Photochemistry of 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic Acid (= Ciprofloxacin) in Aqueous Solutions

by **Mariella Mella**, **Elisa Fasani**, and **Angelo Albini***

Department of Organic Chemistry, University of Pavia Via Taramelli 10, I-27100 Pavia

Dedicated to Professor *André M. Braun* on occasion of his 60th birthday.

The 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid (= ciprofloxacin; **1**) undergoes low-efficiency ($\Phi = 0.07$) substitution of the 6-fluoro by an OH group on irradiation in H_2O *via* the $\pi\pi^*$ triplet (detected by flash photolysis, λ_{max} 610 nm, τ 1.5 μs). Decarboxylation is a minor process ($\leq 5\%$). The addition of sodium sulfite or phosphate changes the course of the reaction under neutral conditions. Reductive defluorination is the main process in the first case, while defluorination is accompanied by degradation of the piperazine moiety in the presence of phosphate. In both cases, the initial step is electron-transfer quenching of the triplet ($k_q = 2.3 \cdot 10^8$ M^{-1} s^{-1} and $2.2 \cdot 10^7$ M^{-1} s^{-1}, respectively). Oxoquinoline derivative **1** is much more photostable under acidic conditions, and in this case the F-atom is conserved, and the piperazine group is stepwise degraded ($\Phi = 0.001$).

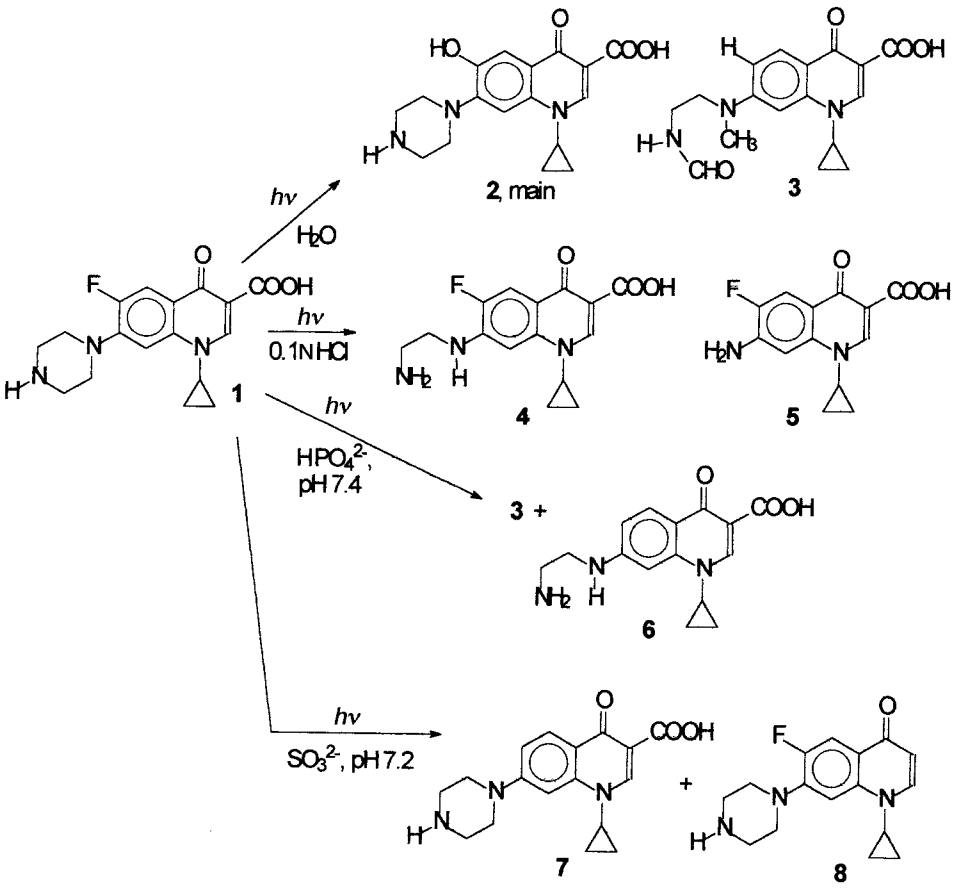
1. Introduction. – A large number of 1-alkyl-7-(dialkylamino)-6-fluoro-1,4-dihydro-4-oxoquinolinecarboxylic acids with various substituents at the benzo moiety are used in therapy (3rd-generation fluoroquinolone antibacterials; quinolone = quinolinone). Overall, these are considered well-tolerated drugs; however, one of the important exceptions is phototoxicity. This adverse effect has been reported for all of these derivatives, though to a different degree, and in fact, it is recommended that exposure to UV light should be minimized during therapy [1]. It is unusual that a class of drugs presents light-related adverse effects of such seriousness (genotoxicity and tumorigenesis have been reported [2]) and generality, and this has stimulated an intense effort towards the elucidation of the biological mechanism underlying to such effects. Different hypotheses are being considered at the moment, involving some form of oxygen sensitization, the generation of radicals, or the formation of a reactive intermediate, such as a carbene or an ion, by photofragmentation of the drug [3].

In parallel with biologically oriented work, photochemical studies have also been carried out and have significantly contributed to the rationalization of this effect. The reported photochemistry is highly dependent on the structure and on conditions. The main processes observed are degradation of the alkylamino side chain [4–6] and substitution of the ring F-atom [4][6][7]. The latter process is quite efficient with some derivatives of the series. Processes observed only in some cases are decarboxylation [5d][8] as well as loss of the 1-alkyl chain, when this is a cyclopropyl group [6a][7b]. Product studies have been supplemented by photophysical investigations, mainly by laser flash photolysis [4d,e][8–10], aimed to establishing the complex mechanism involved in the above photoreactions.

