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Physicochemical properties of the fluoroquinolone antimicrobials. III. 1-Octanol/water partition coefficients and their relationships to structure

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Summary

The distribution of nalidixic acid and nine fluoroquinolones between 1-octanol and aqueous buffers has been studied at 25°C, pH 5, 7 or 9 and an ionic strength of 0 15 The partition coefficients were determined by analysis of both the organic and aqueous phases using either liquid chromatography or spectrophotometry. The partitioning of the compounds studied was generally consistent with that of zwitterions so that the highest partitioning was observed when the pH of the aqueous solution was close to that of the isoelectric point (pH 7) and decreased at both higher (9) and lower (5) pH values However, deviation from ideal partitioning behavior was observed at pH 5 that was attributed to ion-pairing between the cationic form of the solute and anionic buffer species. Relationships between the theoretical partition coefficients obtained by a functional group approach and the experimental values obtained here were perturbed by the complex ionic equilibria of the compounds

Introduction

The fluoroquinolones are an important class of antimicrobials whose potential clinical usefulness continues to expand (Desplaces et al., 1986; Mitscher et al., 1989). Numerous structurally related quinolones have been synthesized (Fig. 1) and several including norfloxacin, ofloxacin and ciprofloxacin are in routine clinical use throughout the world. Despite widespread clinical application one of the unexplained features of these drugs is the poor relationship between activity in vitro and in vivo and Mitscher et al. (1989) have proposed that these pharmacological differences may be due in large part to differences in the physicochemical properties of the compounds, resulting in different mass-transport properties, rather than differences in binding to the target receptor, DNA gyrase.

No transport system has been reported for the fluoroquinolones and it is reasonable to assume that simple Fickian diffusion is the main mechanism for the transport of fluoroquinolones across biological membranes and can be expected to be

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influenced by solute hydrophobicity. Partitioning between 1-octanol and water is widely accepted as an appropriate model (Fujita et al., 1964) for solute hydrophobicity and to date there has been no systematic investigation of the relationship between fluoroquinolone hydrophobicity and structure.

Because the fluoroquinolones are zwitterionic with pI values of about 7 (Ross and Riley, 1990, 1992a), the partitioning may be expected to be influenced by the pH of the aqueous phase. An understanding of the dependence of fluoroquinolone partitioning on pH is important because coadministration of antacids or other drugs that change the pH of the gut is known to influence the oral absorption of the quinolones (Flor et al., 1985, 1990; Hoffken et al., 1985a,b, 1988; Frank et al., 1986; Lener et al., 1987; Schentag et al., 1988; Frost et al., 1989a,b; Grasela et al., 1989; Nix et al., 1989a,b; Polk, 1989; Polk et al., 1989; Brouwers et al., 1990). Furthermore, the antimicrobial activity of the fluoroquinolones in the urine is known to be influenced by pH as well as by the addition of salts containing cations such as magnesium, calcium and iron (Barbhaiya et al., 1982; Ratcliffe and Smith, 1983; Pohlod and Saravolatz, 1984; Kumada et al., 1985; Smith and Ratcliffe, 1985; Blaser and Luthy, 1988; Gurdal et al., 1990; Perez-Giraldo et al., 1990). It is not yet clear whether the interactions with antacids are due to shifts in pH, complexation with metal ions or both. The results of the present study will help to answer this question. Results of investigations conducted in these laboratories on the com-

