicillin were purchased from Sigma Chemicals. 6-Aminopenicillanic acid (6-APA) was obtained from Fluka Chemicals. All samples were analysed by low-angle *X*-ray powder diffraction using a Phillips PW1720 diffractometer. For those penicillins with previously reported structures from single-crystal *X*-ray studies, the powder diffraction patterns were indexed and the lattice parameters and unit-cell symmetries determined. Where necessary, samples were recrystallized until full agreement with the published values was attained (Table 1). Penicillin atomic co-ordinates were obtained from the Cambridge Crystallographic Data Base and were displayed, manipulated, and plotted with the MODEL program of the CHEMGRAF graphics package on a VAX 11/750 computer.

Solid-state ^{13}C n.m.r. spectra were recorded at 50.32 MHz on a Bruker CXP200 spectrometer. Powdered samples (*ca.* 150 mg) were packed in rotors of the Andrew–Beams design constructed from perdeuteriated poly-methyl (methyl acrylate). MAS frequencies of between 2 and 3 kHz were achieved with these rotors spinning on a nitrogen gas bearing. Small deviations of the spinning axis from the magic angle were found significantly to lower resolution and introduce artefacts.[14] The ^{79}Br magnetic resonance spectrum of a small quantity of KBr included with the penicillin sample was therefore used to set accurately the magic angle for each sample.[15] The combined techniques of high power proton decoupling and single contact cross polarization (CP)[16] were employed. A proton decoupling field of 1.1 mT and contact time of 0.75 ms were used. Free induction decays were defined typically by 8K data points over a 20 kHz sweep width. They were accumulated over 500—5 000 transients with a recycle delay of 3 s for an acceptable signal-to-noise ratio. In certain experiments the non-quaternary suppression (NQS) pulse sequence was employed to suppress selectively protonated carbon resonances.[17] A delay for 40 μs prior to acquisition, during which proton decoupling was suspended, was found to be effective. All ^{13}C chemical shift data were externally referenced to the upfield resonance of solid adamantane at 29.23 p.p.m. relative to tetramethylsilane. All spectra were recorded at room temperature (*ca.* 300 K).

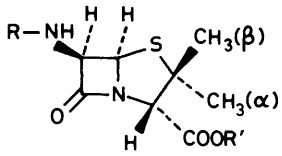
Results and Discussion

^{13}C CP/MAS spectra of sodium ampicillin in the amorphous and crystalline states are shown in Figure 1. In the amorphous sample it is possible to resolve a number of resonances, but the

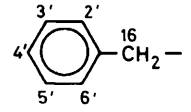
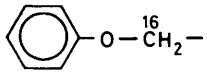
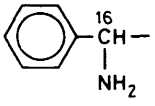
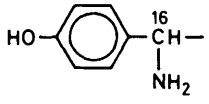
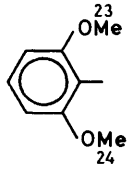
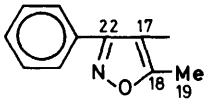
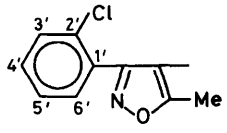
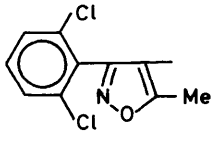
Table 1. List of penicillins and related compounds studied



(1)-(8)



(9)

	R	R′	Name	Crystal structure reference
(1)		Na K Procaine	penicillin G	*a* *b* *b*
(2)		H	penicillin V	*c*
(3)		H(.3H$_2$O) H Na	ampicillin	*d* *e* *f*
(4)		H(.3H$_2$O)	amoxycillin	*g*
(5)		Na	methicillin	
(6)		Na	oxacillin	*h*
(7)		Na	cloxacillin	
(8)		Na	dicloxacillin	
(9)	H	H	6-aminopenicillanic acid (6-APA)	*i*

[a] G. L. Clark, N. I. Kay, K. J. Pipenburg, and N. E. Schultz in 'The Chemistry of Penicillin,' Princeton University Press, 1949, p. 367. [b] D. D. Dexter and J. M. Van der Veen, *J. Chem. Soc., Perkin Trans. 1*, 1978, 185. [c] S. Abrahamson, D. C. Hodgkin, and E. N. Maslen, *Biochem. J.*, 1963, **86**, 514. [d] (i) D. Hall, D. C. Hodgkin, and M. N. G. James, *Nature*, 1968, **220**, 168. (ii) Atomic co-ordinates from M. N. G. James, personal communication. [e] M. O. Boles and R. J. Girven, *Acta Crystallogr., Sect. B*, 1976, **32**, 2279. [f] M. Sunada, K. Mishijima, K. Sugimoto, and S. J. Morimoto, *Takeda. Res. Lab.*, 1970, **79**, 488 (powder diffraction only). [g] M. O. Boles, R. J. Girven, and P. A. C. Gane, *Acta Crystallogr., Sect. B*, 1978, **34**, 461. [h] P. Blanpain, G. Laurent, and F. Durant, *Bull. Soc. Chim. Belg.*, 1977, **86**, 767. [i] (i) R. D. Diamond, D.Phil. Thesis, University of Oxford, 1964. (ii) Z. Galdecki and M. Werfel, *Acta Crystallogr., Sect. B*, 1978, **34**, 590.