The 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid (=ciprofloxacin; **1**) is the most frequently used antimicrobial of this family and has been early reported to be photounstable [11]. Mass-spectrometry evidence has suggested that a photodimer of unknown structure is formed [12], while further studies have led to the isolation of products resulting from the degradation of the piperazinyl side chain [13]. On the basis of our studies on the photochemistry of related molecules [4][8], the fact that defluorination had not been detected was difficult to account for. This fact as well as our continuing interest in the field and the importance of compound **1** as a drug encouraged us to carry out a detailed study of its photochemistry.

2. Results. – 2.1. *Product Studies.* Product studies were carried out by irradiating Ar-flushed $3 \cdot 10^{-4}$ M aqueous solutions of oxoquinoline derivative **1** [14] by means of a *Pyrex*-filtered medium-pressure Hg arc (*Scheme 1*). The course of the reaction was monitored by HPLC (see *Exper. Part*), showing the formation of a main peak and several minor components. Extensively photodecomposed (*ca.* 80%) solutions were

Scheme 1



analysed. The main product **2** was obtained by reversed-phase chromatography after extraction of residual starting material and minor products by stirring with ethyl carbonochloridate in $CHCl_3$. The skeleton and the substitution pattern of **2** was unchanged as compared to **1**, but the F-atom was lost. Analytical and spectroscopic properties showed that an OH group was present at position 6 of the quinoline moiety of **2**. In a separate experiment, extraction of the irradiated aqueous solution with $CHCl_3$ and recrystallization of the residue from the organic phase gave the most abundant product **3** among the minor components. Compound **3** was again an F-free compound, with position 6 unsubstituted and a deep-seated degradation of the piperazine moiety to a [2-(formylamino)ethyl]methylamino group. The initial pH of the solution was 6.2, and it decreased to 4.5 during the irradiation. The photochemistry was also checked after addition of $5 \cdot 10^{-4}$ M $NaHCO_3$, in order to maintain the pH at 7.2 during the course of the reaction, and the products formed and the rate of reaction were practically the same as above.

The irradiation was then carried out in 0.1N HCl. The reaction was much slower under this condition, and the product distribution was different, as clearly shown by the HPLC trace. At *ca.* 50% conversion, the 7-[(2-aminoethyl)amino]-6-fluoroquinoline derivative **4** (*Scheme 1*) was the main product. At longer irradiation times, product **4** was degraded in turn, and the 7-amino-6-fluoroquinoline derivative **5** accumulated. Both products **4** and **5** have been previously detected by *Torniainen et al.* [13c,d]. These were formed only to a very minor extent under neutral conditions.

Following the approach we used with related molecules, the photochemistry of **1** was explored in saline solutions at neutral pH. In phosphate buffer (pH 7.4, 0.01M total phosphate), the reaction was slower than in neat water, and product **2** was not formed. On the contrary, compound **3**, obtained in a small amount by irradiation in neat H_2O, was now the main product. Several other products were formed, one of which was isolated as the *N*,*N*′-bis(ethoxycarbonyl) derivative of product **6**[1]) (*Scheme 1*).

The photochemistry in sulfite buffer (pH 7.2, 0.01M total sulfite) was again different. Functionalization and extraction allowed to obtain the main product, identified as the F-free derivative **7**[1]), and one of the minor ones, the fluorinated quinoline derivative **8**[1]) (*Scheme 1*), both of them isolated as the corresponding *N*-(ethoxycarbonyl) derivatives.

2.2. *Kinetic Measurements.* The reaction quantum yield was measured by using $1 \cdot 10^{-4}$ M solutions of **1** and limiting the conversion to *ca.* 25%. The extent of conversion of **1** and the formation of the F^- anion were measured. The measurements were extended to the further conditions used in the preparative experiments. The results (see *Table*) show that addition of sulfite or phosphate as well as of acids strongly decreased the value of Φ. Furthermore, liberated fluoride corresponded to *ca.* 95% of the substrate consumed in neutral H_2O and remained $>65\%$ in the presence of sulfite or phosphate buffer, but dropped to 25% in 0.1N HCl.

The effect of the above salts was also evaluated by measuring the yields of the main product in the presence of phosphate (compound **3**) or of sulfite (compound **7**) *vs.* the yield of the main product in their absence (compound **2**), under different conditions. As

---

[1]) In *Scheme 1*, the initially formed products **6**–**8** are shown; the actually isolated corresponding derivatives are described in the *Exper. Part.*

Table. *Reaction Quantum Yield of the Conversion of* **1** *and Quantity of Fluoride Anion Formed under Different Reaction Conditions*

	Neat H_2O[b])	Phosphate buffer (0.007M)	Sulfite buffer (0.002M)	HCl (0.1N)
Φ_r	0.07	0.02	0.03	0.001
% F^-[a])	95	82	65	25

[a]) With respect to the number of mol-equiv. of consumed compound **1**. [b]) No difference in neat H_2O (pH 6.2) or in the presence of $NaHCO_3$ solution (pH 7.2) was observed.

shown in *Fig. 1*, the ratios [**3**]/[**2**] and [**7**]/[**2**] linearly depended on the concentration of the added salt.

