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Lipophilicity of antibacterial fluoroquinolones

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Summary

The octanol/water partition coefficients of nine antibacterial fluoroquinolones and nalidixic and oxolinic acids were investigated. The pH-partition profile of amphoteric fluoroquinolones obtained between pH 4 and 10 showed maximum partitioning at the isoelectric point. From the two microspecies (zwitterionic and nonionic forms) which exist predominantly at this pH, the nonionic form is assumed to be transferred into the octanol phase. A relationship is derived between the apparent and true partition coefficients, valid for ampholyte compounds capable of forming zwitterions and having nonionic microspecies present in significant amounts. On the bases of true partition coefficients, three groups of examined fluoroquinolones are distinguished: lipophilic compounds (e.g., pefloxacin and amifloxacin), molecules of intermediate lipophilicity (such as ciprofloxacin and ofloxacin, etc.) and hydrophilic derivatives (e.g., norfloxacin and lomefloxacin, etc.). The influence of structural modification on the lipophilicity of these drugs is discussed.

Introduction

The recent development in the synthesis of highly potent, orally active antibacterial fluoroquinolones has led to several clinically useful agents against infectious diseases (pefloxacin, norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin; see structures in Fig. 1). The common structural element of these compounds is a 3-carboxyl-4-oxoquinolone ring system substituted with a fluorine atom at position 6 and a piperazinyl group at position 7.

Excellent reviews have appeared summarizing the synthesis (Albrecht, 1977; Jack, 1986), mechanism of action (Smith, 1986; Stein, 1988; Rosen, 1990), antibacterial spectrum (Wolfson and Hooper, 1985; Stein, 1988; Rosen, 1990) and pharmacokinetics (Lode et al., 1987) of fluoroquinolones. The structure-activity relationships have been widely investigated (Chu and Fernandes, 1989; Janknegt and Hekster, 1989; Domagala et al., 1991; Hagen et al., 1991). Results of QSAR studies revealed that hydrophobic, electronic and steric parameters of the compounds play equally important roles in their biological activity (Koga

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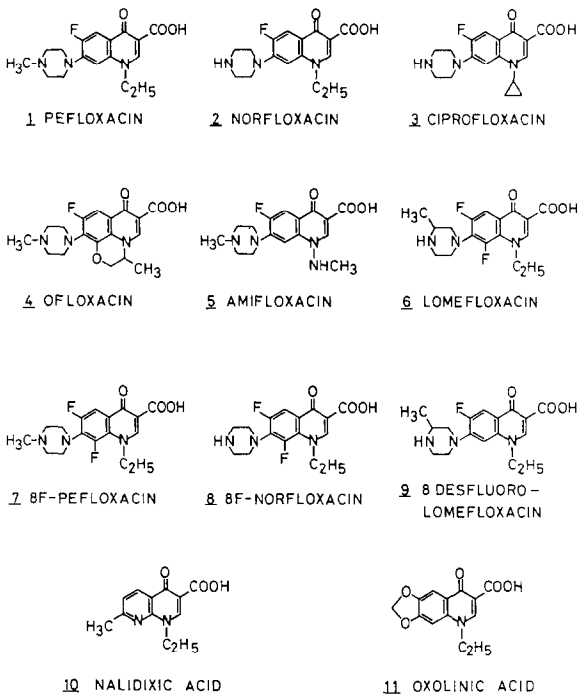

Fig. 1. Structure of model compounds.

et al., 1980; Fujita, 1984; Chu and Fernandes, 1989; Ohta and Koga, 1991). While receptor binding (interaction with the DNA-gyrase enzyme of the bacteria) is governed by the electronic (F, Swain-Lupton-Hansch inductive electronic parameter) and steric (L, Verloop's STERIMOL length; E_S, Taft's steric parameter) properties of N_1, C_6, C_7 and C_8 substituents, the lipophilicity of compounds seems to determine their penetration into the bacterial cell (Fujita, 1984; Stein, 1988; Ohta and Koga, 1991). Moreover, this latter physicochemical property exerts an influence on the absorption, distribution, storage and elimination of fluoroquinolone drugs. Despite these findings, the data currently available in the literature regarding their octanol/water partition coefficients are only sparse, deviating and contradictory (see Table 1). Fluoroquinolones like amphoteric molecules are ionized over the range of pH values (Takács-Novák et al., 1990). The partition coefficient determined at a given pH is an apparent value, so that the comparison of the lipophilicity of fluoroquinolones on the bases of log P_{app} data obtained from different sources is quite a difficult task.

