Degradation Kinetics of the New Antibacterial Fluoroquinolone Derivative, Orbifloxacin, in Aqueous Solution

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The degradation kinetics of orbifloxacin [1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid] was investigated as a function of pH (1.5—10.5), temperature (100—120 °C) and buffer concentration (0.05—0.2 M) by means of high-performance liquid chromatography.

The degradation of orbifloxacin in aqueous solution followed apparent first-order kinetics under all experimental conditions. No appreciable effect of buffer on the degradation of orbifloxacin was observed for any of the buffer species used in this study. The log k–pH profiles indicated specific-acid and specific-base catalyses and there were inflection points near pH 6 and 9 corresponding to the pK_{a1} and pK_{a2} values. From the Arrhenius plots, the activation energies for k'_H, k'_{H_2O}, k_{H_2O}, k''_{H_2O} and k''_{OH} were found to be 31.9, 36.9, 23.5, 26.5 and 19.0 kcal/mol, respectively. Arrhenius data obtained from this study showed that the degradation of orbifloxacin at room temperature was negligible at all pH values studied conditions (pH 1.5—10.5).

Key words degradation kinetics; fluoroquinolone derivative; orbifloxacin; stability

The antibacterial fluoroquinolone derivatives are widely used in the treatment of infections and their mechanism of action is inhibition of deoxyribonucleic acid (DNA) gyrase.[1-3] The stabilities of several fluoroquinolone derivatives have been studied under severe conditions such as in strongly acidic and alkaline solutions and they have been found to be stable in aqueous solution.[4,5] However, there has been no report of any precise kinetic study of the degradation of fluoroquinolone derivatives.

In this report, the degradation kinetics of orbifloxacin, [1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid], a new antibacterial fluoroquinolone agent, was studied in terms of pH (1.5—10.5), temperature (100—120 °C) and buffer concentration (0.05—0.2 M) to obtain information for formulation studies.

Experimental

Materials Orbifloxacin, 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline (**1**) and 1-cyclopropyl-6,8-difluoro-1,4-dihydro-5-hydroxy-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid (**2**) were prepared in our laboratories. All other chemicals used in this experiment were of reagent grade.

Kinetic Study The kinetic study of orbifloxacin was performed in aqueous solutions of various pH (1.5—10.5) at a total buffer concentration of 0.1 M and an ionic strength of 0.5. Citrate buffer (pH 1.5—3.5), acetate buffer (pH 4.4—5.6), phosphate buffer (pH 6.5—8.6), borate buffer (pH 9.0—9.5) and carbonate buffer (pH 10.5) were used as solvents. Separately, five buffer solutions (pH 3.0 citrate buffer, pH 5.0 acetate buffer, pH 7.2 phosphate buffer, pH 9.0 borate buffer and pH 10.5 carbonate buffer) of 0.05 and 0.2 M total buffer concentration were prepared for investigating the catalytic effect of the buffer species. The ionic strength of each buffer was adjusted to 0.5 with sodium chloride. Orbifloxacin was dissolved in these buffers and these solutions (0.1 mg/ml) were used as the sample solutions. The pHs of these sample solutions were measured at 25, 40, 50 and 70 °C and these values were plotted against the reciprocal of the absolute temperature, $1/T$, and the pH values of these sample solutions at 100, 110 and 120 °C were obtained by extrapolation. Two milliliters of each solution was sealed into 10 ml ampoules and stored in constant-temperature ovens at selected temperatures (100, 110 and 120 °C). At appropriate time intervals, a portion of the sample solution was withdrawn, mixed with the same volume of internal standard solution (0.1 mg/ml methyl *p*-hydroxybenzoate in methanol) and assayed by HPLC.