The steady-state measurements were complemented by some time-resolved investigations. Laser flash photolysis of an aqueous solution of **1** evidenced a conspicuous transient in the μs range at *ca.* 610 nm (*Fig. 2*). This was not observed in O_2-equilibrated solutions. The transient was apparent at pH 7.2 (with $NaHCO_3$) and at pH 6.2, but under acidic conditions, the intensity was strongly reduced with a very weak signal at $pH \leq 4$ (*Fig. 3*), nor was any other transient observed in the range 360–650 nm. In the same pH range, the fluorescence shifted to the red (λ_{max} from 408 to 446 nm). Under neutral conditions, the transient was quenched by addition of both phosphate and sulfite. The measured quenching constants by these salts were $k_q = 2.2 \cdot 10^7$ M^{-1} s^{-1} and $2.3 \cdot 10^8$ M^{-1} s^{-1}, respectively.

3. Discussion. – 3.1. *Preamble.* The present investigation showed that, under acidic conditions, the main photochemical paths for ciprofloxacin (**1**) was degradation of the piperazinyl side chain to give products **4** and **5**, previously identified by *Tornianinen et al.* [13c,d]. This was a quite inefficient reaction ($\Phi = 1 \cdot 10^{-3}$). On the other hand, defluorination was by far the main process under neutral conditions, and led to

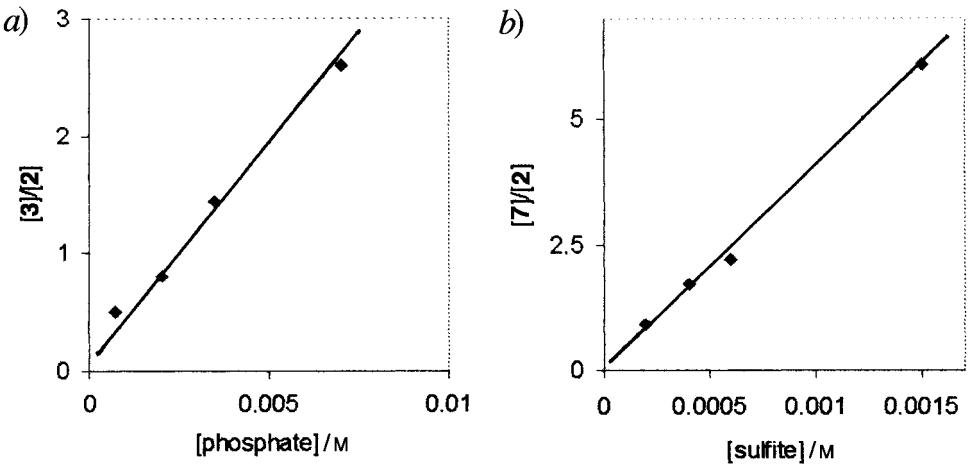


Fig. 1. *Product distribution* a) [**3**] vs. [**2**] *and* b) [**7**] vs. [**2**] *on irradiation of compound* **1** *as a function of the concentration of phosphate and sulfite buffer, respectively*

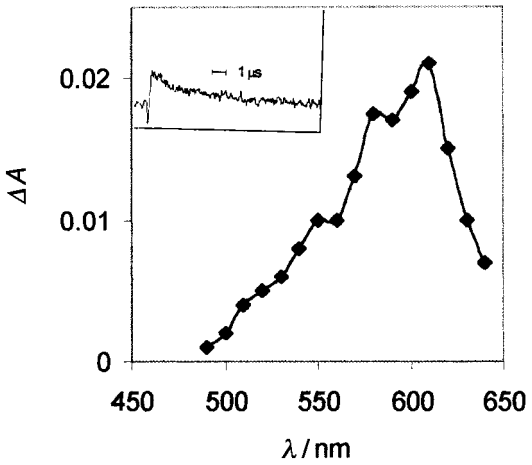


Fig. 2. *End-of-pulse absorption upon flashing an aqueous solution of compound* **1**. Inset: Time profile at 590 nm.

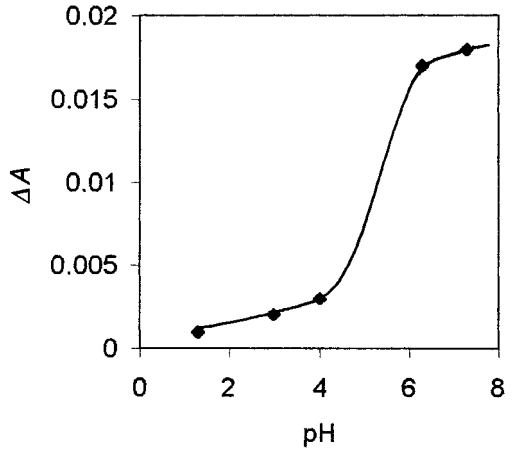


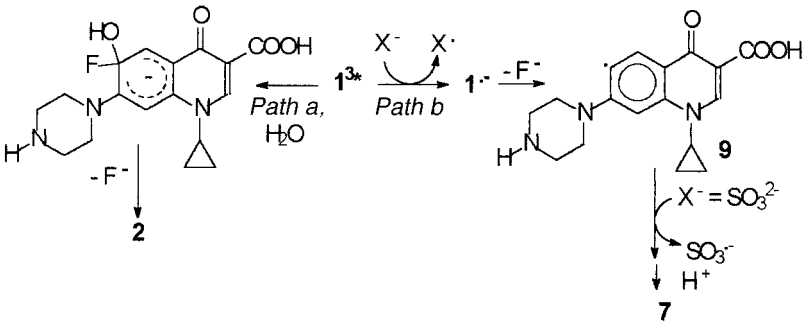
Fig. 3. *Intensity of the end-of-pulse 590-nm absorption upon flashing an aqueous solution of compound* **1** *at different pH values*

photoproducts of different structure depending on conditions, in particular in the presence of sulfite and phosphate.

