Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 1, pp. 49–59, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Characterization of the complexation of fluoroquinolone antimicrobials with metal ions by nuclear magnetic resonance spectroscopy

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Abstract: The complexation of the fluoroquinolone antimicrobials is important because it has been implicated in reduced oral bioavailability and reduced antimicrobial activity when the drugs are co-administered with antacids or multi-vitamin preparations containing iron. The complexation of two model compounds, lomefloxacin and norflaxacin was studied using NMR. With aluminum ions, exchange between free and bound drug molecules was slow on the NMR timescale. Two complexes, proposed to have stoichiometries of 2:1 and 3:1 (drug:metal) based on peak widths and variable temperature studies, were observed. The crystal structure of lomefloxacin, which shows intermolecular self association previously reported to be crucial to the drug's mode of action, is also reported. Because the metal ion complexes could not be crystallized, the crystal structure of uncomplexed lomefloxacin together with the NMR data on the aluminum complexes were used in the molecular modelling of the lomefloxacin–aluminum complexes.

Keywords: *^{13}C-NMR; ^{1}H-NMR; X-ray crystallography; fluoroquinolones; aluminum; magnesium; complexation; crystal structure, molecular modelling.*

Introduction

Fluoroquinolones such as lomefloxacin and norfloxacin are orally active antimicrobials structurally related to nalidixic acid (Fig. 1). Complexation with metal ions such as calcium, magnesium, bismuth and aluminum ions, commonly found in antacid and multivitamin preparations has been reported to reduce the oral bioavailability of such fluoroquinolones [1–17]. The antimicrobial activity of fluoroquinolones in urine is also known to be influenced by the presence of salts containing cations such magnesium, aluminum, calcium and iron [17–26]. Despite the numerous clinical observations and the studies conducted *in vitro* to demonstrate the effects of metal-ion complexation on biological activity of this important class of antimicrobials, very little is known about the physicochemical basis of the interactions of the quinolones with metals or the structures of the putative complexes. Recently, Ross *et al.* have reported that complexation of fluoroquinolones increases their aqueous solubility [27] and reduces their 1-octanol–water partition coefficients [28]. Much of the lack of structural information on the quinolone–metal complexes probably lies in the difficulty in growing crystals, which in turn arise from the increased solubility of the complexes compared with the uncomplexed compounds [27].

Nalidixic acid has been reported to bind metal ions at the carboxylate moiety; however, conflicting reports regarding the participation of the 4-keto functionality in the binding have been published. Behrens and Mendoza-Diaz [29] prepared solid complexes of nalidixic acid and several metals (including Ca^{2+}, Mg^{2+}, and Fe^{3+}) and, using infrared studies, concluded that the 4-keto function did not participate in the complexation of metal ion with the drug molecule. Using ^{13}C-NMR, Mendoza-Diaz and Pannell [30] demonstrated that Cu^{2+} coordinated with nalidixic acid only through the carboxylate moiety, while [Cu(phenanthroline)]$^{2+}$ complexed with nalidixic acid via a carboxylate-keto chelation.

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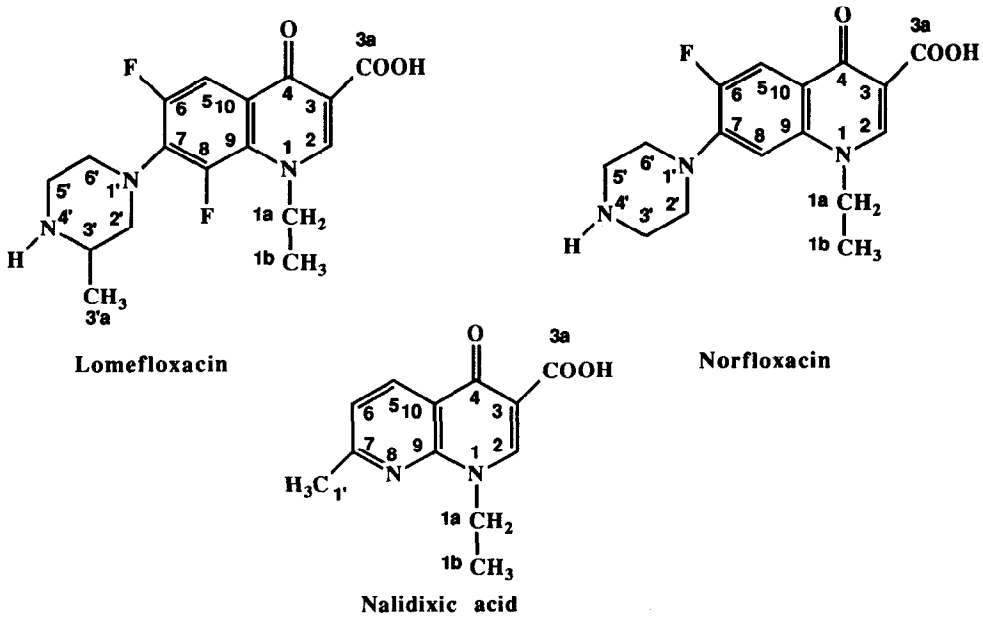


Figure 1
Structure of lomefloxacin, norfloxacin and nalidixic acid.

Experimental

Materials

Lomefloxacin mesylate was obtained from G.D. Searle and Co. (Skoie, IL, USA) and norfloxacin was obtained from Merck, Sharp and Dohme (West Point, PA, USA). All solvents were HPLC grade and obtained from commercial sources. All metal salts (in their chloride forms) were ACS reagent grade or better and obtained from commercial sources. All other chemicals were reagent grade obtained from commercial sources. Water was purified in a Milli-Q Water System (Millipore Corp., Bedford, MA, USA) and stored in glass containers until use.