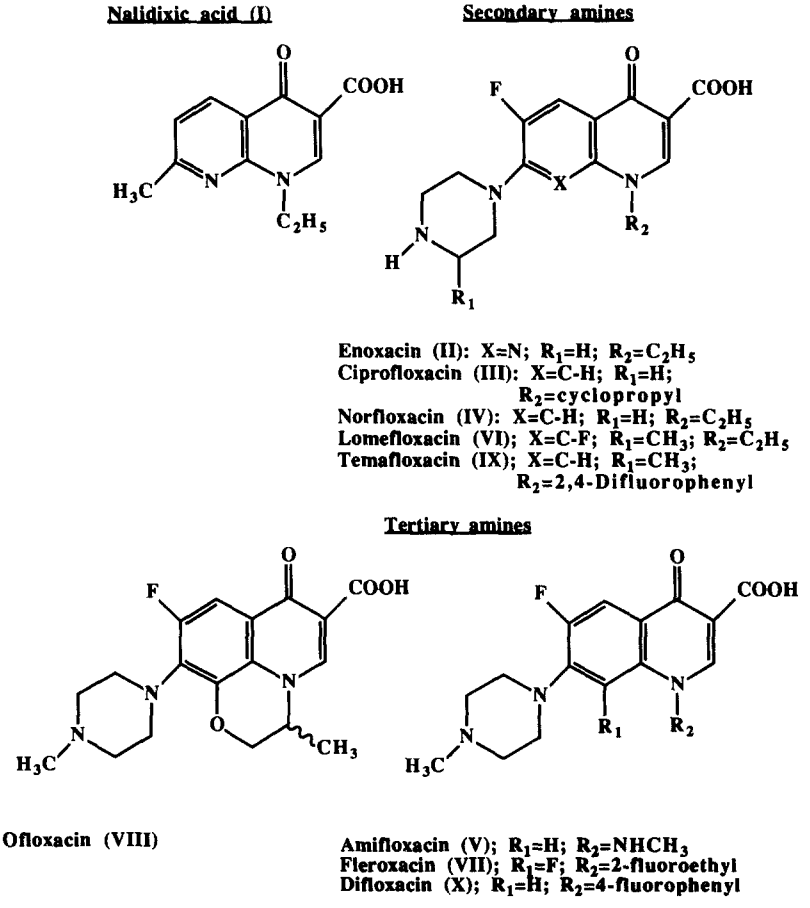

Fig 1 Structures of the quinolone antimicrobials included in this study

plexation of the fluoroquinolones and the effects of complexation on partitioning will be communicated elsewhere (Ross and Riley, 1992b; Ross et al., 1993).

Materials and Methods

Materials

All the quinolones (Fig. 1) were supplied by their respective manufacturers: amifloxacin (Sterling-Winthrop, Rensselaer, NY), ciprofloxacin HCl (Miles Laboratories, West Haven, CT), difloxacin HCl (Abbott Laboratories, North Chicago, IL), enoxacin (Warner-Lambert Co., Ann Arbor, MI), fleroxacin (Hoffmann-LaRoche Inc., Nutley, NJ), lomefloxacin mesylate and lomefloxacin HCl (G.D. Searle and Co., Skokie, IL), ofloxacin (Ortho Laboratories, Raritan, NJ), norfloxacin (Merck, Sharp, and Dohme, West Point, PA), and temafloxacin HCl (Abbott Laboratories). Nalidixic acid was purchased from Sigma Chemical Co (St. Louis, MO). All solvents were HPLC grade and obtained from commercial sources. Tris was Gold Label (99.9$^+$%) purchased from Aldrich Chemical Co (Milwaukee, WI). All buffer components 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) and stored in glass containers until use.

Data analysis and statistical considerations

For all tests, a value of $p \leq 0.05$ was considered significant. Statistical analyses were conducted with the software package StatView SE$^+$® (Abacus Concepts, Inc., Berkeley, CA) on a Macintosh® (Apple Computer, Inc., Cupertino, CA) personal computer.

Apparatus

All pH measurements were made using an Orion SA 520 pH meter (Orion Research, Inc., Boston, MA) and a Tiny Combination pH electrode (Microelectrodes, Inc., Londonderry, NH) or a calomel pH combination glass electrode (Markson, Phoenix, AZ). Samples were assayed for quinolone content by LC using either a Waters chromatographic pump (Waters Associates, Milford MA), or a Beckman Model 110A pump (Beckman Instruments, Inc., Fullerton, CA), a Waters Model U6K injector or an Altex 210 injector (Beckman Instruments, Inc.) fitted with a 20 μl loop, a Waters Model 440 absorbance detector with a 280 nm filter, a Spectroflow 757 Absorbance Detector (Kratos Analytical, Ramsey, NJ), or a Unimetric FS 970 L.C. Fluorometer (Unimetrics Corp., Ramsey, NJ) and an Omni-Scribe Recorder (Houston Instrument, Austin, TX). Spectrophotometric determinations were conducted using a Hewlett-Packard 8451A Diode Array Spectrophotometer (Hewlett-Packard Co., San Diego, CA).

