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Photoreactivity of Fluoroquinolones: Nature of Aryl Cations Generated in Water

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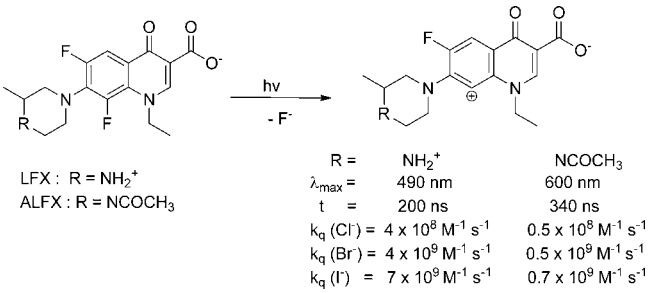
ABSTRACT



The nature of stabilized aryl cations generated from photodehalogenations of fluoroquinolones in aqueous media has been studied by comparing the photophysical and photochemical behavior of lomefloxacin (LFX) and its N(4′)-acetylated form (ALFX). Photoproduct studies, laser flash photolysis, and emission measurements have shown that this small peripheral modification produces important changes in the properties of the singlet aryl cations generated. Also, in basic medium, a new photodehalogenation pathway for 6,8-dihalogenated fluoroquinolones has been observed.

Fluoroquinolones (FQs) are antibacterial agents that develop their pharmacological activity through the inhibition of the bacterial topoisomerase II, an enzyme involved in the replication and repair of bacterial DNA.[1] In recent years FQs have drawn much attention due to their antitumoral activity.[2] In *vitro* and in *vivo* studies have corroborated the anticancer effects of quinolone antibiotics supporting the observation that FQs reduce all-cause mortality among cancer patients.[3] The mechanism suggested for the direct FQ antitumor effect has been the inhibition of mammalian topoisomerases (I and II) and DNA polymerase. In this context, the genotoxic effects exhibited by FQs in eukaryotic systems are enhanced by UV irradiation, which confers to these molecules a potential property as photochemotherapeutic agents.[4] This photoinduced genotoxicity has remarkably been detected in dihalogenated FQs such as fleroxacin (FLX,), BAY y3118 (BAY), and lomefloxacin (LFX), a compound proposed as a standard for photomutagenic action (structures are shown in Scheme 1).[5]

The DNA photoinduced damage has been associated with the alkylating reactivity of an intermediate arising from FQ photodehalogenation.[6] In fact, formation of covalent bindings between LFX and biomolecules such as guanosine monophosphate has been observed.[7a]

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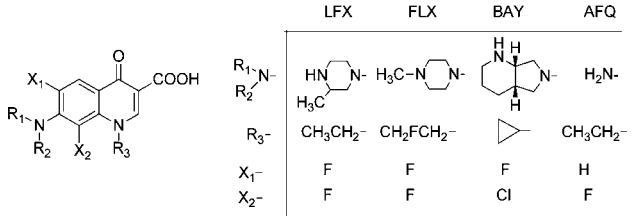
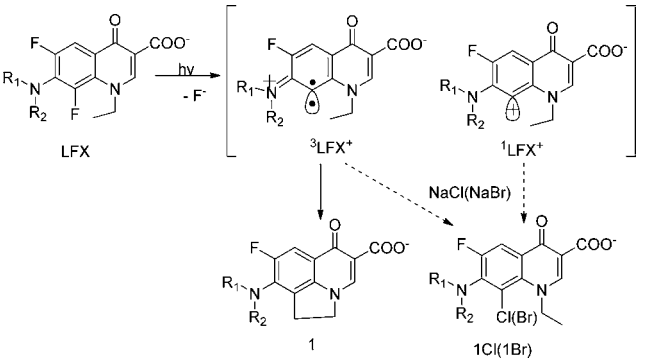
Scheme 1. Structure of Photogenotoxic Fluoroquinolones and the Model Compound AFQ



