Structual Studies on Penicillin Derivatives: ^{13}C Nuclear Magnetic Resonance Studies of Some Penicillins and Related Sulphoxides

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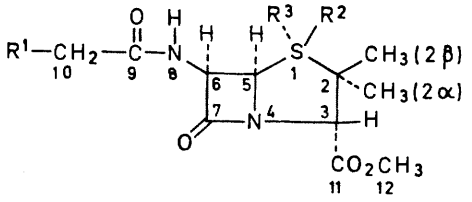
Summary ^{13}C Chemical shift assignments are made for several penicillin and penicillin sulphoxide derivatives: shifts for γ-situated carbons are best explained if a sizeable steric effect is attributed to the S→O bond.

^1H NUCLEAR MAGNETIC RESONANCE has been shown to be a useful technique for defining thiazolidine ring conformation and sulphoxide bond configuration in penicillins and their corresponding sulphoxide derivatives.[1] ^{13}C N.m.r. can also be used in this respect since ^{13}C chemical shifts reflect not only the electronic environment at a carbon nucleus but also steric and conformational effects in a molecule.[2-4] We now report ^{13}C chemical shift assignments for the different carbon atoms in methyl-(I) and phenoxymethyl-(IV) penicillins and their related sulphoxides (II, III, and V).

MHz), respectively, obtained on a solution of methylpenicillin *S*-sulphoxide (II) in dimethyl sulphoxide. In contrast to primary, secondary, and tertiary carbons,



(I) R^1 = H, R^2 = lone pair, R^3 = lone pair
(II) R^1 = H, R^2 = O, R^3 = lone pair
(III) R^1 = H, R^2 = lone pair, R^3 = O
(IV) R^1 = PhO, R^2 = lone pair, R^3 = lone pair
(V) R^1 = PhO, R^2 = O, R^3 = lone pair

TABLE 1

^{13}C *Chemical shift assignments*[a] *for penicillin derivatives* (I)—(V)

	(I)	(II)	(III)	(IV)	(V)
C-2 ..	63·6	74·1	68·1	64·0	74·9
C-3 ..	69·7	65·1	63·9	70·0	65·8
C-5 ..	67·1	75·5	78·7	67·4	76·0
C-6 ..	58·3	55·5	57·0	58·4	54·8
2β-CH_3 ..	30·0	18·7	23·4	31·0	19·0
2α-CH_3 ..	26·1	17·6	15·3	26·6	18·0
C-12	51·7	52·2	52·2	52·1	52·8
C-10 ..	20·8	22·1	21·6	65·6	67·0
C-7[b] ..	—	168·1	167·3	167·6	167·3
C-9 ..	—	169·2	169·5	167·6	167·7
C-11 ..	—	173·8	170·2	172·6	173·8

[a] In p.p.m. from the ^{13}C resonance of Me_4Si. Chemical shifts were measured relative to external Me_2SO and corrected to Me_4Si as internal reference by the relationship $\delta_C(Me_4Si) \times 40{\cdot}4 - \delta_C(Me_2SO)$.
[b] The bracket indicates that the relative assignment of C-7 and C-9 have not been established. Reverse assignment is also possible.

^{13}C assignments made in this study for different carbons in (I)—(V) are recorded in Table 1. In the Figure, B and C illustrate typical continuous wave decoupled (C.W.D.) and noise decoupled (N.D.).^{13}C N.m.r. spectra (at 25·15 quaternary carbons give singlet signals under both C.W.D. and N.D. conditions due to the absence of direct ^{13}C–^1H coupling. Thus, quaternary C-2 carbons in (I)—(V) were simply assigned by comparison of their N.D. and C.W.D.

spectra (see Figure, B and C). Relative assignment of the tertiary (C-3, C-5, and C-6) and primary (methyl carbons)

