Studies on the Interaction of Pyridone Carboxylic Acids with Metals

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The stability constants of metal complexes for several pyridone carboxylic acid drugs (ofloxacin, norfloxacin and lomefloxacin) were determined by potentiometry and spectrophotometry. The values of aluminum complexes, magnesium complexes and calcium complexes were Ca < Mg ≪ Al. The stability constants of lomefloxacin complexed with divalent transition metal ions were determined and these values followed the Irving–Williams series (Mn < Fe < Co < Ni < Cu > Zn). The stability constants of metal complexes for several pyridone carboxylic acids synthesized were also determined and compared with those for pyridone carboxylic acid drugs. The stability constants of these compounds gradually increased with an increasing pK_a value of the carboxyl group of pyridone carboxylic acid. In the case of aluminum complexes, the complexes Al(OH)L and $Al(OH)_2L$ were formed under weak acidic conditions and the dissociation constants for the hydrolysis of the aluminum complexes were determined. The participation of the carboxyl group and the carbonyl group in the chelating reaction was confirmed by the measurement of carbon-13 nuclear magnetic resonance of the aluminium complex and the magnesium complex. These results suggest that when pyridone carboxylic acids are administered with metallic antacid containing aluminum hydroxide and magnesium oxide, aluminum complexes AlL, Al(OH)L or $Al(OH)_2L$ are formed and the adsorption of the drugs in the intestines is reduced.

Keywords stability constants; metal complex; pyridone carboxylic acid; lomefloxacin; ofloxacin; norfloxacin; aluminum complex; magnesium complex; transition metal; ^{13}C-NMR

Introduction

Pyridone carboxylic acid (Fig. 1) is an antibacterial agent against gram-negative urinary tract infections.[1] It has been considered that its mechanism of action is the inhibition of deoxyribonucleic acid (DNA) gyrase[2] and metal ions are closely related to this action.[3] Furthermore, it has recently been reported that when these drugs were administered with metallic antacid, the drug concentration in blood plasma and the recovery of the drug in urine were decreased.[4,5] The stability constants of metal complexes are useful for the consideration of this phenomenon because this is presumed to be caused by the formation of a metal complex in the intestines.[6] However, there are few studies for these metal complexes except for nalidixic acid.[7,8] Considering the close relationship between the metal ions and pyridone carboxylic acids, we determined the stability constants of metal complexes formed from several pyridone carboxylic acids and several metal ions, and elucidated the fundamental property of those metal complexes. In addition, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) study showed that pyridone carboxylic acids give metal complexes in D_2O by the addition of aluminum ion or magnesium ion.

Experimental

Materials Lomefloxacin hydrochloride (1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid hydrochloride) was supplied from Hokuriku Seiyaku Co. Lomefloxacin was prepared by neutralizing lomefloxacin hydrochloride. Ofloxacin and norfloxacin were obtained by extraction from Tarivid tablets (Daiichi Seiyaku Co., Ltd.) and Baccidal tablets (Kyorin Seiyaku Co., Ltd.). 1-Ethyl-1,4-dihydro-4-oxo-3-pyridine carboxylic acid (**1**) and 1,4-dihydro-4-oxo-3-quinolinecarboxylic ethylester were obtained from Shionogi Research Laboratories. 2-Hydroxynicotinic acid was purchased from Aldrich Chemical Company, Inc. Manganese(II), iron(II), cobalt(II), nickel(II), copper(II) and zinc(II) aqueous solutions were prepared using their metal chloride (Wako Pure Chemical Industries, Ltd.). *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 2-(*N*-morpholino)-ethanesulfonic acid (MES) were from Dojindo Laboratories. All reagents used were of analytical-reagent grade and the water used was purified with a Milli-Q system (Milipore) and decarbonated by boiling.