3.2. *Triplet State.* The mechanistic evidence obtained and the comparison with the results previously reported for related fluoroquinolinones offered a rationale for the complex photochemistry of **1** observed. A transient was well apparent in neutral aqueous solution, and wavelength distribution (λ_{max} 610 nm), time profile (τ 1.5 µs, see *Fig. 2*), and sensitivity to O_2 were analogous to what has been observed with structurally related norfloxacin (aza-substituted at position 8) and ofloxacin (with an alkoxy group at position 8) [9][10]. In view of the similarity and of the evidence obtained in those cases, the transient was confidently assigned to the T-T absorption.

3.3. S_N *(Ar) Reaction.* The close correspondence between the modifications in the course of the photoreaction and the quenching of this transient (see below) allowed to conclude that the triplet state was the reactive state of **1** in neutral water. Substitution of an F-atom by an OH group at the ring occurred with low efficiency (Φ 0.07), finally giving product **2**. Analogously to what has been observed with norfloxacin [4c], and more generally with other photoinduced nucleophilic aromatic substitutions [15], the process reasonably occurred *via* an addition-elimination mechanism (S_N2Ar^* mechanism, see *Scheme 2*, *Path a*), indicating the much stronger electrophilicity of the triplet with respect to the ground state.

Scheme 2



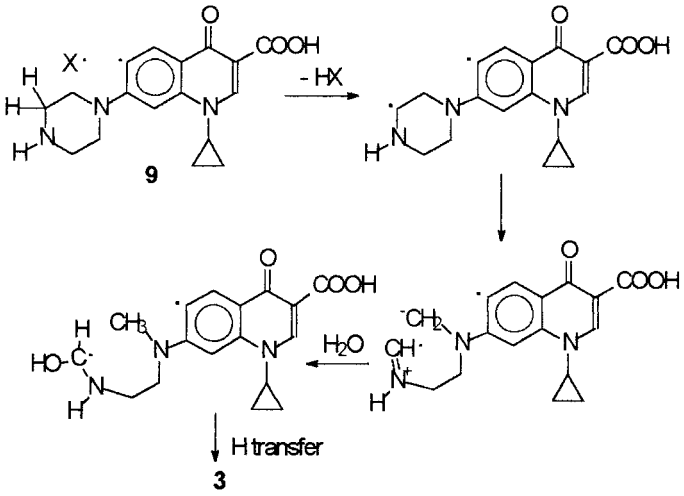
3.4. *Reduction of the Triplet.* Both T-T absorption and formation of hydroxyquinoline derivative **2** from **1** were efficiently quenched both by sulfite and by phophate (see below for a quantitative evaluation). This was again analogous to what has been observed with norfloxacin, and we offer the same rationalization based on the reduction of the triplet (*Scheme 2*, *Path b*, $X^- = SO_3^{2-}$ or HPO_4^{2-}). The triplet-reduction potential, $E_{red}(T) = E_{red} + E_{el}(T)$, was *ca.* 1.5 V *vs.* NHE for compound **1** and related fluoroquinolinones ($E_{red} = -1.3$ V, $E_{el}(T)$ *ca.* 2.8 eV) [3b][9][16]. This made reduction by sulfite ($E(SO_3^{2-\bullet}/SO_3^{2-}) = 0.63$ V, $E(SO_3^{-\bullet}/HSO_3^-) = 0.84$ V) [17] a markedly exothermic process and reduction by phosphate ($E(HPO_4^{-\bullet}/HPO_4^{2-})$ not available, but estimated to be significantly lower than 1.9 V) [9][18] probably close to thermoneutral. Such an evaluation was in accord with the highly efficient quenching of $\mathbf{1}^{3*}$ by sulfite ($2.3 \cdot 10^8$ M^{-1} s^{-1}) and the still remarkable quenching by phosphate ($2.2 \cdot 10^7$ M^{-1} s^{-1}).

The radical anion $\mathbf{1}^{\bullet-}$ formed from the electron-transfer step underwent defluorination to yield an aryl radical **9**. In the presence of a good reducing agent such as sulfite, the sequence was completed by a second electron-transfer step and protonation leading to F-free quinoline derivative **7** (see *Scheme 2*). In fact, with related fluoroquinolinones, we found such a reductive defluorination to occur both by irradiation in the presence of sulfite and by cathodic reduction [19].

This reaction did not take place in the case of phosphate, where the main product, **3**, resulted from reductive ring defluorination coupled with oxidative degradation of the piperazinyl group. Since no products resulting from only one of these processes was isolated (in contrast to other conditions, see below), a stepwise mechanism leading to the final product should be proposed. A speculative mechanism is depicted in

Scheme 3, where the phosphate radical anion ($X^{\bullet} = HPO_4^{\bullet 2-}$) co-formed in the electron-transfer step (and known as H-abstracting species [20]) abstracted a H-atom from the side chain in the cage, and the diradical underwent ring cleavage and H_2O addition to finally give the (formylamino)ethyl derivative **3** or, through a more deep-seated degradation, diamino derivative **6**, the major and the minor product, respectively, isolated in phosphate buffer. Both an analogue of **3** (from a related 1,8-naphtyridinone, enoxacin) and an analogue of **6** (from a 6,8-difluoroquinolinone, lomefloxacin) have been previously isolated [4c].

