Effect of Solvents on NMR Spectra of Penicillins

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Abstract □ High-resolution NMR spectra of penicillin G and ampicillin in deuterium oxide, water, deuterated dimethyl sulfoxide, and deuterated dimethyl sulfoxide–deuterium oxide were examined. The α-amino group of ampicillin could not be observed in these solvents but was observed using the pivaloyl derivative of ampicillin in deuterochloroform. No evidence for intramolecular hydrogen bonding between the protons of the α-amino group and the oxygen of the β-lactam carbonyl group was obtained. The amino group does not restrict the rotation of the side chain, which has been shown to be important in interaction with phospholipids.

Keyphrases □ Penicillins—effect of solvents on NMR spectra □ Solvents—effect on NMR spectra of penicillins □ NMR spectroscopy—effect of solvent on spectra of penicillins

Assignments of the protons in penicillins were reported by several workers (1-4). In a recent study on the interaction of penicillins with phospholipids (5), an attempt was made to observe the signal due to the α-amino group of ampicillin to assess the possibility of intramolecular hydrogen bonding. This was not possible in deuterium oxide (D_2O), and the spectra were observed in deuterated dimethyl sulfoxide [$(CD_3)_2SO$] and a dimethyl sulfoxide–deuterium oxide [$(CD_3)_2SO$–D_2O] mixture.

EXPERIMENTAL

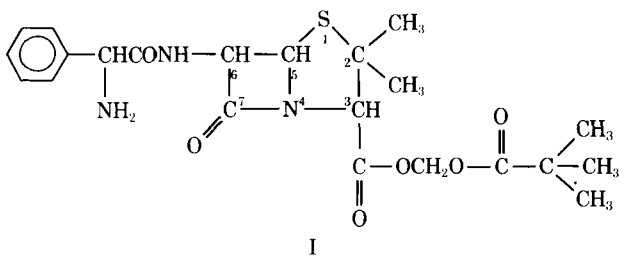
Materials—Potassium penicillin G[1], sodium ampicillin[2], and pivaloyl ampicillin[2] (I) were laboratory reference grade.

Deuterium oxide (99.95%), deuterated dimethyl sulfoxide, and deuterochloroform ($CDCl_3$) were used as solvents; tetramethylsilane was used as the external reference.

NMR Measurements—The spectra were obtained at 31° using a spectrometer[3] operating in a field-sweep mode.

RESULTS AND DISCUSSION

In their original report, Green *et al.* (2) observed anomalous behavior for the β-lactam protons at 60 MHz; further studies at 100 MHz (3) indicated that their original interpretation was incorrect. Penicillin G appeared to give a double doublet at τ 4.35 for the C_6-proton and a single doublet at τ 4.45 for the C_5-proton. Coupling of the amide and C_6-protons is probably responsible for the double doublet. Accidental coincidence of chemical shifts was postulated as being responsible for the two-proton singlet at τ 4.49 for ampicillin. Kan *et al.* (6) did not report the presence of a double doublet for the C_6-proton of penicillin G at 220 MHz.



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The chemical shifts (Table I) indicate that, whereas a doublet was observed for each proton of the β-lactam ring in penicillin G, only one singlet was observed for both protons in ampicillin. The presence of an electron-attracting group such as $-NH_2$ may transfer its effect to the β-lactam ring to produce the observed differences. Collapse of this doublet was also observed for penicillin G solutions in dimethyl sulfoxide and its mixture was deuterium oxide (Table I). In both antibiotics, the proton most affected by changes in solvent was the C_3-proton; upfield shifts of 41 and 33 Hz were observed for penicillin G and ampicillin, respectively, when deuterium oxide was replaced by dimethyl sulfoxide.

An amide signal for ampicillin could only be detected in deuterated dimethyl sulfoxide as a broad signal at τ 1.32. The same signal for penicillin G could be detected in water (τ 1.45, broad), deuterated dimethyl sulfoxide (τ 1.28, doublet, J_{BX} = 8 Hz), and deuterated dimethyl sulfoxide–deuterium oxide (τ 1.27, doublet, J_{BX} = 7 Hz). This lends further evidence to differences in coupling of the amide group to the C_6-proton on substitution of the amino group into the side chain. In deuterium oxide, the amide group was unobservable for both antibiotics. Hydrogen bonding with the solvent cannot be eliminated in view of the presence of this signal for both penicillin G and ampicillin in dimethyl sulfoxide, when the amide signal would shift to be coincident with that due to deuterium oxide. However, exchange of the amide proton with deuterium oxide is a more likely explanation since the signal in deuterated dimethyl sulfoxide disappears upon addition of a drop of deuterium oxide. The exchange appears less rapid in penicillin G since the amide proton can be observed initially in the mixed solvent, which may be due to its coupling with the C_6-proton.