NMR studies

The spectra of lomefloxacin, norfloxacin, and their complexes were recorded at room temperature (22 ± 1°C) on a Bruker AM-500 NMR spectrometer operating at 500.13 MHz (for ^{1}H), 125.77 MHz (for ^{13}C), 470.56 MHz (for ^{19}F) and 130.32 MHz (for ^{27}Al). The ^{13}C spectra were obtained with proton broad-band decoupling and both ^{1}H and ^{13}C spectra were referenced to external TSP (3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid). The two-dimensional, heteronuclear correlation (HETCOR), heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra were taken with standard Bruker pulse programs. A series of solutions was prepared in D_2O in which the concentration of metal ion was increased incrementally while the drug concentration was held constant. Adjustment of pD was made using μl additions of DCl or NaOD solutions so that all solutions in a series had a constant final pD (±0.05).

X-ray data collection, structure solution and refinement

A colourless crystal of lomefloxacin (free amino acid form), ~0.1 × 0.3 × 0.3 mm, obtained by recrystallization from a saturated aqueous solution, was mounted on a glass fibre oriented approximately along [0 1 0]. Cell constants were determined by using 25 centred reflections widely scattered throughout reciprocal space ($68 > 2\theta > 70°$). Preliminary counter data indicated a monoclinic system with systematic absences, $h + k = 2n + 1$ in hkl and $l = 2n + 1$ in $h0l$, uniquely determining the space group as $C2/c$. Two octants (h: $0 \rightarrow 24$, k:$0 \rightarrow 15$,l:$-12 \rightarrow 12$) of data out to $2\theta = 112.5°$ were collected using a Rigaku AFC5R diffractometer (Cu Kα, graphite monochromator) with θ–2θ scan model [2θ scan angle: $(0.30\tan\theta + 1.73)°$, scan speed: 8.0° min^{-1}]. Stationary background counts were recorded on each side of the reflection. The

ratio of peak counting time to background counting time was 2:1. The total number of reflections measured was 2651. Lorentz and polarization factors were applied. Three reflections monitored every 150 measurements did not show significant decay of the crystal. An empirical absorption correction, based on azimuthal scans of several reflections, was applied which resulted in transmission factors ranging from 0.85 to 1.00. After merging equivalent reflections, a total of 2574 independent F_0^2 data were obtained with an R_{int} for merging of 0.024. The $\sigma^2(F_0^2)$ was defined as follows: $\sigma^2(F_0^2) = \sigma^2_{count} + (0.10F_0^2)^2$. The 2516 reflections with $F_0^2 > 0.01\sigma(F_0^2)$ were used in subsequent calculations. The structure was solved by direct methods using MITHRIL [31]. Two partially occupied water molecules were found in the difference map. The occupancy factors (0.20(1) for O(W1) and 0.39(1) for O(W2)) were refined with the positional and isotropic thermal parameters. All hydrogen atoms except for those of partially occupied water molecules were located from difference Fourier maps computed after anisotropic refinement of non-H atoms, and were introduced into the refinement with isotropic thermal parameters. The parameters were refined by a full-matrix least-squares method. The function minimized was $\sum w(|F_0| - |F_c|)^2$ with $w = 4F_0^2/\sigma^2(F_0^2)$. Refinement was converged to $R = 0.064$, $wR = 0.062$, $S = 1261$ for all data. The maximum Δ/σ in the last full-matrix least-squares refinement cycle was 0.51. The final difference map was featureless (± 0.05 e Å^{-3}). Atomic scattering factors were taken from the literature [32]. All calculations were performed on a μ-VAX II using KUDNA (Cyrstallographic Computing System: KUDNA, Department of Chemistry, University of Kansas, 1984) and TEXSAN (TEXRAY Structure Analysis Package, Molecular Structure Corporation, Texas, 1985) software.

Crystal data

$C_{17}H_{19}F_2N_3O_3 \cdot 0.59H_2O$, $M_r = 361.98$, monoclinic, $C2/c$, $a = 22.826(3)$, $b = 14.184(1)$, $c = 11.464(1)$ Å, $\beta = 93.81(2)°$, $V = 3703.4(6)$ Å^3, $Z = 8$, $D_x = 1.298$ g cm^{-3}, Cu Kα, $\lambda = 1.5418$ Å, $\mu = 9.14$ cm^{-1}, $F(000) = 1519$, $T = 298(2)$ K, 309 parameters refined, final $R = 0.064$ for 2391 observed reflections ($R^2 > 0.01\ \sigma(R^2)$).

Results and Discussion

NMR spectra of lomefloxacin and norfloxacin

The ^{13}C-NMR and ^{1}H-NMR spectral assignments for lomefloxacin and norfloxacin are given in Tables 1 and 2, respectively. The two aryl protons in lomefloxacin were distinguishable because the signal of the hydrogen (H5) *ortho* to the 6-position fluorine appeared as a doublet centred at 7.7 ppm. The lowfield signal at 8.7 ppm was attributed to the H2 of the quinolone ring. The 1a methylene substituent appeared as a quartet at 4.6 ppm. No attempt was made to distinguish between the 2′, 6′ and 3′, 5′ protons on the piperazinyl ring which appeared as a multiplet centred at 3.5 ppm. The peak at 2.8 ppm was attributed to the methane sulphonate (mesylate) anion. The triplets at 1.5 and 1.4 ppm were assigned to the methyl hydrogens on the 1b, and 3′a carbons, respectively. The protons attached to the 1b carbon were assigned to the downfield signal due to their proximity to the aromatic ring.

