Concerning the Coordination Site of the Antibiotic Nalidixate Ion Towards Cu^{2+} Ions Using ^{13}C NMR

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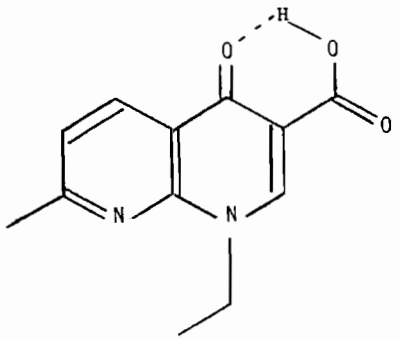
Abstract

The nalidixate ion exhibits the capacity to act as a chelate towards Cu^{2+} ions in aqueous solution, as determined by ^{13}C NMR. The site of binding depends upon the nature of the other ligands present in solution. In the absence of added ligands, chelation via the 3-carboxylate group is observed, while interaction with $[Cu(phen)]^{2+}$ is via chelation with both the 3-carboxylate and 4-oxo groups.

Introduction

Nalidixic acid (HNal), structure **1**, is a drug used to control several Gram-negative microorganisms, especially those that are responsible for infections of the urinary system [1]. It has been found that HNal inhibits the DNA replication system and two distinct modes of action have been suggested [2, 3]. The first suggests that the target of the drug is an enzyme of the DNA-topoisomerase group, DNA-gyrase, which controls the packing and supercoiling of DNA in prokaryotic cells [2]. The second proposal suggests that the activity of HNal involves a direct linkage to DNA mediated by a transition metal ion such as Cu^{2+} or Fe^{3+} [3]. In either case it is suggested that HNal acts as a bidentate chelating agent [4, 5]. It is therefore of importance to investigate the capacity of HNal to coordinate to metal ions that are well known as possessing biological significance.

The crystal structure of the complex $[Cu(phen)(Nal)H_2O]^+NO_3^- \cdot 3H_2O$ (phen = phenanthroline) has been reported, illustrating that the copper ion is coordinated by the Nal^- via the 3-carboxylate and 4-oxo groups, forming a six-membered chelate ring [6]. However, solid-state infrared and ESR studies of $[Cu_{aqu}]^{2+}$ complexes formed with HNal indicated no interaction between the metal ion and the 4-oxo group [7]. This difference may be due to solid-state effects upon the spectroscopic analysis or, more fundamentally, because of differences between the



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coordination properties of $[Cu_{aqu}]^{2+}$ *vs.* $[Cu(phen)]^{2+}$. In the aqueous ion, only O atoms would be coordinated to the metal ion, *i.e.* relatively hard ligands. In the case of the $[Cu(phen)]^{2+}$ ion, a relatively soft polarizable ligand (phenanthroline) is present and may change the nature of the metal acid in that it prefers to bind to more polarizable ligands.

In order to probe the solution binding of the two forms we have used a technique employed by Eichorn *et al.* [8] involving the paramagnetism of Cu^{2+}. In NMR spectra, nuclei close to the Cu^{2+} binding site will be diminished in intensity and finally collapse preferentially to more distant nuclei [8].

Experimental

Nalidixic acid was recrystallized prior to use to yield melting point of 228 °C. Nalidixate solutions, 0.01 M, were prepared by dissolving HNal in a NaOH solution in D_2O for direct NMR examination.

^{13}C NMR were recorded on a Bruker NR NMR–FT 200 MHz spectrometer, and spectra were recorded on the parent nalidixate solution. Subsequent to recording the parent solution spectrum, small amounts of Cu^{2+} and $[Cu(phen)]^{2+}$ ions were added to the same solution such that the final concentration of Cu was in the range 10^{-7}–10^{-8} mol/l.

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The spectrum of the parent Na nalidixate is recorded in Fig. 1, and representative spectra of the Cu-treated solutions are illustrated in Fig. 2. A complete spectral analysis is provided in Table I.

Results and Discussion

Coordination of the Cu ion to the carboxylate ion alone would be expected to cause an initial broadening, diminution and finally disappearance of the resonances C-14 and C-3, whereas chelation via the carboxylate and keto group would be predicted to equally effect resonances of atoms C-9, C-4, C-3 and C-14.

