Determination of the Configuration of Some Penicillin *S*-Oxides by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The ^{13}C n.m.r. spectra of a range of penicillins and some sulphoxides and sulphones derived from them are presented. Along the series sulphide, β-sulphoxide, α-sulphoxide, sulphone there is a clear pattern in the changes of the shifts of C-2, -3, -5, and -6 and the C-2 methyl groups. This means that ^{13}C n.m.r. spectroscopy can be used to determine the configuration of sulphoxides, and using this technique we have shown that oxidation of 6,6-dibromopenicillanic acid by peroxy-acid gives mainly the α-sulphoxide, whereas methyl penicillanate and its 6α-chloro- and 6α-bromo-derivatives give mainly the β-sulphoxides.

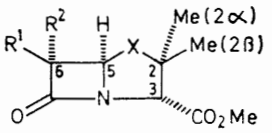
We wished to determine the configurations of certain penicillin *S*-oxides, including the epimeric sulphoxides derived from 6,6-dibromopenicillanic acid, the configurations of which we believed to have been incorrectly assigned.[1] Archer *et al.*[2] had measured the ^{13}C n.m.r. spectra of methyl 6β-acetamidopenicillanate (I) and its α- and β-*S*-oxides, (II) and (III) respectively, and of methyl 6β-phenoxyacetamidopenicillanate (IV) and its β-*S*-oxide (VI), and found that the shifts in resonance positions on going from compounds (I) and (IV) to their β-sulphoxides were markedly different from the shifts on going from compound (I) to its α-sulphoxide. We have established the generality of this observation and have used the method to assign configurations to some penicillin *S*-oxides.

In order that ^{13}C n.m.r. spectroscopy could be used with greater confidence to determine the configurations of penicillin *S*-oxides, we first measured the spectra of some further penicillins and derived *S*-oxides of known configuration. We also extended the work to include some penicillin *SS*-dioxides. The compounds selected were methyl 6β-phenoxyacetamidopenicillanate α-*S*-oxide (V) and the derived sulphone (VII), and methyl 6β-phthalimidopenicillanate (VIII) and its α-*S*-oxide (IX) and *SS*-dioxide (X). The spectra of compounds (VIII), (IX), and (X) were measured for solutions in dimethyl sulphoxide, but those of compounds (V) and (VII) and all subsequent compounds were measured for solutions in acetone. Archer *et al.* made their measurements for dimethyl sulphoxide solutions, so to facilitate comparison with our other spectra we remeasured the spectra of compounds (IV) and (VI) with acetone as solvent. Our results are summarised in the Table. The signals were assigned on the basis of splitting patterns, expected shifts,[2-4] and coupling constant values. The assignments of the 2α- and 2β-methyl groups are made by analogy

[1] J. P. Clayton, *J. Chem. Soc.* (*C*), 1969, 2123.
[2] R. A. Archer, R. D. G. Cooper, P. V. Demarco, and L. R. F. Johnson, *Chem. Comm.*, 1970, 1291.
[3] S. Kukolja, N. D. Jones, M. O. Chaney, T. K. Elzey, M. R. Gleissner, J. W. Paschal, and D. E. Dorman, *J. Org. Chem.*, 1975, **40**, 2388.
[4] K. Tori, T. Tsushima, Y. Tamura, H. Shigemoto, T. Tsuji, H. Ishitobi, and H. Tanida, *Tetrahedron Letters*, 1975, 3307.

with the work of Archer *et al.*[2] The shift values for compounds (IV), (V), (VIII), and (IX) are similar to those reported for spectra obtained with dimethyl sulphoxide [2] or deuteriochloroform as solvent,[3,4] and solvent effects are, therefore, small. Concentration effects would also be expected to be small; indeed the shift values of the β-sulphoxide (VI) measured over the range 0.25—2.2M did not vary by more than 0.2 p.p.m. from those found for a 1.0M-solution. We measured coupling constants directly by use of a gated undecoupling Fourier transform technique. The values differ considerably from those of Archer *et al.*, who measured only residual values by use of off-resonance decoupling and compared these with theoretical values. We find that the coupling constants show little variation in size unless groups directly attached to the carbon atoms in question are altered; they are, therefore, a valuable aid in making assignment

