Generation of Detectable Singlet Aryl Cations by Photodehalogenation of Fluoroquinolones

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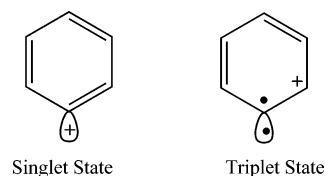*Received: January 30, 2006; In Final Form: February 21, 2006*

Nanosecond laser flash photolysis (λ_{exc} = 355 nm) of neutral aqueous solutions of lomefloxacin (LFX, a 8-fluorinated 7-amino-4-quinolone-3-carboxylic acid derivative) produces a detectable transient species, which shows an absorption maximum at 490 nm and can be assigned to an aryl cation. This intermediate has a lifetime of ca. 200 ns in net water, reacts with Br$^-$ and Cl$^-$ with rate constants of 3.6×10^9 M^{-1} s^{-1} and 4.1×10^8 M^{-1} s^{-1}, respectively, and shows a lack of reactivity toward molecular oxygen. From the photolysis of BAY y3118 (BAY, a 8-chlorinated analogue), an aryl cation is also generated, showing absorption maximum at 480 nm (lifetime of ca. 1 μs in net water) and a reaction rate constant of 9×10^9 M^{-1} s^{-1} with Br$^-$. The existence of these highly reactive species arising from direct photolysis of LFX and BAY can justify the photogenotoxic properties associated with these antibacterial drugs likely due to direct reaction of their cations with DNA.

Photolysis of aromatic halides and benzene diazonium salts has been the subject of extensive investigations because of their wide range of industrial and biological applications. They are used in photolithography and photopolymerization, as well as in photoaffinity labeling studies.[1–3] It has been well established that aryl cations generated upon photolysis of both precursors can correspond to a localized carbocation (singlet ground state) or to a charge delocalized species with a carbene character at the divalent carbon (triplet ground state) (Chart 1).[4–6]

Since aryl cations are highly reactive intermediates, there are only few reports where they have been detected by time-resolved spectroscopic methods. At room temperature in fluid solutions all the observed transients exhibit carbene character.[4,7–9]

Photodegradation of both aromatic halides and benzene diazonium salts has revealed many mechanistic facets of aryl cations.[4–11] In general, although multiplicity can depend on the nature of the substituent, diazonium salts generate products arising from singlet ground state, while heterolysis of halogenated compounds leads to triplet phenyl cations.[4,9] In this context, photochemical and photophysical studies with lomefloxacin (LFX) and fleroxacin (FX), two fluoroquinolone antibiotics (Scheme 1), have proposed an aminoaryl cation with carbene character to justify their photodehalogenation processes.[12–14] Thus, although it has been observed that drug molecules having a halogen atom at the C-8 position are easily photodegraded in neutral aqueous solutions, no direct evidence for the formation of the proposed reactive species has been obtained. However, photoproducts of halide substitution at the C-8 position have been identified, which support the involvement of aryl cations. Accordingly, 8-chloro derivatives of LFX and orbifloxacin (OFX) were obtained in the presence of chloride ions, and the 8-bromo derivative of sitafloxacin (SFX) was produced in buffered solutions containing bromide ions.[15–17]

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CHART 1. Electronic Configuration of the Phenyl Cation

Singlet State   Triplet State

This type of reactive intermediate, if formed, could be the key to explain not only the photocarcinogenic properties attributed to LFX but also the photomutagenicity observed for FX and BAY y3118 (BAY).[13,18,19] Structures are shown in Scheme 1.

Laser flash photolysis (LFP) spectroscopic techniques have been previously applied to study LFX, one of the 8-halogenated 7-amino-4-quinolone-3-carboxylic acid derivatives. Two transients were observed: the solvated electron (λ_{max} = 720 nm) plus another species (λ_{max} = 500 nm) tentatively attributed to the triplet excited state (^{3}LFX) based on its quenching by oxygen.[20]

With this background, the purpose of the present work has been to perform a more detailed nanosecond LFP study on LFX in an effort to detect the putative reaction intermediates in the photodehalogenation process. Thus, the experiments were carried out in neutral aqueous solutions both under aerobic (air or O$_2$) and anaerobic (N$_2$ or N$_2$O) conditions.

