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Photochemical Degradation of Marbofloxacin and Enrofloxacin in Natural Waters

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The photochemical fate of Marbofloxacin (MAR) and Enrofloxacin (ENR), two Fluoroquinolones (FQs) largely used as veterinary bactericides known to be present in surface waters, was investigated in aqueous solution. The degradation of these pollutants (5–50 μg L^{-1} starting concentration) was complete in about 1 h by exposure to solar light (summer) and obeyed a first-order kinetics. The structure of the primary photoproducts was determined. Those from ENR arose through three paths, namely, oxidative degradation of the piperazine side-chain, reductive defluorination, and fluorine solvolysis. More heavily degraded products that had been previously reported were rationalized as secondary photoproducts from the present ones. As for MAR, this underwent homolytic cleavage of the tetrahydrooxadiazine moiety to give two quinolinols. All of the primary products were themselves degraded in about 1 h. The photoreactions rates were scarcely affected by Ca^{2+} (200 mg L^{-1}), Mg^{2+} (30 mg L^{-1}), Cl^{-} (30 mg L^{-1}), and humic acid (1 mg L^{-1}), but increased in the presence of phosphate (20 mg L^{-1}). The fastest degradation of ENR occurred at pH about 8 where the zwitterionic form was present, while in the case of MAR the cationic form was the most reactive.

Introduction

Drugs are important pollutants. A large amount of such substances is not removed by wastewater (*1*) and sewage (*2*) treatment plants or is directly poured into water bodies in countries lacking effluent treatments. For these, as for other exogenous substances, photochemical reactions contribute to the transformations in the environment and have been used for their mineralization (*3–5*). Indeed, for many pharmaceutically active compounds, photodegradation is the main removal pathway in surface waters (*6*). Antimicrobials, particularly Fluoroquinolones (FQs), are largely used for livestock, both for the treatment of infections and as preventive agent because of their good oral intake and large activity spectrum. The chemical stability of the quinolone ring and the resistance to hydrolysis make FQs persistent

pollutants. Concern about accumulation of these drugs has been expressed because it has been proven that these cause bacterial resistance, and the fate of these compounds has not been fully clarified (*7–10*). In fact, FQs have been detected in surface (*11–16*), ground (*17*) and tap (*18*) waters at nanograms *per* liter level. There are no specific limits for antibiotics (*19*), but the 1996–1997 guideline by the European Agency for the Evaluation of Medicinal products (EMEA) set a threshold value for residues of veterinary drugs at 0.1 mg kg^{-1} in the soil and 0.1 μg L^{-1} in groundwater (*20*). A revised guideline for veterinary medicinal products has been published in 2008 (*21*).

At present, little is known about FQs photodegradation under actual environmental conditions, and it has not been ascertained whether the photoproducts are more or less susceptible to biodegradation than the starting drug and whether these are toxic (*3*) or promote bacterial resistance, especially when the active part of the parent molecule remains unaltered (*22*). Studies of the photodegradation path in natural waters are available for a number of FQs, namely, Difloxacin, Sarafloxacin (*23*), Ciprofloxacin (*3, 22, 24*), Lomefloxacin (*25*), and Flumequine (*26*).

In this work we investigated the photolysis in natural waters of Marbofloxacin (MAR) and Enrofloxacin (ENR). These were chosen because they are largely used in cattle and swine farms, for example, in the South Lombardy plain in Italy their occurrence in surface waters has been recently demonstrated (*16*). Moreover, while the photochemical behavior of ENR in natural waters has been investigated to some degree (*24, 27, 28*), that of MAR has not. The latter drug, recently introduced and rapidly expanding, belongs to the third generation of FQs and has unique pharmacokinetic properties, but enhanced environmental persistence (*29, 30*). Finally, as it will appear in the following, MAR reacts differently from other FQs. Comparing the effect of matrix composition on the photoreaction of the two drugs may thus be particularly informative. The present investigation is part of a general study aimed in the long term at correlating the fate of largely used drugs with any adverse effect on human health and environment.