Partition coefficient determinations

Partition coefficient determinations were made at pH 5, 7, and 9, constant ionic strength (μ = 0.15), and 25 $\pm$ 0.1°C. Stock solutions containing 10 μg ml^{-1} of drug were prepared in the appropriate buffer (acetate pH 5, 0.15 M; phosphate pH 7, 0.05 M; borate pH 9, 0.15 M; μ = 0.15 with NaCl), which was pre-equilibrated with 1-octanol. Equal volumes of the aqueous solution and 1-octanol (pre-equilibrated with the appropriate buffer) were mixed, protected from light by wrapping the vials in aluminum foil, and agitated for 24 h in a shaking water bath at 25 $\pm$ 0.1°C. After equilibrium had been achieved, the samples were centrifuged and the two phases separated. The aqueous layer was diluted with mobile phase and assayed by LC. 1 ml of the octanol layer was back-extracted with 10 ml of mobile phase by vortexing for 1 min. The mobile phase fraction was injected directly onto the LC to determine the concentration of quinolone in the octanol phase. The recovery of each quinolone from 1-octanol into mobile phase was 100% under all conditions studied. For nalidixic acid, the 1-octanol layer was assayed spectrophotometrically to determine the concentration in the octanol phase. All partition coefficient determinations were made in triplicate.

Partition coefficient determinations were also made for lomefloxacin and ofloxacin with 0.0225 M sodium heptanesulfonic acid in the aqueous

phase at pH 5, constant ionic strength ($\mu = 0.15$), and 25 ± 0.1°C. Stock solutions containing 10 μg ml^{-1} of drug were prepared in pH 5, 0.1 M acetate buffer containing 0.0225 M sodium heptanesulfonic acid ($\mu = 0.15$ with NaCl), which was pre-equilibrated with 1-octanol. Equal volumes of the aqueous solution and 1-octanol (pre-equilibrated with the buffer) were mixed, protected from light by wrapping the vials in aluminum foil, and agitated for 24 h in a shaking water bath at 25 ± 0.1°C. After equilibrium had been achieved, the samples were centrifuged and the two phases separated. Both the aqueous and the 1-octanol layers were assayed spectrophotometrically to determine the concentration. All partition coefficient determinations were made in triplicate.

Chromatographic conditions

The LC assays for quinolone concentration were conducted using an MOS Hypersil (C8) reversed-phase column (5 μm, 15 cm × 4.6 mm, id) and UV detection at 280 nm or fluorescence detection (λ_{ex}, drug *; λ_{em}, 418 nm cutoff filter), as described previously (Ross and Riley, 1990). The mobile phase was tetrahydrofuran-acetonitrile-H_3PO_4 (100 mM)-triethylamine-water (10:30:10:0.03:qs100) with a flow rate of 1.5 ml min^{-1}. All injections were made in duplicate.

Results and Discussion

Effect of pH on the partitioning between 1-octanol and water

The thermodynamic partition coefficient is described by the distribution law:

$$K_d = \frac{a_{N_o}}{a_{N_w}} \qquad (1)$$

where K_d is the thermodynamic distribution (or partition) coefficient, a_{N_o} denotes the equilibrium activity of the neutral species in a water-immiscible organic solvent, and a_{N_w} is the equilibrium activity of the neutral species in water.

Because these experiments were performed in dilute solutions ($< 10^{-4}$ M) activity coefficients were neglected (Martin et al., 1983), and the activity terms replaced by concentration. For ionizable compounds such as the quinolones, the total concentration of drug in the aqueous layer is the sum of all the Bjerrum species present:

$$[\text{Drug}]_w = [H_2Q^+]_w + [HQ^\pm]_w + [HQ^0]_w + [Q^-]_w \qquad (2)$$

Because the analytical method used (LC) provided the total concentrations of drug in the two phases, only the experimentally observed distribution coefficient or apparent partition coefficient (D) could be determined:

$$D = \frac{[HQ^\pm]_o + [HQ^0]_o}{[H_2Q^+]_w + [HQ^\pm]_w + [HQ^0]_w + [Q^-]_w} \qquad (3)$$

The distribution of the Bjerrum species in the aqueous phase depends on the hydrogen ion concentration [H^+] and the dissociation constants (K_{a_1} and K_{a_2}). Combining Eqns 2 and 3 with the dissociation constant expressions results in an equation that relates the partition coefficient to the apparent partition coefficient and the hydrogen ion concentration [H^+]:

$$K_d = D\left(\frac{[H^+]^2 + K_{a_1}[H^+] + K_{a_1}K_{a_2}}{K_{a_1}[H^+]}\right) \qquad (4)$$

For a weak acid, such as nalidixic acid, the expression that relates the thermodynamic partition coefficient to the apparent partition coefficient and the hydrogen ion concentration [H^+] is:

$$K_d = D\left(\frac{K_a + [H^+]}{[H^+]}\right) \qquad (5)$$

The partitioning of the compounds in Fig. 1 between 1-octanol and water was studied at pH 5, 7,