Procedure; Synthesis of 1-Ethyl-1,2-dihydro-2-oxo-3-pyridinecarboxylic Acid (2) Potassium *tert*-butoxide (4.5 g) was added to 2-hydroxynicotinic acid (1.4 g) dissolved in dimethylformamide. After stirring for 1.5 h at room temperature, ethyl iodide (8 ml) was added slowly with cooling in ice. After stirring for 6 h at room temperature, the pale yellow solution

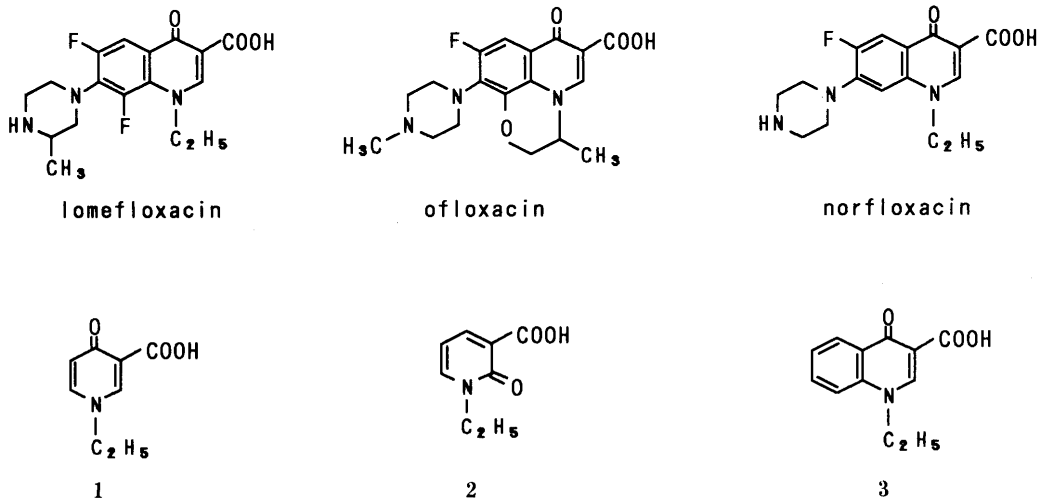


Fig. 1. Structure of Pyridone Carboxylic Acids

was evaporated to dryness. To the residue, chloroform was added and the precipitates were filtered off. After evaporation under reduced pressure, the products were dissolved in water and the solution was made acidic with concentrated HCl. After standing for 24 h at room temperature, the precipitates were filtered off and recrystallized from ethanol. The yields were 61% and the melting point was 174 °C. *Anal.* Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.52; H, 5.49; N, 8.45.

Synthesis of 1-Ethyl-1,4-dihydro-4-oxo-3-quinoline Carboxylic Acid (3) 1,4-Dihydro-4-oxo-3-quinoline carboxylic ethylester (1.4 g) was suspended in dimethylformamide and potassium *tert*-butoxide (4.5 g) was added. After the solute was dissolved with stirring for 1.5 h at room temperature, ethyl iodide (8 ml) was added slowly with cooling in ice. After stirring for 6 h at room temperature, the pale yellow solution was evaporated under reduced pressure, the reaction mixture was made alkaline with 4 M NaOH and heated for 2 h on a steam bath. The solution was neutralized with 4 M HCl, the products precipitated were filtered off and recrystallized from ethanol. The yields were 43% and the melting point was 250 °C. *Anal.* Calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.00; H, 5.11; N, 6.42.

Dissociation Constants by Spectrophotometry All measurements were made at 25 °C in Britton–Robinson's buffer solution (pH 4—8, $\mu=0.1$). The final concentration of drugs was 2.5×10^{-5} M. The absorbances at several pH values were measured at 288 nm (lomefloxacin), 299 nm (ofloxacin) and 284 nm (norfloxacin), respectively.

Stability Constants of Metal Complexes by Potentiometry Drugs (0.1—0.3 mmol) were dissolved in water (40 ml), and the pHs of the solutions were adjusted to about pH 3.4 (Al^{3+}), about pH 4 (Fe^{2+}, Cu^{2+}) or about pH 6 (Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}) by accurately adding a proper amount of 0.1 M HCl or NaOH (in the case of lomefloxacin hydrochloride) to avoid the influence of metal hydroxide. Then, 1 M NaCl (5 ml) was added to each solution and the solutions were diluted to 50 ml with water. The drug solutions (40 ml) were placed in the thermostated cell equipped with a jacket and titrated at 25 °C in an atmosphere of nitrogen with the metal solutions (15 mM) containing 0.1 M NaCl using a Comtite-101 titrator (Hiranuma Sangyo Co., Ltd.) equipped with a glass-Ag/AgCl electrode. In the case of Al^{3+}, the solution was dropped at 200 s intervals because the reaction rate of Al^{3+} complexes was low.