Scheme 3



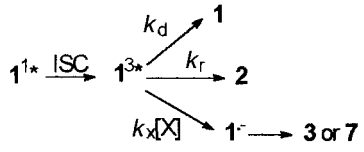
Apart from the details of the mechanistic sequence, the steady-state analysis in *Fig. 1* connects quenching of triplet **1** by the above anions and formation of products **7** and **3**. According to the competition shown in *Scheme 4*, the quantum yield for the formation of product **2** (Φ_2) and that for the formation of either product **7** or **3** (Φ_X) in the presence of the anions (X) are given by *Eqn. 1* and *2*, respectively, and their ratio by *Eqn. 3*.

$$\Phi_2 = \Phi_{isc}\, k_r/(k_d + k_r + k_x\,[X]) \tag{1}$$

$$\Phi_X = \Phi_{isc}\, k_x\,[X]/(k_d + k_r + k_x\,[X]) \tag{2}$$

$$\Phi_x/\Phi_2 = k_x\,[X]/k_r \tag{3}$$

Scheme 4

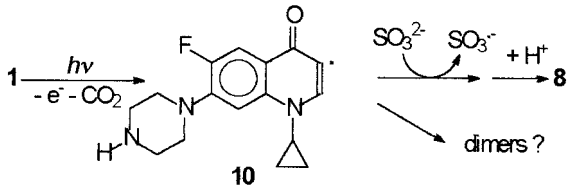


The products ratios [**3**]/[**2**] and [**7**]/[**2**] depended linearly on [X] (*Fig. 1*). These plots give $k_x/k_r = 3.5 \cdot 10^2$ M^{-1} for phosphate and $4 \cdot 10^3$ M^{-1} for sulfite. Previous determinations by the singlet depletion method suggested that $\Phi_{isc} \geq 0.5$ with related fluoroquinolinones [7c][20], although experiments based on energy transfer to acenaphthenone indicated a lower value ($\Phi_{isc} = 0.33$) [10]. Taking the 0.5 value and considering that in the absence of added anions, $\Phi_2 = \Phi_{isc}\ k_r/(k_d + k_r) = 0.07$ (*Table*), the value $k_r/(k_d + k_r) = 0.12$ was obtained. On the basis of the measured rate of triplet decay ($k_d + k_r \approx 7 \cdot 10^5$ s^{-1}, the pseudounimolecular rate of reaction with H_2O (as the solvent) was $k_r \approx 9 \cdot 10^4$ s^{-1}. With the above ratios k_x/k_r, this gives $k_x \approx 3.1 \cdot 10^7$ M^{-1} s^{-1} for phosphate and $3.6 \cdot 10^8$ M^{-1} s^{-1} for sulfite. The reasonable accord with the direct measurements of k_x (see above) supports that products **3** and **7** arise *via* quenching of the triplet by anion X.

Another point is worthy of mentioning with regard to this mechanism. Compound **3** is formed also in the absence of phosphate, albeit in a lower amount ($\leq$1/5th than with 0.002M phosphate). A possible explanation is that H_2O itself is oxidized inefficiently, as expected from the highly positive E_{red}(T), generating the radical anion **1**$^{\bullet-}$ and the OH$^{\bullet}$ radical. The latter species may abstract a H-atom in the same way as the phosphate radical anion (see *Scheme 3*, X$^{\bullet}$ = OH$^{\bullet}$).

3.5. *Decarboxylation*. Another minor product worthy of commenting is compound **8** formed from **1** in the presence of sulfite. This had undergone reductive decarboxylation, not defluorination. With fluoroquinolinones, decarboxylation has been reported only in the case of the 8-(alkylthio) derivative rufloxacin, for which defluorination was *not* the main photoproces (<40%) in H_2O [8] [21]. In the present case, decarboxylated **8** was isolated under conditions in which the triplet of **1** was fully quenched and gave the radical anion. Decarboxylation could not result from this path, and any rate would not be favored under reductive conditions. A possibility was that decarboxylation was a minor process from the singlet (which would not be quenched since τ_S was 1 ns) and gave radical **10** (*Scheme 5*). Under reductive conditions this led to **8**, while in neat H_2O, it might form dimers, that we did not isolate, but had been suggested to be among the products by *Tiefenbacher et al*. on the basis of HPLC/MS evidence [12]. This remained a minor process, however, since 95% of the overall photochemistry in H_2O involved defluorination, not decarboxylation.

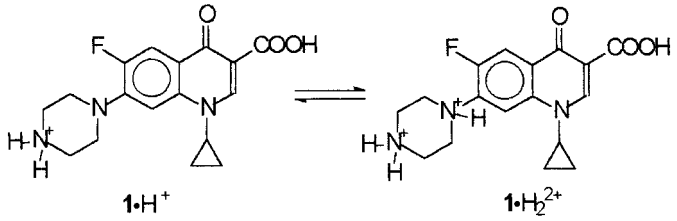
Scheme 5



3.6. *Photochemistry in Acids*. Finally, a different photochemistry was observed under acidic conditions. In this case, no reaction occurred at the heteroaromatic moiety of **1** (the amount of F^- liberated was only 25% of the overall reaction, see *Table*) and the piperazine moiety was stepwise oxidatively degraded to give products **4** and **5** in an inefficient process ($\Phi = 1 \cdot 10^{-3}$). A related – and likewise inefficient – degradation has

been observed with lomefloxacin in 0.1N HCl [4d]. At pH 1, **1** was present in the mono- and dicationic form rather than in the zwitterionic form predominating under neutral conditions (pK_a(**1**) 6.09 [22], see *Scheme 6*). This affected the nature of the excited state (compare the shift in the fluorescence spectrum), which lost the internal charge-transfer character. Under acidic conditions, the triplet was either not formed or had a fully different character, since the T-T absorption, which was apparent under neutral conditions, was not detected (see *Fig. 3*). The same observation has been made for enoxacin [7c]. H-Abstraction either by triplet **1** or by OH$^{\bullet}$ radicals formed by photoinduced electron transfer (*cf. Scheme 2*, $X^{\bullet} = OH^{\bullet}$) presumably causes the observed degradation of the piperazinyl group, analogously to many known oxidative degradations of dialkylanilines by photochemical, electrochemical, or thermal initiation [23].