* λ_{ex} (nm): amifloxacin, 284; ciprofloxacin, 280; difloxacin, 284; enoxacin, 274; fleroxacin, 286; lomefloxacin, 288, nalidixic acid, 258; norfloxacin, 282; ofloxacin, 286; temafloxacin, 282.

and 9, 25°C and constant ionic strength ($\mu = 0.15$ with NaCl). Both the aqueous and octanol phases were analyzed and mass balance verified (±5%). For all the fluoroquinolones, the thermodynamic partition coefficients were calculated using Eqn 4, the apparent partition coefficient determined experimentally at pH 7, and the experimentally determined dissociation constants taken from the recent paper of Ross and Riley (1990). The thermodynamic partition coefficient of nalidixic acid was calculated using Eqn 5 from the apparent partition coefficient determined experimentally at pH 5, and the literature dissociation constant reported by Ross and Riley (1990) (Table 1). The apparent partition coefficients between 1-octanol and water for all of the quinolones studied are shown in Table 1 along with the calculated thermodynamic partition coefficients of the neutral species. Apparent partition coefficient values for those compounds which have been reported previously in the literature are also included in Table 1 for comparison. Although the apparent partition coefficient for ofloxacin reported by Meulemans et al. (1988) (0.437) is very similar to the value obtained in these studies (0.448), the value they obtained for enoxacin (0.219) was somewhat higher than that reported here (0.106).

The apparent partition coefficients reported by Hirai et al. (1986) for enoxacin (0.007), norfloxacin (0.01), ciprofloxacin (0.02), and ofloxacin (0.33) are substantially lower than those reported here (0.106, 0.0811, 0.103, and 0.448, respectively) while the literature value for nalidixic acid (3.92) is slightly greater than the value reported here (2.96). The discrepancies for the fluoroquinolones are probably due to the fact that Hirai et al. only assayed the aqueous phase to determine the apparent partition coefficients. Although the assay of the aqueous phase may be acceptable if the partition coefficient is large, the error associated with this method may be substantial when determining partition coefficients as small as those associated with the fluoroquinolones because at equilibrium, most of the material will reside in

TABLE 1

Thermodynamic partition coefficients (K_d) and apparent partition coefficients (D) as a function of pH between 1-octanol and water for the quinolones studied

Compound	D						K_d	
	pH 5		pH 7		pH 9		Mean [a]	S.D.
	Mean [a]	S.D.	Mean [a]	S.D	Mean [a]	S.D.		
Amifloxacin	0 256	0 021	1.28	0.00	0.0863	0.0006	2.20	0.25
Ciprofloxacin	0 0878	0.0025	0 103 0.02 [b]	0.002	0.0343	0 0002	0.116	0.002
Difloxacin	1.49	0.027	5 70	0.19	0 275	0.005	7.83	0.26
Enoxacin	0.0479	0 004	0.106 0 007 [b] 0.219 [c]	0.002	0 0549	0.0005	0.123	0.002
Fleroxacin	0.0735	0.0030	0 266	0.007	0.0303	0.0027	0.294	0 008
Lomefloxacin	0 118	0.001	0 139	0.004	0.112	0.002	0.146	0.005
Nalidixic Acid	34.5	5 6	2 96 3.92 [b]	0.15	0.0689	0.0214	39.0	6.4
Norfloxacin	0.0570	0 0016	0.0811 0 01 [b]	0 0015	0 0272	0.0015	0.0998	0.0018
Ofloxacin	0.0700	0 0016	0 448 0 33 [b] 0.437 [c]	0.002	0 0846	0 0013	0.526	0.001
Temafloxacin	0.0735	0.0030	0.266	0.007	0.0303	0.0027	0.627	0.008

[a] Mean ± standard deviation, $n = 3$.

[b] Determined by Hirai et al. (1986) at pH 7.2 (25°C) between 1-octanol and 0 1 M phosphate buffer.

[c] Determined by Meulemans et al. (1988) at pH 7.4 (20°C) between 1-octanol and 0.01 M PBS

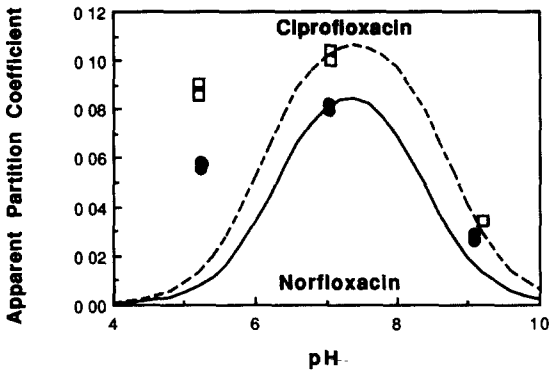

Fig 2. Quinolone apparent partition coefficient (D) between 1-octanol and water as a function of pH. norfloxacin (•) and ciprofloxacin HCl (□) Calculated curve generated by using Eqn 4, and the pK_{a_1}, pK_{a_2} and K_d values from Table 2 All data points for each triplicate determination have been included