Dissociation Constants for Hydrolysis of Aluminum Complex Lomefloxacin (0.25 mmol) was dissolved in water, aluminum ion equivalent to the drug and 5 ml of 1 M NaCl were added and the solution was diluted to 50 ml. The solution (40 ml) was placed in the thermostated cell (25 °C) and titrated with carbonate-free 0.1 M NaOH in an atmosphere of nitrogen.

Stability Constants of Metal Complexes by Spectrophotometry Drugs (25 mM, 2 ml) were dissolved in 2 ml of 1 M HEPES buffer (pH 7.5) containing 0.5 M NaCl and then metal solutions were added at several concentration ratios (Mg^{2+}, Ca^{2+}, Co^{2+} and Ni^{2+}) to make 20 ml with water. Drugs (0.25 mM, 2 ml) were dissolved in 4 ml of 0.5 M MES buffer (pH 6.1) containing 0.25 M NaCl and then metal solutions were added at several concentration ratios (Zn^{2+}, Cu^{2+}) to make 20 ml with water. The absorption spectra of these solutions were measured on a model UV-190 spectrophotometer (Shimadzu). Stability constants were calculated from the difference between the absorbances of the drugs and those of the metal complexes by means of the method presented by Timmers *et al.*[8]

Measurement of ^{13}C-NMR Spectra Lomefloxacin hydrochloride (17—25 mg) was dissolved in D_2O (1 ml) and the calculated amount of metal ion equivalent to the drug was added to the solution. The final concentration was $2.0—6.5\times10^{-2}$ M. Acetonitrile was used as an internal standard and ^{13}C-NMR spectra were recorded on a Varian XL-400 spectrometer.

Results and Discussion

Stability Constants of Metal Complexes by Potentiometry

When a metal ion was added to a pyridone carboxylic acid solution, proton was liberated from the ligand by the metal binding and the pH value of the solution decreased. The titration curve is shown in Fig. 2. Through adding a metal solution accurately, a formation curve with a wide range of the average coordination number was obtained by only one titration. The equations for the stability constant from Bjerrum's theory[9] are as given below.

$$C_H=[H]+[LH]-[OH] \quad (1)$$

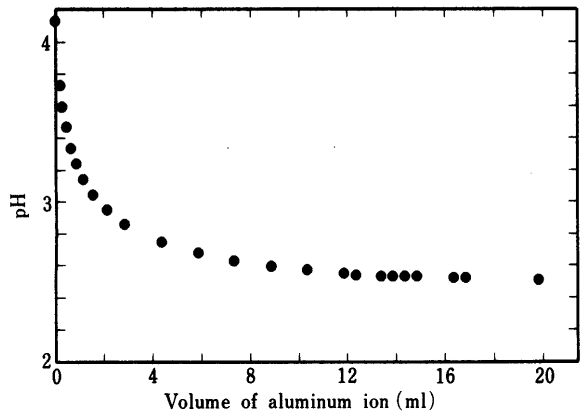


Fig. 2. Titration Curve of Lomefloxacin with Aluminum Ion

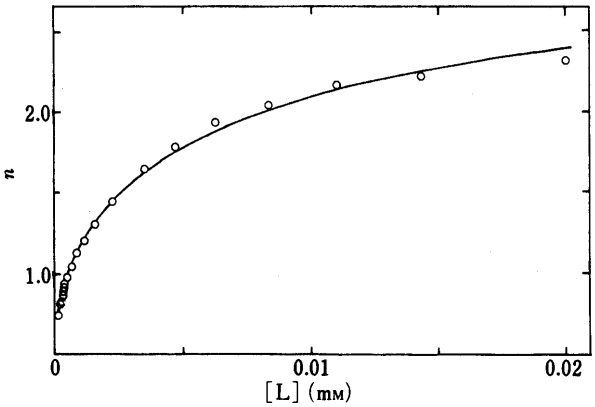


Fig. 3. Formation Curve of Aluminum Complex of Lomefloxacin
[L], free concentration of lomefloxacin; *n*, average coordination number.