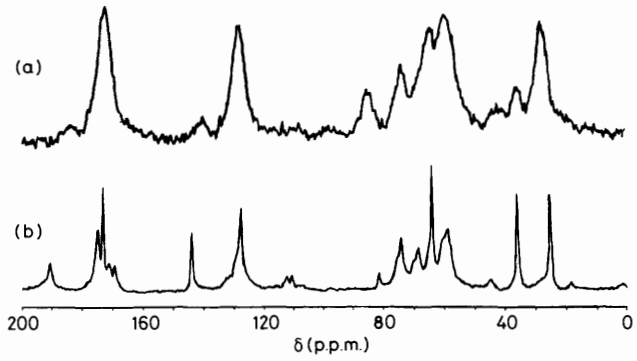


Figure 1. 50.32 MHz ^{13}C CP/MAS spectra of sodium ampicillin recorded as described in the text. MAS 3.2 kHz. (a) Amorphous sample, (b) crystalline sample

linewidths are of the order of 300 Hz. In the crystalline state, however, a dramatic increase in resolution is evident with linewidths typically 50 Hz or less. This difference is attributed to the existence of a range of molecular conformations and intermolecular interactions in the amorphous material which give rise to a dispersion of chemical shifts for each resonance.[14] All data reported below are for crystalline penicillins.

The high resolution attainable for the crystalline penicillins is further illustrated in Figures 2 and 3, where spectra of penicillin G, ampicillin, and 6-aminopenicillanic acid (6-APA) are shown. Comparison of spectra recorded at different spinning frequencies (Figure 2) enables ready identification of spinning sidebands which occur at multiples of the spinning frequency from the isotropic chemical shift. This allows clear resolution of resonances corresponding to virtually all the carbons in each molecule. Most of these resonances are singlets but a number are split into apparent doublets.

The assignment of resonances in the spectra of the penicillins

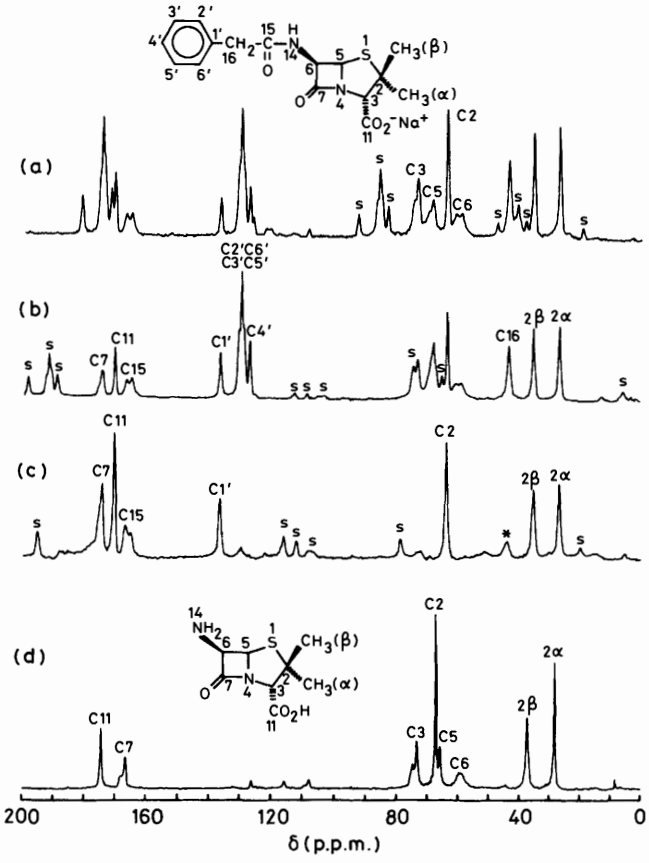


Figure 2. 50.32 MHz ^{13}C CP/MAS spectra of crystalline sodium penicillin G at (a) MAS 2.3 kHz, (b) MAS 3.2 kHz, (c) MAS 3.0 kHz with NQS, (d) spectrum of crystalline 6-APA at MAS 3.0 kHz. The assignments of the various carbon resonances are indicated: s denotes spinning sideband and * resonances of the PMMA rotor

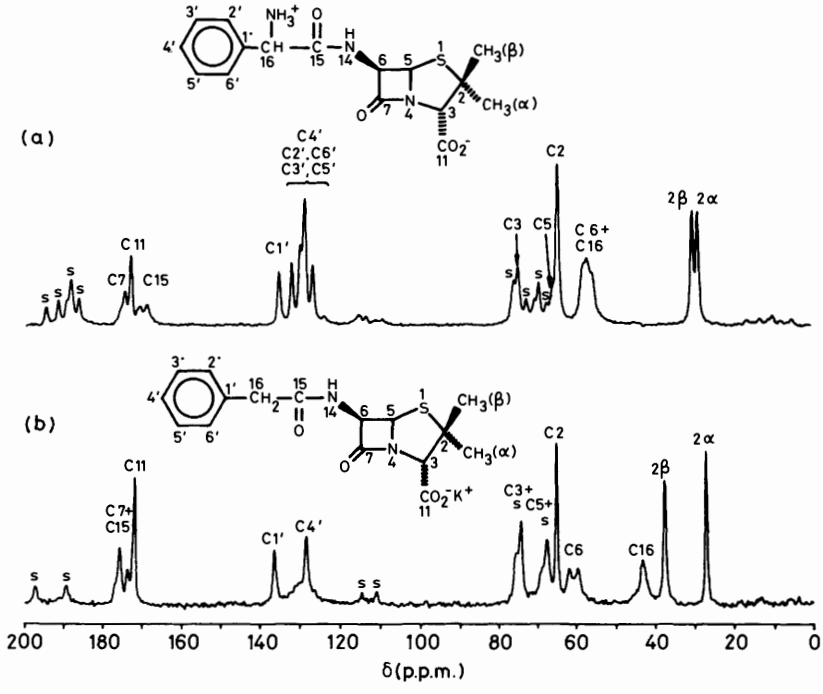


Figure 3. 50.32 MHz ^{13}C CP/MAS spectra of (a) crystalline ampicillin anhydrate at MAS 3.0 kHz, (b) potassium penicillin G at MAS 3.1 kHz. The assignments of the various carbon resonances are indicated. Note the differences between the spectra of the resonances corresponding to the 2β-Me, C-16 and aromatic carbons; s denotes spinning sideband