Assay of Intact Orbifloxacin The degradation rates were monitored by reversed-phase HPLC. The HPLC equipment consisted of a Shimadzu model LC-6A pump, and an SPD-2A UV-detector, a Chromatopack C-R5A integrator and a SIL-6A injector with 100 μl loop. A Develosil ODS-7 column (4 mm i.d. × 250 mm, particle size 7 μm, Nomura Chemical Co., Ltd.) was used in this study and the column temperature was maintained at 40 °C in the column oven. A mobile phase consisting of 0.1 M citrate buffer (pH 3.5)–methanol–dioxane (84 : 12 : 5) was used. The flow rate was 1.5 ml/min and the column eluent was monitored at 290 nm.

Identification of Degradation Products Degradation products in acidic and alkaline solutions were isolated by preparative HPLC and the mass spectra of the degradation products were recorded on a Hitachi B-80 B mass spectrometer *via* direct inlet.

Determination of Dissociation Constants of Orbifloxacin The apparent dissociation constants of orbifloxacin were determined spectrophotometrically at room temperature,[6] using the same buffers in the kinetic study. The apparent dissociation constants of orbifloxacin, pK_{a1} and pK_{a2}, which refer to the dissociation of the 3-carboxylic and 7-dimethylpiperadinyl groups, were 5.60 and 8.90, respectively.

Results and Discussion

Degradation Product of Orbifloxacin Two degradation products were isolated by preparative HPLC and their structures were elucidated by mass spectrometry. In addition, their mass spectra agreed with those of the corresponding authentic compounds.

Degradation of orbifloxacin was found to occur by two different mechanisms depending on the pH of the solution (Chart 1). In acidic solution, the main reaction was decarboxylation at the 3-position to form 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline (**1**). The mechanism of decarboxylation under acidic conditions was studied precisely by Jivani and Stella.[7] The mechanism of decarboxylation was considered to take place by proton addition to the α-carbon and elimination of the carboxylic group. In alkaline solution, the main degradation product was 1-cyclopropyl-6,8-difluoro-1,4-dihydro-5-hydroxy-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid (**2**), suggesting hydrolysis of the C–F bond at the 5-position.

Reaction Order and Rate Constants Figure 1 shows

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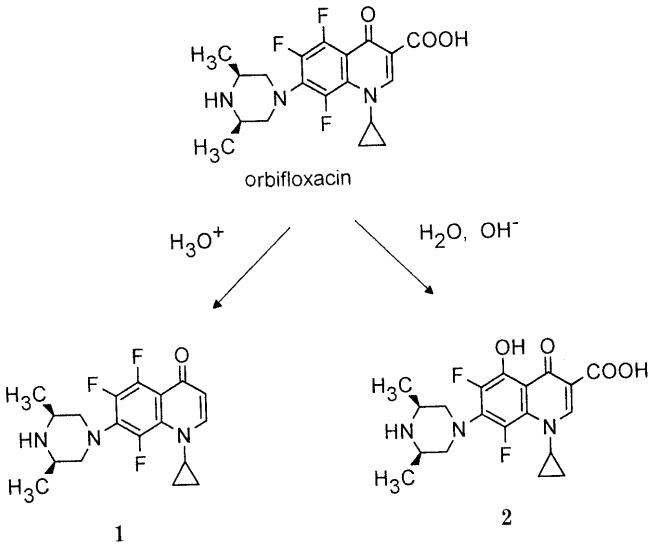

Chart 1. Possible Mechanisms for the Degradation of Orbifloxacin

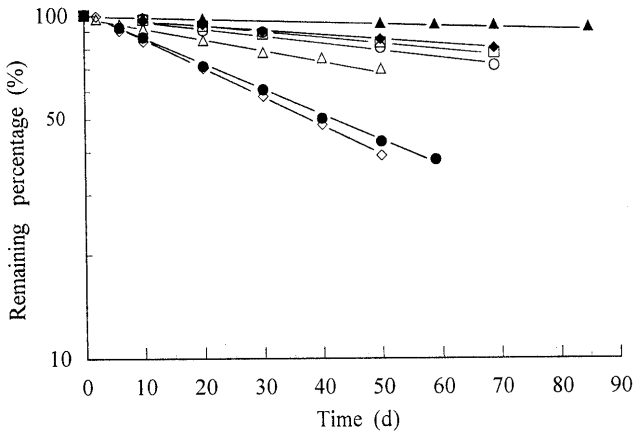

Fig. 1. Apparent First-Order Plots for the Degradation of Orbifloxacin at Various pH Values, 100°C and Ionic Strength of 0.5