In order to determine the involved mechanisms of this new class of photoactivated drugs, a large number of studies concerning the photophysical and photochemical properties of FQs bearing a further halogen atom at position 8 of the quinolinic ring have been carried out during the past few years.[5d,7] All of them have shown an unusual photodehalogenation by heterolysis of the strong C_8–halogen bond.[5d,7] Generation of an aryl cation seems to be the key intermediate of the FQs photobinding properties.[7h,g] However, controversial interpretations about the nature and reactivity of this type of intermediate have been reported in the literature.[7a,f,h] In fact, in studies performed with LFX, the nature of the aryl cation detected was attributed not only to a charge delocalized species with a carbene character $^3LFX^+$ (triplet ground state multiplicity)[7f] but also to a carbocation with singlet multiplicity ($^1LFX^+$).[7h] The involvement of $^3LFX^+$ was supported by a lack of reactivity between the aryl cation and water as a neutral nucleophile and by theoretical calculations, claiming that the triplet carbene generated from photodefluorination of an aminofluoroquinolone AFQ (Scheme 1) is the lowest transient state.[7f] By contrast, the high reactivity of the intermediate toward Br^- and Cl^- and the absence of quenching by molecular oxygen were the most important results supporting the participation of $^1LFX^+$.[7h] Moreover, although in LFX photodegradation it was established that product **1** is generated by an intramolecular reaction of $^3LFX^+$ with a neighboring C–H bond (position β in the N-ethyl group), the intermediate involved in the formation of **1Cl** and **1Br** remains unclear (Scheme 2).[7f,h]

Scheme 2. Photodegradation of LFX in Neutral Aqueous Media with and without Halides



With this background, and considering that acetylation of the piperazinyl ring of norfloxacin as a peripheral change of the heterocyclic skeleton of this FQ produces photophysical changes in the singlet and triplet excited states,[8] the whole aminoalkyl substituent (R_1R_2N–) is expected to be very influential in the photophysical and/or photochemical behavior of 6,8-dihalogenated fluoroquinolones. Thus, the calculations performed for AFQ photodehalogenation should not be extrapolated to FQs with this type of substituent such as LFX, FLX, or BAY. Accordingly, it appeared reasonable to check this hypothesis using LFX and its N(4′)-acetylated derivative (ALFX) to obtain further evidence of the involvement of singlet aryl cations in FQ photodegradation. This issue has been addressed by performing both steady-state and time-resolved studies in aqueous media at pH ca. 7.4 as well as under basic conditions. Lomefloxacin is commercially available, and the preparation of its N-acetyl derivative (ALFX) was achieved following standard acetylation procedures,[9] and its structure was unambiguously assigned on the basis of its NMR spectroscopic data (Supporting Information).

The study was initiated by analyzing the singlet excited state properties of both compounds. Acetylation induced a red shift in the emission (Figure 1) and a slightly different fluorescence lifetime (τ = 1.2 and 1.7 ns for LFX and ALFX respectively). Besides, when the pH was increased to 12, the fluorescence faintly changed for ALFX but almost disappeared in the case of LFX. Therefore, at this pH, a new reaction pathway from ^1LFX must be occurring. An intramolecular electron transfer between the lone pair of N(4′) and the quinolinic ring (path I, Scheme 3), as previously described for norfloxacin,[8a] would explain this result.

Flash photolysis of ALFX and LFX in water also revealed the influence of the N-acetylation of the piperazinyl ring on the spectroscopic and kinetics properties of the detected intermediates arising from LFX. In fact, both FQs under a N_2O atmosphere at pH = 7.4 showed very

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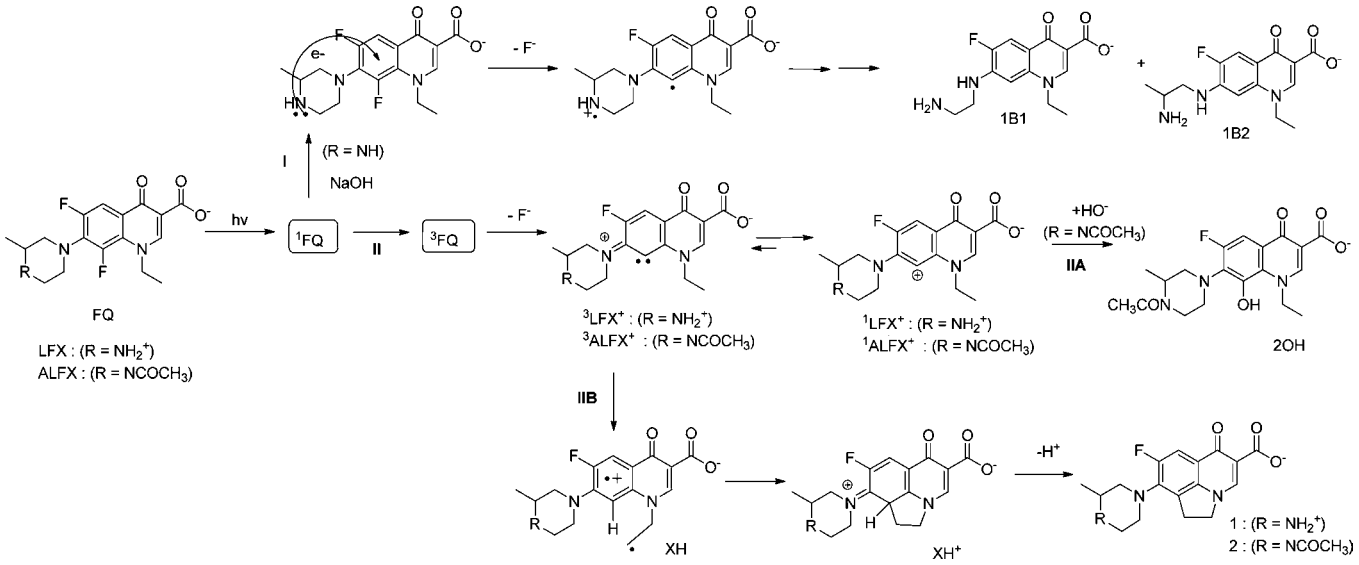
Scheme 3. Photodegradation Pathways of (A)LFX in Aqueous Media