Table 2. ^{13}C Chemical shifts (p.p.m.) and ^{13}C–^{14}N splitting (Hz) in parentheses of various penicillins and related compounds

		2β-Me	2α-Me	C-2	C-3	C-5	C-6	C-7	C-15	C-11	C-1′	C-4′	C-2′, C-6′	C-3′, C-5′	C-16	C-17	C-18	C-19	C-22	C-23, C-24
Penicillin G (Na)[a]	solution	31.7	27.3	65.2	73.9	67.4	58.9	175.3	174.1	174.7	135.3	128.2	130.0	129.7	48.2					
	solid	35.7	27.0	63.9	73.9(65)†	68.9(55)†	60.3(115)‡	175.3(60)†	166.1(85)†	170.7	136.9	127.4	129.2, 130.0	131.0	43.9					
Penicillin G (K)	solid	37.0	26.4	64.6	74.4(70)†	67.7(65)†	60.1(105)‡	u	u	171.7	135.8	127.9	br	br	42.6					
Penicillin G (Pro)	solid	30.8	29.4	67.0	u	u	u	u	u	u	134.7	u	u	u	u					
Ampicillin (Na)[a]	solution	31.1	27.2	65.1	74.0	67.4	58.7	175.5	176.3	175.4	140.1	129.4	130.0	127.9	58.9					
	solid	35.6	24.9	63.9	74.5(60)†	69.3(70)†	u	175.4(55)†	170.4(95)‡	173.2	144.0	127.7	br	br	u					
Ampicillin trihydrate	solid	29.1	28.1	64.5	73.6(70)†	65.1(60)†	54.3(95)‡	u	u	170.5	133.0	130.3	134.0, 130.7	127.7, 124.9	u					
Ampicillin anhydrate	solid	29.9	28.4	64.4	73.3(75)†	65.0(55)†	u	175.2(55)†	170.1(100)‡	173.1	135.4	132.2	129.9, 126.8	129.0	u					
Amoxycillin (Na)[b]	solution	31.3	27.3	65.2	74.0	67.4	58.6	176.1	175.3	175.1	131.5	157.2	129.3	116.9	58.4					
Amoxycillin trihydrate	solid	28.8	27.7	65.2	72.8(75)†	65.7(55)†	u	173.6(55)†	170.5(95)‡	174.2	123.9	158.4	128.3, 124.6	120.4, 118.7	u					
Penicillin V (K)[c]	solution	31.6	26.8	64.8	73.4	66.8	57.5	174.0	169.6	173.8	156.9	122.2	114.9	130.1	66.8					
Penicillin V	solid	35.2	27.7	62.2	u	u	61.1(115)‡	174.7(70)†	169.6(95)‡	167.1	157.0	122.8	118.4, 112.3	130.6	u					
6-APA	solid	36.9	27.9	67.0	73.7(75)†	66.2(75)†	59.0	167.1(60)†		174.3										
Oxacillin (Na)[d]	solution	32.1	27.8	66.0	74.5	67.4	59.2	175.4	162.3	175.4	132.2	128.2	129.9, 129.2	130.7		111.8	164.3	13.3	175.4	
	solid	34.7	25.2	67.9	73.6(80)†	70.2(75)†	61.4(110)‡	u	165.5(85)‡	u	127.7		129.9, 131.4	129.0		110.9	162.1	11.8	u	
Cloxacillin (Na)[d]	solution	32.0	27.6	65.7	74.2	67.0	58.6	175.5	160.0	175.5	133.6	127.1	134.2, 132.2	133.1		112.5	163.5	13.4	175.5	
	solid	35.6	25.3	65.3	72.1(60)†	66.0(70)†	60.0(85)‡	u	164.2(85)‡	u	u	u	u	u		112.7	158.7	11.4	u	
Dicloxacillin (Na)[d]	solution								157.7	175.5	134.4	126.7	136.4	130.2		112.1	162.2	13.7	175.3	
	solid	36.1	25.7	66.7	72.8(65)†	u	59.4(110)‡	u	164.6(95)‡	u	u	u	u	u		110.9	155.4	12.3	u	
Methicillin (Na)[d]	solution	31.0	27.5	65.5	74.4	67.4	58.6	175.9	158.1	175.5	u	133.4	169.5	105.9						57.1
	solid	35.9	26.4	66.1	72.4(65)†	66.8(65)†	u	175.7(65)	166.8(95)‡	173.0	115.1	132.6	159.3	107.3, 108.0						58.1, 59.2

u = Overlapping or unassigned resonance; br = broad resonance; † asymmetric doublet; ‡ symmetric doublet.