Experimental Section

Irradiation Experiments. The chemicals were reagent grade or higher and were used without any further purification. Details are reported in the Supporting Information. Solar irradiation was carried out in Pavia (45°11′ N, 9°09′ E, June–September, 11.00 a.m.–1.00 pm, 25–30 °C). The incident power (W m^{-2}) was measured by means of a HD 9221 (Delta OHM) (450–950 nm) and a Multimeter (CO.FO.ME.GRA) (295–400 nm) pyranometers, as reported in Supporting Information, Figure S1. An open glass container (20 mm depth, exposed surface 280 × 200 mm) was used. Tap water from the municipal waterworks of Pavia (pH 7.8, conductivity 271 μS cm^{-1}, DOC 0.43 mg L^{-1}) and unfiltered water from River Ticino collected at 30–50 cm depth (pH 8.0, conductivity 380 μS cm^{-1}, DOC 1.18 mg L^{-1}) spiked by either ENR or MAR at two different concentration levels (5 and 50 μg L^{-1}) were used. Aliquots (1 mL) of each sample (500 mL) were withdrawn and immediately injected in the HPLC system after 0.45 μm filtration. The same experiments were performed also by using a solar simulator (Solar Box 1500e, CO.FO.ME.GRA) set at a power factor 250 W m^{-2}, equipped with a UV outdoor filter of soda lime glass, IR treated. Details of the analytical procedure are reported in the Supporting Information.

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		k (min^{-1})		*h* (min^{-1})
sample	pH	MAR	ENR	ENR
rw[b]	8.0	0.060(1)	0.15(8)	0.04(2)
tw[c]	7.9	0.061(2)	0.24(3)	0.08(1)
tw[c]	3.2	0.286(3)	0.0130(1)	
tw[c]	10.0	0.00216(5)	0.107(3)	
tw[c] + Ca^{2+} (200 mg L^{-1})	7.9	0.12(1)	0.3(1)	0.1(1)
tw[c] + Mg^{2+} (30 mg L^{-1})	7.9	0.094(4)	0.136(4)	
tw[c] + Cl^{-} (30 mg L^{-1})	7.9	0.088(5)	0.119(3)	
tw[c] + PO$_4^{3-}$ (20 mg L^{-1})	7.5	0.102(8)	1.1(3)	0.171(9)

[a] Kinetic constants obtained from eqs 1 and 2. [b] Solar light. [c] Simulated sunlight.

Identification of Photoproducts. One liter samples of a 7×10^{-4} M ENR solution were irradiated in an immersion well apparatus with a water-cooled medium-pressure mercury arc (125 W) and analyzed by HPLC/ESI/MS (the products were the same as under solar irradiation, Supporting Information, Figure S2). The irradiation of MAR was carried out on a 1 L 2.5×10^{-3} M solution and the products were analyzed by HPLC-ESI-MS. Results are shown in Supporting Information, Tables S1, S2. Further characterizations will be separately reported in a mechanistic study.

Results and Discussion

Rate of degradation and photoproducts distribution were determined on both river and tap water samples spiked with FQs at realistic concentration (5–50 μg L^{-1}), under both natural and simulated solar light. The influence of macro-constituents, natural organic matter (NOM), and pH was investigated.

Photolysis in Tap and River Water. The FQs content was determined through a previously developed analytical method (*16*). MAR and ENR were present in the river Ticino at concentrations (few tens of nanograms *per* liter) negligible with respect to the spikes (5 and 50 μg L^{-1}) used in photodegradation experiments, whereas their concentration in tap water was < LOD.

To reproduce the conditions of surface waters, samples were not filtered (particulate was next to negligible and previous experiments (*16*) had shown that filtration decreased recovery). On the contrary, most previous studies have been carried out in ultrapure (*23, 27, 31*) and river (*22, 23*) water with unrealistic FQs spikes (in the range of milligrams *per* liter). An example of decay profile is reported in Supporting Information, Figure S3, and similar results were obtained for both FQs and both concentrations (5 and 50 μg L^{-1}).

To confirm that the degradation was only due to photochemical reactions, a tap water solution fortified with 5–50 μg L^{-1} of either drug was stored in the dark, at room temperature. No decomposition was detected in a 2 days-monitoring period, thus excluding that other degradation paths, such as hydrolysis, occurred in the course of the experiments. As previously demonstrated (*26*), temperature had no significant effect on the degradation.