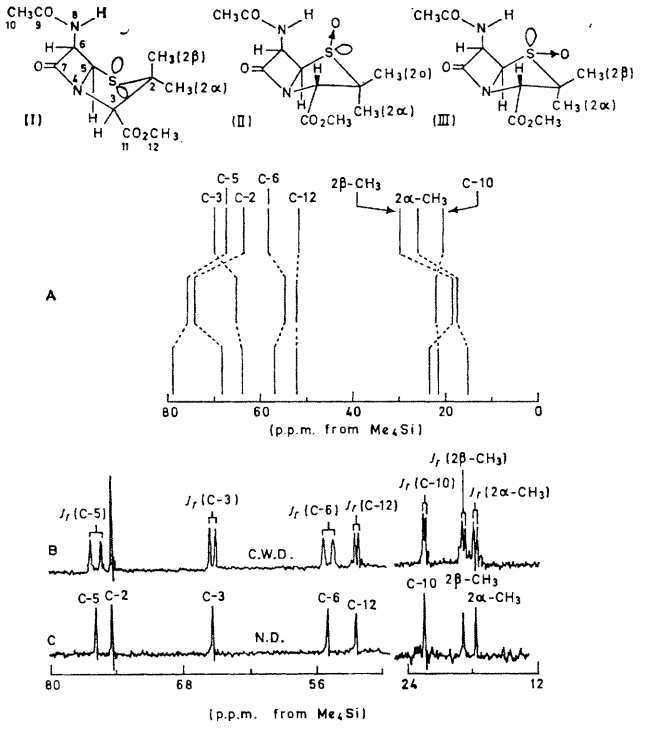


FIGURE. (A) ^{13}C *Chemical shift correlation for the process* (I) → (II) → (III); *compounds* (I)—(III) *are drawn in accord with previously derived*[1a,b] *thiazolidine ring conformational structures.* (B) *C.W. decoupled spectrum of* (II) (0·2 *g*) *in dimethyl sulphoxide solution.* (C) *Noise-decoupled spectrum of* (II). *Spectra shown in Band C were run at* 25·15 *MHz on an* HA-100 *spectrometer equipped with a* V-3530 RF/AF *Sweep unit, a* V-4335-1 8 *mm probe and a* V-3512-1 *heteronuclear spin decoupler. The above spectra are the result of time-averaging* 100 *scans, each at* 50 *s scan time.*

centres, which give rise to doublets and quartets, respectively, in their C.W.D. spectra (see Figure, B), was based on their expected chemical shift behaviour and substantiated by use of equation 1 derived by Ernst[5] which allows the correlation of ^{13}C with corresponding ^{1}H signals through off-resonance decoupling.† From this

$$J_r \simeq \frac{\Delta f \cdot J}{\gamma H_2/2\pi} \qquad (1)$$

equation, it is possible to estimate the residual splitting, J_r, for a ^{13}C resonance produced by application of a decoupling field of strength $\gamma H_2/2\pi$‡ in the proton region of the spectrum if Δf, the difference in frequency between the resonance position of a given ^{1}H signal and the applied decoupling frequency,§ and J, the magnitude of ^{13}C–^{1}H coupling,¶ are known. In molecular systems where proton assignments have been determined, as the case for penicillins (I)—(V) studied herein,[1] ^{13}C assignment is simply accomplished by the comparison, and subsequent matching, of observed and calculated J_r values. Typical results of such calculations are given for compound (II) in Table 2. The agreement between observed and calculated J_r values clearly establishes assignments for all carbons except C-6 and C-5 where, because of the difference in their carbon–proton coupling constant, calculated J_r values coincidentally have similar magnitudes (see Table 2). Ambiguity in the relative assignment of these carbons was clarified by selective heteronuclear spin–spin decoupling at 55·34 MHz. Proton irradiation at δ 5·78, the resonance position of 6-H, caused the collapse to a singlet of the high field CH carbon doublet (δ 55·5), which is accordingly assigned to C-6, and leaves a residual splitting in the low field CH signal (δ 75·5), which is accordingly assigned to C-5. Saturation of 5-H resulted in the reverse observations. Trends shown in the shift behaviour of the different penicillin carbons** are given in the Figure, A.