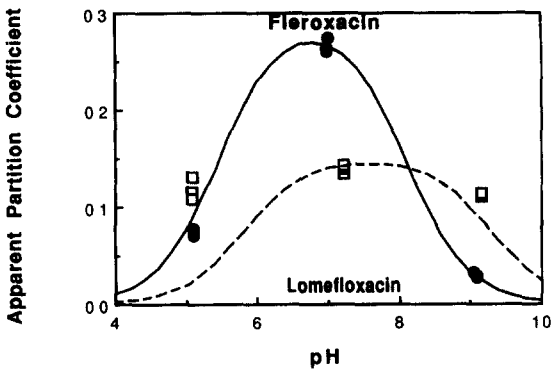

Fig. 3 Quinolone apparent partition coefficient (D) between 1-octanol and water as a function of pH. fleroxacin (•) and lomefloxacin mesylate (□). Calculated curve generated by using Eqn 4, and the pK_{a_1}, pK_{a_2} and K_d values from Table 2. All data points for each triplicate determination have been included

the aqueous phase. To avoid this problem in the present study, the concentration of drug was measured in both phases.

For zwitterionic compounds, such as the fluoroquinolones, an apparent partition coefficient-pH curve is expected to be bell-shaped because the species with the smallest net charge is the most lipophilic and it is the predominant species at the isoelectric point. On either side of the isoelectric point, the contribution of the charged species increases and therefore because the charged species is less lipophilic, the apparent partition coefficient will decrease. In an analogous manner, the apparent partition coefficient of nalidixic acid will decrease with increasing pH because of the increasing concentration of the anionic species which is less lipophilic. Figs 1–5 show the apparent partition coefficients of each of the compounds studied as a function of pH. Although the thermodynamic partition coefficient of nalidixic acid was much higher ($K_d = 39$) than that of the fluoroquinolones studied here ($7.8 \geq K_d \geq 0.1$), at physiological pH, the apparent partition coefficient of nalidixic acid ($D = 3.0$) is comparable to that seen with the fluoroquinolones ($0.1 \leq D \leq 5.7$). The apparent partition coefficient of nalidixic acid decreased with increasing pH, which is consistent with the expected partitioning of a weak acid. The relationship between the partition coefficients for the quinolones was generally consistent with what was expected for zwitterion of pI approx. 7. That is the partition coefficients were generally higher at pH 7 than at pH 5 or 9. However, the apparent partition coefficient values determined experimentally at pH 5 exceeded the predicted values for norfloxacin, temafloxacin, lomefloxacin, enoxacin, and ciprofloxacin, based on their partition coefficient values at pH 7 and their known pK_a values (Table 2) (Ross and Riley, 1992a). All these compounds are secondary amines, while those com-

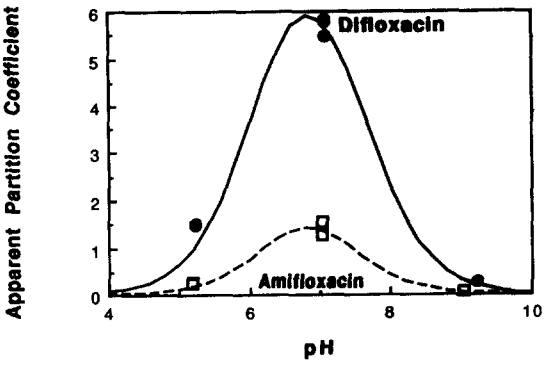

Fig. 4 Quinolone apparent partition coefficient (D) between 1-octanol and water as a function of pH· difloxacin HCl (•) and amifloxacin (□) Calculated curve generated by using Eqn 4, and the pK_{a_1}, pK_{a_2} and K_d values from Table 2 All data points for each triplicate determination have been included.