UV–vis spectra (200–450 nm) showed that absorbance was <0.01 for both FQs at these concentrations (20 mm path) and other components, such as HAs, did not contribute significantly. Thus, first order rate constants were expected to apply. Indeed, all of the decays were fitted by a first order exponential law, as determined by a dedicated software (Figure.P application, Figure.P Software Corporation). The rate constants obtained in this way (see Table 1) are more accurate than the common linear correlation in the ln [C]/[C$_0$]

versus irradiation time plot, which gives an undue weight to the first points. With MAR the data were fitted by a monoexponential (eq 1) law, while with ENR, a biexponential law (eq 2) gave the best fit.

$$y = A \times e^{-kt} \tag{1}$$

$$y = A \times e^{-kt} + B \times e^{-ht} \tag{2}$$

This difference was maintained in all of the samples. Notice further that at these low concentrations absorbance of any component is additive and thus there is no inner filter effect by the photoproducts, although these absorb in the same region.

Matrix Effects: Organic Matter and Salts. The matrix might affect the photochemical processes because of either the presence of photoactive compounds or the interaction between photogenerated reactive species and water components (*6*). Natural organic matter (NOM) is ubiquitous and may affect the photodegradation process, as previously demonstrated for some FQs (*32, 33*). HAs, the hydrophobic fraction of NOM, can both increase the degradation by acting as photosensitizers and inhibit it by competitive absorption of solar radiation, and the overall effect depends on the balance between the opposing contributions (*32*). In the river water we used, HAs were assessed as 1 mg L^{-1}, whereas in tap water the concentration was negligible (in the range of micrograms *per* liter). The degradation proceeded in the same way in the two cases, showing that HAs have no effect. We deemed not appropriate exploring the behavior at (non realistic) higher concentrations, although Schmitt-Kopplin et al. (*31*), working at a high NOM concentration (20 mg L^{-1}), found a decrease in the photolysis rate for ENR. Figure 1 reports the degradation under solar light of MAR and ENR in 500 mL samples of river Ticino and tap waters fortified with 5 μg L^{-1} of drugs.

Further macro-constituents investigated were calcium and magnesium ions, known to give 1:1 complexes with FQs and to influence the photochemistry of Norfloxacin (NOR) (*34*). Ubiquitous chloride was also considered along with phosphate, a potential contaminant known to affect the FQs photolysis (*35*). 50 μg L^{-1} FQs samples with ions at the levels actually detected in surface freshwater (see Supporting Information, Table S3) were examined.

Tap water was convenient because of the invariant composition, greater similarity to surface water than ultrapure water, and because the experimentally determined rate constants (*k, h*) did not differ from those obtained with river water. Table 1 shows that Ca^{2+} (200 mL^{-1}), Mg^{2+} (30 mL^{-1}), and Cl^{-} (30 mL^{-1}) had a negligible effect on the degradation rate of both FQs (rates within a factor of 2), but phosphate had a larger one in the case of ENR (5-fold increase of the rate at 20 mg L^{-1}).

pH Effect. FQs are zwitterions (*36*), and the pH affects both the kinetics and the nature of the photoproducts (*23, 35, 37*). This is relevant because of the large pH range among environmental water, for example, hospital waste-waters are basic (pH ~ 9) (*3*). Thus, 500 mL samples of tap water spiked with 50 μg L^{-1} of either FQs, under acidic (pH 3.2 ± 0.1) and alkaline (pH 10.0 ± 0.1) conditions were irradiated by the simulator. As it appears in Table 1, MAR was most reactive under acidic conditions and rather photostable at pH 10. As for ENR, the fastest degradation occurred under neutral conditions (with biexponential decay, see Table 1), with some decrease under basic and a strong decrease under acidic conditions (monoexponential).