Results show that, under N$_2$, only two transient species are formed within the nanosecond time scale (Figure 1). The first one has a lifetime of ca. 300 ns and a λ_{max} of ca. 720 nm; it is efficiently quenched by oxygen and N$_2$O, which supports its assignment as the solvated electron. The second transient resulted to have a lifetime of ca. 200 ns and λ_{max} of ca. 370 and 490 nm; it was the only species observed under N$_2$O (Figure 1A). However, this intermediate is not quenched by oxygen (see Figure 1B), which is not in agreement with its assignment as the ^{3}LFX.[20] In fact, the described quenching at 490 nm is due
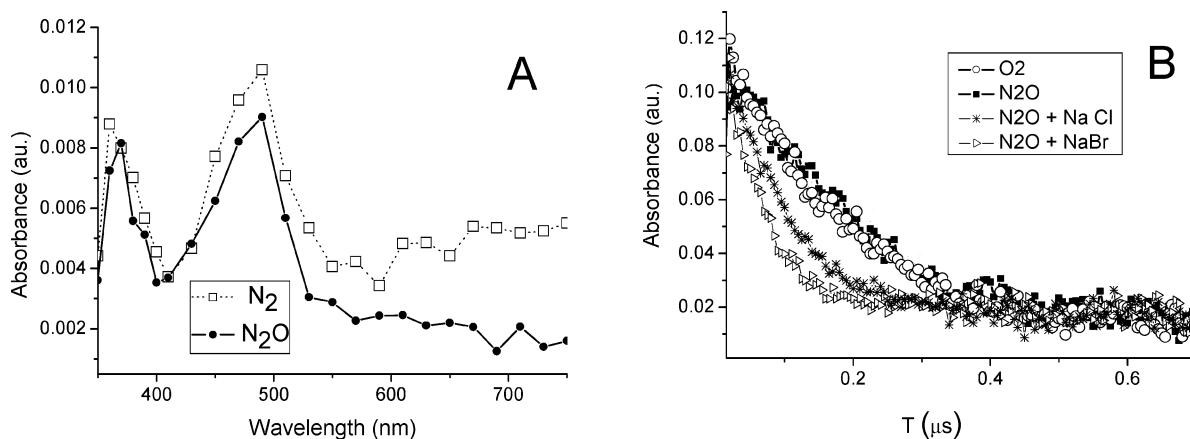
Figure 1. (A) Transient spectra observed 20 ns after flashing (355 nm) 0.1 mM solution of LFX in water at pH ca. 7.4 in the presence of N_2 (□) or N_2O (●). (B) Oscilloscope traces at 490 nm after flashing aqueous solutions of 0.1 mM LFX in the presence of oxygen and N_2O with or without additives such as NaCl or NaBr at 7 mM concentration.

**SCHEME 1. Photolysis of Some 8-Halogenated Fluoroquinolones**
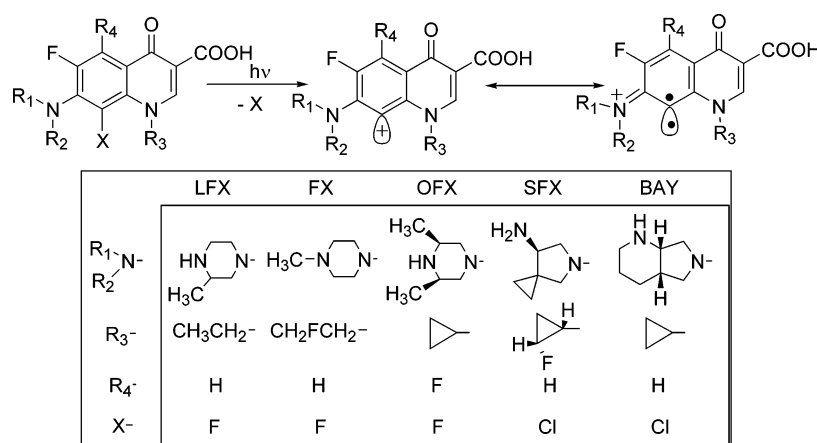
to the solvated electron, as revealed by comparing the experiments performed under N_2 with those under aerobic conditions (data not shown). Though the solvated electron contribution at 490 nm under our LFP experimental conditions is not remarkable (less than 15% of the signal at 5 mJ/pulse energy), as it has been described that LFX photoionization mainly occurs via a biphotonic process,[15] the use of higher energy pulses or the presence of hot spots could result in a higher contribution of the solvated electron. On the other hand, it is well known that an aryl cation with carbene character is very reactive to molecular oxygen.[7-9] Thus, this transient species can be neither a triplet excited state nor an aryl cation with carbene character.