Carbon assignments for lomefloxacin were made using a combination of 1-D and 2-D NMR techniques. The two carbonyl carbons (4 and 3a) were distinguishable using 2-D heteronuclear multiple bond correlation (HMBC), which showed long-range (2 and 3 bond) coupling between carbons and protons. The 4-carbon was coupled to both the H2 and H5 protons while C-3a was coupled only to H2. Fluorine split the signals of both C6 and C8 ($^1J_{CF} = 252$ Hz). Assignments were again made using HMBC because C6 was coupled to H5 while C8 displayed no long-range coupling. The long-range coupling of C10 to H5 and C9 to H5 and H2 helped distinguish these carbons. Using heteronuclear multiple quantum coherence (HMQC) which showed 1-bond coupling between carbons and protons, C5 and C2 were assigned. Of the remaining aryl carbons, C3 and C7, C7 was assigned to the downfield signal due to its attachment to nitrogen (N1′) and fluorine splitting. Of the non-aromatic carbons, C1a was assigned to the signal most downfield due to direct attachment to aryl nitrogen (N1). No attempts were made to make absolute assignments to carbons 2′, 3′, 5′ and 6′ of the piperazinyl ring whose signals ranged from 56.5 to 46.6 ppm. The signals at 18.4 and 18.0 ppm were assigned to C1b and C3′a, respectively. C1b was assigned to the signal more downfield due to its close proximity to the aromatic system.

Table 1
Chemical shifts (ppm) for lomefloxacin mesylate at 25°C and pH 5.3 in D_2O

Carbon atom	^{13}C	^{1}H
4	178.2	
3a	171.5	
6	157.8 (d) J_{CF} = 252 Hz	
2	153.5	8.7
8	149.0 (d) J_{CF} = 252 Hz	
10	136.0 (d) J_{CF} = 14 Hz	
9	130.0 (d) J_{CF} = 7 Hz	
7	123.6 (t) J_{CF} = 9 Hz	
5	109.8 (d) J_{CF} = 22 Hz	7.7 (d) J = 12 Hz
3	109.3	
1a	58.0	4.6 (q) J = 7 Hz
2′, 6′ and 3′, 5′	56.5, 54.8, 49.8, 46.6	3.5 (m)
1b	18.4	1.5 (t) J = 7 Hz
3′a	18.0	1.4 (d) J = 6 Hz

Table 2
Chemical shifts (ppm) for norfloxacin at 25°C and pH 2.1 in D_2O

Carbon atom	^{13}C	^{1}H
4	177.4	
3a	170.9	
6	155.4 (d) J_{CF} = 252 Hz	
2	149.9	8.2
7	147.4 (d) J_{CF} = 10 Hz	
9	139.4	
10	121.0 (d) J_{CF} = 8 Hz	
5	113.2 (d) J_{CF} = 24 Hz	6.8 (d) J = 13 Hz
3	108.0	
8	107.9 (d) J_{CF} = 17 Hz	6.7 (d) J = 7 Hz
1a	53.0	4.2 (q)
2′, 6′ and 3′, 5′	49.0, 45.9	3.5 (m)
1b	16.5	1.3 (t) J = 7 Hz

Two structural differences exist between lomefloxacin and norfloxacin. Norfloxacin has a hydrogen instead of a fluorine substituent at the 8-position and a hydrogen instead of a methyl substituent at the 3′-position of the piperazinyl ring. Norfloxacin proton and carbon assignments were made in a manner analogous to those of lomefloxacin. The C5 and C8 carbons were distinguished by the relative splitting of signal caused by the 6-position fluorine. Because C5 was closer to the fluorine, the C5 assignment was given to the signal with the larger J value. The J values for C5 and C8, as assigned, were 24 Hz and 17 Hz, respectively. Proton assignments for H5 and H8 were possible due to coupling with C5 and C8 in HETCOR experiments which showed 1-bond coupling between protons and carbons. Again C7, C9, and C10 were distinguished by HMBC and the J_{CF} values, as well as C4 and C3a. The proton and carbon assignments given here for norfloxacin agree with assignments made by Buckingham *et al.* [33] with the exception of carbons 4 and 3a, and 7 and 10. Buckingham made no attempt to distinguish between carbons 7 and 10, which were distinguished in this laboratory using HMBC. The assignments made by Buckingham *et al.* for carbons 4 and 3a were reversed from the assignments made here, i.e. C4 and C3a were assigned to signals at 170.9 and 177.4 ppm, respectively. While Buckingham *et al.* made the assignment based on shifts in spectra associated with changes in pH [33], the assignments to C4 and C3a were confirmed here using HMBC.

Metal-ion complexation

Preliminary NMR studies using lomefloxacin with Al^{3+}, Fe^{3+}, or Mg^{2+} showed that Fe^{3+} could not be used because the paramagnetic effect of the Fe^{3+} obscured the drug spectrum. Studies with Mg^{2+} showed a shift in the NMR spectral lines with increasing metal-ion concen-

trations; however, due to fast exchange (on the NMR timescale) between the complexed and free forms, a single averaged peak was obtained and this did not give any structural or stoichiometric information. Studies using Al^{3+} resulted in the appearance of three or four additional peaks with the addition of metal ion to the drug solution due to slow exchange between complexed and free drug. Since this slow exchange provided additional information about the solution equilibria occurring with the metal ions, all subsequent studies were conducted using Al^{3+} as a probe. Because the presence of a chiral centre in some quinolones could result in diasteroisomeric complex formation, two drugs were chosen for study: lomefloxacin, which contained a chiral centre due to the 3′-methyl substituent on the piperazinyl ring and norfloxacin, which is achiral.