Photoreactivity of ENR. The high fluorescence of ENR and its photoproducts allowed a detailed analysis at the concentrations expected in environmental surface waters (5–50 μg L^{-1}), whereas this was not possible for the less
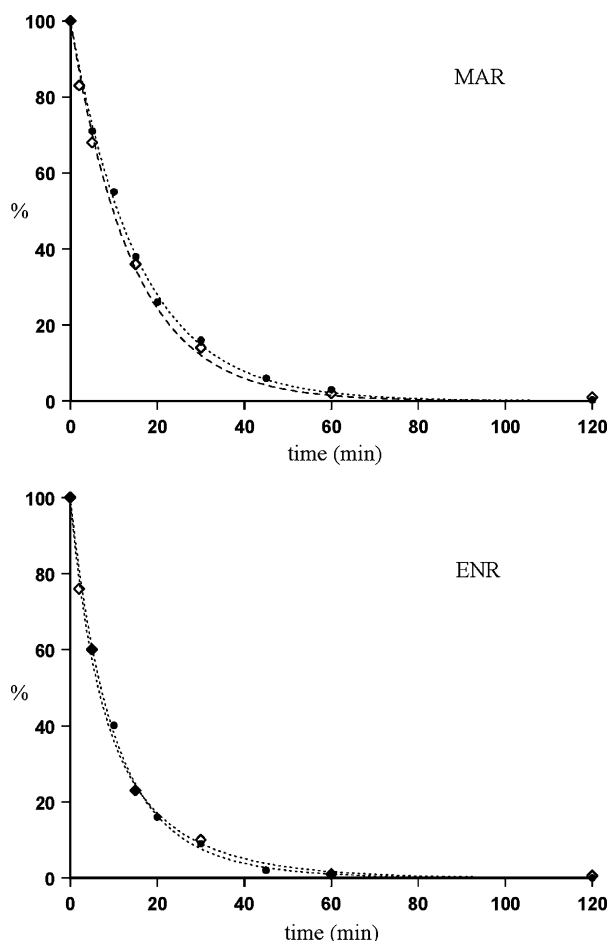
FIGURE 1. Degradation profiles obtained on tap water (diamonds) pH 7.9 and river Ticino water (circles) pH 8.0 fortified with 5 μg L^{-1} of MAR and ENR, under natural solar light.

fluorescent (by a factor of 10) MAR, known to count among the less emitting FQs (*38*). Photolysis of ENR gives products A−E, as shown in Scheme 1. Compound B is known, the others have not been reported for ENR, but are analogous to photoproducts from related FQs (*39*). The mass spectrum of B was identical to that found by Burhenne et al. (*40*) who

reported also the NMR spectrum, and this is sufficient for identification. The other structures were suggested primarily on the basis of mass spectra. That of product E is supported by a MW 14 au higher than ENR MW, by the slow elution (this is the least basic photoproduct) and by the previous identification of the corresponding deacetylated product (*40*). Products A and C had mass spectra almost identical to those of ENR and B, respectively, except for the missing fluorine. As for product D, the mass characteristics suggested substitution of the F atom by a OH group. Since previous work gave no evidence for such a process, the product was isolated by semipreparative HPLC and characterized by ^{1}H, ^{13}C, and ^{13}C-DEPT NMR spectroscopy (see Supporting Information, Tables S4, S5).

Thus, three processes occur, in part combined. These are (i) oxidative degradation of the ethyl-piperazine side chain; (ii) fluorine solvolysis; and (iii) reductive defluorination.

Figure 2(a) shows that in the initial phase the three paths mentioned above had a comparable role leading to products A−E, which, however, were consumed within 1 h, just as ENR. Reductive defluorination to give A was followed by side-chain oxidation to give C (combined yield up to 35%); we were unable to separate the two peaks, but literature evidence (*39*) on related FQs demonstrates that defluorination is a primary process and can be followed by process i. Solvolysis gives compound D, with no detectable products from side chain oxidation, reasonably because the phenol formed undergoes ring oxidative degradation. Side chain oxidation on the intact ENR leads to products B and E through parallel paths. This is reasonable, since these involve oxidation of different methylene groups α to the amino nitrogen, a general, if usually inefficient, process in amine photochemistry.