[a] R. Mondelli and P. Ventura, *J. Chem. Soc., Perkin Trans. 2*, 1977, 1749. [b] J. Everett, personal communication. [c] C. Chang and S. L. Hen, *J. Pharm. Sci.*, 1979, **68**, 64. [d] Ref. 18.

was relatively straightforward. The approach adopted was first to compare the spectra with previously published solution ^{13}C n.m.r. data (Table 2). The similarity of chemical shifts between the solution and solid states permitted immediate assignment of many of the resonances. In the case of sodium penicillin G, for example, direct identification of the well resolved C-2, C-3, C-5, C-6, 2α-Me, and 2β-Me resonances of the fused bicyclic ring system was possible. Further information concerning the assignments was available from the NQS spectra in which the resonances of all protonated carbons, with the exception of the rapidly spinning methyl groups,[9,10] were completely suppressed. Using sodium penicillin G as an example again, clear identification of the non-protonated aromatic carbon resonances was possible; these are entirely consistent with the assignments referred to above (Figure 2). The major uncertainties remaining in the assignment of the spectra of sodium penicillin G are the poorly dispersed carbonyl resonances (C-7, C-11, C-15). Two of these three resonances are apparent doublets with differing lineshapes and splittings; the splittings of the asymmetric and symmetric doublets are 60 and 80 Hz, respectively. The third resonance is a sharp singlet. Similar splittings could be identified in other penicillins; these arise from the incomplete suppression of the ^{13}C–^{14}N dipolar interactions by the MAS experiment.[19,20] The singlet resonance can be directly assigned to C-11, the only one of these three carbons not immediately adjacent to a nitrogen atom. Distinction between C-7 and C-15 was achieved by comparison of the penicillin spectra with the spectrum of 6-aminopenicillanic acid (6-APA) (Figure 2). The carbonyl region of the 6-APA spectrum shows a singlet (C-11) and a resonance asymmetrically split by 65 Hz, which must arise from C-7. The magnitude of the splitting and the resonance lineshape will depend on the geometry of the β-lactam ring.[19] This is closely similar in the compounds studied here with N-4 pyramidally disposed towards its three substituents;[4,21] the resonance with an asymmetric splitting of 60 Hz was therefore assigned to C-7 and the resonance with a symmetric splitting of 80 Hz assigned to C-15 for sodium penicillin G. This approach was employed throughout this study and returned unambiguous assignments for all spectra with resolvable resonances. Chemical shifts for the assigned resonances of the various penicillins are listed in Table 2.

Comparison of the spectra of different penicillins, and of the data given in Table 2, reveals a number of interesting features which are exemplifed in Figure 3. The chemical shifts of the carbons in the common bicyclic ring system (C-2, C-3, C-5, C-6, and C-7) and of the 2α-Me group are remarkably constant over the range of compounds and typically vary by *ca.* 2 p.p.m. about a mean value (Tables 2 and 4). The chemical shift of C-11 varies by up to 5 p.p.m. but this can be attributed to the variation in ionization state of the carboxy group. The sidechains contain carbons at which substitution takes place and this is reflected in their chemical shifts. Two other features of this comparison are shown in Figure 3. First, in certain cases, resonances of several sidechain aromatic carbons cannot be identified in the spectra. Second, there is a large variation in the chemical shift of the resonance of the 2β-Me group within the series of molecules examined although it is remote from the sites of chemical substitution. These two features are discussed below.

The aromatic regions of the spectra of ampicillin and penicillin G in different crystal environments are compared in Figure 4. A change from sodium to potassium as the counterion of penicillin G, and from the free acid to sodium salt of ampicillin has a profound effect on the aromatic resonances. A closer examination of the aromatic carbon resonances (Figure 4) reveals that although all six resonances can be identified in the spectra of sodium penicillin G and ampicillin trihydrate, only two sharp resonances are observed in the spectra of potassium penicillin G and sodium ampicillin; the other four

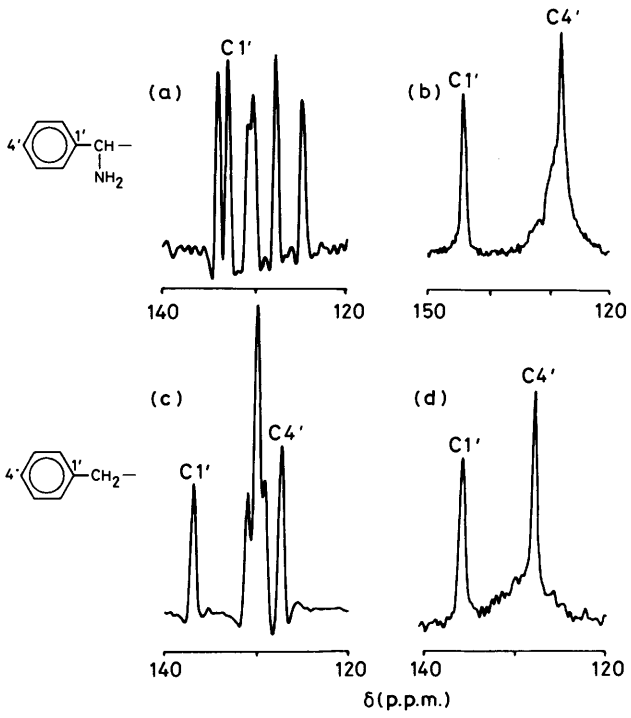


Figure 4. Aromatic regions of 50.32 MHz ^{13}C CP/MAS spectra. (a) Ampicillin trihydrate, (b) sodium ampicillin, (c) sodium penicillin G, (d) potassium penicillin G, MAS ~3 kHz. Assignments were made on comparison with the corresponding NQS/MAS spectra and high-resolution solution spectra

