Unexpected Photoreactions of Some 7-Amino-6-fluoroquinolones in Phosphate Buffer

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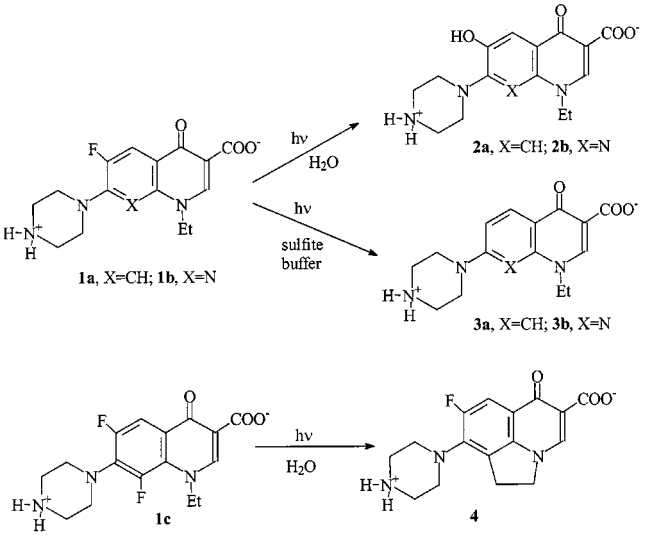
The products of irradiation of some 6-fluoro-7-piperazino-4-quinolone-3-carboxylic acids in phosphate buffer arise from a combination of reductive defluorination of the heteroaryl ring and oxidative fragmentation of the piperazine side chain. This unusual reaction contrasts with the fluorine atom substitution observed in neat water. The results of steady-state and time-resolved experiments are consistent with initiation of the process by electron-transfer quenching of the triplet state of these heterocycles by the phosphate anion. For one of the compounds, a transient band (λ_{max} = 670 nm), previously attributed to the defluorinated cation, must now be reassigned to the radical anion. This intermediate undergoes inefficient reductive defluorination of the ring. In the process, a phosphate radical anion is expected to be formed and to abstract a hydrogen atom from the piperazine group, leading to degradation of the latter.

Introduction

The photochemistry of push−pull aromatics has yet to be explored in depth. These compounds often exhibit interesting photophysical properties (e.g. TICT states),[1] but little photochemistry. On the other hand, it has recently been reported that heterolytic cleavage of the carbon−fluorine bond can occur in some aryl fluorides bearing electron-donating substituents. This reaction would appear to be of interest, since there are very few examples of fragmentations of this strong bond. To date, photoinduced defluorination has been realized for 4-fluorophenol,[2] some polymethoxyfluorobenzenes[3] and 4-fluoroaniline,[4] as well as some 7-amino-6-fluoroquinolones (used in therapy as antibacterial drugs).[5−9] A study of the photochemistry of the latter compounds in neutral water showed that defluorination was the almost exclusive process. For example, both norfloxacin (**1a**, see Scheme 1) and enoxacin (**1b**) gave the corresponding phenols (**2**) through a formal nucleophilic substitution.[5] However, irradiation in sulfite buffer led to reductive defluorination resulting in quinolones **3**. The 6,8-difluoro derivative lomefloxacin (**1c**) reacted similarly, with even higher quantum efficiency than in the previous cases, with the peculiarity that the defluorination occurred selectively at the 8-position and led to cyclization onto the *N*-ethyl chain to give tricyclic **4** rather than a phenol (in sulfite buffer, reductive defluorination also occurred in this case, albeit only as a side process).[5]

Aminofluoroquinolonecarboxylic acids are amphoteric species (p*K* values for protonation are 6.22 for **1a** and 5.49 for **1c**)[10] and it has been observed that the efficiency of their photodegradation is pH dependent, as one might expect.[8,11] The acidity of the medium thus needs to be controlled during the course of the reaction. The aforementioned product studies[5] were carried out under neutral conditions by adjusting the pH to 7.2 through the addition of small amounts of sodium hydrogen carbonate. On the other hand, studies of biologically active substrates are most often carried out in the presence of substantial concentrations of the appropriate phosphate buffers, in order to adjust both the pH and the ionic strength. Generally, it has been assumed that such buffers have no other effect on the chemistry that occurs. Some studies on the photostabilities of the aforementioned fluoroquinolones have been carried out in phosphate buffers (from 0.007 to 0.2 M),[7,8] but in a preliminary investigation we found that the addition of phosphate had a more significant effect than buffering the solution, since the photodecomposition quantum yields of compounds **1a**,**b** changed by more than a factor of ten on



Scheme 1. Products from the photolysis of compounds **1a−c** in water and in bisulfite buffer

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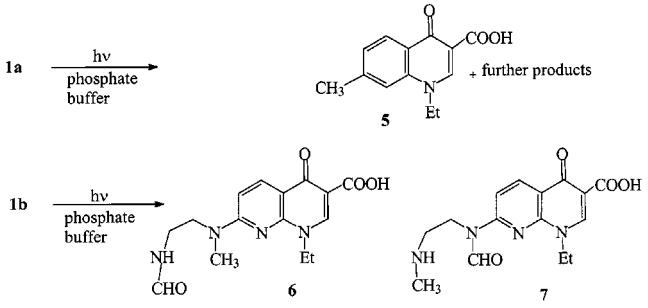
adjusting the phosphate concentration while the pH was kept constant.[6] Thus, it appeared worthwhile to explore whether the products formed might also change under these conditions. As shown herein, the photoreactions of fluoroquinolones **1a**–**c** indeed follow a different path in phosphate buffer and there is evidence that, quite unexpectedly, a different mechanism becomes operative.