Scheme 6



4. Conclusions. – It seems worthwhile to point out two conclusions from this work. The first one refers to the identification of the photochemically labile functions on oxoquinoline derivative **1** and the individuation of a structure/photoreactivity relationship, in view of the worldwide use of this molecule as a drug and of the concern about the photostability and phototoxicity of fluoroquinolinones and drugs in general [24]. The above results confirm that, analogously to related quinolinones [4][6], and differently from what appeared from partial examinations [12][13], the main photoreaction in neutral solution is defluorination, though this may occur with a different mechanism and lead to different end products in the presence of some anions. Fluorine-conserving degradation of the piperazine group is important only under strongly acidic conditions, and then with a low quantum yield ($1 \cdot 10^{-3}$). The 1-cyclopropyl group is unaffected (and indeed a 1-cyclopropyl [6a][7b] and, more generally, a 1-alkyl group [4a,c] has been found to be modified or eliminated in the photoreaction only in quinolinones bearing an extra F-atom at position 8, for which a different mechanism applies), and decarboxylation is only a minor process.

The second one refers to the mechanism. Fluoroquinolinones, and indeed most highly stabilized six-membered heteroaromatics show very little unimolecular photoreactivity (again, 8-fluoro derivatives are an exception [4c][9]). However, as indeed it is intuitively expected, the $\pi\pi^*$ triplet of these heterocycles is a strong electrophile and, since this is a relatively long-lived species, a S_N2Ar^* substitution occurs with some efficiency in H_2O. Obviously the triplet is also a strong oxidant, and is not only efficiently reduced by sulfite but also, though at a rate one order of magnitude slower,

by phosphate (a reaction for which there is little precedent [4e][25]) and even – quite inefficiently – by H_2O.

Importantly, the present data were obtained in Ar-flushed solution. Since most of the reactions proceed *via* the triplet, they would be at least in part quenched in air-equilibrated solutions, where different processes involving O_2 may play a role. Furthermore, the efficiency and the course of the photochemical reaction change completely in phophate buffer, routinely used in (photo)biological studies. These two facts illustrate that standard conditions for the study of thermal stability of drugs can not be applied directly to the study of photostability or phototoxicity.

The photochemistry of (halo)heteroaromatics (frequent among drugs) in H_2O has been explored only to a very limited extent up to now, but a nucleophilic substitution such as that observed here is expected to be a quite common result, and the same holds for the electron-transfer path with inorganic anions. Since an F-atom at position 6 appears to be a required feature for a broad-spectrum antibacterial activity in quinolinones (and has led to the success of ciprofloxacin) [14a][26], photoreactivity (and thus light-induced degradation and toxic side effects) is an unavoidable characteristic of these otherwise well tolerated [1] drugs.

Experimental Part

1. *General*. Column chromatography (CC): *Merck* silica gel (0.04–0.063 mm) and $CHCl_3$/MeOH; for reversed phase, *Fluka* C_{18} reversed-phase silica gel (0.04–0.063 mm) and phosphate buffer (pH 3)/MeOH. HPLC: *Hypersil* column (4.6 × 250 mm) and phosphate buffer (pH 3)/MeCN. IR Spectra: *Perkin-Elmer 881* instrument; in cm^{-1}. NMR Spectra: *Bruker 300* instrument, δ in ppm rel. to $SiMe_4$ (= 0 ppm) as an internal reference, J in Hz; attributions supported by exchange with D_2O, double irradiation, BB, and DEPT-135 experiments when appropriate. Elemental analyses: *Carlo-Erba 1106* instrument.

2. *Photochemical Reactions.* A soln. of cyprofloxacin (**1**, 129 mg) in 1.3 l of bidistilled H_2O ($3 \cdot 10^{-4}$ M) in the presence of the appropriate buffer in an immersion well apparatus was flushed with Ar for 40 min and then irradiated by means of a *Pyrex*-filtered medium-pressure Hg arc (*Helios Italquartz*, 500 W), while maintaining a slow flux of Ar. The course of the reaction was monitored by HPLC and the irradiation continued until *ca.* 80% conversion was reached.

After irradiation in 0.1N HCl followed by extraction with $CHCl_3$, *7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid* (**4**) and *1-cyclopropyl-6-fluoro-7-amino-1,4-dihydro-4-oxoquinoline-3-carboxylic acid* (**5**) were obtained; the properties of these compounds corresponded to those reported by *Torniainen et al.* [13c,d].