resonances are apparently very broad. Similar line broadening has been observed for the resonances of aromatic rings in peptides and proteins, and has been associated with the dynamic behaviour of the molecules.[22–28] In the simplest case of rotation about the two-fold axis of a symmetric ring, the environments of C-2′,C-6′ and of C-3′,C-5′ are interchanged. In the extreme case of fast motion, complete averaging within these pairs of resonances will give a single resonance in each case; such a situation occurs in solution. When the rate of rotation is slower and comparable to the difference in resonant frequencies of the exchanging sites (*ca.* 300 Hz) or particularly to the frequency of the perturbation induced by the proton dipolar decoupling field (*ca.* 50 kHz), the lines can broaden considerably.[14] The linewidths of the C-1′ and C-4′ resonances will be little affected, however, as their environments are not significantly altered by this motional behaviour. Such a situation is apparent for potassium penicillin G and sodium ampicillin; the clear resolution of the resonances in the spectra of sodium penicillin G and ampicillin trihydrate indicates that the timescale of any similar motion of the aromatic rings is much slower. Examination of the crystal structures shows that intramolecular contacts do not offer a simple explanation for the differences in motional properties within the pairs of molecules. It is apparent from the results shown here that the characteristics of these dynamic processes are profoundly dependent on the intermolecular contacts between molecules within the crystals. Further analyses of these phenomena are in progress, in an effort to provide a full description of the nature of these crystal packing effects.

The variation in chemical shift of the 2β-Me resonance noted above is over 6 p.p.m. but is not random. For each penicillin studied, the shift of this resonance can be placed unambiguously into one of two categories (Table 3). In one category, denoted U (upfield), the shift is within 1.5 p.p.m. of 30 p.p.m. and for the

Table 3. Solid-state parameters of penicillins

	^{13}C Chemical shift (p.p.m.)			
	2α-Me	2β-Me	Thiazolidine ring pucker[a]	ψ[b] (°)
Penicillin G (K)	26.9	37.0	C-3	89.5
Penicillin G (Na)	27.0	35.7	C-3	85.1
Penicillin V	27.7	35.2	C-3	83.2
Oxacillin (Na)	25.2	34.7	C-3	89.2
6-APA	27.9	36.9	N-4[c]	93.2
Methicillin (Na)	26.5	36.0		
Cloxacillin (Na)	25.3	35.6		
Dicloxacillin (Na)	27.6	37.9		
Ampicillin (Na)	29.9	35.6		
Ampicillin trihydrate	28.1	29.1	S-1	144.8
Ampicillin anhydrate	28.4	29.9	S-1[d]	152.3
Amoxycillin trihydrate	27.7	28.8	S-1	147.7
Penicillin G (Pro)	29.4	30.8	S-1	136.1

[a] Atom out of best-fit plane defined by remaining four atoms. [b] 2β-Me–C-2–C-3–N-4. [c] First and second best-fit planes in real space are:

$$X + 0.9023Y + 0.4074Z = 7.0046$$
$$X + 0.8881Y + 0.6755Z = 7.1467$$

defined by S-1–C-2–C-3–C-5 and S-1–C-2–N-4–C-5 respectively. Perpendicular distances from these planes (Å) are S-1 0.03; C-2 −0.03; C-3 0.03; N-4 −0.42; C-5 −0.02, and S-1 0.03; C-2 −0.05; C-3 0.36; N-4 −0.09; C-5 0.11. [d] Best-fit plane in real space is $X \times 1.0973Y - 0.9760Z = +5.2781$ defined by C-2–C-3–N-4–C-5. Perpendicular distances from this plane (Å) are S-1 −0.75; C-2 0.025; C-3 0.04; N-4 0.05; C-5 −0.03.

Table 4. Mean chemical shifts (p.p.m.) and standard deviations (in parentheses) of the thiazolidine ring carbons

	2β-Me	2α-Me	C-2	C-3	C-5	C-6	C-7
Solution: all[a]	31.7	27.4	65.4	74.1	67.3	58.6	175.2
	(0.5)	(0.3)	(0.4)	(0.4)	(0.2)	(0.5)	(0.6)
Solid: all	33.9	26.9	65.3	73.4	67.1	60.2[b]	174.8[c]
	(3.1)	(1.4)	(1.6)	(0.8)	(1.8)	(0.8)	(0.6)
Solid: C-3′	35.9	26.3	65.3	73.4	67.9	60.2	175.1[d]
	(0.7)	(1.1)	(1.8)	(0.9)	(1.6)	(0.9)	(0.4)
Solid: S-1′	29.7	28.4	65.3	73.3	65.3	*e*	174.4
	(0.9)	(0.7)	(1.2)	(0.4)	(0.4)	*e*	*e*

[a] See Table 2 for data and references. [b] Ampicillin trihydrate δ 54.3 p.p.m. not included. When included, mean shift = 59.5 p.p.m.; s.d. 2.2. [c] 6-APA δ 167.1 p.p.m. not included. When included, mean shift = 174.0 p.p.m.; s.d. 2.6. [d] 6-APA δ 167.1 p.p.m. not included. When included, mean shift = 174.1 p.p.m.; s.d. 2.8. [e] Insufficient data.