$$C_L=[L]+[LH]+[ML]+2[ML_2]+3[ML_3] \quad (2)$$

$$C_M=[M]+[ML]+[ML_2]+[ML_3] \quad (3)$$

$$K_a=([L][H])/[LH] \quad (4)$$

$$M+L=ML \qquad K_1=[ML]/([M][L]) \quad (5)$$

$$ML+L=ML_2 \qquad K_2=[ML_2]/([ML][L]) \quad (6)$$

$$ML_2+L=ML_3 \qquad K_3=[ML_3]/([ML_2][L]) \quad (7)$$

Combination of Eqs. 3, 5, 6 and 7 leads to 8.

$$n=(K_1[L]+2K_1K_2[L]^2+3K_1K_2K_3[L]^3)/(1+K_1[L]+K_1K_2[L]^2+K_1K_2K_3[L]^3) \quad (8)$$

where C_H, C_L and C_M are the concentrations of proton, ligand and metal added, respectively, n is the average coordination number, and K_1, K_2, K_3 and K_a are the stepwise stability constants and the dissociation constant. The concentrations of the free ligand [L] and n at various total concentrations of the metal ion were calculated by Eqs. 1—4 from the titration curve. When the maxima of the n values were sufficient to form the chelate ML_3 (near 2) for the ligands with the metal (Al^{3+}, Ni^{2+}, Cu^{2+} and Zn^{2+}), the stability constants of the metal complexes were calculated from Eq. 8 by means of the nonlinear least square method using the approximate values as initial parameters. The plot of n against [L] showed excellent fitting for the calculated curves using the stability constants. The example of the aluminum complex of lomefloxacin is shown in Fig. 3, and the calculated curves of other metal complexes fit

with the observed values better than this example. When the maxima of n values were near 1 for the manganese complex and the cobalt complex, the approximation without the K_3 term (the equation by substituting $K_3=0$) fit better. The K_1 and K_2 values were calculated by means of the linear least square method. When the maxima of n values were less than 0.5 for the calcium complexes and the iron complex, K_1 values were calculated from the Eq. 8 by substituting $K_2=0$ and $K_3=0$. In the case of the iron complex, the equilibrium of ML was mainly observed because the measurement was done under acidic conditions to eliminate the influence of the metal hydroxide in a higher pH solution.

The stability constants are summarized in Table I. The values of aluminum complexes are very large compared with those of magnesium complexes and calcium complexes. The order of the stability constants for divalent transition metal complexes are Mn< Fe<Co<Ni<Cu>Zn and this follows the Irving–Williams series.[10] The stability constants of metal complexes for quinolone carboxylic acid drugs were similar to those for **1** and **3**, but the values for **2** were small. This result suggests that 4-pyridone carboxylic acids or 4-quinolone carboxylic acids have stronger activity to metal ions than 2-pyridone carboxylic acids. Although the stability constants of several metal complexes for pyridone carboxylic acids have been measured by spectrophotometry, only the K_1 values have been obtained.[5] We could obtain the stepwise stability constants (K_1, K_2 and K_3) of various metal complexes for several pyridone carboxylic acids. The ratio of ML, ML_2 and ML_3 can be calculated from these values quantitatively in various conditions, and these values give important information when the reactions of metals and drugs *in vivo* are considered.