different transient absorption spectra (Figure 2A and 2B). In the case of LFX, two successive short-lived transients species were detected. The first one, absorbing at λ_{max} = 370 nm and with a τ of ca. 30 ns, has been claimed as the LFX triplet excited state (^{3}LFX).[7f] Nevertheless, this transient species could also be attributed to $^3LFX^+$ because all ^{3}FQ show absorption maxima at long wavelengths[6,8c] and there is an important similarity between the absorption spectrum of this LFX transient species and those observed with other triplet carbenes (see path II, Scheme 3).[10]

The second species detected in LFX experiments (λ_{max} = 490 nm and τ = 200 ns) corresponds to an aryl cation.

However, as mentioned above, the nature of this transient is still an open matter ($^1LFX^+$ or $^3LFX^+$).[7f,h]

Analysis of the results obtained using ALFX also showed two successive intermediates but with absorptions at λ_{max} = 600 nm (the first intermediate τ = 340 ns) and at

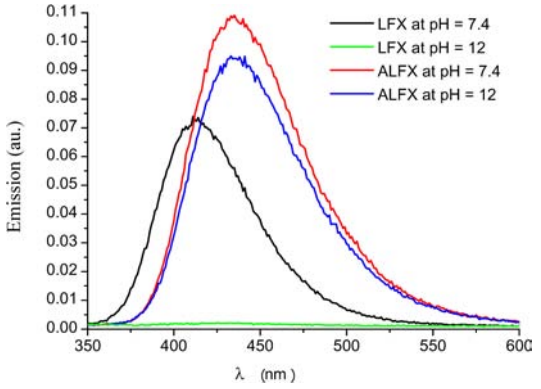


Figure 1. Emission spectra of LFX and ALFX in aqueous media at pH = 7.4 and 12.

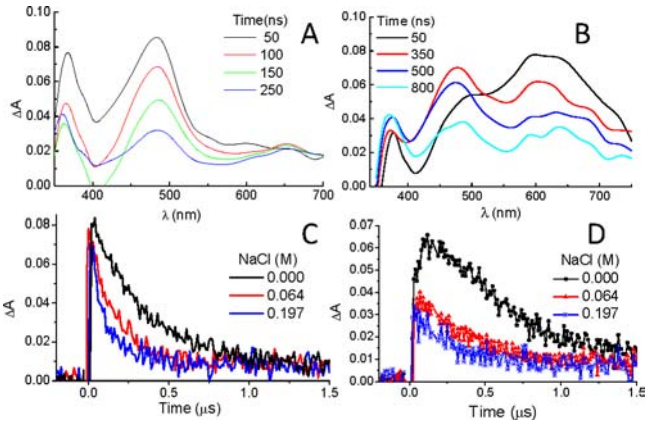


Figure 2. Transient absorption spectra of LFX (A) and ALFX (B) aqueous solutions in N_2O at different times after excitation at 355 nm. Kinetics of ALFX aqueous solutions in the absence and presence of NaCl at 600 nm (C) and 480 nm (D).

λ_{max} = 480 nm (the second one with τ = 440 ns). To characterize these transient species, similar quenching studies to those performed with the intermediates arising from LFX were performed. Thus, it was observed that the two bands are not appreciably quenched or modified by the presence of an O_2 atmosphere. This means that both transient species can be neither a triplet excited state nor an aryl cation with carbene character because it appears to be well established that both types of intermediates are very reactive to O_2.[10,11]

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Table 1. Quantum Yields of Fluorescence and Photodegradation of LFX and ALFX Aqueous Solutions at pH 7.4 and 12

	LFX		ALFX	
pH	Φ_F	Φ_D	Φ_F	Φ_D
7.4	0.08[a]	0.55[a]	0.11	0.6
12	<0.002	0.25	0.095	0.4

[a] Values from the literature.[7h] Experimental data and results analysis in the Supporting Information.