The aim of the present work is to determine the octanol/water partition coefficients of several important fluoroquinolones (see Fig. 1), to study the pH dependence of their partitioning and to elucidate the relationship between the apparent and true partition coefficients valid for zwitterion-type amphoteric molecules. Nine fluoroquinolone derivatives (**1–9**) and the parent molecules nalidixic acid (**10**) and oxolinic acid

TABLE 1

Literature data on the lipophilicity of quinolones

Compound	P_{app} [a] (pH 7.6)	P_{app} [b] (pH 7.4)	P_{app} [c] (pH 7.2)	P_{app} [d] (pH 7.2)	log P_{app} [e]
Pefloxacin		0.03	1.32	0.529	
Norfloxacin	n.m.	0.04	0.01	0.162	
Ciprofloxacin	n.m.	0.07	0.02	0.151	
Ofloxacin	0.212	< 0.01	0.33	0.196	−2.4
Amifloxacin				0.602	−3.01
Lomefloxacin					−1.36
Nalidixic acid	0.563	< 0.01	3.92	0.531	1.87
Oxolinic acid			2.23	0.742	

n.m., not measurable.

[a] Ashby et al. (1985).

[b] Jack (1986).

[c] Hirai et al. (1986).

[d] Chapman and Georgopapadakou (1988).

[e] Ross and Riley (1990).

(**11**) were selected for this study. Their partition coefficients at the isoelectric point and the physiological blood pH were determined using the shake-flask method. The pH-partitioning profile of three compounds was investigated in the 4–10 pH range.

Materials and Methods

Materials

Samples of fluoroquinolones, and nalidixic and oxolinic acids were generously supplied by Chinoin Pharmaceutical Works and were used without further purification. The *n*-octanol was HPLC grade (Aldrich). Britton-Robinson buffer (acetic, phosphoric and boric acids, each at 0.04 M, and 0.2 M sodium hydroxide) was used for the pH range 4–10.

Methods

The partition coefficients were measured using the shake-flask technique (Purcell et al., 1973) at room temperature. The organic and aqueous phases were mutually saturated. After the representative testing of the mass balance and the time of equilibration in the case of compound **1**, the others were measured as follows. The compounds were dissolved in aqueous buffer solution (pH 4–10) and the solutions were equilibrated with octanol for 1 h. The phase ratio (V_w/V_o) varied from 5:20 to 10:2, depending on the expected log P value of the given compound. The samples were centrifuged at $730 \times g$ for phase separation and the concentration of the solute was determined in the aqueous phase by UV spectrophotometry (Specord UV-Vis, Zeiss PMG-II, Jena) at the λ_{max} of each compound. Measurements of the pH were made using a Radelkis OP 211/1 digital pH meter.

The apparent partition coefficients were calculated according to Eqn 1.

$$P_{app} = \frac{A_0 - A_1}{A_1} \cdot \frac{V_w}{V_o} \qquad (1)$$

where A_0 and A_1 represent the absorbance of fluoroquinolone in the aqueous phase before and after partitioning, respectively. Each log P_{app} value is an average of 12 parallel measurements, the standard deviation being indicated in the tables.

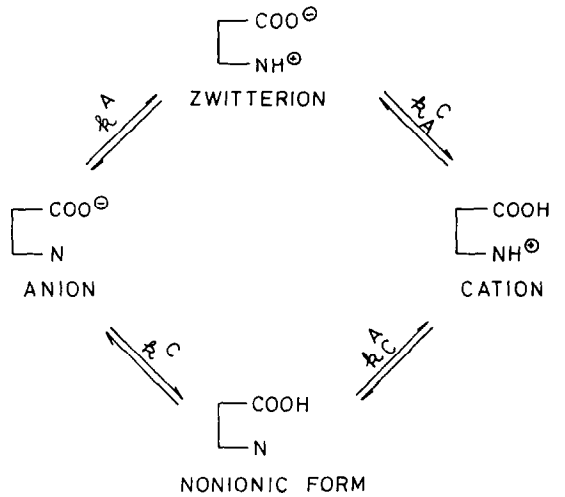

Fig. 2. The protonation scheme of fluoroquinolones (C_N^{COOH} represents the two protonation sites of the compounds without indicating the relevant valency of nitrogen).