Since reduction in electron density at a carbon nucleus results in orbital contraction with concomitant shift to low field,[3] the shifts to low field for β-carbons C-2 and C-5, upon oxidation of the thiazolidine sulphur, is in accord with the expected decrease in electron density at these sites as a result of inductive withdrawal by the sulphoxide oxygen and a hybridization change at sulphur. Shifts to high field for γ-situated carbons 2α-CH_3, 2β-CH_3, C-3, and C-6, however, are more difficult to rationalize since they do not appear to originate solely from known increases in steric interactions due to thiazolidine ring conformational changes[1] in the processes (I)[(IV)]→(II)[(V)] or (I)→(III). Anomalies in the γ-carbon shifts, to a large extent, appear to be satisfactorily explained by attributing a sizeable steric effect to the S→O bond. Thus, while the higher field resonance positions for 2α-CH_3 in (II)[(V)] and (III) relative to (I)[(IV)], can be attributed to steric shielding of these protons by virtue of their conformationally induced 1,3-diaxial relationship to 5-H in (II)[(V)] and (III), no similar explanation is available for the equally high field shifts of the 2β-methyl protons except their proximity to the S→O bond. Accordingly, the 2β-methyl protons which

† Application of the Ernst equation for ^{13}C assignment was first reported by one of us (L.F.J.) at the Eighth National Meeting for the Society of Applied Spectroscopy held at the Disneyland Hotel, Anaheim, California, October 6—10, 1969. Full details regarding the use of this technique for ^{13}C assignment will be reported in *Appl. Spectroscopy*.

‡ In this study $\gamma H_2/2\pi$ was set at 2100 Hz.

§ In this study the applied decoupling frequency was set at δ 2·49, the shift position of the solvent dimethyl sulphoxide methyl signals.

¶ The magnitudes of ^{13}C–^{1}H couplings in penicillins were estimated using the additivity relationships of Malinowski.[6] Since J_{CH} is dependent upon the percentage S character at the carbon participating in the C–H bond,[7] the magnitude of J_{CH} in penicillins will be dependent upon the nature of substituents attached to each carbon. For tetrahedral carbons of the type CHXYZ, the following additivity relationship for ^{13}C–^{1}H coupling has been demonstrated: $J_{CH} = \zeta_X + \zeta_Y + \zeta_Z$, where ζ_X, ζ_Y and ζ_Z are contributions to J_{CH} associated with substituents X, Y, and Z, respectively. Thus, for example, J_{CH} for the C-3 carbon in (II) is calculated using the empirically derived substituent contributions[5] as follows: $J_{C\text{-}3\text{-}H} = \zeta_{CO^-} + \zeta_{N^-} + \zeta_{CMe_3^-} = 47{\cdot}1 + 49{\cdot}6 + 38{\cdot}6 = 135{\cdot}3$ Hz.

** In the case of (I) [and (IV)] unambiguous relative assignments for C-5 and C-6 was not possible using either spin decoupling or the proton–carbon correlation method due to the near degeneracy of these signals in their ^{1}H n.m.r. spectra. Accordingly, assignments shown in Table 1 and the Figure, A, for these carbons are tentative and in accord with expected trends. Since reversal of their assignment affects the magnitude and not the sign of their shift upon proceeding from penicillin to one of its related sulphoxides, error in the relative assignment of these carbons is inconsequential to arguments presented later.

TABLE 2

Calculated and observed residual splittings for methylpenicillin S-sulphoxide (II)

Proton(s)	δ_H[a]	Δf[b]	δC[c]	J_{C-H}[d]	J_r(obs)[d]	J_r(calc)[d]
3-H	4·43	193	65·1	135·3	12·2	12·4
5-H	5·44	294	75·5	151·8	22·7	21·3
6-H	5·76	321	55·7	139·8	22·7	21·4
2β-CH_3	1·57	93	18·7	120·0	6·0	5·3
2α-CH_3	1·17	133	17·6	120·0	7·9	7·6
C-10	1·92	58	22·1	126·8	3·8	3·5
C-12	3·74	124	52·2	142·8	8·3	8·5

[a] In p.p.m. from Me_4Si.
[b] In Hz at 100 MHz.
[c] In p.p.m. from the ^{13}C signal of Me_4Si.
[d] In Hz at 25·15 Hz.

subtend dihedral angles (ϕ) of approximately 60° and 85° to the S→O bond in (II) and (III), respectively, are shifted to high field, relative to (I), by 11·3 and 6·6 p.p.m. Similarly, 2α-CH_3 appears at higher field in (III) (δ 15·3, ϕ *ca.* 40°) relative to (II) (δ 17·6, ϕ *ca.* 180°) by 2·3 p.p.m. in accord with the closer proximity of these protons to the S→O bond in the former case. The shift behaviour of C-6 and C-3 can for the most part be similarly rationalized.

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