^{13}C Nuclear Magnetic Resonance of *N*-Heterocycles. Part 3.[1] ^{13}C Chemical Shift Assignments of the Carbonyl Groups in Penicillins and Cephalosporins[1]

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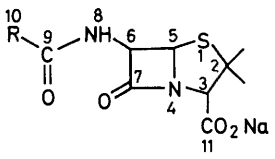
The ^{13}C resonance frequencies of all the carbonyl carbon atoms in several penicillins and cephalosporins have been uniquely assigned for aqueous solutions by use of various experimental techniques. The ^{13}C shift values are compared with other spectroscopic data (i.r. and *X*-ray). The possibility of interpreting the ^{13}C results in terms of charge density distributions is discussed in relation to the mechanism of ring opening of the β-lactams by basic hydrolysis. For those compounds for which spectra have not been reported previously, the ^{13}C frequencies of all the carbon atoms are assigned.

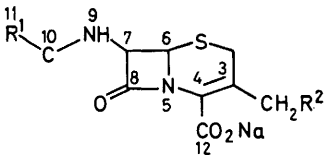
A FEW papers have appeared [2-5] concerning ^{13}C n.m.r. analyses of the important classes of antibiotics reported here, but in none of these have the resonances of carbonyl carbon atoms been defined.‡ This is due in part to the difficulty of detecting the peaks at natural ^{13}C abundance, owing to their very low intensity and also to their proximity to each other. It is important to assign these signals by independent techniques in view of their potential utility as a probe for biosynthetic studies. Moreover the carbon shift values may enable an evaluation of the effective positive charge localized on the β-lactam carbonyl carbon atoms, and of the possibility of nucleophilic attack at this position by hydrolytic agents. This is particularly interesting since the ease of basic hydrolysis of the lactam amide bond in these antibiotics is generally accepted [6] as explaining their antibacterial activity: the ease of β-lactam ring opening, consequent on the lack of planarity of the amide nitrogen centre, should make possible irreversible acylation by the antibiotic of the enzyme which controls the final step in bacterial cell-wall synthesis. In fact the acylation of the cell enzyme is presumed to proceed *via* attack on the β-lactam carbonyl of the antibiotic by a thiol group of the enzyme.[6,7]

Since the chemical reactivity of the β-lactam moiety of penicillins and cephalosporins may reflect their antibiotic activity, a number of correlations have been explored, involving for example i.r. carbonyl stretching

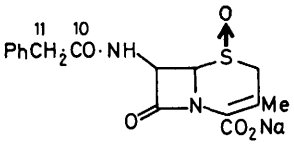


R

(1) $PhCH_2$
(2) $PhO{\cdot}CH_2$
(3) $PhCH{\cdot}NH_2$
(4) $PhCHN_3$ (10*S*)
(5) $PhCHN_3$ (10*R*)
(6) $(Cl)_2C_6H_3$



	R^1	R^2
(7)	$PhCH_2$	H
(8)	$PhCH_2$	OAc
(9)	$PhCH{\cdot}NH_2$	H
(10)	$PhCH{\cdot}NH{\cdot}CHO$	H



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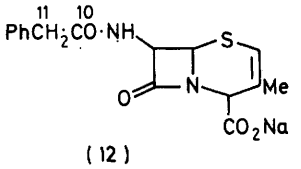

(12)

frequency variations, C–N and C=O bond length differences, hydrolysis rate constants, and CNDO charge

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‡ Only in a biogenetic study of cephalosporin C [N. Neuss, C. H. Nash, P. A. Lemke and J. B. Grutzer, *J. Amer. Chem. Soc.*, 1971, **93**, 2337, 5314 (correction)], the carbonyl carbons have been assigned from the results of incorporation of labelled acetates.

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density distributions.[6,8-11] In addition to the foregoing physical methods (i.r. and *X*-ray), ^{15}N n.m.r. has also been used; the ^{15}N shift values obtained for some penicillins and cephalosporins [12] are in sharp contrast to those expected. A ^{13}C n.m.r. study is therefore of interest.