Results

Product Studies

Irradiation of quinolone **1a** ($2\cdot10^{-4}$ M) in water at pH 7.2 ($5\cdot10^{-4}$ M $NaHCO_3$ added to adjust the pH to neutrality) gave, as mentioned above, phenol **2a**. We then carried out the irradiation of **1a** in 0.1 M phosphate buffer. The process that occurred was again defluorination, the amount of fluoride ion liberated corresponding exactly to the amount of substrate consumed. However, phenol **2a** was not formed and HPLC analysis after 80% photodecomposition revealed the formation of a different main product. We were unable to isolate this product or a functionalized derivative thereof (see below for the functionalization methods). Extraction with chloroform followed by chromatography allowed the isolation of one of the minor products. Spectroscopic characterization showed that the latter compound had undergone complete degradation of the piperazine side chain. It was identified as the 7-methylquinolone **5** (see Scheme 2).



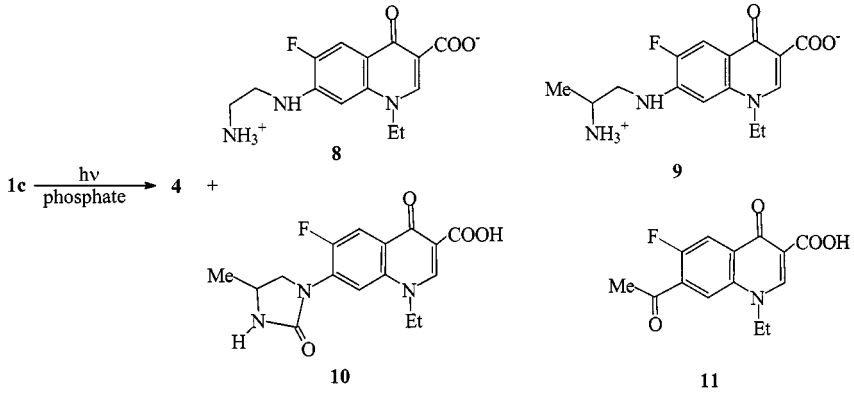
Scheme 2. Products from the photolysis of compounds **1a**,**b** in phosphate buffer

Likewise, irradiation of the analogous 1,8-naphthyridinone **1b** in phosphate buffer did not give phenol **2b** as observed in the absence of this salt, but rather a different main product. This could be extracted with chloroform and purified by crystallization. Analysis of the spectra of this compound showed that the starting structure had been modified in two respects, viz. reductive ring-defluorination had occurred and the piperazine chain had been cleaved (see Experimental Section). The structure of the product could thus be assigned as the *N*-methyl *N*′-formyl derivative **6** (see Scheme 2). Furthermore, extraction of the aqueous phase with 0.1% ethyl chloroformate in chloroform, methylation with diazomethane, and subsequent chromatography allowed the isolation of a further product in a smaller amount. This was shown to be the isomeric *N*-formyl *N*′-methyl derivative **7** (obtained as the *N*-methoxycarbonyl methyl ester; for the sake of simplicity the nonfunctionalized product is indicated in the scheme and in the following discussion).

In the case of difluoroquinolone **1c**, the effect of the medium was less dramatic. In neutral water, the tricyclic derivative **4** was the main product and was accompanied by only minor amounts of other products. In phosphate buffer, the latter products increased at the expense of the former, which nevertheless still predominated. Functionalization, extraction, and separation as in the previous case allowed the isolation of two main and two minor products besides **4**. All of the new products were found to be 6-monofluoro derivatives and had undergone varying degrees of degradation of the piperazinyl moiety. The two main products were shown to be the 2′-aminoethylamino derivatives **8** and **9**, differing in the presence of a methyl group at the 2′-position. As for the minor products, these were found to have different groups in the 7-position, specifically an imidazolinone group in the first case and an acetyl group in the latter (structures **10** and **11**, respectively; see Scheme 3 and the Experimental Section for structural assignments).

Evidence Concerning the Course of the Reaction

The course of the reaction was monitored by HPLC in the case of enoxacin, **1b**, since the product distribution was simpler in this case. The results were straightforward in the



Scheme 3. Products from the photolysis of compound **1c** in phosphate buffer

absence of phosphate: phenol **2b** was formed from the beginning — along with only trace amounts of side products — and the amount increased regularly until over 80% reagent conversion, albeit with decreasing rate. On the contrary, in phosphate buffer, several peaks appeared in the HPLC trace, one of them — corresponding to a trace amount — coincident with that of reduced quinolone **3b**. These did not grow appreciably, except for those due to the finally isolated amides **6** and **7**.