The data recorded in Table I and illustrated in Fig. 1 clearly demonstrate that addition of Cu^{2+} ion to the Na nalidixate solutions causes significant changes in the spectral parameters. In the presence of aqueous

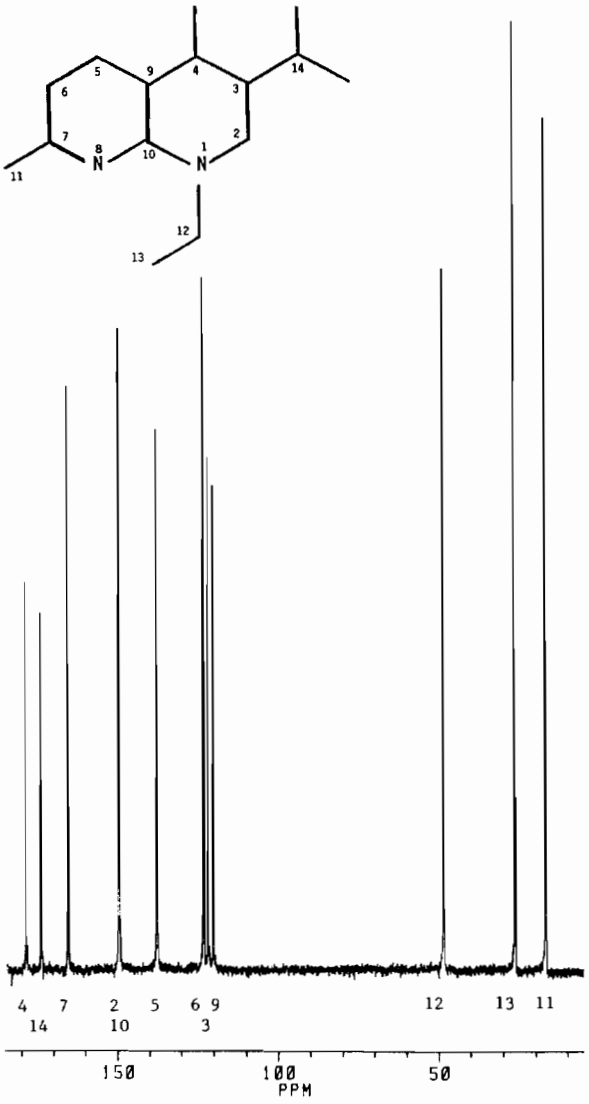

Fig. 1. ^{13}C NMR spectrum of sodium nalidixate in D_2O.

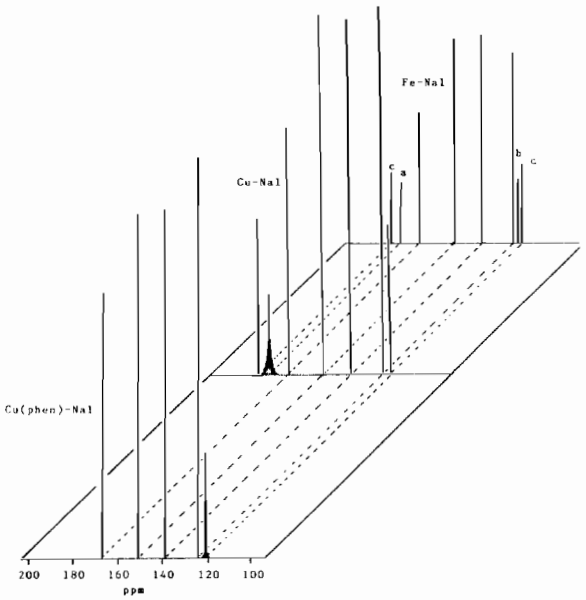

Fig. 2. Partial ^{13}C NMR spectra of the nalidixate ion in the presence of Fe(III), Cu(II) and Cu(phen)(II) ions. The Fe(III) spectrum is added to illustrate a non-specific paramagnetic effect.