We report here the ^{13}C n.m.r. parameters of several penicillins [(1)—(6)] and cephalosporins [(7)—(12)] in D_2O, with assignments of all the carbon frequencies, in particular those of the carbonyl carbon atoms.

EXPERIMENTAL

The ^{13}C Fourier transform spectra at natural abundance were recorded with a Varian XL-100-15 spectrometer. The single frequency selective heteronuclear decoupling (s.f.s.d.) experiments for the assignment of the carbonyl carbon signals were performed with 90–92 dB of decoupling power on the Gyrocode unit. All the compounds were studied as sodium salts in D_2O in order to allow a better comparison with the basic hydrolysis parameters.[9] Concentrations of *ca.* 0.2M were used for obtaining chemical shift values (Tables 1 and 2). Shifts were measured from internal dioxan and converted to the Me_4Si scale by using the observed [13] difference of 67.4 p.p.m. between dioxan and internal Me_4Si; they are accurate to within ± 0.1 p.p.m. For the Fourier transform measurements of long-range coupling constants and for decoupling experiments a 2500 or 1000 Hz spectral window was employed, and more concentrated solutions (solubility permitting) were used. The magnitudes of all J_{CH} values have been taken from proton-coupled spectra in 'gated' decoupling mode, except for $^3J_{C(7)H(3)}$ in compounds (2)—(4). In these cases measurements of this coupling were possible only in s.f.s.d. mode with irradiation at the frequencies of H-6 and H-5 at δ 5.5—5.6, but particular care was taken in the calibrations of the decoupling power in order to avoid the perturbation of the H-3 signal at δ 4.2—4.3. Errors introduced in this mode were checked to be within the limits of error by comparison between the J values measured in both 'gated' decoupling and s.f.s.d mode for all the other compounds. The reported couplings are accurate to within ± 0.5 Hz. The ^1H chemical shift values used for decoupling experiments were obtained with the same solutions as for ^{13}C spectra and are reported in Table 3 LAOCN3 analyses of the spectra show that H-6, H-7, and C-8 patterns of all cephalosporins studied are first-order, as are the corresponding patterns of the penicillin (6), at least with regard to frequencies.

RESULTS

^{13}C Chemical Shift Assignments.—The assignment of the ^{13}C signals (Table 1), except for carbonyl carbon frequencies, was based on the expected chemical shift behaviour and on correlation with published data. Single frequency (s.f.s.d.) and off-resonance proton decoupling were employed where necessary. The values of one-bond coupling constants were largely used for the assignment of tertiary carbon signals with similar chemical shifts, for example C-3, -5, and -6 in penicillins, C-6 and -7 in Δ^3-cephalosporins, and C-4, -6, and -7 in Δ^2-cephalosporins. The effect of substituents on these coupling constants follows the expected trend. This method is particularly useful for penicillins

TABLE 1

^{13}C N.m.r. parameters of penicillins (1)—(6) and cephalosporins (7)—(12) [a]

	C-2	C-3	C-5	C-6	C-10	C-12 [c]	C-13 [c]	Others
(1) [b]	65.2	73.9 (*J* 145)	67.4 (*J* 178)	58.9 (*J* 153)	48.2 (*J* 130)	31.7 (*J* 130)	27.3 (*J* 130)	135.3 (s), 128.2 (*p*), 130.0, 129.7 (*o*, *m*) [d]
(2)	65.4	73.7 (*J* 145)	67.4 (*J* 178)	58.2 (*J* 155)	67.4 (*J* 145)	32.0 (*J* 130)	27.3 (*J* 130)	157.6 (s), 123.1 (*p*), 115.5 (*o*), 130.7 (*m*) [d]
(3)	65.1	74.0 (*J* 145)	67.4 (*J* 178)	58.7 (*J* 156)	58.9 (*J* 140)	31.1 (*J* 130)	27.2 (*J* 130)	140.1 (s), 129.4 (*p*) 130.0, 127.9 (*o*, *m*) [d]
(4)	65.3	74.1 (*J* 145)	67.4 (*J* 178)	59.1 (*J* 156)	67.0 (*J* 148)	31.6 (*J* 130)	27.3 (*J* 130)	135.0 (s), 130.4 (*p*),
(5)	65.2	74.0 (*J* 145)	67.4 (*J* 178)	58.9 (*J* 155)	66.8 (*J* 145)	31.4 (*J* 130)	27.3 (*J* 130)	130.1 (*o*), 128.7 (*m*) [d]
(6)	65.6	73.9 (*J* 145)	66.9 (*J* 180)	58.4 (*J* 155)	111.8 [e]	32.1 (*J* 130)	27.4 (*J* 130)	13.5, 176.1, 157.4, [f]