Different magnetic environments for the protons result from solvent interaction, giving rise to line shifts. The differences in chemical shifts observed between the systems in water and deuterium oxide may be due to differences in the dielectric constant; differences in the dipole moment are negligible. It was reported (7) previously that solvent effects on the NMR spectrum of acetonitrile correlated with dielectric constants.

The signal due to the amino protons of ampicillin was unobservable even in different solvents; the spectrum of the nonzwitteri-

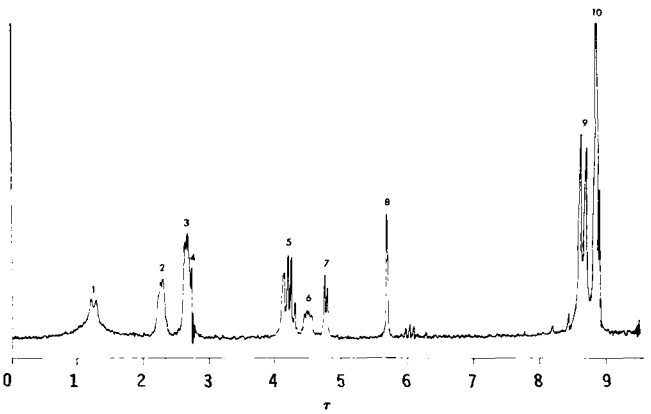
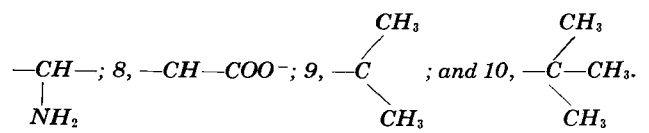


Figure 1—*100-MHz NMR spectrum of pivaloyl ampicillin in deuterochloroform at 31°. Key: 1, —NH—; 2, —NH_2; 3, C_6H_5—; 4, $CDCl_3$; 5, —O—CH_2—O—; 6, —CH—CH—; 7,*  *; and 10,* .

[1] Gift of Glaxo Research Ltd, Greenford, England.
[2] Gift of Beecham Research Laboratories, Betchworth, England.
[3] Varian Associates HA-100.

Table I—Effect of Solvents on 100-MHz Signals[a] of Antibiotic Protons (τ Values) (J Values in Hz in Parentheses)

Antibiotic and Solvent	C_6H_5—	—CH—X[b]	C-6	C-5	C-3	C-2—$(CH_3)_2$	
Penicillin G							
Deuterium oxide	2.60	6.32	4.44 d (4.0)	4.46 d (4.0)	5.69	8.39	8.44
Water	2.45	6.16	—[c]	—[c]	—[c]	8.20	8.35
Deuterated dimethyl sulfoxide	2.73	6.45	4.60 s	4.66 s	6.10	8.44	8.54
Deuterated dimethyl sulfoxide–deuterium oxide	2.72	6.46	4.63 s	4.63 s	6.05	8.44	8.54
Ampicillin							
Deuterium oxide	2.48	5.26	4.45 s	4.45 s	5.72	8.41	8.48
Water	2.44	—[c]	—[c]	—[c]	—[c]	8.24	8.30
Deuterated dimethyl sulfoxide	2.64	5.48	4.57 s	4.57 s	6.05	8.45	8.56
Deuterated dimethyl sulfoxide–deuterium oxide	2.49	5.30	4.46 s	4.46 s	—[c]	8.29	8.40

[a] s = singlet, and d = doublet. [b] X = H in penicillin G, and X = —NH_2 in ampicillin. [c] Not observable under water peak.

onic pivaloyl ampicillin (I) in deuterochloroform was therefore examined (Fig. 1). Decoupling experiments were performed in which signals 2 and 7 were separately irradiated and the changes in each, if any, were observed. The occurrence of intramolecular hydrogen bonding between the α-amino group and the β-lactam carbonyl oxygen would be expected to result in complex signals for the amino protons and the α-CH-proton due to changes in their coupling. Despite the presence of a doublet for the α-CH-proton, irradiation provided no evidence of the complex signals required to postulate intramolecular bonding. James *et al.* (8) found no evidence of intramolecular hydrogen bonding in the crystal of ampicillin trihydrate. A recent study (9) suggested that the nonuniform concentration dependence observed for the β-lactam protons may indicate intermolecular hydrogen bonding, which would aid the postulation of self-association of penicillin G.

It would appear that the amino group does not restrict the rotation of the side chain, which has been shown to be important in interaction with phospholipids (5, 10, 11).

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