The binding of lomefloxacin to Al^{3+} was studied by changing the molar ratio of drug to metal while holding the drug concentration constant at 10 mg ml^{-1}. When Al^{3+} was added, the proton spectra of lomefloxacin showed for additional signals for each signal in the free drug spectrum (Fig. 2). Near the H5 signal, three doublets of the same height (and area) appeared, one doublet was upfield and two doublets were downfield from the free drug signal. Variable temperature studies showed that an additional peak was located under the free drug peak near 7.7 ppm and appeared as a

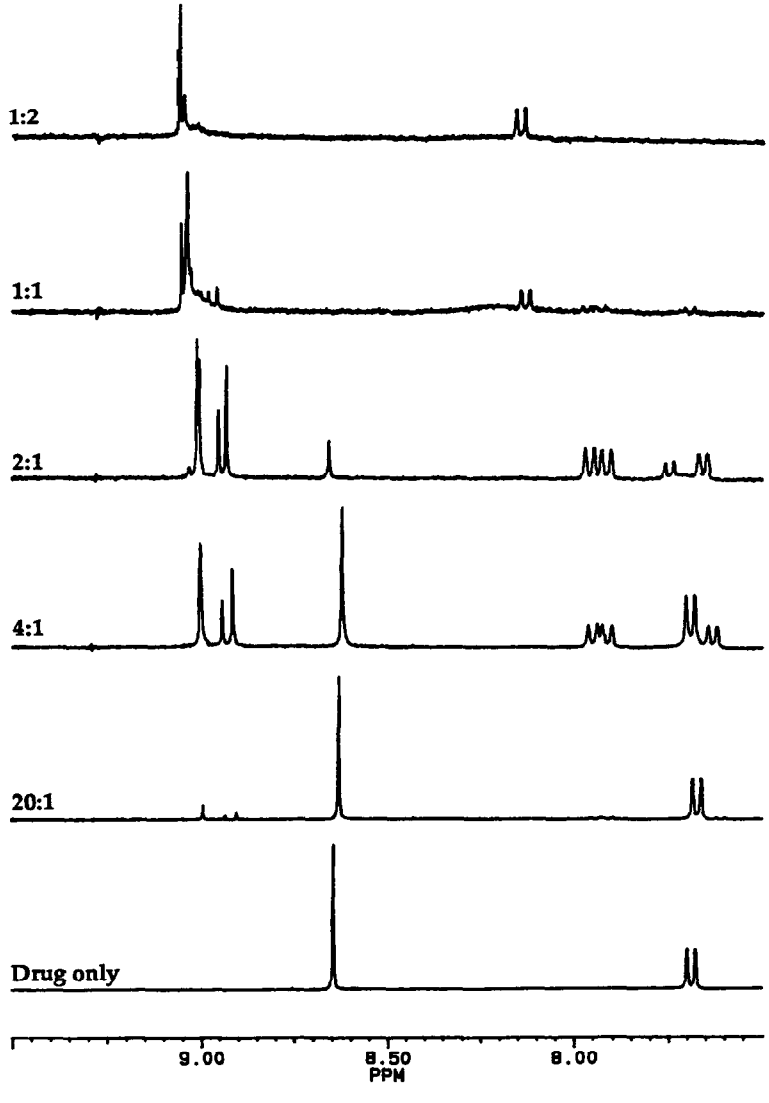


Figure 2
Part of the proton NMR spectra of lomefloxacin mesylate (10 mg ml^{-1}) with $AlCl_3$ added at various molar ratios, showing H-2 and H-5 free and complexed resonances.

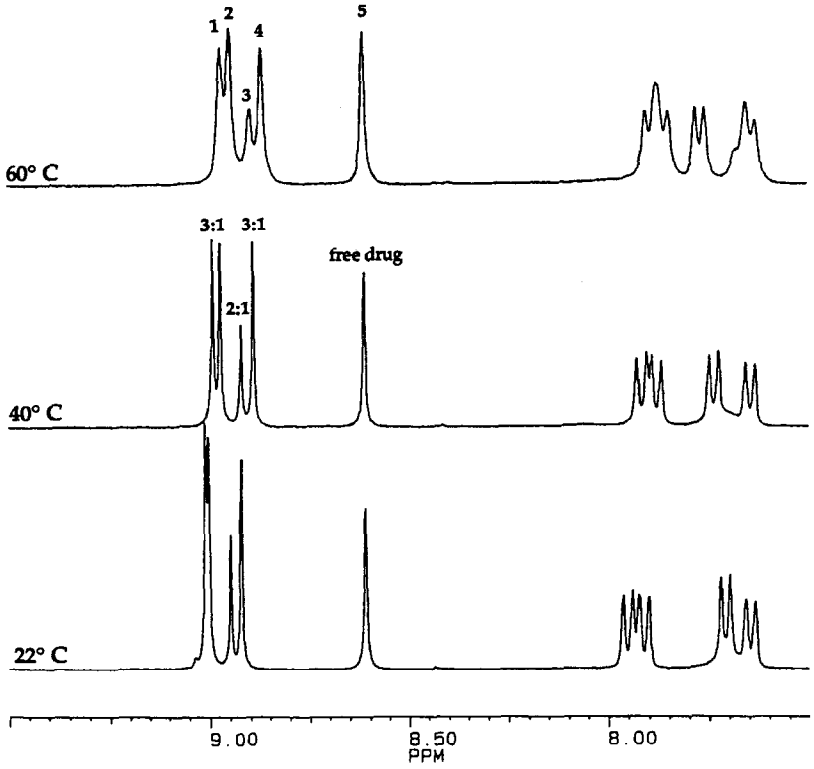


Figure 3
Expanded low-field region of the proton NMR spectrum of lomefloxacin mesylate: $AlCl_3$ (4:1) mixture at pH 5.3 as a function of temperature. Peaks 1, 2 and 4 are assigned to the 3:1 (drug:metal) complex while peak 3 is assigned to the 2:1 (drug:metal) complex. Peak 5 represents free drug.