	C-2	C-3	C-4	C-6	C-7	C-11	C-13	Others
(7)	29.2 (*J* 145)	123.2	127.6	57.7 (*J* 175)	59.6 (*J* 155)	42.8 (*J* 130)	19.3 (*J* 130)	135.6 (s), 128.3 (*p*), 130.2, 129.9 (*o*, *m*) [d]
(8)	26.0 (*J* 140)	117.2	132.5	58.0 (*J* 175)	59.9 (*J* 155)	42.7 (*J* 130)	59.9 (*J* 150)	135.7 (s), 128.4 (*p*), 130.2, 129.9 (*o*, *m*), [d] 174.9 (CO), 20.9 (Me)
(9)	29.1 (*J* 142)	122.9	127.5	57.8 (*J* 176)	59.3 (*J* 156)	58.8 (*J* 140)	19.2 (*J* 130)	139.3 (s), 129.3 (*p*), 130.1, 129.7 (*o*, *m*) [d]
(10)	29.2 (*J* 140)	122.8	127.4	57.8 (*J* 175)	59.5 (*J* 158)	57.3 (*J* 140)	19.3 (*J* 130)	133.3 (s), 130.1, 129.9, 128.2 (*o*, *m*, *p*), [d] 164.4 (CHO), (^1J 200)
(11)	48.6 (*J* 140)	116.1	127.9	62.9 (*J* 172)	59.2 (*J* 154)	42.8 (*J* 130)	19.6 (*J* 130)	135.2 (s), 127.9 (*p*), 1301, 129.8 (*o*, *m*) [d]
(12)	111.8 (*J* 175)	126.4	57.4 [h]	53.4 (*J* 175)	60.4 (*J* 155)	42.8 (*J* 130)	22.2 (*J* 130)	135.5 (s), 128.4 (*p*), 130.3, 129.9 (*o*, *m*) [d]

[a] Shifts are in p.p.m. from Me_4Si (see Experimental section); one-bond coupling constant values in Hz are given in parentheses. [b] Already assigned by L. F. Johnson and C. Jankowski in 'Carbon-13 NMR Spectra,' Wiley–Interscience, New York, 1972. [c] Assigned on the basis of the values given [2] for the methyl ester of penicillin G (2). [d] Phenyl group signals: s is singlet (quaternary carbon), *p* is *para*-, *o* is *ortho*-, and *m* is *meta*-carbon frequency. [e] Isoxazole signal (singlet). [f] Frequencies of isoxazole carbon atoms in the order: Me (doublet), C_α (quartet, ^2J 6.0 Hz), and C_β–N (singlet). [g] Frequencies of the benzene carbon atoms. [h] ^1J 145 Hz.

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where H-5 and -6 have chemical shifts too close for proton s.f.s.d. experiments.

TABLE 2

^{13}C Chemical shift assignments for the carbonyl carbon atoms in penicillins (1)—(6) and cephalosporins (7)—(12)

	β-lactam C=O	CO_2Na	CO·NH
(1)	175.3	174.7 [a]	174.1 [a]
(2)	174.8	174.6	171.4
(3)	175.5	175.4	176.3
(4)	174.4	174.9	171.4
(5)	174.6	174.9	171.3
(6)	175.0	174.6	162.2
(7)	165.3	170.9	176.3
(8)	165.6	169.2	176.1
(9) [b]	164.9	170.9	176.3
(10)	164.4	170.5	173.1
(11)	165.2	169.1	175.6
(12)	166.4	175.2	176.0

[a] Concentration 0.8M; with a 0.2M-solution these two signals overlap at 174.9 p.p.m., whereas the third remains at 175.3 p.p.m. [b] The assignments reported by N. Neuss *et al.* (*J. Amer. Chem. Soc.*, 1971, **93**, 2337) and obtained by direct correlation with those of cephalosporin C are 167.7, 173.3, and 179.9 p.p.m. (values converted from CS_2 reference).