Hydrolysis of Aluminum Complex Aluminum forms the complexes Al(OH)L with monocarboxylic acids under weak acidic conditions.[11] When the 1:1 aluminum complex of lomefloxacin was titrated with 0.1 M NaOH, the titration curve was different from that of lomefloxacin or aluminum chloride and two moles of NaOH were consumed for the hydrolysis of the complex (Fig. 4). The equations of these reactions are as follows.

$$ML \rightleftharpoons M(OH)L + H \qquad K_{A_1}=([M(OH)L][H])/[ML] \tag{9}$$

$$M(OH)L \rightleftharpoons M(OH)_2L + H \qquad K_{A_2}=([M(OH)_2L][H])/(M[OH)L] \tag{10}$$

K_{A_1} and K_{A_2} were calculated from Eq. 11 by means of the least square method.

$$Z/Y=K_{A_1}K_{A_1}+K_{A_1}\cdot X/Y$$

$$X=[H](C_L-C_{NaOH}-[H]+[OH]) \tag{11}$$

$$Y=2C_L-C_{NaOH}-[H]+[OH]$$

$$Z=[H]^2(C_{NaOH}+[H]-[OH])$$

TABLE I. Stability Constants of Metal Complexes of Pyridone Carboxylic Acids Obtained by Potentiometry

	Metal ion	log K_1	log K_2	log K_3
Lomefloxacin	Al^{3+}	7.12	5.47	4.71
(pK_a 5.82)	Ca^{2+}	2.08		
	Mg^{2+}	2.80	2.14	
	Mn^{2+}	3.09	2.77	
	Fe^{2+}	3.76		
	Co^{2+}	3.76	3.23	
	Ni^{2+}	4.21	3.52	1.31
	Cu^{2+}	6.16	4.80	2.45
	Zn^{2+}	3.71	3.02	2.68
Ofloxacin	Al^{3+}	7.13	5.40	5.34
(pK_a 6.05)	Ca^{2+}	2.12		
	Mg^{2+}	2.82	2.66	
Norfloxacin	Al^{3+}	7.03	5.44	5.45
(pK_a 6.24)	Ca^{2+}	2.22		
	Mg^{2+}	2.93	2.65	
2 (pK_a 5.18)	Al^{3+}	6.81	5.11	3.64

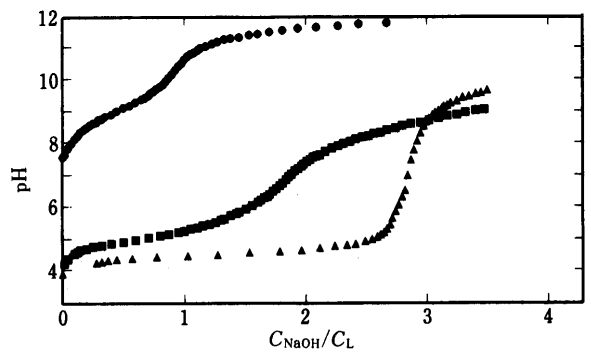


Fig. 4. Titration Curves

●, lomefloxacin (0.25 mmol); ■, aluminum complex of lomefloxacin (0.25 mmol); ▲, aluminum chloride (0.25 mmol). $\mu=0.1$ (NaCl). C_{NaOH}, total concentration of NaOH; C_L, total concentration of the drug titrated.

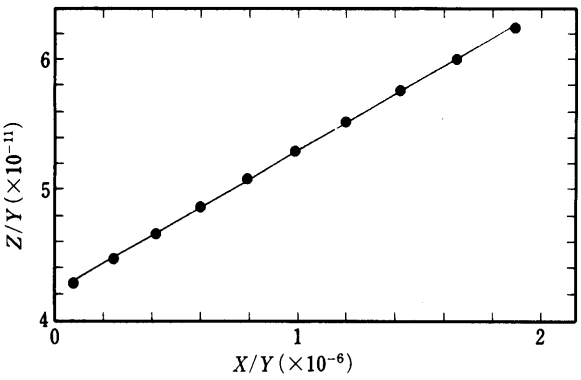


Fig. 5. Determination of Dissociation Constants for Hydrolysis of Aluminum Complex

$X=[H](C_L-C_{NaOH}-[H]+[OH])$; $Y=2C_L-C_{NaOH}-[H]+[OH]$; $Z=[H]^2(C_{NaOH}+[H]-[OH])$; C_L, total concentration of aluminum complex; C_{NaOH}, total concentration of NaOH.