broad band on the upfield peak at 60°C (Fig. 3). Near the H2 signal, three peaks of different heights were observed all downfield from the free drug signal. As the concentration of metal ion was increased, the same pattern persisted. However, at a 2:1 (drug:metal) ratio four peaks were clearly seen associated with the H2 signal. Integration of the H2 signals from the ^{1}H-NMR of lomefloxacin revealed that three peaks had the same intensity while one peak had an intensity approximately 60% that of the other bound peaks (1:1:0.6:1). One of the peaks with the same area was shorter and broader than the other two equal-intensity peaks resulting in two peaks which were the same height and two peaks which were different heights. The intensity ratio of the four peaks did not vary with the drug:metal ratio from 20:1 to 2:1. At and above a ratio of 1:1 (drug:metal), only one peak was seen due to fast exchange between complexes with increasing metal concentration.

The carbon spectra of lomefloxacin changed in an analogous manner with increasing concentrations of Al^{3+}. With the addition of Al^{3+}, the C3a and C4 signals both had four additional peaks upfield from the free drug signals. Two peaks were the same height and the other two peaks differed in height. The C2 signal also had four additional peaks showing the same pattern of height and intensity ratios; however, these peaks were downfield from the free drug signal. Fluorine NMR showed the same pattern of height and intensity (1:1:0.6:1) ratios but offered no additional information than provided by the ^{1}H or ^{13}C-NMR studies.

Aluminum NMR studies were conducted at 10:1, 4:1, and 1:1 (drug:metal) ratios. At a ratio of 10:1 (drug:metal) no free Al^{3+} was present. At a ratio of 4:1, primarily complexed Al^{3+} was evident; however some free Al^{3+} was present. As the concentration of Al^{3+} was increased to a ratio of 1:1 (drug:metal), only the free Al^{3+} signal was evident. Aluminum NMR provided no information on the species involved in complexation.

The binding of norfloxacin to Al^{3+} was also studied by changing the ratio of drug to metal. The addition of Al^{3+} to norfloxacin resulted in four additional peaks, which were most clearly

Table 3
Changes in peak area of ^{1}H-NMR of lomefloxacin and $AlCl_3$ (4:1) at pH 5.3 with changes in temperature. Peaks are identified by numbers in Fig. 3

Temp.* (°C)	Peak area				Contribution to total area (%)		
	1 & 2	3	4	5	3:1 (Pks 1, 2 & 4)	2:1 (Pk 3)	Free drug
60	0.45	0.12	0.21	0.21	66	12	21
40	0.45	0.12	0.21	0.22	66	12	21
22	0.44	0.15	0.20	0.21	64	15	21

*Solvent = D_2O.

seen using HMBC (data not shown). Because a total of four additional peaks was seen with both lomefloxacin and norfloxacin in the presence of Al^{3+}, it appeared that none of the lomefloxacin species could be explained by diastereoisomers. If diastereoisomers for lomefloxacin do exist, then their NMR signals are indistinguishable.

To clarify whether a single complex (such as a 5:2 complex) or two different complexes (such as a 3:1 and 2:1 complex) existed, the ^{1}H-NMR of the 4:1 (drug:metal) mixture was studied as a function of temperature. Table 3 contains the normalized integration data for each of the peaks and Fig. 3 illustrates the changes in the ^{1}H-NMR seen with changing temperature. The results indicated that two types of complexes existed. As the temperature was increased, the total area of the three peaks of the same area (probably due to a 3:1 complex) increased while the area of the peak associated with a different complex (probably a 2:1 complex) decreased slightly. The total area of peaks 1, 2 and 4 (Fig. 3), which can be attributed to a 3:1 complex, increased by 2% while the total area of peak 3, which can be attributed to a 2:1 complex decreased by 3%. These changes are consistent with the stoichiometries proposed for the two complexes, but because the concentration changes over the accessible temperature range (above 60°C broadening of the resonances made accurate integration impossible) were small, they are not completely definitive.

X-ray crystal structure of lomefloxacin and possible structure of the lomefloxacin–aluminum complex

Attempts to grow a crystal of lomefloxacin complexed with magnesium, aluminum or iron were unsuccessful, presumably because the metal-ion complexes were more soluble in the crystallization solvents (water, water–methanol, water–ethanol mixtures) than the uncomplexed compound. During the experiments designed to crystallize the lomefloxacin–aluminum complex a crystal of the free amino acid form of the parent compound was isolated of sufficient size to obtain the X-ray crystal structure of lomefloxacin.

