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The complexation of transition series metal ions by nalidixic acid and related methoxyquinolones: its influence on partition coefficients with reference to antibacterial activity

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Summary

The following formation constants have been determined for 1-ethyl-1,4-dihydro-7-methoxy-4-oxoquinoline-3-carboxylic acid (MQC) and the corresponding homologous acid (MQE), which are congeners of the antibacterial drugs, nalidixic acid and oxolinic acid; proton, copper(II) complexation, magnesium(II) complexation. Consideration of these data, together with the appropriate partition coefficients, suggests that lipid solubility is not a major factor in the mechanism of action of such compounds and supports the involvement of 1 : 1 drug–metal complexes.

Introduction

Nalidixic acid (I, Fig. 1) was first synthesized in 1962 (Lesher et al.) and has proved to be useful clinically in the treatment of urinary tract infections caused by *E. coli, Proteus* and *Klebsiella* species (Harrison and Cox, 1970; Brumfitt and Pursell, 1971; Stamey, 1971). This resulted in the empirical synthesis of many analogues

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(Albrecht, 1977) and the mechanism of action of this group of drugs (ring-fused 1-ethyl-1,4-pyridone-3-carboxylic acids) has been extensively investigated (Crumplin et al., 1980). It appears that the chelation of certain metal ions (Dick and Murgu, 1964; Ruzicka et al., 1975; Yamabe, 1976; Nakano et al., 1978; Timmers and Sternglanz, 1978; Vincent et al., 1981) between the carbonyl and carboxyl groups of these molecules may play an important biological role and the previous report (Cole et al., 1984) considered the complexation of some divalent metal ions between therapeutic concentrations of nalidixic acid and oxolinic acid (II) (Kaminsky and Meltzer, 1968) in plasma using a steady-state computer simulation model. It was concluded that these drugs were substantially bound to calcium and magnesium ions in plasma. However, they exert their antibacterial activity in urine and previous studies (Crumplin et al., 1980) have indicated that ions such as ferrous or copper are involved in the mechanisms of action, which is supported by the suggestion (Yamabe, 1976; Cole et al., 1984) that redox processes (e.g. between ferrous ions and cytochrome c) are an important feature. Other biochemical explanations have involved complex formation between the drug–metal ion—DNA (probably with some base specificity for guanine) (Crumplin et al., 1980; Cole et al., 1984) the formation of mixed ligands (Timmers and Sternglanz, 1978) or bonds between the drug and metalloenzymes (Wright et al., 1981).

Recently, it has been demonstrated that nalidixic acid and oxolinic acid are specific inhibitors of DNA-gyrase, the enzyme responsible for converting double-stranded DNA into a negative superhelical form (Cozzarelli, 1980; Gellert, 1981). The latter has a higher energy which is supplied by the hydrolysis of ATP (Gellert et al., 1976) (a process requiring magnesium ions) and it was observed that oxolinic acid is a more potent inhibitor of this process than nalidixic acid. It is also interesting to note that oxolinic acid is more active than nalidixic acid in vitro (Turner et al., 1968; Atlas et al., 1969).

In order to carry out systematic investigations into the relationship between the chemical structure and biological activity of compounds of this type it is desirable

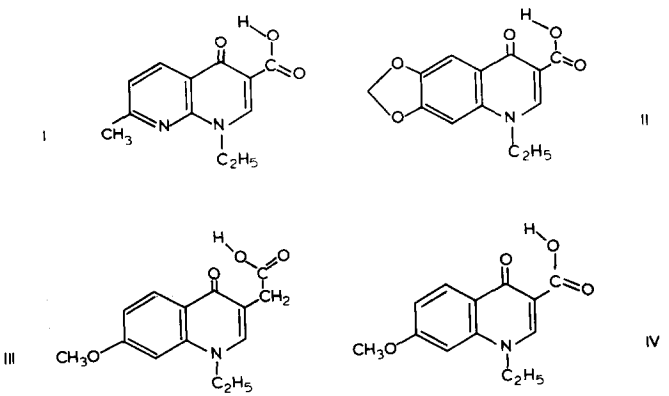

Fig. 1. Formulae of nalidixic acid(I), oxolinic acid(II), MQE(III) and MQC(IV).

that structural variations should be based upon a common heterocyclic nucleus and the 7-methoxyquinoline moiety was chosen (Dreyfuss, 1981) for this purpose. The parent compound (1-ethyl-1,4-dihydro-7-methoxy-4-oxoquinoline-3-carboxylic acid, MQC; IV, Fig. 1) of this series was first synthesized by Albrecht and Kessler (1972) and it is more active than nalidixic acid against *E. coli* and *P. vulgaris*. The carbonyl group at C4 and the carboxyl group at C3 have been implicated (Crumplin et al., 1980; Cole et al., 1984) in the mechanism of action of such compounds particularly in the bidentate chelation of metal ions and it was therefore of considerable interest to investigate the homologue of substance IV, namely, 2-(1-ethyl-1,4-dihydro-7-methoxy-4-oxoquinol-3-yl)-ethanoic acid, MQE (Fig. 1, III).