Results and Discussion

Ionization pattern of fluoroquinolones

The acid-base properties of antibacterial fluoroquinolones in terms of the macro- and microprotonation constants have been recently reported (Takács-Novák et al., 1990). Fluoroquinolones, like molecules with two proton-binding sites, exist in four protonation forms in solution. The scheme of their protonation equilibria and the relevant microconstants which characterize the basicity of individual proton-binding sites (carboxylate and piperazinyl groups) are shown in Fig. 2. The microconstants can be expressed by means of the microspecies concentrations as follows:

$$k^A = \frac{[C_{NH^+}^{COO^-}]}{[C_N^{COO^-}][H^+]} \qquad (2)$$

$$k_A^C = \frac{[C_{NH^+}^{COOH}]}{[C_{NH^+}^{COO^-}][H^+]} \qquad (3)$$

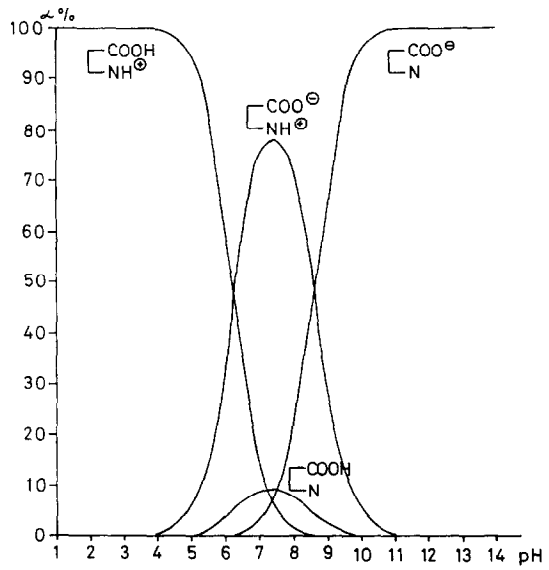

Fig. 3. Microspeciation of norfloxacin (2).

$$k^{C} = \frac{[C_N^{COOH}]}{[C_N^{COO^-}][H^+]} \quad (4)$$

$$k_C^A = \frac{[C_{NH^+}^{COOH}]}{[C_N^{COOH}][H^+]} \quad (5)$$

where the superscript on k denotes the functional group protonating in a given process, the subscript (if any) represents the already protonated group, and A and C refer to the amino and carboxylate functions, respectively.

The above-cited study on the microspeciation (calculation of pH-dependent relative concentrations of different microspecies) of fluoroquinolones demonstrated that: (1) the zwitterionic form predominates at the isoelectric point of molecules near to neutral pH (see Fig. 3) and (2) the ratio of zwitterionic/nonionic forms differs considerably among the examined compounds. Hence, the consideration of microspecies concentration is needed in the investigation of octanol/water partition coefficients of these, and other amphoteric molecules.

pH-dependent partitioning behavior of fluoroquinolones

The apparent partition coefficients of pefloxacin, norfloxacin and ofloxacin determined at different pH values between 4 and 10 are summarized in Table 2 (the number of decimal places is proportional to the accuracy of the data). The pH-partition profile of the compounds has a parabolic shape (see Fig. 4), which reflects the maximum lipophilicity of the compounds at their isoelectric points. On comparing the curves for the pH-partition behavior and the microspeciation of fluoroquinolones, it becomes evident that plots of the concentration of zwitterionic and nonionic forms as a function of pH show similar maximum shapes within the same pH range. From

TABLE 2

Apparent log P values of fluoroquinolones at different pH values

pH	log P_{app}		
	Pefloxacin	Norfloxacin	Ofloxacin
4.00	−1.56 ± 0.06		−2.1 ± 0.20
5.00	−0.67 ± 0.04	−1.7 ± 0.20	−1.31 ± 0.06
6.00	0.13 ± 0.03	−1.23 ± 0.09	−0.70 ± 0.06
7.00	0.23 ± 0.05	−1.00 ± 0.03	−0.39 ± 0.05
8.00		−1.19 ± 0.08	−0.63 ± 0.02
8.50	−0.45 ± 0.06	−1.26 ± 0.05	
9.00		−1.63 ± 0.09	−1.16 ± 0.08
10.00	−1.6 ± 0.14		−2.0 ± 0.13

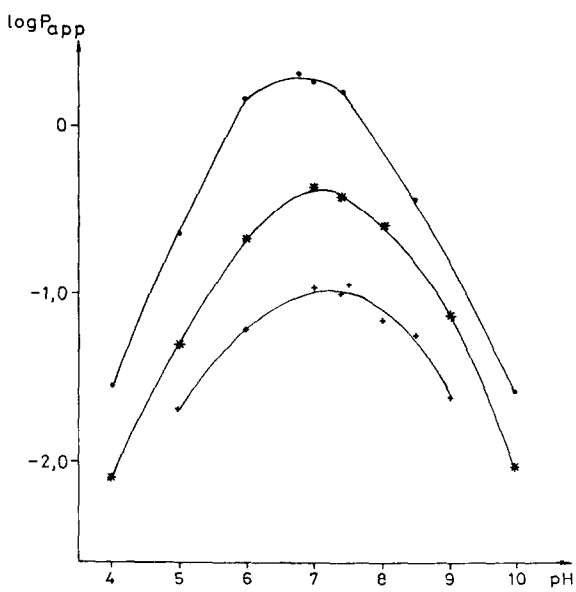

Fig. 4. pH-partitioning profiles of fluoroquinolones: (●) pefloxacin (1), (*) ofloxacin (4), (+) norfloxacin (2).