The most important conclusions from our results and those of Archer *et al.*[2] is that, along the series sulphide, β-sulphoxide, α-sulphoxide, sulphone, there is a clear pattern in the changes in the shifts of C-2, -3, -5, and -6, and the C-2 methyl groups. This is shown in Figure 1, for the series of compounds (IV)—(VII). It is evident from the results discussed below that the shifts in the 6,6-dibromo-series (XI)—(XIV) follow a similar pattern (see Figure 2), even though some of the shift positions are substantially different from those in the first series. Hence, the pattern remains, despite substantial alterations in the substitution at C-6. Assignment of configurations to sulphoxides of unknown configuration will be simplest when both epimers are to hand, but if only



	R1	R2	X		R1	R2	X
(I)	AcNH	H	S	(XII)	Br	Br	S⋯⋯O
(II)	AcNH	H	S⋯⋯O	(XIII)	Br	Br	S——O
(III)	AcNH	H	S——O	(XIV)	Br	Br	SO_2
(IV)	PhO·CH_2·CO·NH	H	S	(XV)	H	H	S
(V)	PhO·CH_2·CO·NH	H	S⋯⋯O	(XVI)	H	H	S⋯⋯O
(VI)	PhO·CH_2·CO·NH	H	S——O	(XVII)	H	H	S——O
(VII)	PhO·CH_2·CO·NH	H	SO_2	(XVIII)	H	Cl	S
(VIII)	Phthalimido	H	S	(XIX)	H	Cl	S——O
(IX)	Phthalimido	H	S⋯⋯O	(XX)	H	Br	S
(X)	Phthalimido	H	SO_2	(XXI)	H	Br	S⋯⋯O
(XI)	Br	Br	S	(XXII)	H	Br	S——O

^{13}C N.m.r. data

Comp.	Concentration [a] (M)	Chemical shifts [b] C-2	C-3	C-5	C-6	2α-Me	2β-Me	Coupling constants [c] 3-C	5-C	6-C
(IV)	2.2	64.8	70.6	68.3	59.1	26.6	32.1	145	180	154
(V)	1.7	69.8	65.7	80.6	58.2	16.0	24.3	147 [d]	173	153
(VI)	1.0	75.4	66.9	77.0	56.0	18.4	19.3	148	177	151
(VII)	1.5	65.1	64.3	66.3	56.8	17.7	20.0	141	175	152
(VIII) [e]	1.3	65.1	70.4	66.2	58.5	27.2	30.4	145 [d]	178	152
(IX) [e]	1.3	70.2	64.9	80.3	54.9	15.8	24.2	145	172	150
(X) [e]	0.7	64.1	63.4	63.7	57.5	18.6	19.3	150	178	159
(XI) [f]	1.3	64.7	70.0	80.6	58.9	25.9	33.0	147	183	
(XII) [f]	1.1	71.4	65.8	90.2	48.2	16.2	23.8	148	180	
(XIII)	0.3	75.0	66.5	84.2	45.4	18.3	19.8	147	179	
(XIV)	1.1	65.2	63.6	73.5	43.7	18.3	19.6	153	183	
(XV)	4.2	65.6	70.4	60.9	46.6	26.5	31.6	147	177	142
(XVI)	0.2	70.7	64.5	73.4	40.8	15.7	24.0	*g*	*g*	*g*
(XVII)	1.2	73.9	66.2	71.5	36.2	18.4	20.0	145	175	145
(XVIII)	1.8	65.2	70.2	71.6	63.8	25.7	33.4	147	184	169
(XIX)	1.1	73.6	66.0	79.8	54.7	18.0	19.7	149	180	170
(XX)	2.2	65.1	70.1	70.6	49.8	25.6	33.3	150	175	169
(XXI)	0.2	68.9	63.4	83.1	43.2	15.9	23.6	*g*	*g*	*g*
(XXII)	1.8	73.4	65.9	79.5	40.2	17.9	19.7	148	180	167

