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Inter- and Intramolecular Photochemical Reactions of Fleroxacin

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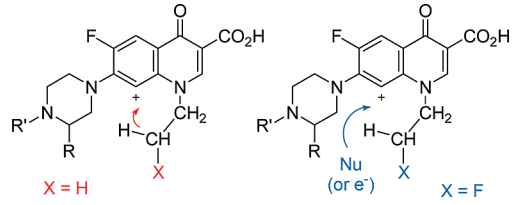
ABSTRACT



In the cation formed by photoinduced C−F bond cleavage in fleroxacin, intramolecular reaction with the *N*−ethyl chain is prevented by the electron-withdrawing effect of fluorine and intermolecular attack by nucleophiles is facilitated.

Some components of the important class of fluoroquinolone (FQ) antibacterials are characterized by an uncommonly high photolability and phototoxicity.[1−3] Indeed, some "2nd generation" derivatives bearing a second fluoro atom substituent in **8**, besides the ubiquitous fluoro in **6**, had inhibited or limited the therapeutic use due to this fact.

Clarifying the chemistry underlying these negative side effects has proven to be a hard task. Much work has been devoted to the case of lomefloxacin (**1**, Scheme 1) because it is strongly photo*geno*toxic.[4] Apparently, this cannot be traced back to the generation of singlet oxygen, generally assumed to be responsible for such effects. In fact, compound **1** outbalances other FQs not as O_2 sensitizer [$\Phi(^1O_2) = 0.07$][5] but for the efficient photodecomposition ($\Phi = 0.55$,[2] for most FQ*s* < 0.1). Excitation of this drug is known to cause *in vitro* DNA cleavage,[4] which suggests irreversible binding to DNA.[6]

Determining the nature of such chemical binding would help in understanding the photogenotoxic effect and thus to devise better drugs, but more importantly may suggest how to tune the effect so that related molecules could be envisaged that have a photo*therapeutic* effect. In the case of **1**, extensive experimental and computational studies have been carried out[3] and support the mechanism depicted in Scheme 1 with triplet aryl cation $^3\mathbf{2}^+$ as the intermediate. As it generally occurs with triplet aryl cations, this is similar to a triplet carbene and reacts via hydrogen abstraction from the *N*-ethyl chain giving a distonic diradical that cyclizes (path *a*). A weak nucleophile such as water does not interfere, but anions Cl^- and Br^- trap the cation and attack at position 8 (path

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(2) Fasani, E.; Barberis Negra, F. F.; Mella, M.; Monti, S.; Albini, A. *J. Org. Chem.* **1999**, *64*, 5388.

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(6) Notice, however, that photoinactivation of topoisomerase IIα has been supported for related FQs, not for **1**, see: Perrone, C. E.; Takahashi, K. C.; Williams, G. M. *Toxicol. Sci.* **2002**, *69*, 16.

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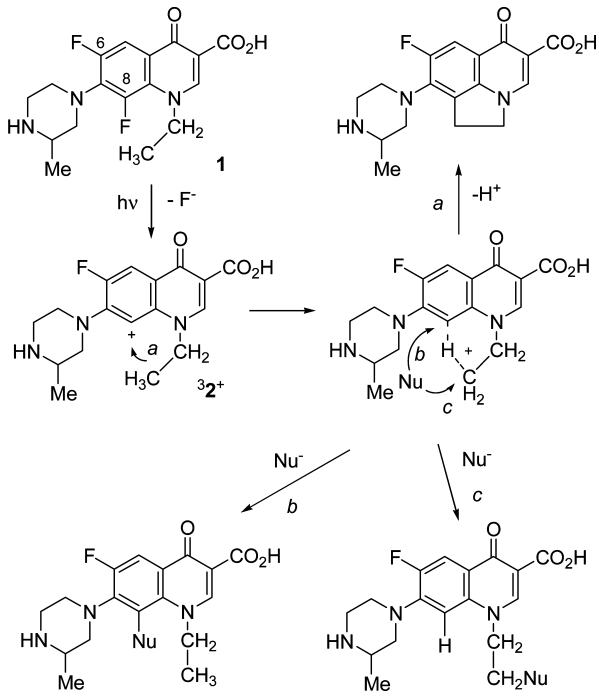
Scheme 1. Photochemical Reactions of Lomefloxacin (**1**) in Water