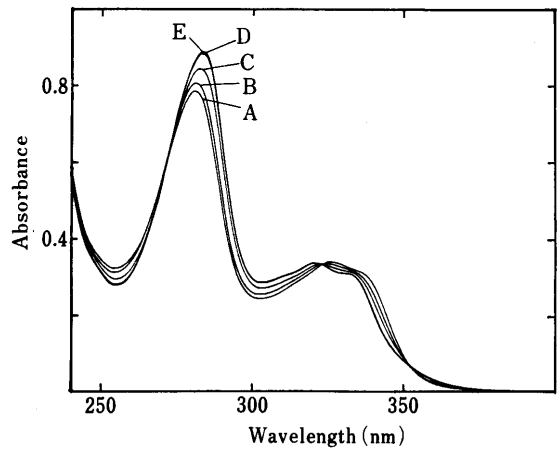


Fig. 6. Absorption Spectra of Lomefloxacin and Its Magnesium Complex

Concentration of lomefloxacin: 2.6×10^{-5} M. Concentration of magnesium ion; A, 0 mM; B, 0.5 mM; C, 2.5 mM; D, 25 mM; E, 50 mM.

where C_L and C_{NaOH} are the total concentration of the drug and NaOH, respectively. A plot of Z/Y against X/Y was found to be linear (Fig. 5), and pK_{A_1} and pK_{A_2} were 4.97 and 5.41, respectively. These results showed that the hydrolysis did not occur when the stability constants of aluminum complexes were determined by potentiometry under the acidic conditions (< pH 3.4). However, Al(OH)L or $Al(OH)_2L$ complexes are formed in the intestines because of the neutral conditions and these complexes may inhibit the absorption of the drugs.

Stability Constants of Metal Complexes by Spectrophotometry The absorption spectra of pyridone carboxylic acids were changed with increasing metal ion at a constant pH. The example of magnesium complex of lomefloxacin is shown in Fig. 6. The results indicate that the conditions of excess of metal ion gave a 1 : 1 metal complex. The stability constants at pH 7.5 or pH 6.1 were obtained from the absorbance differences (ΔA) between the drugs and the metal complexes. The results are shown in Table II. The stability constant of magnesium complex of nalidixic acid agreed with the value of the reference[8] and the other values almost agreed with the values obtained by means of potentiometry. In the case of **3**, because it is slightly soluble in the neutral solution, the stability constants of metal complexes, which have small stability constants and can not be formed in acidic conditions, could not be obtained by means of potentiometry. However, the stability constants of its calcium complex and magnesium complex, which have small values, could be determined by means of spectrophotometry.

TABLE II. Stability Constants of Metal Complexes of Pyridone Carboxylic Acids Obtained by Spectrophotometry

	Metal ion	$\log K_1$
Nalidixic acid	Mg^{2+}	3.0 (3.0[a])
Lomefloxacin	Ca^{2+}	1.7
	Mg^{2+}	2.9
	Zn^{2+}	3.6
	Co^{2+}	3.8
	Ni^{2+}	4.3
	Cu^{2+}	5.8
1 (pK_a 6.05)	Ca^{2+}	1.8
	Mg^{2+}	3.0
2	Ca^{2+}	1.6
	Mg^{2+}	2.4
3 (pK_a 6.31)	Ca^{2+}	2.0
	Mg^{2+}	2.9

a) Timmers and Sternglanz.