Figure 4 shows the structure of lomefloxacin perpendicular to the unsaturated rings and Fig. 5 shows a stereo-view of the crystal structure. The bond lengths and angles were unexceptional (Table 4)* and consistent with those of the related compounds nalidixic acid [34] and silver pefloxacin [35] with one important exception. In the crystal of nalidixic acid the hydrogen atom of the carboxylic acid is hydrogen bonded to the adjacent 4-keto moiety [34]. In marked contrast the hydrogen atom of the carboxylic acid in lomefloxacin was found to be hydrogen bonded to the 4′-nitrogen atom (Fig. 1) (N17 in Fig. 4) of an adjacent piperazine ring system. Also, the N-ethyl groups of adjacent molecules were in close contact. The head-to-tail stacking of lomefloxacin, with the carboxylic acid groups positioned above and below the piperazine rings of adjacent molecules is best seen in the stereo-view of the unit cell shown in Fig. 5. In a model proposed for the binding of fluoroquinolones to the complex of DNA and DNA gyrase (the molecular basis of action for these antibacterials), these same interactions play a crucial role [36]. However, it is interesting to note that intramolecular hydrogen bonding of the carboxylic acid to the adjacent 4-keto in aqueous solution has been

*It should be noted that the standard numbering system for crystal structures was used in Table 4 and Fig. 4. The standard IUPAC system for numbering is used in Fig. 1 and elsewhere in the text.

Table 4
Intramolecular distances and bond angles for lomefloxacin with the estimated standard deviations in the least significant figure given in parentheses

Distances Atom–atom*	Distance (Å) (SD)	Atom–atom*	Distance (Å) (SD)
N1–C2	1.350 (2)	N14–C15	1.455 (3)
N1–C10	1.389 (2)	C15–H15B	1.06 (2)
N1–C21	1.491 (3)	C15–H15A	1.08 (3)
C2–H2	0.99 (3)	C15–C16	1.502 (3)
C2–C3	1.351 (3)	C16–H16	1.07 (2)
C3–C4	1.428 (3)	C16–N17	1.494 (3)
C3–C11	1.497 (3)	C16–C25	1.498 (3)
C4–O20	1.246 (2)	N17–H17	0.81 (2)
C4–C5	1.476 (2)	N17–C18	1.495 (2)
C5–C6	1.404 (3)	C18–H18A	0.84 (2)
C5–C10	1.409 (3)	C18–H18B	1.04 (2)
C6–H6	0.93 (2)	C18–C19	1.512 (3)
C6–C7	1.354 (3)	C19–19A	0.94 (2)
C7–F23	1.354 (2)	C19–H19B	1.00 (2)
C7–C8	1.402 (3)	C21–H21B	0.90 (2)
C8–C9	1.382 (3)	C21–H21A	0.96 (2)
C8–N14	1.410 (2)	C21–C22	1.486 (4)
C9–F24	1.340 (2)	C22–H22C	0.88 (4)
C9–C10	1.404 (3)	C22–H22B	0.96 (3)
C11–O13	1.236 (2)	C22–H22A	1.09 (3)
C11–O12	1.262 (3)	C25–H25A	0.97 (3)
O13–H13	1.08 (7)	C25–H25B	0.98 (3)
N14–C19	1.454 (2)	C25–H25C	1.26 (5)
Angles **Atom–atom–atom***	**Angle (°)**	**Atom–atom–atom***	**Angle (°)**
C2–N1–C10	118.9 (2)	C8–C9–C10	123.8 (2)
C2–N1–C21	116.7 (2)	N1–C10–C9	124.5 (2)
C10–N1–C21	124.4 (1)	N1–C10–C5	118.3 (1)
H2–C2–N1	121.1 (1)	C9–C10–C5	117.1 (2)
H2–C2–C3	112 (1)	O13–C11–O12	123.5 (2)
N1–C2–C3	126.7 (2)	O13–C11–C3	119.9 (2)
C2–C3–C4	118.5 (2)	O12–C11–C3	116.7 (2)
C2–C3–C11	117.0 (2)	H13–O13–C11	117 (3)
C4–C3–C11	124.5 (2)	C8–N14–C19	119.3 (1)
O20–C4–C3	125.9 (2)	C8–N14–C15	116.1 (2)
O20–C4–C5	119.0 (2)	C19–N14–C15	111.8 (1)
C3–C4–C5	115.1 (2)	H15B–C15–C15A	104 (2)
C6–C5–C10	120.2 (2)	H15B–C15–N14	102 (1)
C6–C5–C4	117.6 (2)	H15B–C15–C16	116 (1)
C10–C5–C4	122.3 (2)	H15A–C15–N14	108 (1)
H6–C6–C7	122 (1)	H15A–C15–C16	114 (1)
H6–C6–C5	118 (1)	N14–C15–C16	110.9 (2)
C7–C6–C5	119.5 (2)	H16–C16–N17	100 (1)
C6–C7–F23	119.2 (2)	H16–C16–C25	115 (1)
C6–C7–C8	123.3 (2)	H16–H16–C15	107 (1)
F23–C7–C8	117.6 (1)	N17–C16–C25	109.5 (2)
C9–C8–C7	116.1 (2)	N17–C16–C15	110.2 (2)
C9–C8–N14	125.5 (2)	C25–C16–C15	114.0 (2)
C7–C8–N14	118.5 (2)	H17–N17–C16	102 (1)
F24–C9–C8	115.9 (2)	H17–N17–C18	112 (1)
H18A–C18–H18B	112 (2)	H21A–C21–C22	105 (1)
H18A–C18–N17	107 (1)	H21A–C21–N1	113 (1)
H18–C18–C19	111 (1)	C22–C21–N1	113.2 (2)
H18B–C18–N17	102 (1)	H22–C22–H22B	110 (3)
H18B–C18–C19	114 (1)	H22C–C22–H22B	110 (3)
N17–C18–C19	110.3 (2)	H22C–C22–H22A	97 (3)
N17–C18–C19	110.3 (2)	H22C–C22–C21	110 (3)
H19A–C19–H19B	102 (1)	H22B–C22–H22A	130 (2)
H19A–C19–N14	117 (1)	H22B–C22–C21	111 (2)
H19A–C19–C18	103 (1)	H22A–C22–C21	98 (2)
H19B–C19–N14	111 (1)	H25A–C25–H25B	100 (2)
H19B–C19–C18	116 (1)	H25A–C25–H25C	128 (3)
N14–C19–C18	107.9 (2)	H25A–C25–C16	114 (2)
H21B–C21–H21A	96 (2)	H25B–C25–C25C	108 (2)
H21B–C21–C22	111 (2)	H25B–C25–C16	107 (1)
H21B–C21–N1	117 (2)	H25C–C25–C16	99 (2)