[a] Solutions in acetone unless indicated otherwise. [b] In p.p.m. downfield from Me_4Si. Values for the phenoxyacetamido- and phthalimido-groups were similar to those reported in refs. 3 and 4. Values for C-7 and the ester carbonyl were in the range δ 162—172 and for the ester methyl δ 52.0—53.3. [c] In Hz, between ^{13}C and the directly attached proton; J_{CH} for the ester methyl groups was 148 Hz in each case; J_{CH} for the methylene carbon in the phenoxyacetamido-group was in the range 147—149 Hz. [d] Approximate values; signals partially obscured. [e] Solutions in dimethyl sulphoxide. [f] Spectra measured for the acid. [g] Not measured.

one epimer is available the most characteristic shifts, *i.e.* those of the 2α- and 2β-methyl groups, should be employed. In the sulphide series both signals appear downfield from δ 25. In the α-sulphoxide series one methyl group resonates at δ 23—25 and the other at δ 15—16.5, and in the β-sulphoxide series both resonate at δ 17.5—20. In the sulphone series the resonance positions of these two groups are similar to those in the β-sulphoxide series, but the compounds can be readily distinguished by use of other signals, notably that of C-5, which moves upfield on going from sulphide to sulphone and downfield on going from sulphide to either sulphoxide.

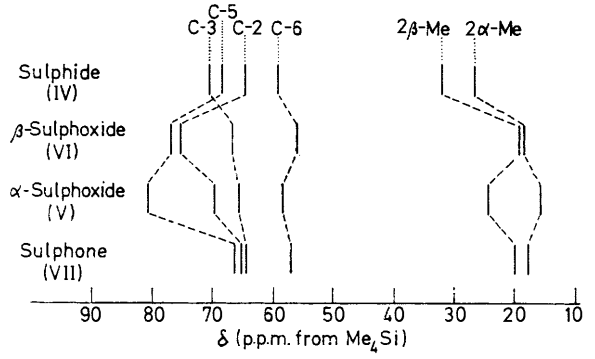


FIGURE 1 Chemical shift correlations for the series (IV) ⟶ (VI) ⟶ (V) ⟶ (VII)

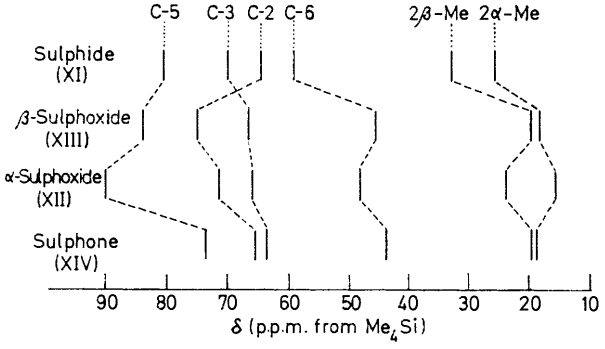


FIGURE 2 Chemical shift correlations for the series (XI) ⟶ (XIII) ⟶ (XII) ⟶ (XIV)