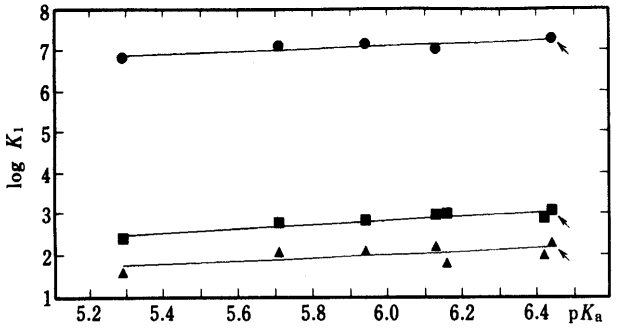
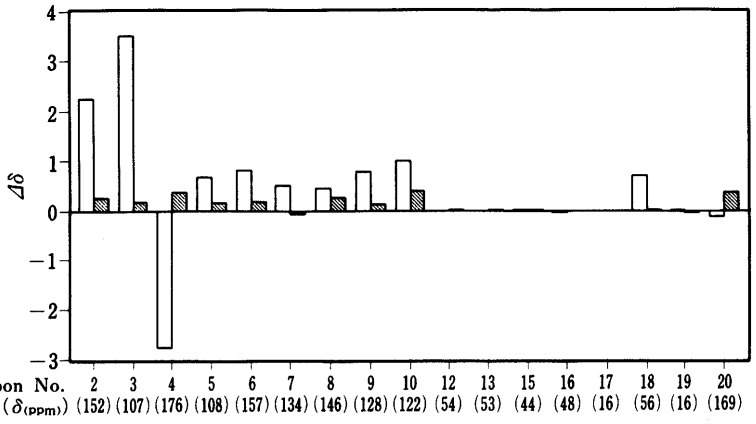


Fig. 7. Relationship between Dissociation Constants of Pyridone Carboxylic Acids and Stability Constants of Metal Complexes

●, aluminum complex; ■, magnesium complex; ▲, calcium complex. (←: The values were from a new pyridone carboxylic acid derivative which is an unreported compound.).

Relationship between Stability Constants and pK_a Values The differences in the stability constants among each pyridone carboxylic acid are small. However, as shown in Fig. 7, the $\log K_1$ values increased with an increase of pK_a values of the carboxyl group of drugs and the relation was almost linear. The stability constant of a pyridone carboxylic acid can be approximately estimated from the pK_a value of the carboxyl group.

^{13}C-NMR Spectra for Metal Complexes of Pyridone Carboxylic Acids Mendoza-Diaz *et al.* demonstrated that when Cu^{2+} binds to nalidixic acid, the carbons near the binding site (carboxyl group) are relaxed rapidly by the paramagnetic ions and their NMR lines are broadened.[12] Al^{3+} binding induces diamagnetic shifts for the carbon signals.[13] In our study, the formation of metal complexes was confirmed by comparing the ^{13}C-NMR spectra of lomefloxacin with that of the aluminum complex or magnesium complex in D_2O. Figure 8 shows the differences of each carbon resonance between lomefloxacin and the aluminum complex or magnesium complex ($\Delta\delta$). The addition of metal ions to the lomefloxacin solutions causes a significant change in the spectral parameters about the carboxyl group (C-20) and the carbonyl group (C-4). In the case of the magnesium complex, the resonance for atom C-20 (169.0 ppm) and C-3 (106.7 ppm) are dramatically broadened, while that for atom C-4 (176.1 ppm)



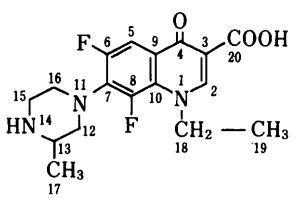


Fig. 8. Difference of ^{13}C-NMR between Lomefloxacin and Its Metal Complexes

□, aluminum complex; ▨, magnesium complex.