*See Fig. 4 for numbering system.

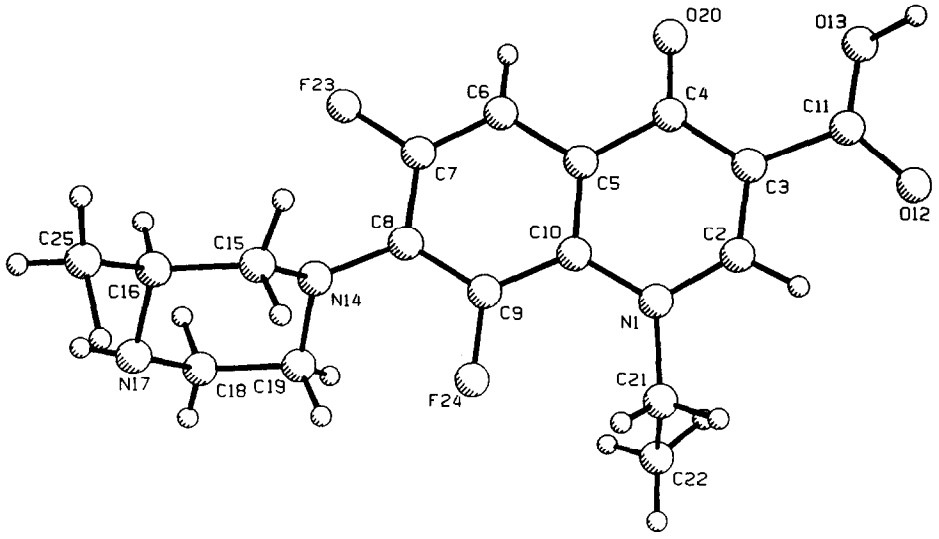


Figure 4
Projection of lomefloxacin drawn perpendicular to the unsaturated rings. See Table 4 for intramolecular distances and angles. The numbering system shown here refers only to the crystal structure. The standard IUPAC numbering system is used throughout the rest of the paper.

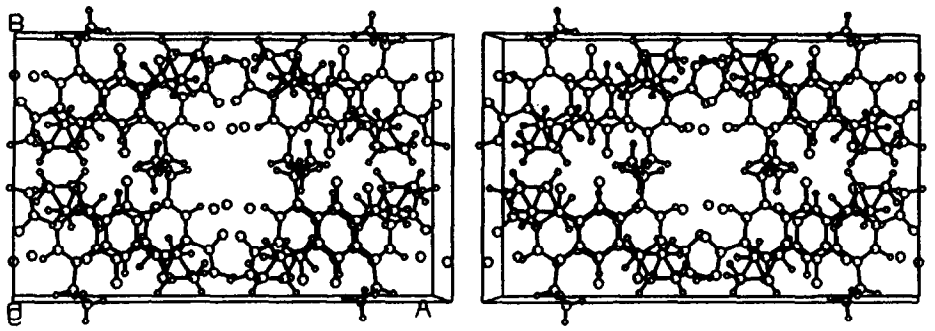


Figure 5
Stereo-view of the crystal structure of lomefloxacin.

implicated in the pK_a value of the carboxylic acid being approximately 6, which is approximately 2 pK_a units higher than would be expected for carboxylic acids that are not similarly hydrogen bonded [37, 38]. Furthermore, Bailey *et al.* [39] have shown that the homo-acid of nalidixic acid, 2-(1-ethyl-1,4-dihydro-7-methyl-4-oxoquinol-3-yl)-ethanoic acid, which does not form an intramolecular hydrogen bond is biologically inactive (Prof. John Midgley, Strathclyde University, personal communication, 1991). The fact that the pK_a value of the carboxylic acid of the fluoroquinolones is essentially the same as the pK_a value for nalidixic acid [37, 38] suggests that intramolecular hydrogen bonding in the fluoroquinolones occurs in dilute solution; however, the X-ray crystal data suggest that intermolecular hydrogen bonding is energetically favoured in the solid state.

Using the X-ray crystal structure of lomefloxacin (Figs 4 and 5, Table 4) and information from the Cambridge Data Base about Al^{3+}–carbonyl complexes, molecular modelling was used to propose a structure (Fig. 6) for the lomefloxacin–aluminum complex, which was consistent with the NMR data. The Cambridge Data Base contained 14 X-ray crystal structures of complexes of Al^{3+} with carbonyl ligands. Of these seven were α- and six were β-diketones. Of the 14 complexes, eight of the ligands formed octahedral complexes with the aluminum ion. All the structures had bidentate binding between the Al^{3+} and the ligand.