The buffer strongly quenched the formation of phenol **2b**: at $1\cdot10^{-2}$ M the rate of consumption of **1b** was reduced to one-third of that in water, but formation of **2b** was reduced to one-tenth of that in the absence of phosphate. At a buffer concentration in excess of $1.5\cdot10^{-2}$ M, product **2b** was below the limits of detection and the overall reactivity was diminished by a factor of more than five (see also ref.[6]). A plot of the ratio of the reaction quantum yield in the absence ($\Phi°$) vs. in the presence (Φ) of phosphate showed a linear correlation according to Equation (1) for both **1a** and **1b** in the range $6.7\cdot10^{-3}$ to $6.7\cdot10^{-2}$ M.

$$\Phi°/\Phi = 1 + K_{SV}\,[H_2PO_4{}^{2-}] \quad (1)$$

This is shown in Figure 1. Values of $K_{SV} = k_q\tau = 194$ M^{-1} for **1a** and 179 M^{-1} for **1b** were derived from the plots. However, at a higher concentration, the quantum yield for the decomposition of **1a** reached a plateau value, as can be seen in Figure 1, where the last point is seen to deviate markedly from linearity; measurements at a higher buffer concentration showed no further decrease in Φ. As regards **1c**, the reaction was slower than in water in this case as well, but the effect was smaller, with a retardation of just 30% at $7\cdot10^{-2}$ M buffer concentration.

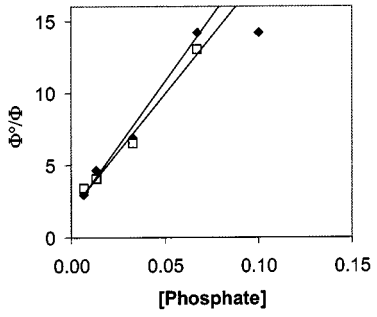


Figure 1. Quantum yield in the absence vs. in the presence of phosphate buffer at pH 7.4 ($\Phi°/\Phi$) for the photoreaction of quinolones **1a** (◆) and **1b** (□); linear correlations in the range $7\cdot10^{-3}$ to $7\cdot10^{-2}$ M are shown

Hydroxyaminoquinolone **(2b)** was found to be one order of magnitude less reactive than **1b** upon further irradiation both in neat water and in phosphate buffer. In a separate experiment, **1b** was first partially (50%) converted into **2b** by irradiation, then phosphate was added making the buffer $1.5\cdot10^{-2}$ M and the irradiation was continued. Under these conditions, residual **1b** reacted further, producing the same HPLC trace as in the above experiments, while **2b** proved to be rather photostable (≈ one-tenth of the reactivity of **1b**).

In a previous laser flash photolysis study, we showed that the heterocycles **1a**,**b** (as well as **1c**, although in this case the signal was much less intense and shorter-lived) gave rise to a transient absorption in the 500−650 nm region and we assigned these absorptions to the respective triplet states.[5,8] With naphthryridinone **1b**, we also reported that a different transient band was seen in phosphate buffer (a broad band centred at 670 nm).[8] Such a transient band is identical to that detected for the same substrate in sulfite buffer (compare Figure 7 in ref.[8] and Figure 1 in ref.[5]).

Discussion

Previous work on the present aminofluoroquinolones has reported some unusually clean reactions involving the C−F bond. Thus, photosubstitution to give the corresponding aminohydroxy derivatives was seen in neat water (with the variation that in the case of **1c** an intramolecular alkylation occurred in the replacement of the fluorine atom), while photoreduction was observed in sulfite buffer.[5] Such reactions are notable in that the mild conditions under which the fluorine substitution or reductive elimination are accomplished contrast with the general inertness of C−F bonds towards thermal reactions (even photochemically, there are only a few precedents for substitution and none for reduction). It was also found that reductive defluorination could be induced at a cathode in the case of **1b** and **1c**, although not with **1a**.[12] Again, there are only a few precedents in the literature of the cathodic reduction of aryl fluorides.[13,14] In parallel with the present study, devoted to the organic chemical aspects of the mechanism in phosphate buffer, a systematic investigation of the luminescence of these heterocycles and of the transient species formed has been carried out by means of pico- and nanosecond flash photolysis both in the presence and absence of inorganic salts and the results have been submitted separately.[15] In support of the following mechanistic discussion, some key data are anticipated (see below).

The present study reveals a different and intriguing chemistry in phosphate buffer. Under these conditions, the process is again 100% C−F bond heterolysis. However, the chemical outcome is different, since reductive ring-defluorination is accompanied by degradation of the piperazine side chain. The effect of phosphate is in many respects the same as that previously observed with sulfite buffer. The reaction in the sulfite medium has a low quantum yield (of the order of 0.01, whereas Φ_{F^-} in neat water is 0.06 for **1a** and 0.13 for **1b**).[5,6] It involves electron transfer to the triplet state of the quinolones followed by inefficient defluorination at the radical anion stage [see Equation (2) and Equation (3), where Het = quinolone residue]. Indeed, in the case of **1b**, flash photolysis provided direct proof of the quenching of the triplet state and the formation of a second transient species, which was identified as the radical anion $\mathbf{1b}^{-\cdot}$.[5]

$$Het{-}F^{3}* + SO_3{}^{2-} \rightarrow Het{-}F^{-\cdot} + SO_3{}^{-\cdot} \quad (2)$$

$$Het{-}F^{-\cdot} \rightarrow Het^{\cdot} + F^- \quad (3)$$

In the case of **1c**, where the quantum yield is higher ($\Phi_{F^-} = 0.55$) and the reaction predominantly proceeds

through the singlet excited state, sulfite has only a limited effect.[5]