When ALFX was submitted to LFP in the presence of halides, the first intermediate was found to be highly reactive. Bimolecular rate constants (k_q) of 0.5×10^8, 0.5×10^9, and 0.7×10^9 M^{-1} s^{-1} were determined for its interaction with Cl^-, Br^-, and I^- respectively (Figure 2 C). With these results it can be asserted that the species absorbing at λ_{max} = 600 nm is a singlet aryl cation $^1ALFX^+$ (path II, Scheme 3). Besides, the fact that cation $^1ALFX^+$ is quenched by halides with k_q ca. 10 times lower than those reported in the literature for $^1LFX^+$ (k_q with Cl^-, Br^-, and I^- are 4×10^8, 4×10^9, and 7×10^9 M^{-1} s^{-1} respectively)[7f,h] is evidence of the importance of the acetylation of the piperazinyl ring in the reactivity of this type of aryl cation.

The LFP experiments performed using LFX aqueous solutions at pH = 12 did not show any transient species. Nevertheless, in the case of ALFX, the intermediates absorbing at λ_{max} = 600 nm ($^1ALFX^+$) and at λ_{max} = 480 nm were detected (transient absorption spectra are shown in the Supporting Information). This important difference observed between both FQs under basic conditions clearly confirmed the intramolecular pathway described above for ^1LFX, which is blocked for ^1ALFX (path I, Scheme 3). Under basic conditions it was also observed that the hydroxyl anion quenches $^1ALFX^+$ with a rate constant of 0.2×10^9 M^{-1} s^{-1}. Moreover, increasing amounts of OH^- as well as of halides at neutral pH result in rapid generation of the second intermediate but with a decrease of its absorbance (Figure 2C and 2D). Therefore, the structure of this species would be attributed to the diradical cation XH but not to the cation XH^+ because the latter would be also quenched by OH^- and this fact does not take place (path IIB, Scheme 3).

The photophysical results were complemented by photoproduct studies in order to confirm the intermediates involved in the photolysis of ALFX. Irradiations of ALFX and LFX in deaerated aqueous solutions at pH ca. 7.4 and 12 were performed in a multilamp photoreactor using UVA light at λ_{max} = 350 nm. Kinetics studies revealed that photodegradation quantum yields (ϕ_D) of LFX and ALFX are very similar at pH 7.4 (Table 1). Product analysis of LFX and ALFX photolysis showed mainly the formation of **1**[12] and **2** respectively, which arise from their triplet aryl cations (Scheme 3). Nevertheless, under basic conditions, the ϕ_D values of LFX and ALFX showed important differences (Table 1). Moreover, while **1B1** and **1B2** were the photoproducts detected for LFX,[7b] **2** and **2OH** were obtained from the photolysis of ALFX. The structures of **2** and **2OH** were unambiguously assigned (key analytic and spectroscopic data are listed in the Supporting Information). In this context, in agreement with path IIA in Scheme 3, the increase of pH produces the formation of higher amounts of photoproduct **2OH** and a rapid quenching of $^1ALFX^+$. By contrast, the different type of compounds obtained from LFX at pH = 12 would be produced by the intramolecular electron transfer of the N(4′) lone pair of the piperazinyl substituent and the quinolinic ring (path I in Scheme 3), which clearly discards the suggested involvement of the LFX triplet excited state or any aryl cation.[7b]

In summary, the contribution of the aryl cations $^1LFX^+$ and $^1ALFX^+$ to the (A)LFX photodehalogenations has clearly been demonstrated in the present investigation and all findings are in agreement with the photophysical and photochemical pathways shown in Scheme 3. Additionally, with these processes taken into account, the photochemistry of other 6,8-dihalogenated quinolones such as fleroxacin[7i] and BAY y3118[7h] as well as the alkylating properties described for this type of FQ can be better understood through the involvement of aryl cations with singlet multiplicity.

On the other hand, results have revealed the impact of N-alkyl peripheral substituents on the photophysical and photochemical properties of 6,8-dihalogenated quinolones. Thus, by changing the substituents of fluoroquinolones, it is possible to modulate not only their pharmacological properties but also the reactivity of their aryl cations, which may be used to design a new family of photoactivated drugs for photochemotherapy.

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Supporting Information Available. Spectroscopic data and experimental details. This material is available free of charge via Internet http://pubs.acs.org.

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The authors declare no competing financial interest.