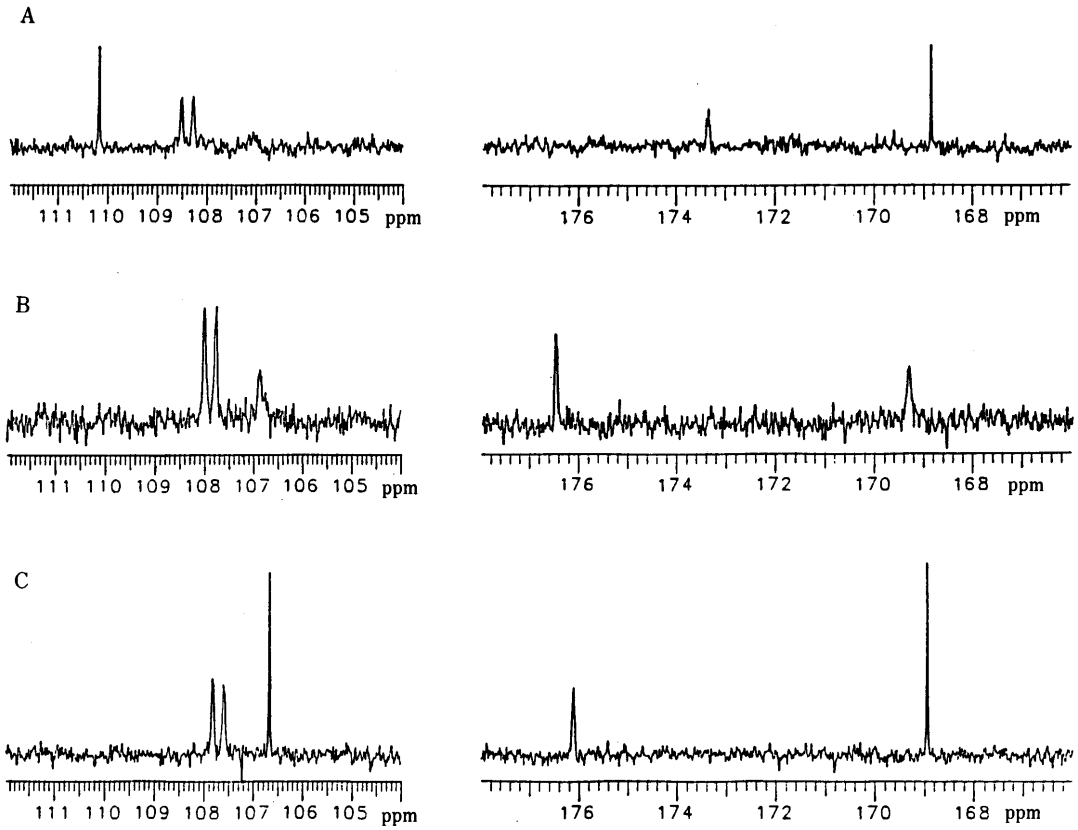


Fig. 9. ^{13}C-NMR Spectra of Lomefloxacin and Its Metal Complexes
A, aluminum complex; B, magnesium complex; C, lomefloxacin.

is not so much affected (Fig. 9B). On the other hand, the resonance of atom C-4 is significantly changed by the addition of the aluminum ion (Fig. 9A). This shows that the aluminum ion is tightly bound to the carbonyl group compared with the magnesium ion.

The NMR data also show little or no change of the piperazine group by the addition of the aluminum ion or the magnesium ion. From the pK_a value (9.06), the NH-group (N-14) under the measurement conditions is protonated. However, the spectral results show little effect of the metals on dissociation of the proton from the NH-group. Therefore, we consider that the chelation of the ligand occurs at the one site between the carbonyl and the carboxyl group, which is the reason why Bjerrum's theory could be applied to this compound.

Conclusion

The chelation equilibriums of a pyridone carboxylic acid with various metals were examined and the stability constants of the metal complexes were measured. These values followed the Irving–Williams series (Mn < Fe < Co < Ni < Cu > Zn). The stability constants of aluminum, magnesium and calcium complexes for several pyridone carboxylic acids were also determined and the values (log K_1) increased linearly with an increase of the pK_a values of the carboxyl group of drugs. However, the differences were small and substituent groups at 7-position did not exert much influence on the stability constants. These results showed that pyridone carboxylic acids similarly bonded to metals and especially binding to Al^{3+} was stronger than that to other metals. The aluminum complex (AlL) was found to form complexes Al(OH)L and $Al(OH)_2L$ under weak acidic conditions by considering the dissociation constants for the hydrolysis of the aluminum complex. The formation of these aluminum complexes in the intestines may have much influence on the absorption of pyridone carboxylic acids, when the drugs are administered with metallic antacid containing aluminum hydroxide and magnesium oxide simultaneously.

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