In phosphate buffer, the reaction quantum yield is one order of magnitude lower than in water, just as in sulfite buffer, and is sensitive to oxygen. The photoproducts are completely different (with respect to neat water) with quinolones **1a**,**b**, while these effects occur to a somewhat lesser extent with **1c**. Thus, the triplet state may also be involved in this case. Steady-state measurements of the photoreaction quantum yields at various buffer concentrations (see Figure 1) can be submitted to a Stern−Volmer treatment [Equation (1)] and lead to values of $K_{SV} = k_q\tau$ = 194 and 179 M^{-1} for **1a** and **1b**, respectively.

Thus, steady-state experiments support the view that the triplet is quenched by phosphate. That a static or dynamic quenching of the singlet state contributed significantly was excluded both by the low K_{SV} values obtained from fluorescence intensity measurements ($K_{SV(fluo)}$ = 16 and 15 M^{-1} for **1b** and **1a**, respectively)[15] and by the lack of any marked effect of the buffer on the initial intensity of the triplet absorption at a concentration range up to 0.01 M, over which τ_T decreased sharply (up to a factor of 10 with **1b**).[15] Finally, the rate constants for phosphate quenching of the triplet states were measured for both **1a** and **1b** (k_q = 0.8 and $9.8\cdot10^8$ M^{-1} s^{-1}, respectively; the signal was too weak in the case of **1c**).[15]

The similar effects of the sulfite buffer and particularly the formation of an identical transient species from the quenching of triplet **1b** with both sulfite and phosphate suggest that the first step is electron transfer to give the radical anion in the latter case as well. Phosphate is certainly a poorer reducing agent than sulfite. However, there is some literature precedent that it can act as such towards excited states.[16]

We expect the reduction of **1b**^{3}* by the HPO_4^{2-} anion to be close to thermoneutral on the basis of the following data. For the sake of simplicity, the discussion is limited to the more reducing HPO_4^{2-} anion (see note below), although the monoanion $H_2PO_4^-$ is also present in the mixture. Quenching constants are always referred to the total phosphate concentration. This introduces an error, but we do not feel that the data are yet suitable for a really thorough discussion of these details.

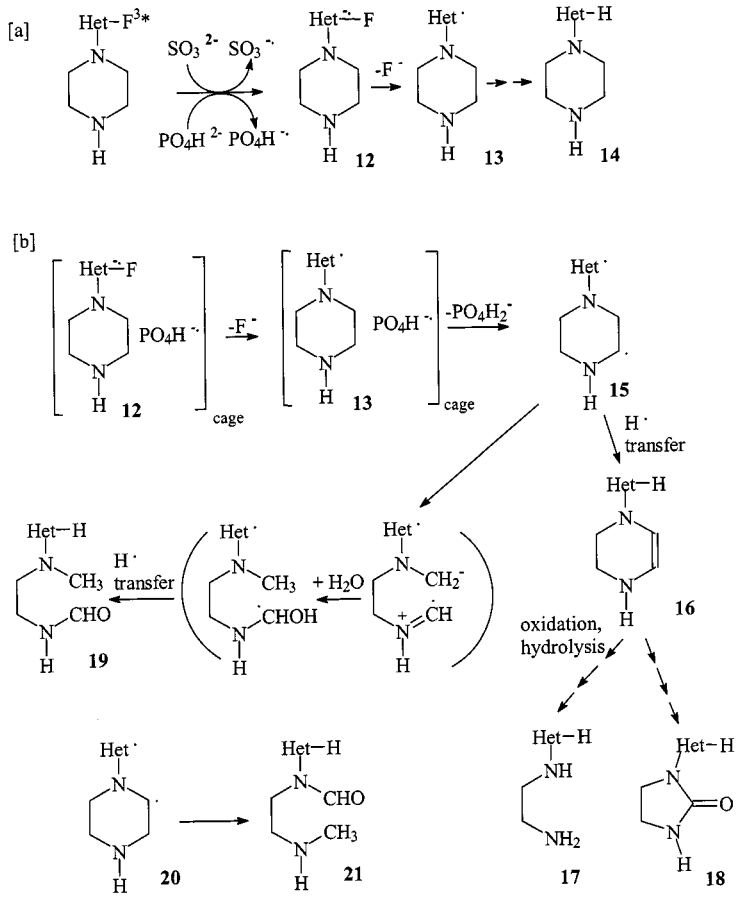
The reduction potential of triplet **1b** may be estimated on the basis of the ground state E_{red} (−1.35 V vs. Ag/AgCl)[12] by adding the favourable contribution represented by the triplet energy (ca. 2.8 eV),[8] thus giving E_{red} (**1b**^{3}*) ≈ 1.45 V. The potential of the couple $HPO_4^{-\cdot}/HPO_4^{2-}$ has not yet been reported, but we estimate it to be significantly lower than 1.9 V vs. NHE. This is in agreement with the weaker oxidative power of $HPO_4^{-\cdot}$ as compared to $H_2PO_4^{\cdot}$ (the potential of the couple $H_2PO_4^{\cdot}/H_2PO_4^-$ is close to 2.4 V vs. NHE)[17] and is inferred by taking into account the fact that $HPO_4^{-\cdot}$ is capable of oxidizing bromide to bromine with a rate constant $6.5\cdot10^6$ M^{-1} s^{-1}.[18] This relatively slow rate is consistent with a potential lower by several tenths of a volt than that of the Br/Br^- couple, viz. 1.92 V vs. NHE,[17] on the basis of the Marcus (or Rehm−Weller) equations for electron-transfer rates.[19]