b). On the other hand, softer anions such as I^- or π nucleophiles such as pyrrole act as electron donors and the ensuing radical coupling leads to products functionalized at the side chain (path *c*).

The aggressive cation $^3\mathbf{2}^+$ may be responsible for phototoxicity, but this implies that an intermolecular reaction with a biomolecule competes with the intramolecular trapping by the *N*-ethyl group (path *a*). The association of the drug with DNA *in vivo* is obviously determining (the association with bacterial gyrase and topoisomerase explains the FQs action), but tackling the mechanism in solution by some structural change may evidence any chemoselectivity. In view of the above, an obvious move is a modification of the *N*-ethyl group.

Fleroxacin (**3**) appeared a suitable candidate. This is a FQ known to be even more phototoxic than **1**, while producing less $^1O_2$ [$\Phi(^1O_2) = 0.03$].[5,7] **3** differs from **1** for bearing a *N*-(2-fluoroethyl) group. Thus, with **3** the photodefluorination from C_8 would not be affected, since the chromophore is unchanged, but the chemistry of the cation would, since fluorine substitution at the side chain hinders electrophilic H abstraction (path *a*). As it will be shown below this change did indeed dramatically affect the photochemistry occurring and the results strengthened the mechanistic picture.

Fleroxacin **3** turned out to be rather photoreactive (Φ = 0.22). Flash photolysis in water showed a differential absorption in the region 350−500 nm that underwent biexponential decay (Figure 1a).[8]

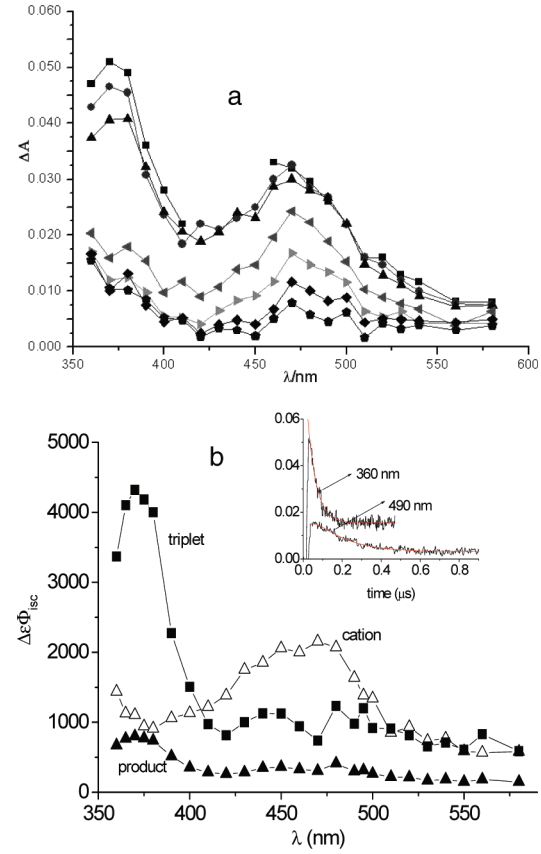


Figure 1. (a) Transients following excitation at 355 nm of a N_2O saturated 1.4×10^{-4} M solution of **3** in 1.0×10^{-3} M $NaHCO_3$ buffer of pH 7.2 at 25 °C.; upper trace, 40 ns, lower trace, 420 ns after the flash. (b) Calculated components by deconvolution of the previous transient absorption; for the attribution, see text. (Inset) Decays at 360 and 490 nm and biexponential best fit with $\tau_1 = 41$ ns and $\tau_2 = 154$ ns.