TABLE 3

^1H N.m.r. parameters [a]

Compd.	H-3	H-5 [b]	H-6 [b]	H-10	$J_{5,6}$/Hz
(1)	4.32	5.49	5.55	3.63 [b]	4.0
(2)	4.39	5.66	5.68	4.54 [b]	4.0
(3)	4.23	5.47	5.47	4.65	4.0
(4)	4.28	5.47	5.57	5.24	4.0
(5)	4.19	5.44	5.50	5.21	4.5
(6)	4.15	5.52	5.69		4.2
	H-4	H-6	H-7	H-11 [c]	$J_{6,7}$/Hz
(7)		5.00	5.57	3.65	4.0
(9)		4.93	5.62	4.74	4.0
(11)		4.78	5.83	3.73	4.5
(12)	4.60	5.27	5.40	3.66	4.0

[a] Shifts are in p.p.m. (δ) from internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate in D_2O solution. [b] The values for these protons may be reversed. [c] Broad singlet.

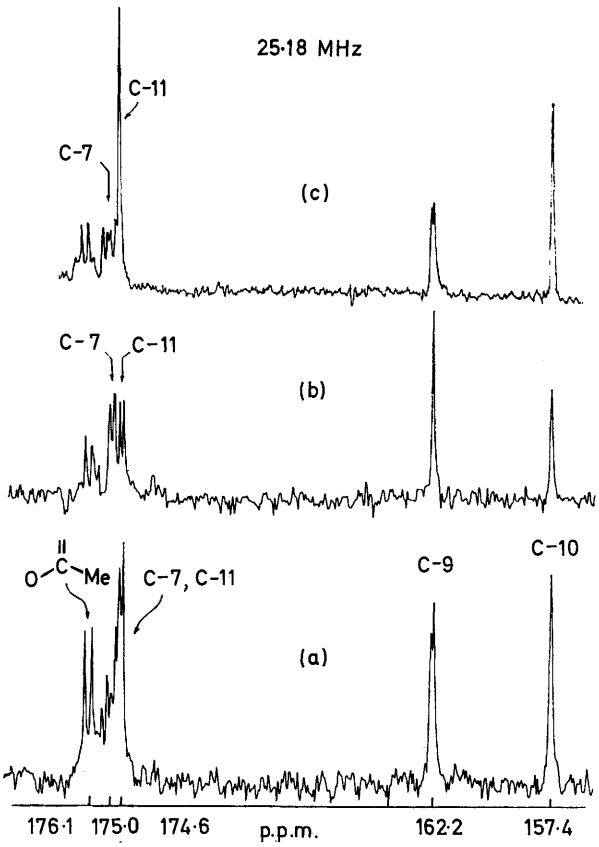
TABLE 4

Long-range C,H-coupling constants (Hz) for penicillins (1)—(6) and cephalosporins (7)—(12)

	$^3J_{C(7)H(3)}$	$^3J_{C(7)H(5)}$	$^2J_{C(7)H(6)}$	$^3J_{C(9)H(6)}$	$^2J_{C(9)H(10)}$ [a]	$^2J_{C(11)H(3)}$
(1)	5.0	16.0 [b]		2.0 [c]	6.5	4.0
(2)	5.0	15.5 [b]		2.0 [c]	3.8	4.5
(3)	5.0	12.0 [b]		*d*	*d*	4.0
(4)	5.0	*d*	*d*	2.5 [c]	4.0	4.0
(5)	*d*	*d*	*d*	2.0 [c]	5.0	*d*
(6)	5.0	7.0 [e]	5.0 [e]	3.0		4.0
	$^3J_{C(8)H(4)}$	$^3J_{C(8)H(6)}$	$^2J_{C(8)H(7)}$	$^3J_{C(10)H(7)}$	$^2J_{C(10H(11)}$ [a]	$^2J_{C(12)H(4)}$
(7)		6.5 [e]	6.0 [e]	2.5	6.5	
(8)		6.0	6.0	3.0	6.5	
(9)		6.5 [e]	5.5 [e]	4.0 [c]	4.0 [c]	
(10)		6.5 [e]	5.5 [e]	4.0	4.0	
(11)		7.5	6.5	2.5	6.5	
(12)	5.0	5.0	5.0	3.0	7.0	6.5