The assignment of the 670 nm transient band exhibited by **1b** in phosphate buffer — identical to that in sulfite buffer — to the radical anion **1b**$^{-\cdot}$ is a revision of our previous assignment of this band to the defluorinated cation,[8] proposed prior to our studies in neat water and in sulfite buffer.

As for the chemistry occurring under these conditions, defluorination at the radical anion stage (formula **12** in Scheme 4) is certainly an inefficient process. Previous literature shows that other nucleofugal groups (e.g. cyano in fluoro- and polyfluorobenzonitriles) are preferentially cleaved when simultaneously present with fluoro in the cathodic reduction of such aromatics.[14,20] It is therefore not surprising that a large proportion of the radical anions undergoes back electron transfer to give the starting heterocycle, and that the quantum yield in both buffers is lower than that in neat water. This fragmentation is the key step in both cathodic reduction and photolysis in both buffers (notice that in the case of difluorinated **1c**, the fluorine atom in the 8-position is selectively cleaved under all three conditions) and gives radical **13**. In a reducing environment, as at a cathode or in sulfite buffer, the radical is simply reduced to defluorinated quinolone **14** and no further process intervenes. In phosphate, however, the overall process involves fluorine loss but does not correspond to simple defluorination. The reasons are twofold: first, the environment is non-reducing, the only potential hydrogen donor being the piperazine group, and second, the electron-transfer step in this case generates an aggressive inorganic intermediate, the radical anion $HPO_4^{-\cdot}$. It has previously been demonstrated that this species is an efficient hydrogen abstractor ($k_H = 7-9\cdot10^7$ $M^{-1}s^{-1}$ with pentoses in water).[21]

The presence of easily abstractable hydrogens in the piperazinyl chain, in particular those α to the aliphatic amino nitrogen (N-4′), determines the following course of the reaction. We suggest that fluoride loss and hydrogen transfer to the phosphate radical anion occur "in cage" prior to diffusion. This is indicated by the fact that the products of simple defluorination (**14** in Scheme 4; **3** in Scheme 1) are formed only in trace amounts upon photolysis in phosphate buffer and that only a minor product, in which the piperazine moiety has been degraded but the fluorine atom has not been lost, can be isolated (see product **5** in Scheme 2). Furthermore, the experiment in which a mixture of **1b** and **2b** was irradiated in phosphate buffer, where the former was found to be degraded much more rapidly, lends further support to the view that $HPO_4^{-\cdot}$ arises only from the reduction of triplet **1b** and reacts mainly "in cage" by attack on the same molecule in the vicinity in which it was generated.

This is in accordance with the fact that, despite the high intrinsic rate for intermolecular H-abstraction (see above), the low concentration of the heterocycles makes attack on the piperazine chain a relatively slow process (rates ≤ $2\cdot10^4$ s^{-1}), which, in turn, makes other decay paths of the phosphate radical anion competitive. This explains the fact that all of the principal end products have undergone both de-



Scheme 4. Proposed mechanism for the photochemistry of piperazinofluoroquinolones **1a−c** in phosphate buffer (Het = quinolone moiety); protonation equilibria are not explicitly indicated

fluorination and side-chain degradation and not just one of these processes.

According to this mechanism, diradical **15** is generated. A possible rationalization of the formation of the isolated products is shown in Scheme 4b, where two paths are considered, both of which are apparently favoured by the aqueous medium. In the first, hydrogen transfer gives enediamine **16**, which would easily undergo hydrolysis and oxidation reactions. This would explain the degradation of the piperazine chain to an aminoethylamino group (see formula **17**, corresponding to products **8** and **9** in the case of **1c**, according to whether the methyl-substituted or -unsubstituted α-carbon is attacked initially) or to an imidazolinone ring (see formula **18**, corresponding to product **10** from **1c**). In the second path, hydrogen transfer is accompanied by C−C bond fragmentation and the addition of water. A hypothetical mechanism is outlined in Scheme 4, where the *N*-methyl *N*-formylaminoethyl aminoquinolone **19** is formed via a hydroxyalkyl diradical, although the sequence of events could well be different. Compound **19** corresponds to amide **6** obtained as the main product from **1b** (Scheme 2). Less favoured hydrogen abstraction from the position α to the aromatic amino nitrogen would lead to the related diradical **20** and thence to the isomeric amide **21** (corresponding to compound **7** obtained from **1b** as a minor product).