The objectives of the present work were as follows: (1) investigation of the complexation of divalent metal ions by compounds III and IV with regard to that by nalidixic acid; (2) correlation of the antibacterial activity of nalidixic acid, MQC (IV) and MQE (III) with data obtained for their metal speciation calculated from computer simulation models. In the latter case chelation of a metal between the carbonyl group at C4 and the carboxyl group at C3 would involve the formation of a 7-membered ring; an arrangement which is far less stable (Bell, 1977; Albert, 1979) than the corresponding 6-membered ring which would be obtained with the metal ion and MQC; and (3) an investigation of the variations in the solubilities in organic solvents of nalidixic acid, MQC and MQE and their respective complexes with metals with regard to the pK_a values of the parent carboxylic acids.

Materials and Methods

Materials

All the chemicals used were of reagent grade and solvents were distilled before use. Standard stock solutions of metal ions were prepared as their chloride salts and analyzed as previously described (Cole et al., 1984). MQC (IV) was synthesized by the method of Albrecht and Kessler (1972) and the preparation of MQE (III) is reported elsewhere (Dreyfuss, 1981). Both compounds (and the corresponding intermediates) displayed the requisite physical, chemical and spectral characteristics.

Measurement of formation constants (Linder et al., 1984)

Potentiometric titrations were performed on MQE at 37°C, $I = 150$ mmol $\cdot$ dm^{-3} [NaCl] so that the formation constants thus obtained could be used directly in the computer simulation model of blood plasma (Cole et al., 1984). MQC was not sufficiently soluble in totally aqueous media and so conventional potentiometric methods were precluded: measurements were performed in dioxan–water (1 : 4 and 1 : 1) and the results thus obtained were extrapolated to aqueous conditions.

Measurement of partition coefficients

These were determined in the following manner. The pH of a stock solution of each carboxylic acid in sodium hydroxide was adjusted to a value of 6 by the addition of 0.1 M HCl solution. The resultant solution was agitated with an equal

volume of carbon tetrachloride (using an ultrasonic vibrator) for 90 min at 37° C. The absorbance of the residual ligand in the aqueous layer was measured at the appropriate λ_{max} [nalidixic acid, 326 nm (ϵ 10,200); MQC, 317 nm (ϵ 10,000); MQE, 318 nm (ϵ 10,000)] using a Perkin-Elmer model 402 ultraviolet spectrophotometer. The experiments were repeated using a range of mixtures of the metals and ligands in order to produce 1 : 1 complexes.

Results and Discussion

Complexation of drugs with metals may increase the lipophilicity of the drug (and hence aid the passage of the drug through the cell membrane) and increase the bioavailability (Albert, 1979). The partition coefficients of nalidixic acid, MQC, MQE and of their complexes with Cu(II), Zn(II) and Mg(II) were determined for carbon tetrachloride–water at pH 6.0 and 37°C (Table 1). The value of 2.51 for nalidixic acid is similar to that previously reported (Nakano et al., 1978) for this compound.

Rather surprising differences were observed in the distribution behaviour: the partition coefficient of MQE itself was almost twice that of nalidixic acid and ca. 100 × that of its lower homologue, MQC. Considering the pK_a values of these compounds, at pH 6 it may be calculated (Florence and Attwood, 1981) that the percentages of MQE, nalidixic acid and MQC existing in the anionic form are approximately 97, 53 and 33, respectively, and it might be expected that the value of the partition coefficients would increase in this order. The observed results (Table 1) indicate that other factors are important and are consistent with a progressive decrease in the strength of the intramolecular hydrogen bond (see below) in the

TABLE 1

PARTITION COEFFICIENT VALUES FOR NALIDIXIC ACID, MQE AND MQC

Compound	Partition coefficient value [a]
Nalidixic acid	2.51
Cu(II) nalidixate	1.20
Mg(II) nalidixate	1.05
Zn(II) nalidixate	0.62
MQE	4.55
Cu(II) MQE	0.14
Mg(II) MQE	0.15
Zn(II) MQE	0.24
MQC	0.05
Cu(II) MQC	0.05
Mg(II) MQC	0.15
Zn(II) MQC	0.10

[a] CCl_4–water (pH 6), 37°C, 90 min.

order MQC, nalidixic acid, MQE accompanied by a progressive decrease in aqueous solubility of these compounds.

It may be observed from the data in Table 2 that MQE (pK_a 4.5) is a stronger acid than MQC (pK_a 6.32; cf. acetic acid, pK_a 4.75; phenylacetic acid, pK_a 4.25 (Merck Index, 9th edn.)). This may be attributed to: (1) The weaker intramolecular H-bond formed by the C3 carboxyl group and C4 carbonyl moiety of MQE (a 7-membered ring) as opposed to the corresponding 6-membered ring of MQC; and (2) the inability of the lone pair of electrons on N1 to destabilize the anion of MQE (in contrast to that of MQC where the lone pair is conjugated with the carboxyl group). The corresponding values measured for nalidixic acid and oxolinic acid are 5.94 (Cole et al., 1984) and 6.91 (Timmers and Sternglanz, 1978), respectively, and the difference was explained by Timmers and Sternglanz (1978) in terms of the electron-releasing oxygen substituent at C7 of oxolinic acid increasing the partial negative charge on the oxygen atom at C4 thereby increasing the strength of the intramolecular hydrogen bond with the neighbouring carboxyl group at C3.