the two microspecies, the partitioning of the less polar nonionic (neutral) form into the octanol phase can be assumed, whereas a predominant zwitterion transfer into the lipid phase with low dielectric constant appears unlikely due to the lower tendency of the organic solvent to solvate the dipolar ion than that of water. Purich et al. (1973) suggested that two types of ampholytes can be distinguished depending on whether or not significant amounts of uncharged species are generated in their isoelectric region. Maximum partitioning into the lipid phase occurs when an uncharged species is present in commensurable concentration relative to the zwitterionic form, as was found in the case of tetracyclines (Colaizzi and Klink, 1969).

Relationship between true and apparent partition coefficients of amphoteric molecules

On the basis of the above consideration, the true partition coefficient of amphoteric fluoroquinolone molecules can be defined by Eqn 6:

$$P = \frac{[C_N^{COOH}]_o}{[C_N^{COOH}]_w} \tag{6}$$

and the apparent (measured at a given pH) partition coefficient follows in a similar fashion:

$$P_{app} = \left([C_N^{COOH}]_o\right)\left([C_N^{COO^-}]_w + [C_{NH^+}^{COO^-}]_w + [C_N^{COOH}]_w + [C_{NH^+}^{COOH}]_w\right)^{-1} \tag{7}$$

Expressing the equilibrium microspecies concentrations in the aqueous phase by the microprotonation constants using Eqns 2–5 yields:

$$P_{app} = \left([C_N^{COOH}]_o\right) \times \left(\frac{[C_N^{COOH}]_w}{k^C[H^+]} + \frac{k_C^A[C_N^{COOH}]_w}{k_A^C} + [C_N^{COOH}]_w + k_C^A[C_N^{COOH}]_w[H^+]\right)^{-1} \tag{8}$$

By combination of Eqns 6 and 8, the relationship between the true and apparent partition coefficients is given as follows:

$$\log P = \log P_{app} + \log\left(1 + \frac{1}{k^C[H^+]} + \frac{k_C^A}{k_A^C} + k^A[H^+]\right) \tag{9}$$

Eqn 9 reflects the fact that the measured partition coefficient should be corrected for the ratio of anionic/nonionic, zwitterionic/nonionic and cationic/nonionic microspecies in order to obtain the partition coefficient of an unionized form (true partition coefficient or P_u). A similar conclusion was drawn for the partitioning of morphine by Schill and Gustavii (1964).

It should be noted that the above model does not consider the partitioning of the ionized species (P_i) regarding the low lipophilicity of fluoroquinolones. However, the contribution of the ionized species to the apparent partition coefficient may be significant and cannot be neglected in a number of cases, as reported by Irwin et al. (1988) and Quigley et al. (1989).

The lipophilicity of antibacterial fluoroquinolones

The apparent log P values of nine fluoroquinolone derivatives were measured at their isoelectric point and the values are summarized in Table 3. Since the isoelectric points of the compounds under study varied in the range of pH 6.50–7.53, a common pH (physiological blood pH) was also selected for the experimental determination of log P (data in Table 3). As is evident, a significant difference occurs in log P_{app} only when the isoelectric point deviates considerably from pH 7.4. For example, the log P_{app} value of compound **5** is reduced from 0.23 to 0.05 on increase in pH from 6.5 to 7.4 due to a decrease in the concentration of the nonionic species from 36.5 to 25.3%.

The true partition coefficients ('intrinsic lipophilicity') of the compounds as calculated from the log P_{app} values are listed in Table 4 in a

TABLE 3

Apparent log P values of fluoroquinolones at the isoelectric point and blood pH values

Compound	Isoelectric point [a]	log P_{app} at isoelectric point	log P_{app} at pH 7.4
(1) Pefloxacin	6.91	0.27 ± 0.04	0.18 ± 0.05
(2) Norfloxacin	7.37	−1.03 ± 0.04	−1.03 ± 0.04
(3) Ciprofloxacin	7.53	−1.08 ± 0.05	−1.11 [b] ± 0.04
(4) Ofloxacin	6.97	−0.39 ± 0.05	−0.44 ± 0.02
(5) Amifloxacin	6.50	0.23 ± 0.03	0.05 ± 0.02
(6) Lomefloxacin	7.14	−0.80 ± 0.10	−1.03 ± 0.09
(7) 8-Fluoropefloxacin	6.73	−0.09 ± 0.03	−0.21 ± 0.05
(8) 8-Fluoronorfloxacin	7.44	−1.13 ± 0.09	−1.13 ± 0.09
(9) 8-Desfluorolomefloxacin	7.19	−0.75 ± 0.07	−0.80 ± 0.10

[a] Takács-Novák et al. (1990).