The signal could be analyzed as involving three subsequent transients (Figure 1b). The initial signal (■, $\lambda_{max} = 370$ nm) was safely attributed to triplet **3** ($\tau = 40$ ns, a short lifetime just as for $^3\mathbf{1}$, due to the fast C_8−F bond cleavage). This evolved into a second transient (△, $\lambda_{max} = 475$ nm, $\tau = 150$ ns), attributed to cation $^3\mathbf{4}^+$. This had a spectrum similar to that of $^3\mathbf{2}^+$ but was somewhat longer-lived, consistent with the idea that H abstraction from the *N*−ethyl chain was now suppressed. Coherently, the products isolated after irradiation[9] involved different paths.

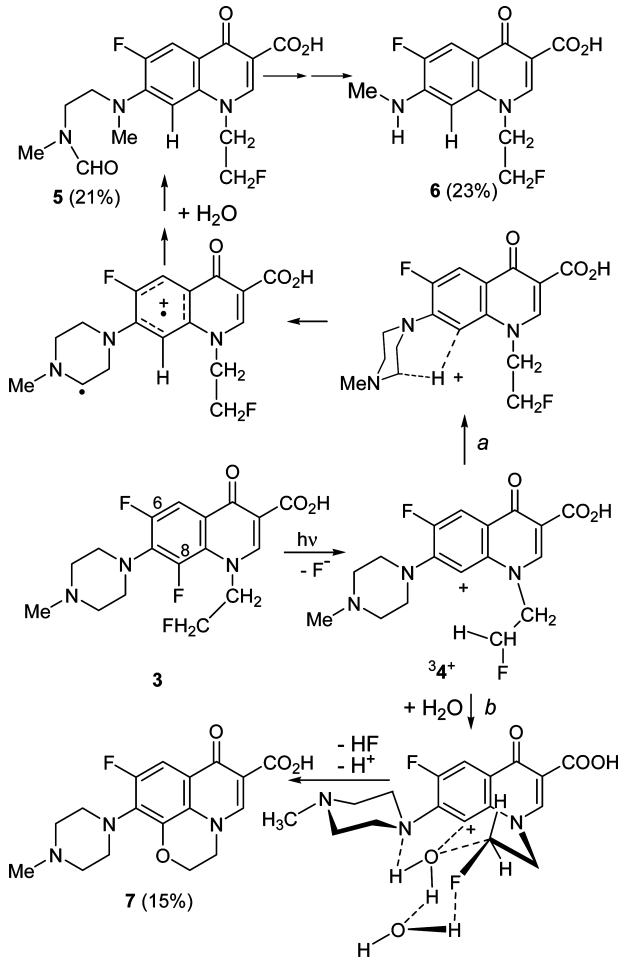
Two of these, amide **5** and amine **6**, reasonably resulted from the alternative hydrogen abstraction from the piperazine side-chain (geometrically less convenient, see Scheme 2, path a). Analoguous products had been previously found from other FQs and rationalized as involving C−C bond cleavage

(7) Yabe, K.; Goto, K.; Jindo, T.; Sekiguchi, M.; Furuhama, K. *Toxicol. Lett.* **2005**, *157*, 203.

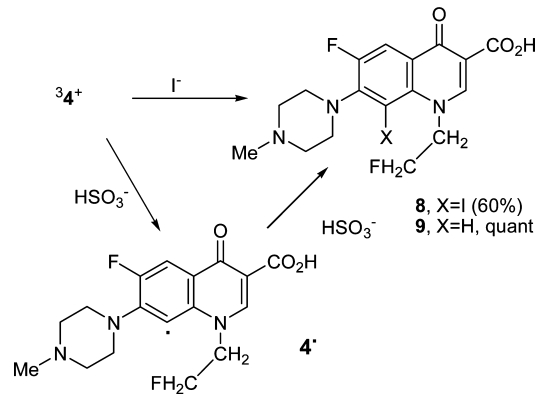
(8) For the conditions of the flash photolysis experiments, see ref 2.