We then used ^{13}C n.m.r. spectroscopy to determine the configuration of some sulphoxides of doubtful or unknown configuration. Clayton [1] found that oxidation of 6,6-dibromopenicillanic acid [acid corresponding to the ester (XI)] with sodium periodate gave two sulphoxides in the ratio 73 : 27. We obtained the same compounds but in the ratio 91 : 9 by using *m*-chloroperbenzoic acid in tetrahydrofuran. A pure sample of the major component can readily be isolated (as the ester) from the peroxy-acid oxidation product, but a pure sample of the minor component is best obtained albeit in low yield, from the oxidative bromination of 6β-aminopenicillanic acid, which gives the same isomers as before but in reversed proportions.[1] Clayton found the ratio to be 27 : 73; we found it to be 10 : 90. The ^{13}C n.m.r. spectra of these isomers are summarised in the Table and in Figure 2. These show that the major component from the peroxy-acid oxidation is in fact the α-sulphoxide [acid corresponding to (XII)] and the minor component the β-sulphoxide [acid corresponding to (XIII)]. These are the reverse of the assignments made by Clayton [1,4] on the basis that the β-sulphoxide would be the epimer which has its C-3 proton signal at lower field in the ^1H n.m.r. spectrum.[6] This method does not, however, appear to be completely reliable as the resonance positions of the C-3 protons in the spectra of the sulphoxides derived from methyl 6α-phthalimidopenicillanate differ by only 0.02 p.p.m., and it is that of the α-isomer which is at lower field.[7] The α-sulphoxide is not only the kinetically favoured product from oxidations with peroxy-acid or periodate; it is also the thermodynamically more stable product. Thus, when a solution of the acidic reaction product in methyl ethyl ketone or the esterified reaction product in benzene is heated under reflux, the α : β ratio shifts [8] from 91 : 9 to 73 : 27 or to 75 : 25, respectively, and when a solution of the β-sulphoxide ester in benzene is heated under reflux the α : β ratio in the product is 56 : 44. It is not clear why the oxidative bromination of 6β-aminopenicillanic acid should give mainly the β-sulphoxide, but the total yield of sulphoxides was only 5%.

Three further oxidations were investigated. Oxidation of methyl penicillanate (XV) with *m*-chloroperbenzoic acid gave predominantly one isomer (79%), which from its ^{13}C n.m.r. spectrum (see Table) is the β-sulphoxide (XVII). The spectrum of the crude oxidation product also contained low intensity signals which can be confidently assigned to the α-sulphoxide (XVI).

Methyl 6α-chloro- and 6α-bromo-penicillanates, (XVIII) and (XX), respectively, behaved similarly when oxidised with *m*-chloroperbenzoic acid. The former gave two isomers in the ratio 88 : 12, and the latter two isomers in the ratio 92 : 8. The ^{13}C n.m.r. spectrum indicated (see Table) that in each case the major component was the β-sulphoxide, (XIX) and (XXII), respectively. The ^{13}C n.m.r. spectrum of the crude oxidation product from the 6α-bromo-compound (XX) contained low intensity signals in the positions expected for the α-sulphoxide (XXI).

To summarise, the course of the oxidations of penicillanic acid derivatives by peroxy-acid depends on the substituents at C-6 as follows. When there is no 6β-substituent, as in methyl penicillanate and its 6α-phthalimido-,[7] 6β-chloro-, and 6α-bromo-derivatives, the β-sulphoxide is the major product. The steric effects of 6β-bromo- and 6β-phthalimido-[7] substituents are sufficient to cause the α-sulphoxide to be, respectively, the major and the only product. The N–H group of a 6β-

[5] J. P. Clayton, J. H. C. Nayler, M. J. Pearson, and R. Southgate, *J.C.S. Perkin I*, 1974, 22.

[6] P. V. Demarco and R. Nagarajan in 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York and London, 1972, p. 349.

[7] R. D. G. Cooper, P. V. Demarco, and D. O. Spry, *J. Amer. Chem. Soc.*, 1969, **91**, 1528.