Thus, reductive defluorination of the aromatic ring and oxidative degradation of the side chain both involve the phosphate anion, which acts as an electron donor in the first step, thereby forming the corresponding radical anion, which acts as a hydrogen abstractor in the latter step. This process is less efficient than direct reaction of the triplet state in water, but only slightly so in the case of **1b**. In agreement with this fact, the formation of product **2b**, arising directly from the triplet state, is quenched in proportion to the flash-photolytically measured k_q for this state, but the overall reaction of **1b** is retarded by phosphate to a smaller extent (the steady-state measured K_{SV} is smaller than $k_q\tau$ from measurement of the transient band).

Finally, out-of-cage reactions are, as discussed above, minor paths with the phosphate radical anion, although other radicals may contribute to the overall consumption of the reagent. Their role is indicated by the isolation of minor photoproducts in which the piperazine side-chain has been substituted by an alkyl or acyl group (products **5** and **11**; the latter retaining a fluoro substituent). This suggests that C-centred radicals are formed from the degradation of the

piperazine group, which then diffuse and trigger further degradation processes.

Conclusion

The work described herein shows the unexpected efficiency of the phosphate anion as a reducing agent for excited states, which, coupled with the radical reactivity of the phosphate radical anion, leads to a photoreaction of fluorinated aminoquinolones that has no precedent in the photochemistry of heterocycles.

With regard to studies of drug photochemistry[22] and its biological relevance, a subject which is attracting increasing attention, two points are worth mentioning. First, this work suggests that care should be taken in comparing photoreactivity data with photobiological studies (e.g. phototoxicity determinations), since the latter, in contrast to the former, are usually carried out in phosphate buffer. As is apparent in the present case, this may lead to major deviations in the photochemical reactivity since the phosphate anion participates in the photochemical reaction, rather than acting merely as a buffer as is often naively assumed. Second, the highly efficient interaction of phosphate ions with triplet fluoroquinolones suggests that partially alkylated phosphates may behave similarly. In the presence of such substrates, e.g. of phosphorylated sugars, radical processes of the type discussed above may be initiated. Thus, it may be that the reported DNA photocleavage caused by fluoroquinolones,[23] a phenomenon that is related to the remarkable phototoxicity of these molecules, widely used as bactericidal drugs, involves a reaction with the phosphorylated deoxyribose units. Clearly, more work is needed in this field.

Experimental Section

Materials and Instrumentation: Norfloxacin (**1a**) and enoxacin (**1b**) were purchased from Sigma Chemicals Co. (Milan) and were used without further purification. Lomefloxacin hydrochloride, from the same supplier, was dissolved in water (0.02 M solution) and 1 M NaOH was added until the solution was neutral. The free base **1c** (m.p. 231−234 °C) was extracted with chloroform. All other chemicals and solvents were reagent grade or better. The pH 7.4 phosphate buffer was prepared using 29.38 g $Na_2HPO_4{\cdot}12H_2O$ and 2.48 g KH_2PO_4 per litre to give a 0.1 M solution. − NMR spectra were recorded on a Bruker 300 instrument, mass spectra on a Finnigan LCQ instrument. − Elemental analyses were carried out on a Carlo Erba instrument.

Preparative Irradiations: $1-2 \times 10^{-4}$ M solutions of the drugs in water or the appropriate buffer (1.4 L) were purged with nitrogen for 1 h and then irradiated in an immersion well apparatus by means of a Pyrex-filtered 500 W medium-pressure mercury arc (Helios Italquartz) at 17 °C. The course of each reaction was monitored by HPLC (see below). When a convenient (50−90%) fraction of the starting material had been consumed, the aqueous solution was adjusted to neutrality and treated as detailed in each specific case.

Photolysis of 1a: The irradiated aqueous solution was extracted with chloroform (3×0.45 L), and the combined organic phases were washed with water and dried. Chromatography of the residue on a silica gel column (100 g, Millipore 60 Å, 35−70 μm, chloroform/methanol mixture from 99:1 to 90:10 as eluent) gave a single characterized product (**5**) in a trace amount. The use of $CHCl_3$ containing EtOCOCl for the extraction (see below) did not allow the isolation of further products.

1-Ethyl-1,4-dihydro-7-methyl-4-oxoquinoline-3-carboxylic Acid (5): Colourless solid following chromatography. − MS: *m*/*z* = 231 [M^+]. − ^{1}H NMR ($CDCl_3$): δ = 1.6 (br. t, *J* = 7 Hz, 3 H), 2.6 (s, 3 H), 4.6 (m, 2 H), 7.4 (br. s, 1 H), 7.42 (br. d, *J* = 9 Hz, 1 H), 8.4 (d, *J* = 9 Hz, 1 H), 8.75 (s, 1 H), 14.0 (br. s, 1 H). − ^{13}C NMR ($CDCl_3$): δ = 14.4, 22.3, 49.5, 108.6, 115.8, 124.3, 127.1, 127.7, 139.2, 145.4, 147.6, 167.2, 178.2.