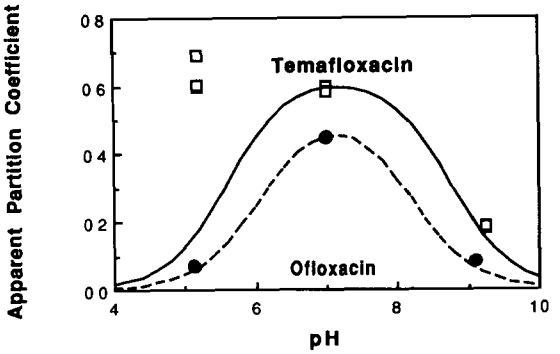

Fig. 5 Quinolone apparent partition coefficient (D) between 1-octanol and water as a function of pH· ofloxacin (●) and temafloxacin HCl (□). Calculated curve generated by using Eqn 4, and the pK_{a_1}, pK_{a_2} and K_d values from Table 1. All data points for each triplicate determination have been included.

pounds whose predicted values were similar to the experimental values determined were all tertiary amines.

TABLE 2

The 1-octanol / water partition coefficients and the dissociation constants for the quinolones studied

Compound	Calculated log P [a]	Measured log K_d [b]	pK_{a_1} [d]	pK_{a_2} [e]
Amifloxacin	−1.75	0.34	6 28	7.39
Ciprofloxacin	−0.75	−0.94	6.09	8.74
Difloxacin	−0 42	0 89	6.06	7 63
Enoxacin	−0 91 [c]	−0 91	6.31	8.69
Fleroxacin	−1 27	−0.53	5.46	8 10
Lomefloxacin	0.26	−0.83	5.82	9.30
Nalidixic acid	0.78	1.59	5.95	[f]
Norfloxacin	−1 00 [c]	−1 00	6.30	8.38
Ofloxacin	−0 04	−0 28	6.05	8.22
Temafloxacin	0 53	−0.20	5.61	8 75

[a] Log 1-octanol/water partition coefficient values were calculated using the method described by Leo et al. (1971, 1975). Norfloxacin was used as the parent compound for all calculations except in the case of nalidixic acid for which enoxacin was used as the parent compound

[b] Values for the thermodynamic 1-octanol/water partition coefficient were taken from Table 1

[c] By definition

[d] pK_{a_1} values for dissociation of carboxylic acid (Ross and Riley, 1992a)

[e] pK_{a_2} values for dissociation of protonated amino groups (Ross and Riley, 1992a)

[f] Not relevant

The increased partitioning, compared with that predicted by theory, observed at pH 5 with those compounds containing a secondary amine group was attributed to ion-pairing between the cationic form of the drug and the anionic buffer species (acetate or chloride) and increased partitioning of the formed ion-pair into the octanol phase. Several studies have shown that the acetate anion (Thompson and Kraus, 1947; Murthy and Zografi, 1970; Asmus and Freed, 1979) and chloride (Tomlinson et al., 1978) can form ion-pair with amines. The observation that the fluoroquinolones which are secondary amines have increased partitioning at pH 5 as compared with the tertiary amines further suggests that ion-pairing is a possible explanation because it has been observed that the order of ion-pair formation constants of amines is 3° < 2° < 1° (Witschonke and Kraus, 1947; Davis and Paabo, 1960; Marcus and Kertes, 1969). The order of ion-pairing has been attributed (Witschonke and Kraus, 1947; Davis and Paabo, 1960; Marcus and Kertes, 1969) to the number of hydrogen atoms available to hydrogen bond with the anion and stabilize the ion-pair. An alternative explanation for the different propensity of amines to form ion-pairs is the different degrees of steric hindrance (Marcus and Kertes, 1969).

To determine if the fluoroquinolones form ion-pairs at pH 5, partitioning experiments between 1-octanol and water were conducted in the presence of 0.0225 M heptanesulfonic acid, a strong ion-pairing agent (Tomlinson et al., 1978), added to the aqueous phase. The change in apparent partition coefficient in the presence of heptanesulfonic acid was determined for lomefloxacin, a secondary amine, and ofloxacin, a tertiary amine. In the presence of the ion-pairing agent at pH 5, the apparent partition coefficient of lomefloxacin was increased from 0.118 to 2.03 while the apparent partition coefficient of ofloxacin was increased from 0.0700 to 1.15. It should be noted that the apparent partition coefficient predicted by theory (Eqn 4) for lomefloxacin was 0.0258. Thus, the ion-pairing agent increased the apparent partition coefficient of lomefloxacin approx. 80-fold compared with the predicted value in the absence of the ion-pairing

agents; however, the ion-pairing agent increased the apparent partition coefficient of ofloxacin only 16-times that predicted in the absence of the ion-pairing agent. In carrying out these calculations the assumption was made that ion-pairing did not occur at pH 7. The assumption was reasonable in view of the facts that primarily the zwitterionic species is present at pH 7 and, unlike chloride or acetate, the phosphate anion is a weak ion-pairing agent (Tomlinson et al., 1978). These findings suggest that the fluoroquinolones do have a propensity to ion-pair at pH 5. The fact that lomefloxacin had a greater increase in partitioning over that predicted as compared to ofloxacin is in agreement with the observation that ion-pairing of amines increases: $3° < 2° < 1°$ (Witschonke and Kraus, 1947; Davis and Paabo, 1960; Marcus and Kertes, 1969).