other, denoted D (downfield), it is within 2 p.p.m. of 36 p.p.m. More dramatically, perhaps, the separation of the 2α-Me and 2β-Me resonances is between 1.0 and 1.5 p.p.m. for those penicillins in class U but between 7.5 and 10.7 p.p.m. for those in class D (Table 4). Examples of these categories are shown in Figure 3. The 2β-Me group is conserved in all the penicillins and is not attached to an atom at which substitution takes place. The nearest site at which chemical differences exist between the molecules is at the carboxy group. Neither the state of ionization of this group nor the nature of the counterion correlates with the two observed shift classes U and D. For example, members of class D include penicillin V (protonated free acid) and potassium penicillin G, whereas ampicillin trihydrate (zwitterionic free acid) and procaine penicillin G are members of class U. It is of particular interest to note from the results for penicillin G that different salts of the same molecule can fall clearly into different categories (Table 3). This implies

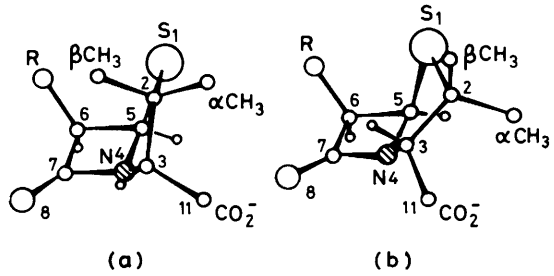


Figure 5. Penicillin thiazolidine ring conformations (ref. 4). (a) C-3, (b) S-1

that the shift must reflect a specific intermolecular or intramolecular conformational feature. This was directly investigated by examining the available *X*-ray crystal structures of the penicillins.

An examination of the molecular packing within the crystals reveals that the 2β-Me carbon atom has no intermolecular contacts within 6 Å which could in any way be correlated with the shift classes U and D. One specific interaction considered in detail was the aromatic ring current shift. The minimum separation of a 2β-Me carbon and the centroid of an intermolecular aromatic ring in any of the crystals is *ca.* 5 Å. The aromatic ring current perturbation of a chemical shift at such a distance will be <1 p.p.m.,[29] which is far smaller than the observed differences between the 2β-Me chemical shift classes U and D. Furthermore, this effect would be expected to induce similar perturbations in the chemical shifts of the C-2 and 2α-Me resonances. The relative orientations of the sidechain with respect to the fused bicyclic ring system in the different structures were also examined. Some of the penicillin structures have an extended molecular conformation but others have the sidechain folded over the central bicyclic ring nucleus.[18,21] No obvious correlations could be found between the sidechain conformations and the shifts of the 2β-Me resonances. For example, sodium oxacillin and sodium penicillin G have extended and compact sidechains respectively. Both, however, have 2β-Me shifts of class D. As with the intermolecular interactions, the intramolecular separation of the aromatic ring and the 2β-Me group was insufficiently small for significant ring current shifts to be anticipated.

The fused bicyclic β-lactam–thiazolidine ring system is common to all the penicillins. Single-crystal *X*-ray analyses have shown the nitrogen (N-4) contained in the strained β-lactam ring to be pyramidally disposed towards its C-3, C-5, and C-7 substituents and the geometry of this ring to be essentially unchanged in the series of compounds considered here.[4,21] In contrast, the five-membered thiazolidine ring has been found to exist primarily in one of two non-planar conformations (Figure 5). These are denoted C-3 or S-1 indicating the atom significantly deviating from the approximate plane defined by the remaining four atoms.[21] The penicillins studied here are classified by this method in Table 3. Examination of Table 3 shows that all penicillins with C-3 puckers have class D 2β-Me shifts and all penicillins with S-1 puckers have class U 2β-Me shifts. However, although we have observed class U and D 2β-Me shifts in ampicillin anhydrate and 6-APA, respectively, the thiazolidine puckers of these compounds have been determined to be C-2 and N-4 out-of-the-plane, respectively. This prevents a more general correlation between 2β-Me shift class and thiazolidine ring pucker from being established.

In the light of these data, we have re-analysed the atomic co-ordinates of the thiazolidine ring in ampicillin anhydrate and have found the ring pucker to be best characterized by the deviation of S-1 and *not* of C-2 from the approximate plane

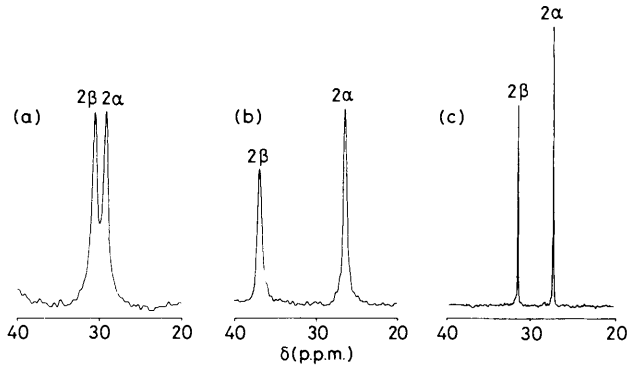


Figure 6. Solution and solid-state ^{13}C n.m.r. spectra of penicillin G. (a) 50.32 MHz $^{13}CP/MAS$ spectrum of crystalline procaine penicillin G, (b) 50.32 MHz ^{13}C CP/MAS spectrum of crystalline potassium penicillin G, (c) 62.9 MHz proton-decoupled ^{13}C n.m.r. spectrum of a solution of potassium penicillin G (80 mg cm^{-3} in D_2O)