Photolysis of 1b: The irradiated solution was extracted with chloroform (3×0.45 L), and the combined organic phases were washed with water, dried, and concentrated. The residue was recrystallized from methanol to give **6**.

1-Ethyl-7-[*N*-(2-formylaminoethyl)-*N*-methylamino]-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxyilic Acid (6): Light-yellow crystals, m.p. > 260 °C (methanol). − $C_{15}H_{18}N_4O_4$: calcd. C 56.59, H 5.70, N 17.60; found C 55.3, H 5.8, N 14.2. − ^{1}H NMR ([D_6]DMSO, 60 °C): δ = 1.4 (t, *J* = 7 Hz, 3 H), 3.2 (s, 3 H), 3.4 (q, *J* = 6.5 Hz, 2 H), 3.8 (t, *J* = 6.5 Hz, 2 H), 4.5 (q, *J* = 7 Hz, 2 H), 7.05 (d, *J* = 9 Hz, 1 H), 7.95 (br. t, exch., *J* = 6.5 Hz, 1 H), 8.0 (s, 1 H), 8.3 (d, *J* = 9 Hz, 1 H), 8.9 (s, 1 H), 15.8 (br. s, 1 H). − ^{13}C NMR ([D_6]DMSO, 60 °C): δ = 18.7, 36.7, 40.6, 50.4, 53.1, 111.5, 111.9, 114.4, 139.1, 151.4, 153.3, 163.1, 165.4, 170.0, 180.4.

The aqueous phase was extracted with chloroform (3×0.45 L) containing 1% ethyl chloroformate, and the combined organic phases were washed with $NaHCO_3$ until neutral, then with water, and then dried. Freshly prepared ethereal diazomethane was added; after 30 min the excess reagent was decomposed with acetic acid and the reaction mixture was concentrated. The residue was chromatographed on a silica gel column (60 g, Millipore 60 Å, 35−70 μm, chloroform/methanol mixture from 99:1 to 90:10 as eluent) to give the *N*-ethyloxycarbonyl derivative methyl ester of compound **6** as well as Methyl 1-Ethyl-7-{*N*-[2-(*N*′-ethyloxycarbonyl-*N*′-methylamino)ethyl]-*N*-formylamino}-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**7′**, , the *N*-ethyloxycarbonyl methyl ester of compound **7**, see Scheme 2).

Methyl 1-Ethyl-7-{*N*-[2-(*N*′-ethyloxycarbonyl-*N*′-methylamino)-ethyl]-*N*-formylamino}-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (7′): Light-yellow solid following chromatography. − MS: *m*/*z* = 390 [M^+]. − ^{1}H NMR [$(CD_3)_2CO$]: δ = 1.25 (t, *J* = 7 Hz, 3 H), 1.43 (t, *J* = 7 Hz, 3 H), 3.2 (s, 3 H), 3.7 (s, 3 H), 3.85−4.0 (m, 4 H), 4.25 (q, *J* = 7 Hz, 2 H), 4.45 (q, *J* = 7 Hz, 2 H), 6.8 (d, *J* = 9 Hz, 1 H), 8.3 (d, *J* = 9 Hz, 1 H), 8.53 (s, 1 H), 9.15 (s, 1 H). − ^{13}C NMR [$(CD_3)_2CO$]: δ = 14.8, 15.8, 37.5, 39.0, 47.0, 49.0, 51.7, 64.5, 105.9, 115.4, 138.1, 148.8, 150.5, 155.0, 160.2, 163.8, 166.4, 174.0.

Photolysis of 1c: The irradiated aqueous solution was treated with 1% EtOCOCl in $CHCl_3$ and then with CH_2N_2 as in the case of **1b** (see above). The residue was chromatographed on a silica gel column (120 g, Millipore 60 Å, chloroform/methanol mixture from 99:1 to 90:10 as eluent) to give the following compounds, all as *N*-ethyloxycarbonyl derivative methyl esters (indicated with primed numbers), along with some functionalized starting material (**1′**).

Methyl 1-Ethyl-7-[*N*-(2-ethyloxycarbonylaminoethyl)amino]-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (8′): Colourless

crystals, m.p. 237 °C (ethanol). − $C_{18}H_{22}FN_3O_5$ (379.4): calcd. C 56.98, H 5.85, N 11.08; found C 56.9, H 5.8, N 10.8. − ^{1}H NMR ($CDCl_3$): δ = 1.2 (d, *J* = 7 Hz, 3 H), 1.4 (t, *J* = 7 Hz, 3 H), 3.35 (m, 2 H), 3.5 (m, 2 H), 3.8 (m, 2 H), 4.05 (q, *J* = 7 Hz, 2 H), 4.25 (q, *J* = 7 Hz, 2 H), 5.5 (s, exch., 1 H), 5.9 (s, exch., 1 H), 6.7 (d, *J* = 6 Hz, 1 H), 7.75 (d, *J* = 12 Hz, 1 H), 8.4 (s, 1 H). − ^{13}C NMR (CD_3OD): δ = 15.6, 15.7, 40.6, 44.3, 51.5, 53.5, 62.5, 97.5, 109.0, 111.9 (d, J_{C-F} = 20 Hz), 115.2, 140.2, 145.1, 150.7 (d, J_{C-F} = 7 Hz), 152.3 (d, J_{C-F} = 243 Hz), 160.2, 165.1, 176.5.