●, pH 2.0; ▲, pH 3.5; ◆, pH 5.0; □, pH 7.0; ○, pH 8.8; △, pH 9.5; ◇, pH 10.5.

that the decrease in concentration of orbifloxacin with time followed appearent first-order kinetics at pH values ranging from 1.5 to 10.5. The first-order plots obtained under all experimental conditions exhibited good linear relationships with correlation coefficients of over 0.98. The apparent first-order rate constants (k) were obtained from these slopes.

Effect of Buffer Concentrations The catalytic effects of the buffer systems were determined at constant pH, temperature, and ionic strength; only the buffer concentration was varied from 0.05 or 0.2 M. No appreciable effect on the degradation of orbifloxacin was observed for any of the buffer species used in this study.

log k–pH Profile The log$k$–pH profiles for the degradation of orbifloxacin at different temperatures are shown in Fig. 2. These results show that orbifloxacin is stable at around pH 3—4 and unstable under strongly acidic and alkaline conditions. The degradation rates decreased with an increase in the pH value below 3.5 with a slope of about unity, which indicates specific hydrogen-ion catalysis of cationic species. The degradation rates increased gradually with an increase in the pH value over 3.5 and were almost constant from pH 5 to 8.

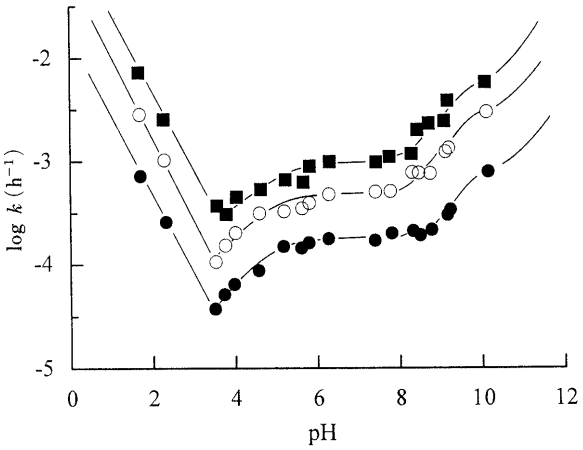

Fig. 2. log$k$–pH Profiles for the Degradation of Orbifloxacin at Various pH Values and Temperatures

●, 100°C; ○, 110°C; ■, 120°C.

These results can be explained by an increase in the neutral species (or zwitterion of orbifloxacin) attacked by water molecules or the kinetically equivalent specific hydroxide-ion catalysis of the cationic species. The shape of the profiles between pH 8 to 9 indicates the water-catalysis of the anionic species or the kinetically equivalent hydroxide-ion catalysis of the neutral species (or zwitterion). Two inflection points in the log$k$–pH plots near pH 6 and 9 indicate that the dissociation of the carboxylic acid and dimethylpiperadinyl groups in orbifloxacin influence the degradation rate. The linear increase in log$k$ with an increase in the pH value above 9 indicates specific hydroxide-ion catalysis of the anionic species.

Thus, the log$k$–pH profiles could be fitted to the following relationship:

$$k=(k'_{H}\cdot a_{H}+k'_{H_2O})\cdot\frac{a_{H}^2}{a_{H}^2+K_{a1}\cdot a_{H}+K_{a1}\cdot K_{a2}}+k_{H_2O}\cdot\frac{K_{a1}\cdot a_{H}}{a_{H}^2+K_{a1}\cdot a_{H}+K_{a1}\cdot K_{a2}}+\left(k''_{H_2O}+k''_{OH}\cdot\frac{K_{W}}{a_{H}}\right)\frac{K_{a1}\cdot K_{a2}}{a_{H}^2+K_{a1}\cdot a_{H}+K_{a1}\cdot K_{a2}} \quad (1)$$