3. *Irradiation in Neat Water*. The irradiated soln. was stirred in $CHCl_3$ (650 ml) for 5 h. The aq. phase was evaporated and the residue submitted to reversed-phase CC: *1-cyclopropyl-1,4-dihydro-6-hydroxy-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid* (**2**). Colorless crystals. M.p. 292–293°. ^{1}H-NMR ($(CD_3)_2SO$): 1.15–1.4 (*m*, 4 H); 3.2–3.7 (*m*, 8 H); 3.8 (*m*, 1 H); 7.45 (*s*, H–C(8)); 7.65 (*s*, H–C(5)); 8.3 (br. *s*, NH); 8.55 (*s*, H–C(2)); 9.5 (br., OH); 10.7 (*s*, OH). ^{13}C-NMR ($(CD_3)_2SO$): 7.8 (CH_2); 36.0 (CH); 43.0 (CH_2); 46.2 (CH_2); 105.9 (CH(5)); 106.5 (C(3)); 109.0 (CH(8)); 120.4 (C); 136.1 (C); 145.7 (C(7)); 146.2 (CH(2)); 149.3 (C(6)); 166.8 (COOH), 176.4 (C(4)=O). Anal. calc. for $C_{17}H_{19}N_3O_4$: C 61.99, H 5.82, N 12.76; found: C 62.3, H 6.0, N 12.5.

The org. phase was stirred in H_2O (50 ml) for 2 h, dried, and evaporated. Recrystallization from MeOH gave *1-cyclopropyl-7-{[2-(formylamino)ethyl]methylamino}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid* (**3**). Colorless crystals. M.p. 248–249°. ^{1}H-NMR ($(CD_3)_2SO$, 60°): 1.1–1.4 (*m*, 4 H); 3.15 (*s*, 3 H); 3.35 (*q*, J = 7, 2 H); 3.65 (*t*, J = 7, 2 H); 3.78 (*m*, 1 H); 7.05 (*dd*, J = 2, 9, H–C(6)); 7.15 (*d*, J = 2, H–C(8)); 7.9 (br., NH); 8.05 (*s*, CHO); 8.12 (*d*, J = 9, H–C(5)); 8.58 (*s*, H–C(2)); 15.5 (br., COOH). ^{13}C-NMR ($(CD_3)_2SO$, 60°): 11.7 (CH_2); 38.6 (CH_2); 39.6 (CH); 45.2 (Me); 54.6 (CH_2); 100.3 (CH); 110.1 (C(3)); 116.7 (CH); 118.5 (C); 131.1 (CH); 147.4 (C); 151.9 (C(2)); 158.9 (C(7)); 166.1 (CHO); 170.7 (COOH); 180.6 (C(4)=O). Anal. calc. for $C_{17}H_{19}N_3O_4$: C 61.99, H 5.82, N 12.76; found: C 61.8, H 5.8, N 12.7.

4. *Irradiation in Phosphate Buffer.* The irradiated soln. was stirred in $CHCl_3$ (650 ml). The org. phase was dried and evaporated and the residue recrystallized as above to give **3**.

The aq. phase was again extracted with 350 ml of $CHCl_3$ containing 1% of ethyl carbonochloridate, the org. layer washed with H_2O, dried, and evaporated, and the residue taken up with $CHCl_3$ (20 ml) and treated with diazomethane/Et_2O. The soln. was evaporated and the residue submitted to CC: *methyl 1-cyclopropyl-7-{(ethoxycarbonyl){2-[(ethoxycarbonyl)amino]ethyl}amino}-1,4-dihydro-4-oxoquinoline-3-carboxylate*, the *N,N′*-bis(ethoxycarbonyl) methyl ester derivative of **6**[1]). Impure oil. IR: 1725. ^{1}H-NMR ($CDCl_3$): 1.15 (*m*, 2 H); 1.2 (*t*, *J* = 7, 3 H); 1.3 (*t*, *J* = 7, 3 H); 1.4 (*m*, 2 H); 3.48 (*m*, 2 H); 3.5 (*m*, 1 H); 3.9 (*m*, 2 H); 3.95 (*s*, 3 H); 4.1 (*q*, *J* = 7, 2 H); 4.25 (*q*, *J* = 7, 2 H); 5.05 (br. NH); 7.35 (*dd*, *J* = 2, 9, H–C(6)); 7.8 (*d*, *J* = 2, H–C(8)); 8.45 (*d*, *J* = 9, H–C(5)); 8.6 (*s*, H–C(2)). ^{13}C-NMR (CDCl): 8.1 (CH_2); 14.4 (Me); 14.5 (Me); 34.4 (CH); 39.8 (CH_2); 49.6 (CH_2); 52.0 (Me); 60.7 (CH_2); 62.2 (CH_2); 110.7 (C(3)); 114.0 (CH(8)); 123.3 (CH(6)); 126.3 (C(4a)); 128.5 (CH(5)); 140.9 (C(8a)); 145.7 (C(7)); 149.0 (CH(2)); 155.2 (CON); 166.2 (COO); 173.6 (C(4)=O).

5. *Irradiation in Sulfite Buffer.* The irradiated soln. was stirred in 350 ml of $CHCl_3$ containing 1% of ethyl carbonochloridate for 5 h. The org. layer washed with H_2O (50 ml), washed, dried, and evaporated, and the residue taken up with $CHCl_3$ (20 ml) and treated with diazomethane/Et_2O. The soln. was evaporated and the residue separated by CC into the derivatives of **7** and **8**.