[b] Value of log P_{app} determined as −1.03 at 37 °C by Takahasi et al. (1986).

decreasing order. These data show that the true partition coefficients are considerably higher than the log P_{app} values. The difference between them varies from compound to compound as a result of the different microspecies concentration ratios of fluoroquinolones present at their isoelectric points. The following conclusions can be drawn from the log P values in relation to the effect of structural modification on lipophilicity of these drugs.

(1) The lipophilicity of tertiary amine type molecules is higher than that of secondary amines. The difference in the log P values is greater than 0.5, the usual contribution of a CH_3 group (see **1** vs **2**, and **7** vs **8**). This finding can be explained with respect to the enhanced basicity of secondary amine fluoroquinolones relative to tertiary amine forms, which results in an increase in the extent of interaction with the aqueous phase.

(2) The introduction of a second fluorine atom at position 8 decreases the lipophilicity of fluoroquinolones. The tendency observed in the cases of pefloxacin vs 8-fluoro-pefloxacin; norfloxacin vs 8-fluoro-norfloxacin and 8-desfluorolomefloxacin vs lomefloxacin contrasts with the lipophilicity-increasing effect of the fluorine atom that is produced in other molecules (Hansch and Leo, 1979) and brings to attention the existence of a specific electronic interaction of the 8-fluoro group with the quinolone ring system (Takács-Novák et al., 1990).

(3) The presence of an oxazine ring anellated to the quinolone system in ofloxacin (**4**) decreased the lipophilicity relative to pefloxacin (**1**) by 0.72 log P value. The higher intrinsic water solubility of ofloxacin among the fluoroquinolones has recently been re-

TABLE 4

Lipophilicity order of quinolone antibacterials on the basis of their true log P values

Compound	log P [a]	
(10) Nalidixic acid	1.46 [c]	
(11) Oxolinic acid	0.68 [c]	
(1) Pefloxacin	1.07	1.09 [b]
(5) Amifloxacin	0.65	
(4) Ofloxacin	0.35	0.31 [b]
(9) 8-Desfluorolomefloxacin	0.34	
(7) 8-Fluoropefloxacin	0.30	
(3) Ciprofloxacin	0.28	
(2) Norfloxacin	−0.02	−0.01 [b]
(6) Lomefloxacin	−0.30	
(8) 8-Fluoronorfloxacin	−0.57	

[a] Calculated from log P_{app} data at the isoelectric point using Eqn 9.

[b] Averaged log P values calculated from all log P_{app} data determined at pH 4–10 using Eqn 9.

[c] Data determined at pH 4.00 and corrected according to $P = P_{app}(1 + 10^{pH - pK_a})$.

ported (Ross and Riley, 1990). However, its lipophilicity in relation to these compounds is intermediate in value due to the simultaneously enhanced solubility in the organic phase.

(4) It is interesting to note the almost identical log P_{app} values of norfloxacin (**2**) and ciprofloxacin (**3**), while their true partition coefficients closely reflect the difference in lipophilicities caused by changing the N_1 substituent from ethyl to cyclopropyl. Similarly, the effect of structural difference between pefloxacin (**1**) and amifloxacin (**5**) on lipophilicity is expressed only in the true log P values.

On the basis of intrinsic lipophilicity, the examined fluoroquinolones can be divided into three groups: (i) lipophilic compounds, like compounds **1** and **5**; (ii) molecules of intermediate lipophilicity such as compounds **3**, **4**, **7** and **9**; (iii) hydrophilic derivatives, for example, compounds **2**, **6** and **8**.

This important property of drugs governs their ability to pass through the bacterial outer membrane and thereby exert antibacterial activity. The mechanism of cell penetration has not been completely elucidated. Two different pathways are believed to be possible for drug penetration depending on the hydrophobicity of quinolones. For compounds of relatively high hydrophobicity, the phospholipid bilayer is considered as the route, while for hydrophilic compounds, the porin pores of the membrane are assumed to play this role (Hirai et al., 1986). Further work is required in order to clarify the precise mechanism of penetration. Our results make it possible to carry out a more profound study of this question on the basis of true partition data.

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