To achieve an unambiguous assignment of this intermediate, LFX was submitted to LFP in the presence of 2-propanol and nucleophilic anions such as Cl^- or Br^- under N_2O.

The assays were performed maintaining the ionic strength (0.5 M $NaClO_4$) and the pH (7.4, 2 mM phosphate buffer, PB). The lifetime of this transient was not affected by 0.1 M 2-propanol while rate constants of 3.6×10^9 M^{-1} s^{-1} and 4.1×10^8 M^{-1} s^{-1} were determined for its reaction with Br^- and Cl^- respectively (1×10^{-4} to 5×10^{-2} M concentrations of NaCl and NaBr were used, Figure 1B). No transients associated with those reactions could be detected by time-resolved absorption spectroscopy. All these results appear to support assignment of this species as the singlet ground-state aryl cation. Its lack of reactivity toward molecular oxygen further supports this hypothesis. However, this seems to be in contradiction with the fact that photoproduct **1** must arise from the carbene "triplet cation" (see structures in Scheme 2). A reasonable explanation

SCHEME 2. Photodegradation Mechanism of LFX in Aqueous Solutions
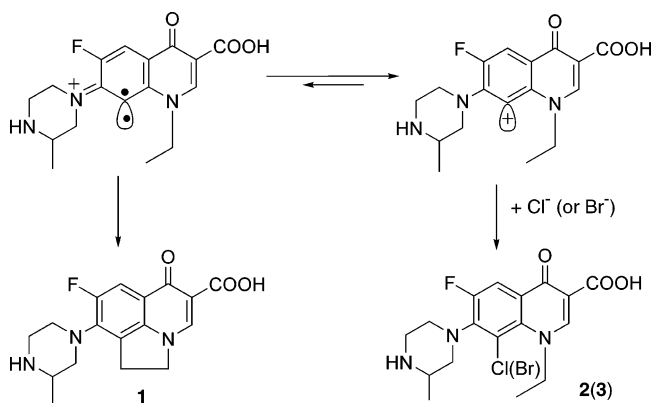
could be that the energy levels of the singlet ground state of the aryl cation and its triplet state are sufficiently close to each other. In fact, singlet–triplet intersystem crossing equilibrations have been established in carbenes such as diphenylcarbene or fluorenylidene.[21] To check this possibility, the temperature dependence of the aryl cation decay was examined in water at pH 7.4. Measurements of decay kinetics (k) at different temperatures allow to establish activation energies (E_a) and preexponential factor (A) for a given process according to Arrhenius eq 1.

$$k = A \, x \, e^{(-E_a/(RxT))} \tag{1}$$

Letters

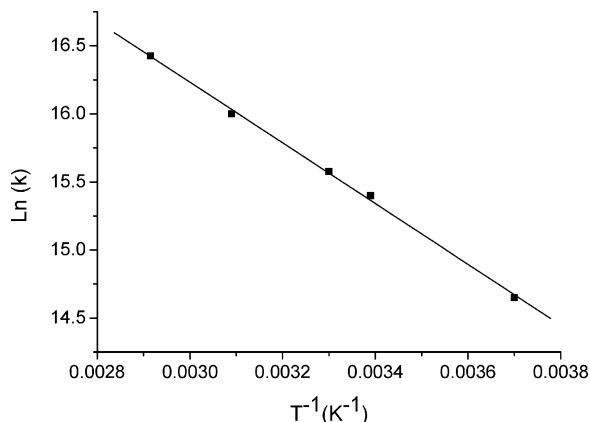*J. Phys. Chem. B, Vol. 110, No. 13, 2006* **6443**

Figure 2. Arrhenius plot for LFX (0.1 mM) determined in water at pH 7.4 under N$_2$O atmosphere.