[a] For compounds (1), (2), (7), (8), (11), and (12), where C-10 (or C-11) bears two protons (AA'), the observed values of this coupling constant may be an average of two couplings, *i.e.* $J = |J_{AX} + J_{AX^*}|/2$. [b] Only the sum of the two coupling constants could be measured, since C-7 gives a second-order pattern. [c] Value deduced from the width of the signal. [d] Not determined. [e] Values within any horizontal line may be reversed.

The assignment of the carbonyl carbon frequencies Table 2) was performed by the proton s.f.s.d. technique. Only low power was used since the couplings present are long-range and therefore small. One example is given in the Figure. In some cases, when this method was unsuccessful, the long-range coupling constants (Table 4) or the multiplicities of the signals were used. The carboxylate carbon signal is a singlet in Δ^3-cephalosporins, but a doublet in penicillins and Δ^2-cephalosporins. The magnitudes of the two-bond couplings responsible for this interaction lie in the expected range. The β-lactam carbonyl carbon atom in Δ^3-cephalosporins interacts with two protons on the four-membered ring, whereas in penicillins (and in Δ^2-cephalosporins) it is coupled also with H-3 (or H-4 respectively). Incidentally the values of the two-bond $[^2J_{C(8)H(7)}]$ and three-bond $[^3J_{C(8)H(6)}]$ coupling constants in the β-lactam ring of the cephem system are equal or very similar. The

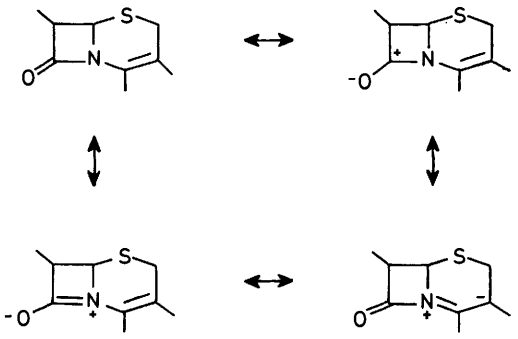


^{13}C N.m.r. spectra of compound (6): (a) proton-coupled, (b) selective irradiation at the frequency of H-5 and H-6 (δ 5.60) (decoupling of C-7 and C-9), (c) selective irradiation at the frequency of H-3 (δ 4.15) (decoupling of C-7 and C-11)

second-order pattern of the signals for the analogous protons in penicillins prevents deduction of the coupling constant values for this series. However it is more interesting to compare the three-bond couplings $^3J_{C(7)H(3)}$ in the penicillins (1)—(6) with $^3J_{C(8)H(4)}$ in the Δ^2-cephalosporin (12), because they involve the β-lactam nitrogen atom. Although this nitrogen centre has been shown [6] to be significantly pyramidal in the former and planar in the latter series, the above couplings are, surprisingly, identical in the two systems. The side-chain amide carbonyl carbon atom gives a complex peak, often poorly resolved, except for compounds (6) and (10), in which it gives a doublet and a double doublet, respectively.