Table 5. ^{13}C Chemical shifts and predicted thiazolidine conformational populations for penicillins in solution

Compound	2β-Me Chemical shift (p.p.m.)	Predicted populations (%)[a] S-1′	C-3′
Penicillin G (Na)	31.7	68	32
Ampicillin (Na)	31.1	77	23
Amoxycillin (Na)	31.3	74	26
Penicillin V (K)	31.6	69	31
Methicillin (Na)	31.0	79	21
Oxacillin (Na)	32.1	61	39
Cloxacillin (Na)	32.0	63	37
Dicloxacillin (Na)	32.5	55	45

[a] Based on 2β-Me shifts of δ 35.9 and 29.7 p.p.m. for C-3′ and S-1′ conformations respectively. $\delta_{obs} = P_{C\text{-}3'}\,\delta_{C\text{-}3'} + P_{S\text{-}1'}\,\delta_{S\text{-}1'}$. δ_{obs} is the observed 2β-Me shift, $\delta_{C\text{-}3'}$, $\delta_{S\text{-}1'}$ are the C-3′ and S-1′ 2β-Me shifts, and $P_{C\text{-}3'}$, $P_{S\text{-}1'}$ are the fractional populations of C-3′, S-1′ puckers where $P_{C\text{-}3'} + P_{S\text{-}1'} = 1$.

defined by the remaining four atoms (Table 3). An alternative criterion for defining the thiazolidine ring pucker is the torsion angle 2β-Me–C-2–C-3–N-4 (ψ) and two distinct angular domains are found. Table 5 shows that 136° < ψ < 153° is clearly associated with the S-1 group and 85° < ψ < 94° with the C-3 group; we have described these domains as S-1′ and C-3′ respectively. Notably, we have ψ 152.3 and 92.3° in ampicillin anhydrate and 6-APA, respectively, which are characteristic of the S-1′ and C-3′ pucker groups respectively. An examination of Table 3 reveals a complete correlation between the 2β-Me shift classes and thiazolidine ring puckers as defined by the torsion angle 2β-Me–C-2–C-3–N-4 (ψ).

We have observed a few large chemical shift changes in addition to those of the 2β-Me resonances. In particular, the C-15 chemical shifts of sodium penicillin G in the solution and solid states are 174.1 and 166.1 p.p.m. whereas for the potassium salt the analogous shifts are 173.1 and *ca.* 174 p.p.m. (exact assignment was not possible due to partial overlap of the C-15 and C-7 resonances). Similarly, whereas the chemical shift of the C-6 resonance is typically 60 ± 1.5 p.p.m. over the series of compounds studied here, in ampicillin trihydrate this resonance is observed at 54.3 p.p.m. These appear to reflect features of the crystal packing which are unique to the individual compound; we have at present, however, insufficient data to correlate these unambiguously with any intramolecular or intermolecular structural features.

The basis for the much larger dependence of the 2β-Me chemical shift on the thiazolidine ring conformation compared with the chemical shifts of the other ring carbons is difficult to establish. Examination of the two different conformations (Figure 5) suggests that the origin could lie in the different geometrical relationships between the 2β-Me group and the β-lactam carbonyl group; the 2β-Me–C-7 separations in the C-3′ and S-1′ puckers are *ca.* 3.5 and 4.5 Å, respectively. Theoretical understanding of ^{13}C shifts is as yet inadequate for problems of this type.[30] It is, however, possible to make use of the empirical correlation to predict the conformations of molecules of unknown structure. On this basis, the thiazolidine rings in the crystals of sodium methicillin, sodium ampicillin, sodium cloxacillin, and sodium dicloxacillin are all predicted to have 85° < ψ < 94°, that is a C-3′ pucker (Table 3). A single-crystal *X*-ray analysis of the methyl ester of methicillin has shown the thiazolidine ring conformation to have ψ 147.8° (S-1′).[31] The analysis presented here therefore predicts a difference between the thiazolidine conformations of the methyl ester and the sodium salt of this penicillin. We await a single-crystal analysis of this salt to test this prediction.

Perhaps the most important use of the solid-state ^{13}C chemical shift data of the penicillin crystals is the comparison with the shifts observed in solution. An examination of the mean chemical shifts for the various bicyclic ring carbons in the crystalline and solution states reveals a remarkable correlation between the two sets of data (Table 4); with the exception of the 2β-Me shift, the agreement is within 1 p.p.m. The 2β-Me chemical shifts observed in solution are, however, intermediate between the values found in the crystalline state to be characteristic of the C-3′ and S-1′ conformations (Figure 6). This observation is consistent with a rapid equilibrium between the two conformations for molecules in solution; the observed 2β-Me chemical shift would thus be a weighted average of the chemical shifts corresponding to the two puckers. In the light of the data in Table 4 we can with confidence take values of the 2β-Me chemical shift in solution to be very close to the mean values found for molecules with the different puckers in the crystals. It is therefore possible to estimate the relative populations of the two conformations for each compound in solution. These data are given in Table 5 and indicate that the S-1′ pucker is marginally favoured over the C-3′ pucker in solution; the energy difference of the two ring conformations is therefore *ca.* 2 kJ mol^{-1}. The stability of the S-1′ with respect to the C-3′ pucker is less pronounced for the isoxazole-derived penicillins, but the differences are small.