Methyl 1-Ethyl-7-{*N*-[2-(ethyloxycarbonylamino)propyl]amino}-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (9′): Light-yellow solid following chromatography. MS: *m*/*z* = 393 [M^+]. − ^{1}H NMR (CD_3CN): δ = 1.25 (t, *J* = 7 Hz, 3 H), 1.28 (d, *J* = 7 Hz, 3 H), 1.45 (d, *J* = 7 Hz, 3 H), 3.2 and 3.4 (two m, 2 H), 3.7 (s, 3 H), 3.9 (sept, *J* = 7 Hz, 1 H), 4.05 (q, *J* = 7 Hz, 2 H), 4.25 (m, 2 H), 5.65 (s, exch., 1 H), 5.7 (d, exch., *J* = 7 Hz, 1 H), 6.85 (d, *J* = 7 Hz, 1 H), 7.98 (d, *J* = 12 Hz, 1 H), 8.4 (s, 1 H). − ^{13}C NMR (CD_3CN): δ = 13.8, 13.9, 17.4, 45.4, 48.5, 48.8, 50.8, 60.3, 96.1, 108.9, 110.0 (d, J_{C-F} = 20 Hz), 118.6, 137.6, 141.4 (d, J_{C-F} = 14 Hz), 149.6 (d, J_{C-F} = 230 Hz), 148.2, 156.7, 165.7, 172.4.

Methyl 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-2-oxotetrahydro-1-pyrazolyl)-4-oxoquinoline-3-carboxylate (10′): Light-yellow solid following chromatography. − MS: *m*/*z* = 347 [M^+]. − ^{1}H NMR ($CDCl_3$): δ = 1.4 (d, *J* = 7 Hz, 3 H), 1.55 (t, *J* = 7 Hz, 3 H), 3.7 (s, 3 H), 3.75 (ddd, *J* = 2.5, 8, 10 Hz, 1 H), 4.02 (m, 1 H), 4.22 (q, *J* = 7 Hz, 2 H), 4.28 (ddd, *J* = 2.5, 8, 10 Hz, 1 H), 5.05 (s, exch., 1 H), 8.15 (d, *J* = 5 Hz, 1 H), 8.21 (d, *J* = 12 Hz, 1 H), 8.51 (s, 1 H). − ^{13}C NMR ($CDCl_3$): δ = 14.1, 21.1, 45.3, 49.1, 51.0, 53.8 (d, J_{C-F} = 10.5 Hz), 109.7, 11.7 (d, J_{C-F} = 22.5 Hz), 113.9 (d, J_{C-F} = 22.5 Hz), 126.3, 131.9 (d, J_{C-F} = 12 Hz), 135.0, 148.6, 152.4 (d, J_{C-F} = 262.5 Hz), 158.6, 166.2, 172.7.

Methyl 7-Acetyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (11′): Light-yellow solid following chromatography. − MS: *m*/*z* = 291 [M^+]. − ^{1}H NMR ($CDCl_3$): δ = 1.6 (t, *J* = 7 Hz, 3 H), 2.7 (d, *J* = 5 Hz, 3 H), 3.9 (s, 3 H), 4.4 (q, *J* = 7 Hz, 2 H), 8.15 (d, *J* = 5 Hz, 1 H), 8.3 (d, *J* = 11 Hz, 1 H), 8.6 (s, 1 H). − ^{13}C NMR ($CDCl_3$): δ = 14.4, 31.4 (d, J_{C-F} = 8 Hz), 45.1, 51.9, 110.3, 114.9 (d, J_{C-F} = 26 Hz), 118.9 (d, J_{C-F} = 3 Hz), 133.4, 145.3, 149.3, 158.4 (d, J_{C-F} = 253 Hz), 125.1 (d, J_{C-F} = 7.5 Hz), 165.7, 172.5, 194.7 (d, J_{C-F} = 5 Hz).

Small-Scale Experiments: Small-scale experiments were carried out by irradiating 5 or 10 mL portions of aqueous solutions of the heterocycles (0.1 mM) in septum-capped quartz tubes. These were purged with argon for 1 h and then irradiated in a merry-go-round apparatus by means of two phosphor-coated lamps (emission centred at 310 nm). The substrate decomposition was monitored by HPLC. The photon flux was measured by ferrioxalate actinometry.

Laser Flash Photolysis: As will be reported in detail in a separate publication,[15] photolyses were carried out using an Nd-YAG from JK Lasers [pulse 20 ns full-width at half-maximum (FWHM), 355 nm] (for details of the detection system, see ref.[24]). The laser beam was focused on a 3 mm high and 10 mm wide rectangular area of the cell and the first 2 mm were analysed at a right-angle geometry. The energy used was ca. 2−4 mJ/pulse. Spectral resolution was 2 nm. The sample absorbance was 0.2−0.5 at 355 nm over 1 cm. Oxygen was removed by vigorous bubbling with a steady stream of argon, which had previously passed through a water trap to prevent evaporation of the sample. Care was taken to renew the solution after each laser shot. The temperature was 295 ± 2 K.

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