TABLE I. ^{13}C NMR Parameters for Nal^-Na^+, Nal^-Cu^{2+} and $Nal^-Cu(phen)^{2+}$ (δ in ppm relative to TMS)

C atom	Nal^-Na^+	Nal^-Cu^{2+}	$Nal^-Cu(phen)^{2+}$
2	150.2	150.3	150.1
3	122.2		
4	179.1	179.1	
5	138.2	138.1	138.2
6	123.7	123.5	123.7
7	166.0	165.8	166.0
9	120.6	120.2	120.5(w)[a]
10	150.0	149.9	150.1
11	26.9	26.8	26.8
12	49.0	48.9	48.9
13	17.1	17.0	17.0
14	174.0		

[a]w = weak

Cu^{2+} ions, resonances of atoms C-14 and C-3 are dramatically diminished and broadened, while those for atoms C-4 and C-9 are virtually unaffected. Addition of $[Cu(phen)]^{2+}$ not only causes a change in the C-14 and C-3 resonances but *also* those for C-4 and C-9. These results demonstrate that in solution the Cu ion coordinates to $[Nal]^-$ via two distinct modes: via the carboxylate group in the case of aqueous Cu^{2+}; and via a carboxylate–keto chelation in the case of $[Cu(phen)]^{2+}$.

To discuss the differences in binding it is constructive to examine the detailed structure of the 4-carboxylate/3-oxo region of the nalidixate ion (Fig. 3). Upon deprotonation of HNal, the electronic

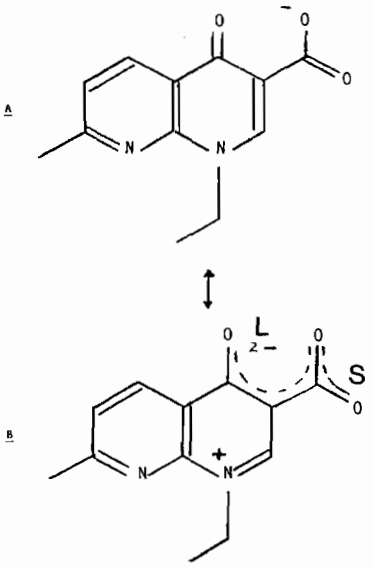

Fig. 3. Resonance contributors to the nalidixate ion structure.

pi-system changes, and the presence of the N-1 atom permits a charge delocalization to occur that produces a multi-atom delocalized system with two potential chelating cavities, L and S. Both sites are available for metal binding and, while the crystal structure noted above clearly implies binding to cavity L, there are many examples of metal ions chelating to carboxylate groups alone [9].

The difference in coordination modes between the two copper ions must result from their distinctive electronic and acid characteristics. As noted above, one possibility is that the relative softness of the Cu^{2+} ion decreases upon addition of phenanthroline and thereby increases the selectivity of the Cu^{2+} ion for softer ligands, hence the incorporation of the 4-oxo group into the coordination sphere of the metal. A second, related, explanation concerns the capacity of the more polarizable $[Cu(phen)]^{2+}$ to preferentially bind to the nalidixate enol form (Fig. 3b). This latter explanation has been used by Greisser and Sigel to explain stability constants of mixed ligand complexes [10]. Whichever of the two concepts is more appropriate, both will result in considerable delocalization of the 4-oxo group pi-system, as noted in Fig. 3b, which needs an efficient pi-accepting ligand (*i.e.* phenanthroline) on the copper atom. *This will only be possible with the participation of the N-1 atom in a resonance contribution, as noted in the Figure.* Such incorporation will result in a significant decrease of the C2–N1 bond length, increase in the C2–C3 length, and equivalence of the two chelating C–O bond lengths, which themselves will be longer than the 'free' CO group. Close comparison of the structures of $[Cu(phen)(Nal)]^{+}Cl^{-}$ and free nalidixic acid confirm just such predictions [6, 11]. In the case of the free acid, the proton hydrogen bonds to the 4-oxo group, *i.e.* chelates, but without the possibility of stabilizing extra negative charge via pi-bonding, hence the unimportance of the resonance contribution illustrated in Fig. 3b for the free acid.

This study clearly demonstrates the capacity of $[Nal]^{-}$ to bind the same metal ion in two distinct modes, via either the L or S cavities. Thus, depending upon the exact location of the transition metal ion in a biological system, the drug will have very different binding geometries, binding constants, etc., and therefore different activities. This will be important in the various parameters chosen to model drug activity. The role of N-1 in this class of drugs may well be determined by the particular metal–ligand binding needed for therapeutic activity since, as noted above, it is only with the N atom present that chelation of the metal ion via both the 4-oxo and 3-carboxylate group may be obtained.

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