(9) Preparative irradiations were carried out on 6 × 800 mL 2.5×10^{-4} M aqueous solutions of **3** by using a 125 high-pressure mercury arc through quartz. The solutions were evaporated, the residue taken up by benzene-methanol 8:2 and treated by (trimethylsilyl)diazomethane (2.0 M solution in hexanes). The products were separated by chromatography (eluent: chloroform/methanol) as the methyl esters and characterized. Key analytic and spectroscopic data are listed in the Supporting Information.

Scheme 2. Photochemical Reactions of Fleroxacin (**3**) in Water



Scheme 3. Photochemistry of Fleroxacin (**4**) in the Presence of Iodide and Bisulfite



and water addition at the radical level to form formyl derivatives such as **5**. This is followed by oxidative dealkylation to give **6**, an ubiquitous phenomenon with dialkylarylamines.[10,11]

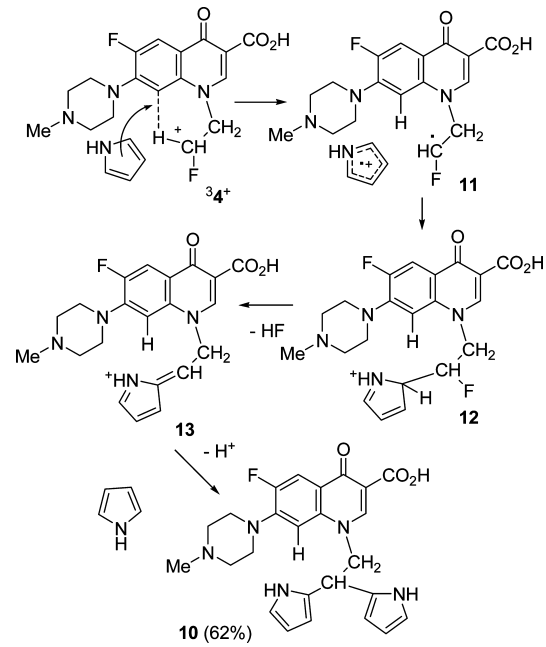
As for the third product, analytic and spectroscopic evidence showed that it resulted from water addition and HF elimination and supported the formula of oxazinoquinolinone **7**. Thus, cation $^3\mathbf{4}^+$ is trapped by water, whereas this is not the case with $^3\mathbf{2}^+$. The difference is explained by the greater charge localization at C_8 in cation $^3\mathbf{4}^+$, due to the lack of the stabilizing interaction with the nucleophilic C−H bonds characteristic of $^3\mathbf{2}^+$, and by the cooperative effect of the formation of the strong HF bond when the fluoride anion is detached.

Cation $^3\mathbf{4}^+$ was effectively quenched by iodide, with $k(I^-) = 3.5 \times 10^9\ M^{-1}s^{-1}$ as determined by flash photolysis. Irradiation with 0.005 M KI gave the straightforward substitution product (**8**, see Scheme 3). This is in contrast to the behavior of cation $^3\mathbf{2}^+$, for which the main process with I^- is attack at the side chain (Scheme 1, path *c*). This result is again an indication of the preference for ionic coupling at C_8 in the case of fleroxacin, which extends also to a soft anion such as iodide.

A clean reduction to product **9** took place in the presence of bisulfite (0.02 M), reasonably via reduction of the cation to radical **4**, followed by further reduction to the anion and protonation.