[8] R. D. G. Cooper, L. D. Hatfield, and D. O. Spry, *Accounts Chem. Res.*, 1973, **6**, 32 (see p. 36).

acetamido- (or substituted acetamido-) substituent can form a hydrogen bond with the incoming reagent or with the sulphoxide group of the product, and this effect is sufficient to overcome the steric effect, with the result that the β-sulphoxide is the only product.[9]

EXPERIMENTAL

Except where indicated otherwise the known compounds referred to below had m.p.s and i.r. spectra in agreement with those reported. ^{1}H N.m.r. spectra were measured for solutions in [$^2H_6$]acetone at 100 MHz, with a Varian HA100 spectrometer and Me_4Si as internal standard. ^{1}H N.m.r. data for certain known compounds are reported because they differ from those given previously, which were obtained with other solvents. ^{13}C N.m.r. spectra were measured at 20 MHz by using a Varian CFT20 spectrometer with proton noise decoupling and gated undecoupling; the references were solvent acetone (δ 30.39) or dimethyl sulphoxide (δ 40.50) as appropriate. A small proportion of deuteriated solvent was added to enable an external deuterium lock to be used.

Methyl 6β-*Phenoxyacetamidopenicillanate* (IV), *Methyl* 6β-*Phthalimidopenicillanate* (VIII), *and their Derivatives*.—The penicillin (IV) and its α- and β-*S* oxide, (V) and (VI), and the penicillin (VIII) and its α-*S* oxide (IX) and *SS*-dioxide (X) were either already available or were prepared by literature methods. The sulphone (VII) was obtained (49% yield) by treating the penicillin (IV) with 6 equiv. of *m*-chloroperbenzoic acid in methylene chloride at 20 °C for 3 h.

6,6-*Dibromopenicillanic Acid* [*corresponding to the Ester* (XI)] *and Derivatives*.—This acid, prepared by Clayton's procedure,[1] usually had m.p. 129—130°, $\nu_{max.}$ (Nujol) 3 250br and 1 760br cm^{-1}, but on one occasion it had m.p. 150°, $\nu_{max.}$ (Nujol) 3 300—2 500vbr, 1 805, and 1 735 cm^{-1}. The latter sample appeared to be a different crystal form; both samples gave the same solution i.r. spectrum [$\nu_{max.}$ ($CHCl_3$) 3 300—2 500vbr, 1 802, and 1 725 cm^{-1}] and the same ^{1}H n.m.r. spectrum, which was the same as that reported.[1] A portion of the acid was treated with diazomethane and the ester (XI) was oxidised with *m*-chloroperbenzoic acid (1.2 equiv.) in tetrahydrofuran at 20 °C. Crystallisation of the product from benzene–petroleum (b.p. 40—60 °C) gave the α-sulphoxide (XII), m.p. 127—128° (lit.,[1] 133—135°). A sample of the β-sulphoxide [acid corresponding to the ester (XIII)], m.p. 160—160.5° (from chloroform) (lit.,[1] 157—158°) was obtained by oxidative bromination of 6β-aminopenicillanic acid.[1] Treatment of the sulphoxide with diazomethane gave the ester (XIII).

Oxidation of 6,6-dibromopenicillanic acid with *m*-chloroperbenzoic acid (1 equiv.) in tetrahydrofuran at 0—25 °C for 70 min gave a mixture of the α- and β-sulphoxides in 90% yield. A portion of the mixture was esterified with diazomethane and the product analysed by ^{1}H n.m.r. spectroscopy (3- and 5-H signals). The α : β ratio was 91 : 9. When the ester mixture in benzene was heated under reflux for 3 h the α : β ratio changed to 75 : 25. A second portion of the acidic sulphoxide mixture was heated in benzene under reflux for 3 h, then methylated and analysed as before. The α : β ratio was still 91 : 9, but when methyl ethyl ketone was used in place of benzene the ratio changed to 73 : 27. A solution of the β-sulphoxide ester (XIII) in benzene was heated under reflux for 3 h and the product analysed as before. The α : β ratio was 56 : 44. A portion of the crude product (5% yield) from the oxidative deamination of 6β-aminopenicillanic acid was esterified and the ^{1}H n.m.r. spectrum measured. The α : β ratio was 10 : 90.