Conclusions

Comparison of the ^{13}C n.m.r. spectra of a series of closely related molecules in defined crystalline states has proved to be a powerful method of interpreting the spectra. Differences both in the conformation of the fused bicyclic ring system and in the dynamics of the aromatic sidechains have been apparent in the study of the penicillins reported here. Both differences can be attributed to the effects of intermolecular interactions within the crystals rather than to intrinsic differences in intramolecular interactions. The solid-state spectra enable the n.m.r. parameters for different conformations to be defined. Indeed, comparison of the ^{13}C n.m.r. spectra of the crystalline materials with spectra of the molecules in the solution state has provided strong evidence that the marked differences in conformation of the bicyclic ring system observed for different molecules in the solid state do not exist in solution. Rather, the data are consistent with a rapid equilibrium in solution between different puckers of the thiazolidine ring, the relative populations of which can be estimated. The similarities in the relative populations of the different thiazolidine ring puckers in solution undermines

attempts to correlate static crystallographic features of these molecules with pharmacological activity in solution.

Acknowledgements

This work is supported in part by the S.E.R.C. We acknowledge valuable discussions with C. K. Prout, Laboratory of Chemical Crystallography, and J. Everett and A. E. Bird, Beecham Pharmaceuticals, Brockham Park. We thank M. A. Howarth for assistance with the computing.

References

1 'β-Lactam Antibiotics, Chemistry and Biology,' eds. R. B. Morin and M. Gorman, Academic Press, New York, 1981.
2 D. J. Waxman and J. L. Strominger, in 'β-Lactam Antibiotics, Chemistry and Biology,' eds. R. B. Morin and M. Gorman, Academic Press, New York, 1981, vol. 3, p. 209.
3 N. C. Cohen, *J. Med. Chem.*, 1983, **26**, 259.
4 A. Balsamo, P. Domiano, B. Macchia, F. Macchia, and M. Nardelli, *Eur. J. Med. Chem.*, 1980, **15**, 559.
5 C. M. Dobson, L. O. Ford, S. E. Summers, and R. J. P. Williams, *J. Chem. Soc., Faraday Trans. 2*, 1975, **71**, 1145.
6 R. J. D. Cooper, P. V. DeMarco, J. C. Cheng, and N. D. Jones, *J. Am. Chem. Soc.*, 1969, **91**, 1408.
7 J. Everett, personal communication.
8 S. J. Opella, *Annu. Rev. Phys. Chem.*, 1982, **33**, 533.
9 R. E. Wasylishen and C. A. Fyfe, *Annu. Rep. NMR Spectrosc.*, 1982, **12**, 1.
10 C. A. Fyfe, 'Solid State NMR for Chemists,' C.F.C. Press, Guelph, 1983.
11 C. F. Brewer, J. S. Blanchard, and S. England, *Carbohydr. Res.*, 1982, **102**, 294.
12 R. Tabeta and H. Saito, *Biochemistry*, 1985, **24**, 7696.
13 G. S. Harbison, S. O. Smith, J. A. Pardoen, J. M. L. Courtin, J. Lugtenburg, J. Herzfeld, R. A. Mathies, and R. G. Griffin, *Biochemistry*, 1985, **24**, 6955.
14 D. L. VanderHart, W. L. Earl, and A. N. Garroway, *J. Magn. Reson.*, 1981, **44**, 361.
15 J. S. Frye and G. E. Maciel, *J. Magn. Reson.*, 1982, **48**, 125.
16 A. Pines, M. G. Gibby, and J. S. Waugh, *J. Chem. Phys.*, 1973, **59**, 569.
17 S. J. Opella and M. H. Frey, *J. Am. Chem. Soc.*, 1979, **101**, 5854.
18 P. C. Blanpain, J. B. Nagy, G. H. Laurent, and F. V. Durant, *J. Med. Chem.*, 1983, **23**, 1283.
19 J. G. Hexem, M. H. Frey, and S. J. Opella, *J. Chem. Phys.*, 1982, **77**, 3847.
20 R. K. Harris, P. Jonsen, and K. J. Packer, *Magn. Reson. Chem.*, 1985, **23**, 565.
21 R. M. Sweet, in 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York, 1972, p. 281.
22 M. H. Frey, J. A. DiVerdi, and S. J. Opella, *J. Am. Chem. Soc.*, 1985, **107**, 7311.
23 M. H. Frey, S. J. Opella, A. L. Rockwell, and L. M. Gierasch, *J. Am. Chem. Soc.*, 1985, **107**, 1946.
24 D. M. Rice, R. J. Wittebort, R. G. Griffin, E. Meirovitch, E. R. Stimson, Y. C. Meinwald, J. H. Freed, and H. A. Scheraga, *J. Am. Chem. Soc.*, 1981, **103**, 7707.
25 E. Oldfield, R. A. Kinsey, and A. Kintanar, *Methods Enzymol.*, 1982, **88**, 310.
26 C. M. Gall, T. A. Cross, J. A. Di Verdi, and S. J. Opella, *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 101.
27 J. Schaefer, E. O. Stejskal, R. A. McKay, and W. T. Dixon, *J. Magn. Reson.*, 1984, **57**, 85.
28 R. A. Kinsey, A. Kintanar, and E. Oldfield, *J. Biol. Chem.*, 1981, **256**, 9028.
29 C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, 1958, **29**, 1012.
30 D. J. Craik, *Annu. Rep. NMR Spectrosc.*, 1983, **15**, 1.
31 P. Blanpain, M. Melebeck, and F. Durant, *Acta Crystallogr., Sect. B*, 1977, **33**, 580.

Received 24*th March* 1986; *Paper* 6/577