TABLE 1: Photolysis of LFX (0.1 mM) in Water under N$_2$ at pH ca. 7.4

salt[a]	temperature[b]	LFX	1	2 or 3
			Yield (%)	
	20[c]	59	22	
	70	55	35	
NaCl (30 mM)	20[c]	53	4	39
NaCl (30 mM)	70	52	16	28
NaBr (3 mM)	20[c]	53	3	42
NaBr (3 mM)	70	51	15	31

[a] Experiments carried out in presence and absence of salts (NaCl and NaBr) at two temperatures (20 and 70 °C). [b] The irradiations were performed using the laser beam at 355 nm as the light source (75 single pulses of 10 ns duration with an energy of 10 mJ/pulse). Photoproducts were identified using a 1100 Series LC/MSD Agilent instrument by API-ES(H$^+$) ionization. [c] Similar results were obtained when the solutions were irradiated bubbling oxygen or when these assays were performed maintaining the ionic strength (0.5 M NaClO$_4$).

The obtained plots are shown in Figure 2; from them, log(A/s^{-1}) values of 10.1 and activation energy of 17.6 kJ/mol were estimated.

Accordingly, LFX in net water loses a fluoride anion to give photoproduct **1** by insertion into the neighboring C−H bond of the *N*-ethyl group or photoproduct **2** by attack of a chloride anion, when this nucleophile is present (Scheme 2).[15]

The transient spectroscopy results were complemented by additional product studies. Thus, irradiations (λ = 355 nm) of LFX in net water were done in the presence and absence of salts (NaCl and NaBr) at two temperatures (20 and 70 °C) (Table 1).

These results are in agreement with expectations. Irradiations without salts give rise to photoproduct **1** mainly, while in the presence of anions such as Cl$^-$ or Br$^-$, the major photoproducts are **2** or **3** respectively (Scheme 2). Additionally, the temperature effect is clearly evidenced by the increase of the amount of **1** at 70 °C.

The LFX cation in water is mainly present as the singlet ground state cation, which is the intermediate detected by laser flash photolysis. However, intersystem crossing to the triplet carbene can easily occur upon thermal activation due to the low singlet−triplet (carbene) energy gap. Thus, the singlet reacts with Cl$^-$ or Br$^-$ to form compound **2** and **3**, respectively, and the carbene produces **1** by intramolecular reaction with the neighboring C−H bond (position β in the *N*-ethyl group).

The mechanism shown in Scheme 2, in addition to explaining photoproducts formation, could justify the photocarcinogenic properties attributed to LFX. If the photodegradation pathway proposed for LFX was similar for all 8-halogenated fluoroquinolones, then the photomutagenicity of LFX, FX, and BAY would be related to the direct reaction of their aryl cations with DNA. Therefore, an additional LFP study was performed on BAY to detect its aryl cation intermediate. The results confirmed this expectation; under N$_2$O, only a transient absorption species was formed (τ = 1 μs) with a λ_{max} of ca. 480 nm (data not shown); it was not quenched by oxygen but was found to be highly reactive toward Br$^-$ (k_r = 9 × 10^9 M^{-1} s^{-1}).

Summarizing, singlet aryl cations have been observed by nanosecond laser flash photolysis of neutral aqueous solutions of LFX and BAY. To our knowledge, this is the first time that such a species has been detected directly in fluid solutions. Steady state photolysis studies are consistent with the singlet ground state character of the detected aryl cations. On the other hand, the existence of this highly reactive species arising from direct photolysis of LFX and BAY can justify not only the photocarcinogenic and photomutagenic properties of these drugs but also those of other 8-halogenated fluoroquinolones.

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