where k'_{H} is the second-order rate constant for the hydrogen-ion-catalyzed degradation of the cationic species; k''_{OH} is the second-order rate constant for the hydroxide-ion-catalyzed degradation of the anionic species; k'_{H_2O}, k_{H_2O} and k''_{H_2O} are the first-order rate constants for the water-catalyzed (or spontaneous) degradation of cationic, neutral and anionic species, respectively; a_{H} is the hydrogen-ion activity as measured by a glass electrode; and K_{a1} is the dissociation constant of the carboxylic acid group of orbifloxacin. The ionization constants of water, K_{W} (12.26 at 100 °C; 12.12 at 110 °C; and 11.99 at 120 °C), were obtained from the literature.[8] As shown in Fig. 2, the line represents the theoretical curve calculated from Eq. 1 and the points indicate experimental results. The catalytic rate constants obtained from the best fits to the observed log$k$–pH profiles are given in Table 1. The reasonable agreement indicates that Eq. 1 adequately describes the degradation kinetics.

Dependence of Degradation Rate on Temperature The

Table 1. Catalytic Rate Constants and Arrhenius Parameters for the Degradation of Orbifloxacin

	k'_H ($M^{-1}h^{-1}$)	k'_{H_2O} (h^{-1})	k_{H_2O} (h^{-1})	k''_{H_2O} (h^{-1})	k''_{OH} ($M^{-1}h^{-1}$)	pK_{a1}	pK_{a2}
Temperature (°C)							
100	4.04×10^{-2} a)	1.82×10^{-5} a)	1.66×10^{-4} a)	7.49×10^{-4} a)	3.18×10^{-2} a)	4.68 a)	9.44 a)
110	1.48×10^{-1} a)	6.39×10^{-5} a)	4.38×10^{-4} a)	1.88×10^{-3} a)	6.09×10^{-2} a)	4.51 a)	9.55 a)
120	3.59×10^{-1} a)	2.30×10^{-4} a)	8.31×10^{-4} a)	4.61×10^{-3} a)	1.17×10^{-1} a)	4.49 a)	9.68 a)
25	8.58×10^{-7} c)	6.52×10^{-11} c)	6.06×10^{-11} c)	9.30×10^{-11} c)	5.06×10^{-5} c)	5.60, b) 5.72 c)	8.90, b) 8.33 c)
E_a (kcal/mol)	31.85	36.89	23.45	26.47	18.96		
ln A	39.83	38.84	22.98	28.51	22.13		

a) Based on the kinetics for the degradation of orbifloxacin (Eq. 1), where the pK_w values used are 12.26 at 100 °C, 12.12 at 110 °C and 11.99 at 120 °C. b) Determined by the spectrophotometric method. c) Estimated from the data obtained from the kinetic study of the degradation of orbifloxacin.

catalytic rate constants and Arrhenius parameters are listed in Table 1. The activation energies for k'_H, k'_{H_2O}, k_{H_2O}, k''_{H_2O} and k''_{OH} were found to be 31.9, 36.9, 23.5, 26.5 and 19.0 kcal/mol, respectively. The values of the dissociation constants, pK_{a1} and pK_{a2}, at 100 °C to 120 °C were determined by the best fits to the log k–pH profiles (Table 1) and the dissociation constants at 25 °C are estimated from the van't Hoff equation. The pK_{a1} and pK_{a2} at 25 °C derived kinetically (5.72 and 8.33, respectively) agreed comparatively well with the value of the dissociation constants (5.60 and 8.90) determined spectrophotometrically at room temperature (Table 1).

The stability of orbifloxacin in aqueous solution at selected temperature can be predicted from the Arrhenius parameters listed in Table 1. The degradation rate constants predicted over the pH range 1.5 to 10.5 at room temperature (25 °C) are estimated to be 6.41×10^{-13}—$1.23\times10^{-10}\,h^{-1}$. Orbifloxacin appears to be very stable at room temperature.

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