Pyrrole was then tested and found to quench effectively $^3\mathbf{4}^+$ [k(pyrrole) = $5 \times 10^8\ M^{-1}s^{-1}$ by flash photolysis]. Irradiation in the presence of 0.05 M pyrrole suppressed the products obtained in neat water and gave a new main product, which was isolated (62%) and found to contain two equivalents pyrrole nuclei attached at $CHCH_2N$ group. The

Scheme 4. Photochemistry of Fleroxacin (**4**) in the Presence of Pyrrole



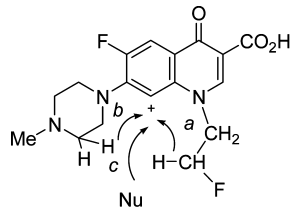
(10) Fasani, E.; Mella, M.; Monti, S.; Albini, A. *Eur. J. Org. Chem,.* **2001**, 391.

(11) Fasani, E.; Rampi, M.; Albini, A. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1901.

characterization supported the assignement of structure **10** to this product (see Scheme 4).

This surprising result was explained by the operation of an electron transfer (ET) path with this π nucleophile followed by coupling within the radical ion pair (**11**, Scheme 4). Elimination of HF (for a precedent, see ref 12) then led to the stabilized pyrrolemethine cation **13** and to the final product via electrophilic substitution.

A neutral π reagent such as pyrrole thus acts as electron donor rather than as nucleophile. The lack of fast ionic attack at C_8 restores the role of the interaction with the side chain, despite its weakness, so that the radical center is formed on the chain, not at C_8. It thus appears that the mode and efficiency of intermolecular reactions of triplet aryl cations can be tuned by structural variations. In $^3\mathbf{2}^+$, the geometrically favored interaction with the nucleophile C−H bond in the chain makes intramolecular reaction fast (path *a*, scheme 1).



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The introduction of a fluorine atom in $^3\mathbf{4}^+$ loosens the "shielding" of C_8 by the ethyl group so that the charge is more localized at that carbon. Therefore, even with a weak nucleophile such as water *intermolecular* trapping of the cation (path *c* in formula **14**) better competes with H abstraction. The latter path remains majoritary (about 3 to 1), but in this case involves the piperazine side-chain, rather than the geometrically, but not electronically, convenient ethyl group (path *b* rather than path *a*).

(12) For related elimination reactions, see, for example: Kwong-Chip, J. M.; Tidwell, T. T. *Tetrahedron Lett.* **1989**, *30*, 1319.

Furthermore, the borderline between the ionic coupling at C_8 and the ET/radical coupling mechanism is shifted. This lies between Br^- and I^- with $^3\mathbf{2}^+$, but between I^- and pyrrole with $^3\mathbf{4}^+$. Thus, with the first cation both pyrrole and iodide act as electron donors. This is followed by a radical reaction, intramolecular H transfer from the chain. On the contrary, with $^3\mathbf{4}^+$ ionic attack at C_8 is the exclusive process with iodide and has some role with water. With this cation, the ET path remains a prerogative of a π donor such as pyrrole. Triplet phenyl cations show some selectivity toward nucleophiles, for example, 4-dimethylaminophenyl cation couples three times faster with iodide than with pyrrole.[13] Apparently, the difference is larger in this case, with no detectable ionic attack with pyrrole.[14]

These results further document the unusual chemistry of aryl cations and suggest that also the interaction with biomolecules can be directed to some degree. It is hoped that these chemical data, completed with biological studies, lead to a better understanding of the phototoxicity of this drug family and in perspective to the design of new photoactivated drugs.

Acknowledgment. Partial support of this work by Murst, Rome is gratefully acknowledged.

Supporting Information Available: Experimental procedures and analytical and spectroscopic data for compounds **5**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Dichiarante, V.; Fagnoni, M.; Albini, A. *J. Org. Chem.* **2008**, *73*, 1282.

(14) An alternative mechanism that can be considered is intramolecular electron transfer from the piperazine moiety to the heterocyclic ring. We thank a Referee for pointing to this possibility, and indeed, we think that this may have a role with other FQs, where oxidation of the amine side-chain results. This seem difficult to apply to the present case both because of the difficult C−F bond cleavage from a radical anion and because it would not lead to the observed products, compare ref 2.