The carbon NMR spectrum of lomefloxacin and Al^{3+} revealed that the carboxylic acid

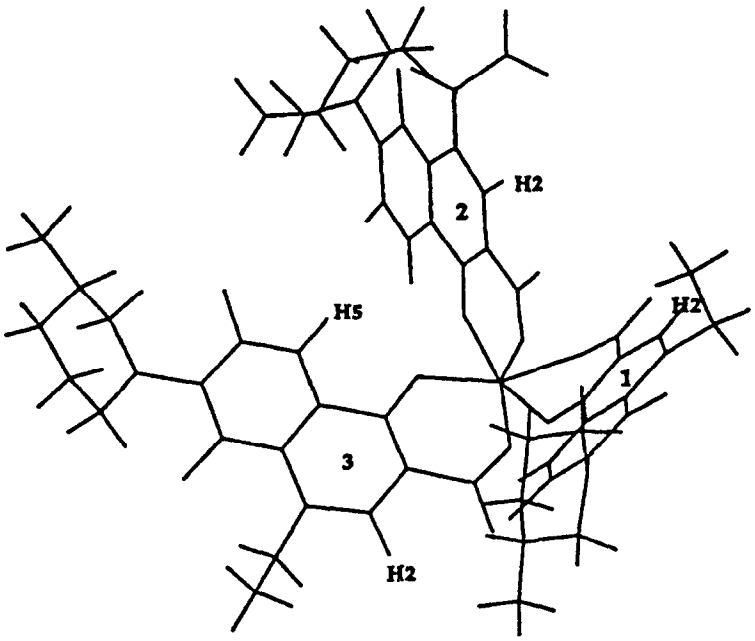
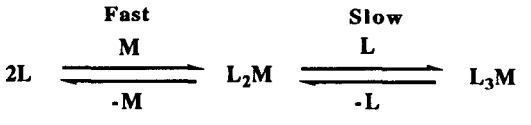


Figure 6
Proposed structure of the 3:1 complex between lomefloxacin and Al^{3+}.

carbon (3a) and the 4-keto carbon signals both had upfield shifts when Al^{3+} was added to the solution, while the aromatic carbons had downfield shifts in the presence of Al^{3+}. The upfield shifts may be due to direct binding of the C3a and C4 oxygens to Al^{3+}. These findings are consistent with those of Karlik *et al.* [40] who studied the binding of Al^{3+} to ATP and also observed upfield and downfield shifts depending on the relationship of the affected atom to the Al^{3+}. The upfield shifts were indicative of direct binding to Al^{3+}, while downshift shifts were related to indirect effects, i.e. the atom associated with the downfield shift was not directly bound to the Al^{3+} [40]. Mendoza-Diaz and Pannell [30] studied the coordination of Cu^{2+} and $[Cu(phenanthroline)]^{2+}$ with nalidixic acid using ^{13}C-NMR. X-Ray analysis of the complex formed between $[Cu(phenanthroline)]^{2+}$ and nalidixic acid showed that the carboxylate and carbonyl functions were involved in binding. This caused a shift of the C3 and C3a resonances together with those attributable to C4 and C9. Since Cu^{2+} only affected the carbon shifts of C3 and C3a, it was concluded that binding of Cu^{2+} only involved the carboxylate function and not the 4-keto function [30]. Because both carbons 3a and 4 of lomefloxacin had an upfield shift in the presence of Al^{3+}, it was likely that both 3a- and 4-carbonyls were directly coordinated with the aluminum ion. A structure of the 3:1 (drug:metal) complex was proposed to consist of three drug molecules bound at the 3a,4-dicarbonyl site of the quinolone molecule with octahedral geometry about the aluminum ion (Fig. 6). This proposed binding is consistent with the findings of Karlik *et al.* [40] and Mendoza-Diaz and Pannell [30] as well as with many of the X-ray structures of the ligands in the Cambridge Data Base. Figure 6 illustrates the proposed 3-D structure of the lomefloxacin–aluminum complex. It should be noted that the H5 on ring 3 in Fig. 6 is affected by the anisotropic field of the adjacent ring and would therefore be more shielded and hence yield a resonance upfield from the free drug signal. The H5 on the other rings are not in an anisotropic field of an adjacent ring and are shifted downfield from the free drug signal. The environments of the H2 on molecules 1 and 2 are similar and would probably account for the two signals most downfield, which are not well separated at 22°C. The third signal (peak 4) is probably due to a third molecule which is in more rapid exchange (as indicated by the wider, shorter peak) possibly due to crowding around the metal centre (Scheme 1).

The signal assigned to the 2:1 complex (peak



Scheme 1
Proposed kinetic scheme for the complexation of lomefloxacin and Al^{3+}.

3) was also relatively broad compared with peaks 1 and 2, which may be due to interchange of the terminal groups between the two drug ligands and racemization at the metal centre as proposed by Pickering *et al.* [41] for aluminum β-diketone chelates. Therefore, the *cis-* and *trans-* forms of the 2:1 complex would be indistinguishable and a single signal would result as seen in these studies.

Acknowledgements — This work was supported by a grant from G.D. Searle and a graduate fellowship for DLR from the American Foundation for Pharmaceutical Education (AFPE). The authors are grateful to Dr Howard Lambert (The Nutrasweet Company), Dr Patricia Frank (G.D. Searle), Dr Arnie Repta (Interx Corporation) and Prof. John Midgley (Strathclyde University) for helpful discussions.

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[Received for review 12 June 1992; revised manuscript received 23 July 1992]