In Scheme 2 products A−E are connected with those identified by Burhenne (*40−42*) and Knapp (*24*), namely, aminoquinolone 1−3, resulting from the stepwise degradation of the side-chain, as well as products of even further degradation, with the oxidation of the benzo ring in pyridones 4, 5. In essence, photodegradation initiated by path (i) involves a stepwise degradation according to the range of oxidability of the functions present, piperazine > benzo ring > pyridone ring. Competitive primary photoprocesses involve the fluoro in 6 and are reduction (iii) and substitution (ii). Mechanistic questions have not been specifically addressed in this work, but the last two processes have been recognized in the FQs photochemistry and attributed to the triplet state,
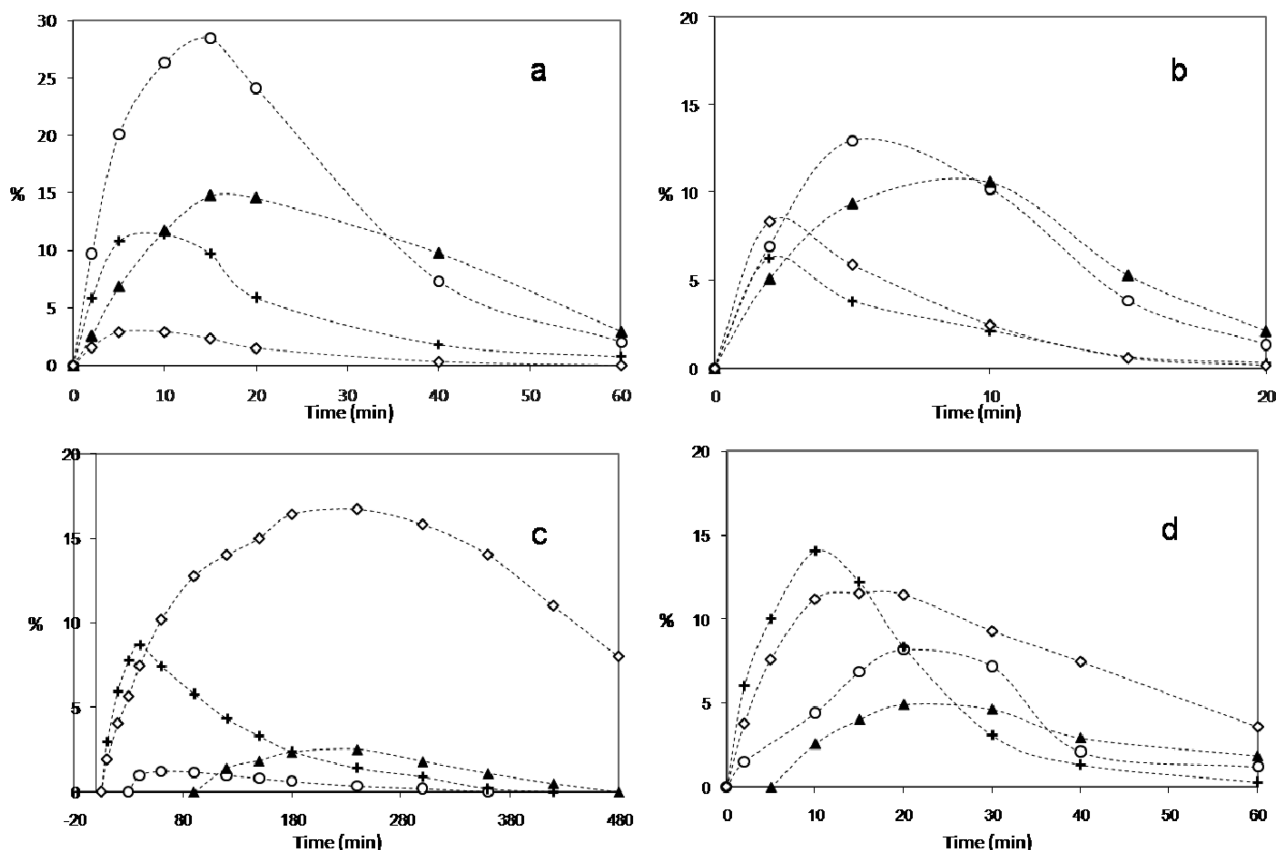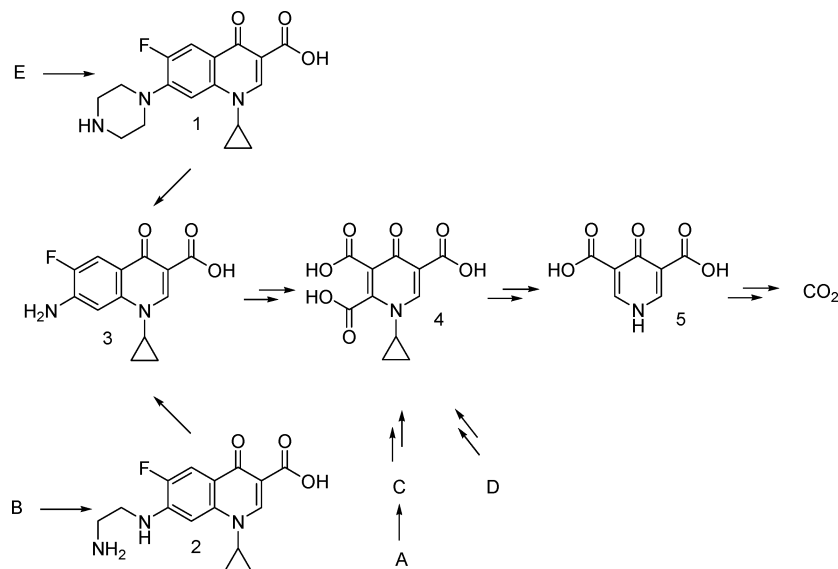
SCHEME 1. Early Events in the Photolysis of ENR
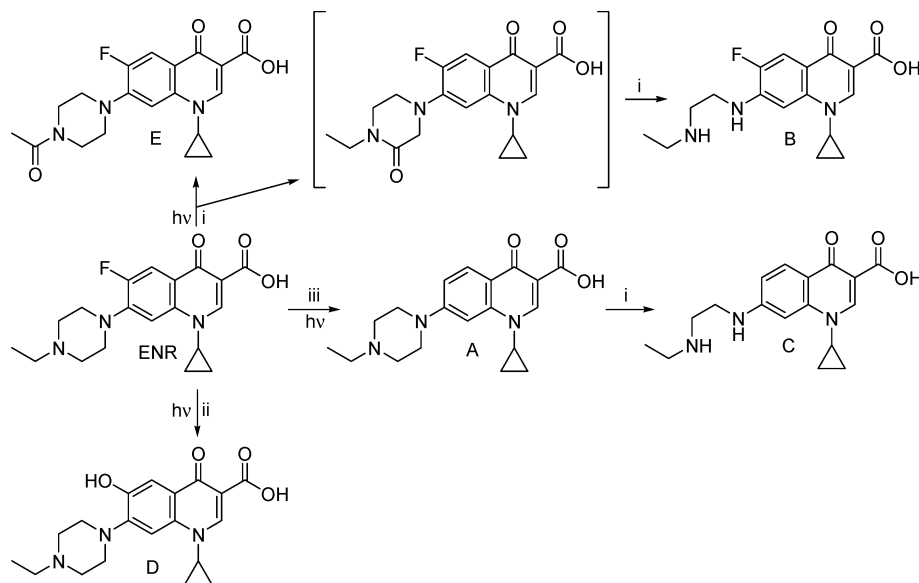
FIGURE 2. Photoproducts distribution profiles obtained by irradiation under simulated sunlight of 500 mL tap water spiked with 50 μg L^{-1} ENR, at different pH: pH 7.8 (a), phosphate buffer pH 7.6 (b), pH 3.2 (c), pH 10.0 (d); photoproducts: A+C (○), B (+), D (▲), E (◇).