Binary formation constant data were obtained for MQE and MQC with copper(II) and magnesium(II) (Table 2) and it may be seen that the values are similar (both in

TABLE 2

FORMATION CONSTANT DATA FOR MQE AND MQC AT 37°C [a,b]

p	q	r	log β	Sum of squares in residuals	MINIQUAD R Factor	No. of data points
MQE						
1	0	1	4.509 (0.001)	3.2×10^{-7}	0.007	195
Copper(II)						
1	1	0	2.507 (0.01)	3.6×10^{-8}	0.006	157
2	1	0	4.071 (0.6)			
1	1	−1	−4.766 (0.4)			
Magnesium(II)						
1	1	0	2.633 (0.03)	5.4×10^{-7}	0.008	197
2	1	0	4.536 (0.07)			
1	1	−1	−7.575 (0.03)			
MQC						
1	0	1	6.316 (0.001)	4.1×10^{-7}	0.006	121
Copper(II)						
1	1	0	6.426 (0.03)	2.9×10^{-7}	0.008	102
2	1	0	10.390 (0.3)			
Magnesium(II)						
1	1	0	3.601 (0.05)	2.5×10^{-8}	0.006	129
2	1	0	6.524 (0.08)			

[a] $I = 150$ mmol·dm^{-3} [NaCl].

[b] $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$ where log β = formation constant and the numbers of metal ions (M), ligands (L) and protons (H) in any species are indicated by q, p and r, respectively.

order of magnitude and in trend) to the corresponding constants obtained previously (Cole et al., 1984) with nalidixic acid. The appropriate constants were utilized in the computer simulation model of the distribution of MQE and MQC between magnesium(II), copper(II) and iron(II) in plasma. Administration of a normal therapeutic dose (1 g) of nalidixic acid to humans produces a concentration of drug of $\sim 10^{-4}$ mol · dm^{-3} in plasma (McChesney et al., 1964; The Pharmaceutical Codex, 11th edn.) and this typical value has been used for all three drugs in the ECCLES computer simulation (Cole et al., 1984). The results are shown in Table 3 and it may be seen that the most prevalent metal complexes formed by MQE and MQC with metals in plasma are those with magnesium(II).

The distribution of MQC between metals in plasma is very similar to that obtained (Cole et al., 1984) previously with oxolinic and nalidixic acids except that a much higher proportion of the former drug is complexed by magnesium(II) (cf. $Mg(OXO)^+$ 39%, $Mg(NAL)^+$ 30% (Cole et al., 1984)). It is clear from Table 3 that MQE exists to a large extent in plasma as the uncomplexed anion and the percentage of drug complexed with magnesium(II) is substantially lower than the other three compounds. This is in accord with previous observations concerning the relative instability of chelate rings involving a 7-membered vis a vis those with a 6-membered one (Bell, 1977; Albert, 1979).

Albrecht and Kessler (1972) found that MQC was very active against *E. coli* and *P. vulgaris* (m.i.c. 0.8 μg/ml and 1.6 μg/ml, respectively). In contrast, MQE is

TABLE 3

COMPUTED FORMATION OF MQE- AND MQC-LOW MOLECULAR MASS METAL ION COMPLEXES IN BLOOD PLASMA COMPUTED FOR TOTAL PLASMA MQE [a] OR MQC [a]

Species	% Total MQE/MQC	% L.M.M. Metal	log β values used
MQE^-	85.4	–	–
$MQEH^\circ$	0.1	–	4.51
$Mg(MQE)^+$	14.0	2.1	2.6
$Mg(MQE)_2^\circ$	0.1	0.0	4.0
$Cu(MQE)^+$	–	0.0	2.5
$Fe(II)(MQE)^+$	–	6.5	3.6 [b]
$Fe(II)(MQE)_2^\circ$	–	1.1	6.9
MQC^-	30.1	–	–
$MQCH^\circ$	2.5	–	6.31
$Mg(MQC)^+$	62.3	8.5	3.6
$Mg(MQC)_2^\circ$	3.0	0.2	6.5
$Cu(MQC)^+$	–	0.0	6.42
$Fe(II)(MQC)^+$	–	19.9	4.6 [b]
$Fe(II)(MQC)_2^\circ$	–	0.5	7.5

[a] Concentration of drug = 10^{-4} mol · dm^{-3}.

[b] Values for log β estimated from constants in Table 2 and a comparison of data obtained for complexes between transition metals and a range of related ligands (Cole et al., 1984; Vincent et al., 1982; and references cited therein).

inactive (mi.c. > 2000 μg/ml) against these and a range of other Gram-positive and Gram-negative organisms (Dreyfuss, 1981). So the results reported here suggest that lipid solubility is not a major factor in the antibacterial activity of this type of compound and constitute further support for the implication (Crumplin et al., 1980; Vincent et al., 1981; Cole et al., 1984) of 1:1 drug–metal complexes in the mechanism of action of such drugs.

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