Effect of structure on partition coefficients between 1-octanol and water

The thermodynamic partition coefficients between 1-octanol and water of the fluoroquinolones studied ranged from 0.0998 for norfloxacin to 7.83 for difloxacin. To determine the relationship between partition coefficient and structure, it seemed reasonable to use a widely accepted method for estimation of partition coefficients for comparison with the values measured here. Several groups of workers (Fujita et al., 1964; Leo et al., 1971, 1975; Harris et al., 1973; Nys and Rekker, 1973; Hopfinger and Battershell, 1976) have developed functional-group approaches for estimating partition coefficients. The first group of workers to establish the additivity concept for a wide variety of functional groups was Hansch and co-workers (Fujita et al., 1964) in the early 1960s using the π-substituent constant defined as:

$$\pi_X = \log P_X - \log P_H \qquad (6)$$

where P_X and P_H are the partition coefficients of the derivative and of the parent compound, respectively. The reference solvent system used was 1-octanol and water. In subsequent work, Leo et al. (1971) compiled an extensive data set from the biochemical and pharmaceutical literature to be used with the π constant.

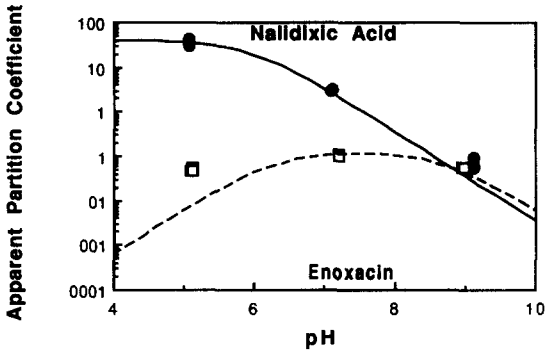

Fig 6 Quinolone apparent partition coefficient (D) between 1-octanol and water as a function of pH: nalidixic acid (●) and enoxacin (□). Calculated curve for enoxacin generated by using Eqn 4, and the pK_{a_1}, pK_{a_2} and K_d values from Table 2 Calculated curve for nalidixic acid generated using Eqn 5, and pK_{a_1} and K_d values from Table 2 All data points for each triplicate determination have been included

The calculation of log P was achieved here according to the modified approach of Leo et al. (1975), using the π-substituent constant. Norfloxacin was used as the parent compound for these calculations, since its structure included only the basic fluoroquinolone features of the 6-fluoro and 7-piperazinyl substituents. Log P values for the other compounds were calculated using the substituent constants for the various changes in the norfloxacin molecule. Enoxacin was used as the parent compound for the 1,8-naphthyridines because no analogous structural change (N in the 8-position of the quinoline ring) was documented in the available references to calculate a π-substituent constant. The results of the calculated partition coefficient values are included in Table 2 along with the measured partition coefficient values for comparison. To determine the relationship between the calculated and observed partition coefficient values, a simple regression was performed. The general regression equation resulting from the analysis correlating the observed log K_d with the calculated log P was:

$$\log K_d = 0.38\ (\pm 0.35)\ \log P - 0.016\ (\pm 0.32) \qquad (7)$$

$$r^2 = 0.13, \quad p = 0.31$$

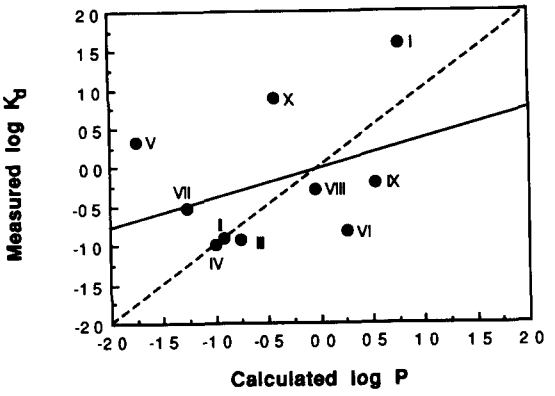

Fig. 7. Results of regression analysis correlating the observed log K_d with the calculated log P. Compounds are identified by the numbers corresponding to the structures in Fig. 1 The regression line is shown by the solid line and fits the equation. log $K_d = 0.38 \log P - 0.016$ The coefficient of correlation (r) is 0 36 The dashed line represents the theoretical line of unit slope and zero intercept