**SCHEME 2. Further Degradation Paths of ENR**

a short-lived species that is partially quenched in air-equilibrated solutions (25).

As for the medium effect, experiments in river water or in tap water containing salts followed the same course. The formation constants of FQs complexes with Ca(II) and Mg(II) are too low (34, 43–46), for these having a significant population and at any rate the change of the electronic structure would be limited. Among anions, Cl⁻ has little effect, but phosphate increases all of the reaction modes (see Figure 2b). A similar effect has been found with other FQs and attributed to a reaction with the drug triplet state (35).

Different ionic states had different spectra (36, 47) and exhibited a different photochemistry (see Scheme 3). In the case of ENR the anionic form FQ(-H⁺) differs only in reacting at a somewhat lower rate than the zwitterionic form (FQ) predominating under neutral conditions. This explains the biexponential rate observed at the "natural" pH of 7.9, since both the zwitterion and the anion are present, absorb light, and react. A dramatic change is observed under acidic conditions (FQH⁺, FQH₂⁺), where the reactions involving the quinolone ring (paths ii and iii) are almost wiped out and only slow side chain degradation occurs. This is understand-
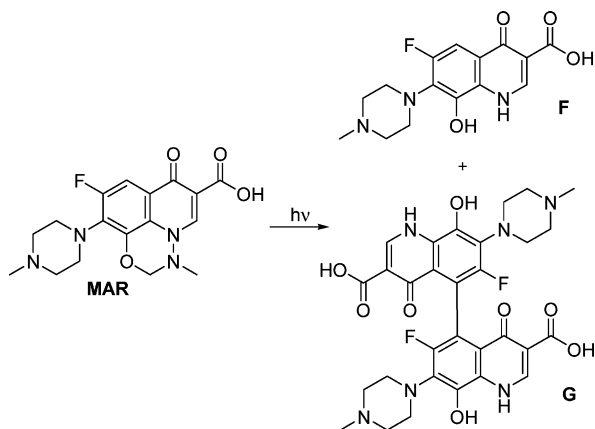
SCHEME 3. Ionic Forms of FQs
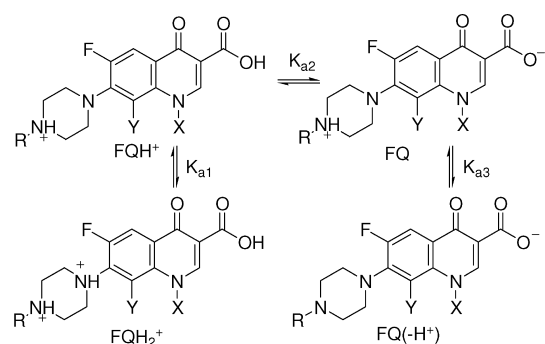
	MAR	ENR
pKa$_1$[47]	≈ 0.7(0.1)	≈ 0.7(0.1)
pKa$_2$[36]	5.51(0.03)-5.69(0.10)	5.88(0.03)-6.09(0.01)
pKa$_3$[36]	8.02(0.20)-8.38(0.13)	7.74(0.03)-7.91(0.05)

SCHEME 4. Photoproducts from Irradiation of MAR

able because partial protonation of the aromatic amino group in the diprotonated form FQH$_2^{2+}$ transforms a donating group into a further electron attracting group on the heterocycle and thus increases the stability of the ring, inhibiting reaction. A similar effect had been previously noticed with other FQs (37).

Photoreactivity of MAR. The products formed by ir-radiation of MAR in water (F, G) are reported in Scheme 4. The reaction follows a path completely different from those of ENR or any other FQ, leaving intact both the amino side chain and the aromatic fluorine and rather involving the degradation of the tetrahydrooxadiazine ring. Previous studies on FQs have shown that the presence of an electron donating group in position 8 hinders defluorination (paths ii and iii above), leaving only the reaction at the alkylamino side-chain (path i, usually inefficient) or different reactions, such as decarboxylation observed with Rufloxacin (a MeS group in 8) (48). MAR has an alkoxy group in 8 and conforms to the rule. However, a rather efficient process occurs, that is, homolytic cleavage of the weak N−N bond, which causes loss of an *N*-methylimine group to yield the observed products.

The reactivity is reduced with respect to ENR, about 1/3 to 1/2, but still sufficient to make photodegradation a significant path. The rate of degradation increases regularly with acidity. Thus, the rate is maximal at pH 3.2 because the significant protonation of the outer hydrazine nitrogen weakens the N−N bond, and minimal at pH 10, where there is no chance of such a process. A consequence of the poor reactivity of the anion is that the photodecomposition of MAR under neutral conditions obeys a monoexponential kinetics, contrary to the ENR case, despite the similar pK_a. Thus, both FQ and FQ(-H$^+$) absorb also in this case, but only the zwitterion reacts significantly.

In conclusion, the above investigation shows that solar irradiation contributes significantly to the degradation of two largely used veterinary FQs in superficial waters in areas nearby cattle and swine farms. The primary photochemical steps have been identified (with MAR a new path has been discovered) and shown to maintain the heteroaromatic ring, degraded however in later stages (41, 42). The primary intermediates lifetime is of the same order of that of the starting material, in some cases even shorter (see Figure 2), and the system seems to proceed toward polyacids or even mineralization. The large number of steps involved and the complex dependence on conditions and on the structure of the various classes of FQs, suggests, however, that detailed studies of the photochemistry and of the ecotoxicity of the intermediates are required for controlling what appears to be a serious contamination. Some generalizations on the photochemical paths reported above hopefully contribute to this effort.

Supporting Information Available
Analytical details, solar irradiation distribution, HPLC profile of ENR photoproducts, mass and NMR spectra of ENR, MAR photoproducts. This material is available free of charge via the Internet at http://pubs.acs.org.

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