DISCUSSION

Table 2 shows that the carbonyl carbon atom of the β-lactam ring in penicillins resonates 10 p.p.m. to lower field than in cephalosporins, and surprisingly shows the same shift in both Δ^3- (active) and Δ^2-cephalosporins (inactive). An increasing low field shift was indeed



SCHEME

expected as the deviation from planarity of the amide nitrogen atom in the lactam ring increased. Steric inhibition of resonance should leave the carbon atom of the amide bond more positive. This is indeed observed for penicillins, where the deviation from planarity is large (0.4 Å from the plane of the nitrogen substituents), but not in Δ^3-cephalosporins, despite the fact that the nitrogen centre is still pyramidal (0.2 Å), as compared with the nearly planar structure of the ceph-2-em system.[6] * However there is some evidence (*X*-ray, u.v., and i.r. data)[6,9] that in ceph-3-em molecules delocalization of the nitrogen lone pair into the adjacent olefinic π-bond system occurs, in spite of the non-planarity of the nitrogen centre. However, quantum-mechanical calculations show that ring strain is approximately the same for the isomeric Δ^2- and Δ^3-cephalosporins.[14] The increasing values of the i.r. carbonyl frequency on going from ceph-2-em to ceph-3-em to 3-acetoxymethyl-Δ^3-cephalosporin (8) presumably indicate an increase in bond order of the C=O lactam bond.[15] It has been suggested [6] that enamine resonance plus the lack of planarity of the nitrogen centre combine to decrease amide resonance in the lactam amide bond of Δ^3-cephalosporins (see Scheme 1). This should leave the carbon atom more susceptible to nucleophilic attack by the enzyme. In fact the i.r. carbonyl stretching frequency correlates fairly well with the rate constants for basic hydrolysis.[9,11]

In contrast with the i.r. results, the similarity of the values of the ^{13}C shifts found for the β-lactam carbonyl carbon atoms in ceph-3-em and ceph-2-em systems indicates that either the charge density or the bond order (or both) at the carbonyl carbon atoms in both systems are approximately the same. A possible interpretation is that the carbon shift is insensitive to minor change in nitrogen hybridization. Alternatively, the existence of enamine-type delocalization involving the lone pair of the amide nitrogen atom may not affect the situation at the carbonyl group, but only that at the nitrogen, since the electron availability at the nitrogen atom is such as to supply the electron density demand by the adjacent π-bond system. A low charge density is thus expected at the β-lactam nitrogen atom of Δ^3-cephalosporins and consequently a downfield effect on the ^{15}N chemical shifts, which is indeed found experimentally, with respect to Δ^2-isomers. In partial support of this suggestion, the C–N bond length † in the β-lactam ring of penicillins and Δ^3- and Δ^2-cephalosporins varies in the expected way, but the C=O bond lengths are approximately constant.

A similar trend in carbon and nitrogen chemical shifts has been observed in other amide–enamine compounds,[1] where no complication arises from the non-planarity of the nitrogen centre, *i.e.* invariance of the carbonyl carbon shift and a large downfield effect on the nitrogen shift. Thus it seems that enamine conjugation does not necessarily inhibit [6] amide conjugation involving the same nitrogen atom.

If this occurs in the present case, with the consequence that the effective positive charge on the carbonyl carbon atom in both cephem systems is approximately the same, Δ^3- (active) and Δ^2-cephalosporins (inactive) should display, despite conformational factors, the same susceptibility to nucleophilic attack. On the other hand, differences in the electronic properties of the β-lactam ring may not be the major factor differentiating the activity of these antibiotics. In particular, whether the electron-withdrawing effect on the carbonyl centre by the enamine nitrogen atom in the ground state (enhanced by electronegative 3-substituents) is more important for the ease of ring opening in ceph-3-em systems than the stabilization, by the αβ-double bond, of the electron density developed on the nitrogen atom in the transition state, is not yet clear. The ^{13}C shifts (if they are meaningful) suggest that the enamine system plays a determining role in the second step only of the amide hydrolysis.

In the particular case of the strongly active cephalosporin (8), quantum-mechanical results suggest that the 3-acetoxymethyl group may be expelled during the nucleophilic attack, thus indicating that leaving group ability may be as important as the inductive effect of the 3-substituent in activating the β-lactam ring in its ground state.[16]

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* For some free β-lactams differently substituted, the ^{13}C carbonyl resonances have been found at 165—166 p.p.m. in CCl_4 solution (A. K. Bose and P. R. Srinivasan, *J. Magnetic Resonance*, 1974, **15**, 592). Comparison with the β-lactam antibiotics may be only orientative, since both solvent and substituent effects must be considered.

† See ref. 6, Table III, p. 297.

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