Treatment of the ester (XI) with 3 equiv. of *m*-chloroperbenzoic acid in methylene chloride at 25 °C for 3 h, followed by a further 3 equiv. at 25 °C overnight, gave, on work-up, the *sulphone* (XIV) (33%), m.p. 180° (decomp.) (from ethanol), $\nu_{max.}$ (Nujol) 1 807 and 1 755 cm^{-1}, δ 1.44 and 1.58 (2α- and 2β-Me), 3.83 (OMe), 4.67 (3-H), and 5.62 (5-H) (Found: C, 27.0; H, 2.9; Br, 39.2; N, 3.6; S, 8.3. $C_9H_{11}Br_2NO_5S$ requires C, 26.7; H, 2.7; Br, 39.45; N, 3.5; S, 7.9%).

Methyl Penicillanate (XV) *and its Oxidation*.—The penicillin (XV), prepared by reduction of 6,6-dibromopenicillanic acid by the method of Clayton,[1] had δ 1.45 and 1.65 (2α- and 2β-Me), 3.05 (dd, *J* 1.5 and 16.1 Hz, 6β-H), 3.68 (dd, *J* 4.2 and 16.1 Hz, 6α-H), 3.77 (OMe), 4.47 (3-H), and 5.35 (dd, *J* 1.5 and 4.2 Hz, 5-H). On treatment with 1.1 equiv. of *m*-chloroperbenzoic acid in methylene chloride at 0—25 °C for 70 min the ester (XV) afforded a mixture of sulphoxides which was analysed by ^{1}H n.m.r. spectroscopy. The α-sulphoxide (XVI) had δ 4.50 (3-H), 4.61 (dd, *J* 2.4 and 4.5 Hz, 5-H), and the β-sulphoxide (XVII) had δ 1.23 and 1.63 (2α- and 2β-Me), 3.10 (dd, *J* 2.1 and 15.9 Hz, 6β-H), 3.40 (dd, *J* 4.3 and 15.9 Hz, 6α-H), 3.79 (OMe), 3.45 (3-H), and 5.21 (dd, *J* 2.1 and 4.3 Hz, 5-H). Based on the intensity of the 3- and 5-H signals the α : β ratio was 11 : 79.

Methyl 6α-*Chloropenicillanate* (XVIII) *and Its Oxidation*.—This compound, prepared by the method of McMillan and Stoodley,[10] had δ 1.51 and 1.66 (2α- and 2β-Me), 3.70 (OMe), 4.52 (3-H), 4.98 (d, *J* 1.5 Hz, 6-H), and 5.28 (d, *J* 1.5 Hz, 5-H). Oxidation as above gave a mixture of sulphoxides. The α-sulphoxide had δ 4.57 (3-H) and 5.16 (d, *J* 1.5 Hz, 6-H) and the β-sulphoxide (XIX) had δ 1.24 and 1.60 (2α- and 2β-Me), 3.78 (OMe), 4.49 (3-H), 5.09 (d, *J* 1.5 Hz, 6-H), and 5.39 (d, *J* 1.5 Hz, 5-H). Based on the 3- and 6-H signal intensities, the α : β sulphoxides ratio was 12 : 88.

Methyl 6α-*Bromopenicillanate* (XX) *and its Oxidation*.—This compound, prepared from 6,6-dibromopenicillanic acid by the method of Cignarella *et al.*,[11] had δ 1.43 and 1.60 (2α- and 2β-Me), 3.69 (OMe), 4.48 (3-H), 5.00 (d, *J* 1.5 Hz, 6-H), and 5.30 (d, *J* 1.5 Hz, 5-H). Oxidation as above gave a mixture of sulphoxides. The α-sulphoxide (XXI) had δ 5.22 (d, *J* 1.5 Hz, 6-H) and 5.69 (d, *J* 1.5 Hz, 5-H) and the β-sulphoxide (XXII) had δ 1.28 and 1.65 (2α- and 2β-Me), 3.82 (OMe), 4.49 (3-H), 5.11 (d, *J* 1.5 Hz, 6-H), and 5.45 (d, *J* 1.5 Hz, 5-H). Based on the intensities of the 5- and 6-H signals the α : β ratio was 8 : 92.

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