The correlation between the calculated and observed partition coefficient values was not significant ($p = 0.31$). Fig. 7 is the graphical representation of the regression results. It was noted that lomefloxacin and temafloxacin had the greatest negative deviation from the theoretical line while amifloxacin, difloxacin, and nalidixic acid had the greatest positive deviation from the line. The apparent K_d is related to the K_d of each of the ampholytic species ($HQ^{\pm}$ and HQ^0) by:

$$K_{d\,app} = K_{d(HQ^{\pm})}\frac{[HQ^{\pm}]}{[HQ^{\pm}] + [HQ^0]} + K_{d(HQ)^0}\frac{[HQ^0]}{[HQ^{\pm}] + [HQ^0]} \quad (8)$$

Therefore, the log P values calculated using norfloxacin as the parent compound would deviate from the measured K_d as a function of the relative concentrations of zwitterionic and neutral species contributing to the total ampholyte concentration (Ross and Riley, 1992a) if the zwitterionic and neutral species had different lipophilicities. Furthermore, if the neutral species were much more lipophilic than the zwitterionic species, difloxacin (3% of the neutral species contributing to the total ampholyte concentration) (Table 1), amifloxacin (7% neutral), and nalidixic acid (100% neutral) would be expected to have larger K_d values while lomefloxacin (0.03% neutral) and temafloxacin (0.07% neutral) would be expected to have smaller K_d values compared to norfloxacin (0.8% neutral) which was the reference compound. Because the deviations of the regression model comparing calculated log P values with observed K_d values followed this pattern, the fraction of neutral species contributing to the total ampholyte concentration was postulated to affect the partition coefficient values. Therefore, a regression was performed comparing the difference between the measured and calculated partition coefficient values (log K_d–log P) and the log$[HQ^{\pm}]/[HQ^0]$ (Table 2). The values of $[HQ^{\pm}]/[HQ^0]$ were normalized to the value of the reference compound, norfloxacin (118). Norfloxacin and enoxacin were excluded from the regression analysis because they were the reference compounds. The result-

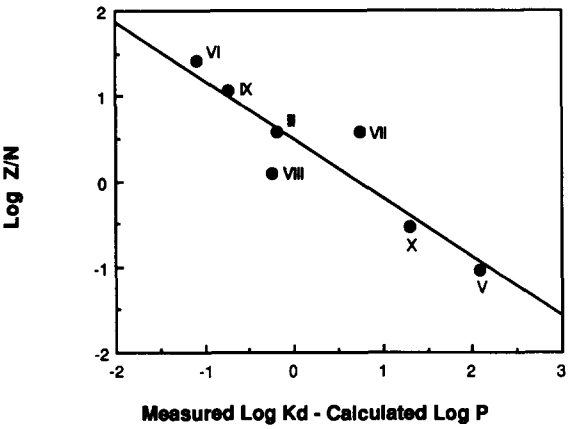

Fig. 8. Results of simple regression analysis which correlated the difference between the measured and calculated log partition coefficient values (log K_d – log P) and the log $[HQ^{\pm}]/[HQ^0]$. The value for $[HQ^{\pm}]/[HQ^0]$ was normalized by dividing by 118 the $[HQ^{\pm}]/[HQ^0]$ value for norfloxacin, the reference compound for the calculations. Norfloxacin and enoxacin were excluded from the regression because they served as reference compounds The regression line is shown by the solid line and fits the equation: log $[HQ^{\pm}]/[HQ^0] = -0\,70(\log K_d - \log P) + 0\,51$. The coefficient of correlation (r) is 0.92.

ing general regression equation was:

$$\log[HQ^{\pm}]/[HQ^{0}] = -0.70\ (\pm 0.14)(\log K_d - \log P) + 0.51\ (\pm 0.15) \quad (9)$$

$$r^2 = 0.84, \quad p = 0.0037$$

Fig. 8 depicts the graphical representation of the regression results. These findings are consistent with the neutral species being more lipophilic than the zwitterionic species and suggest that the ratio of zwitterion to neutral species is a very important feature in describing the lipophilicity of the fluoroquinolones. Because the partition coefficient for this group of compounds is related to the relative amounts of the neutral species present, the π-substituent constant is not an appropriate approach to calculate the partition coefficients of this group of compounds a priori.

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