Data of Methyl 1-Cyclopropyl-7-[4-(ethoxycarbonyl)piperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylate, the *N′*-(ethoxycarbonyl) methyl ester derivative of **7**[1]). Colorless crystals. M.p. 193–194°. IR: 1720. ^{1}H-NMR ($CDCl_3$): 1.15 (*m*, 2 H); 1.35 (*m*, 2 H); 1.3 (*t*, *J* = 7, 3 H); 3.35 (*m*, 4 H); 3.4 (*m*, 1 H); 3.7 (*m*, 4 H); 3.9 (*s*, 3 H); 4.2 (*q*, *J* = 7, 2 H); 7.05 (*dd*, *J* = 2.5, 9, H–C(6)); 7.15 (*d*, *J* = 2.5, H–C(8)); 8.35 (*d*, *J* = 9, H–C(5)); 8.55 (*s*, H–C(2)). ^{13}C-NMR ($CDCl_3$): 8.0 (CH_2); 14.5 (Me); 34.1 (CH); 43.1 (CH_2); 47.6 (CH_2); 51.5 (Me); 61.6 (CH_2); 99.4 (CH(8)); 110.4 (C(3)); 113.9 (CH(6)); 120.9 (C(4a)); 128.8 (CH(5)); 142.1 (C(8a)); 148.6 (CH(2)); 153.6 (C(7)); 155.3 (O–C(=O)N); 166.6 (COO); 173.6 (C(4)=O). Anal. calc. for $C_{21}H_{25}N_3O_5$: C 63.14, H 6.31, N 10.52; found: C 63.2, H 6.3, N 10.5.

*Data of 1-Cyclopropyl-7-[4-(ethoxycarbonyl)piperazin-1-yl]-6-fluoro-quinolin-4-(1*H*)-one* (= *Ethyl 4-(1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinolin-7-yl)piperazine-1-carboxylate*), the *N′*-(ethoxycarbonyl) derivative of **8**[1]) (which in turn is known [27]). Impure oil. IR: 1720. ^{1}H-NMR ($CDCl_3$): 1.15 (*m*, 2 H); 1.3 (*t*, *J* = 7, 3 H); 1.35 (*m*, 2 H); 3.25 (*m*, 4 H); 3.4 (*m*, 1 H); 3.7 (*m*, 4 H); 4.2 (*q*, *J* = 7,2 H); 6.2 (*d*, *J* = 8, H–C(2)); 7.65 (*d*, *J* = 8, H–C(3)); 7.3 (*d*, *J* = 4, H–C(8)); 8.0 (*d*, *J* = 12, H–C(5). ^{13}C-NMR ($CDCl_3$): 8.1 (CH_2); 14.6 (Me); 33.5 (CH); 43.4 (CH_2); 49.9 (CH_2); 61.6 (CH_2); 104.5 (CH(8)); 109.3 (CH(3)); 112.3 (*d*, *J*(C,F) = 22, CH(5)); 121.5 (C(4a)); 138.9 (C(8a)); 141.2 (CH, CH(2)); 144.2 (*d*, *J*(C,F) = 15, CH(7)); 152.7 (*d*, *J*(C,F) = 250, CF(6)); 155.3 (O–C(=O)N); 176.8 (C(4)=O).

6. *Small-Scale Photolyses.* In quartz tubes, a 10-ml portion of the appropriate $1 \cdot 10^{-4}$M aq. soln. of **1** was flushed with Ar for 15 min and then capped. The soln. was irradiated in a merry-go-round apparatus fitted with 6×15 W phosphorus-coated low-pressure lamps, centre of emission 310 nm. The product composition was determined by HPLC with appropriate calibration curves (ofloxacin as the internal standard) and light flux by ferrioxalate actinometry. The fluoride concentration was measured by means of an *Orion-SA-520* potentiometer with a selective electrode (*Orion F-94-09*), after addition to the photolysate (10 ml) of 2 ml of *Orion-TISAB-III* buffer (ammonium acetate, ammonium chloride, 2,2′,2″,2‴-[(cyclohexane-1,2-diyl)dinitrilo]tetrakis[acetic acid] and dilution to 22 ml with bidistilled H_2O.

7. *Laser Flash Photolysis.* Nanosecond flash photolysis experiments were performed by means of a Nd : YAG JK laser (pulse 20 ns full width at half-maximum, λ 266 nm) in the previously described experimental setup [4c]. Ar-Flushed solns. were used with an absorbance of *ca.* 0.5 at 266 nm. The incident pulse energies were < 4 mJ pulse^{-1}. The spectra were reconstructed point-by-point from time profiles taken each 10 nm.

Financial support of this work by the *Istituto Superiore di Sanità*, Rome within the 'Program on the Physical Properties of Drugs and Their Safe Use', is gratefully acknowledged.

REFERENCES

[1] R. Stahlmann, R. Lode, *Drugs* **1999**, *58* (*Suppl. 2*), 37.

[2] H. J. Reavy, N. J. Traynor, N. K. Gibbs, *Photochem. Photobiol.* **1997**, *66*, 368; S. J. Bulera, J. C. Theiss, T. A. Festerling, F. A. de la Iglesia, *Toxicol. Appl. Pharmacol.* **1999**, *156*, 222; M. Makinen, P. D. Forbes, F. Stenback, *J. Photochem. Photobiol. B: Biol.* **1998**, *37*, 182.

[3] a) L. Martinez, C. F. Chignell, *J. Photochem. Photobiol. B: Biol*. **1998**, *45*, 51; b) S. Sortino, G. Condorelli, G. De Guidi, S. Giuffrida, *Photochem. Photobiol*. **1998**, *68*, 652; c) J. E. Rosen, D. Chen, A. K. Prahalad, T. E. Spratt, G. Schluter, G. M. Williams, *Toxicol. Appl. Pharmacol*. **1997**, *145*, 381; d) N. Umezawa, K. Arakane, A. Ryu, S. Mashiko, M. Hirobe, T. Nagano, *Arch. Biochem. Biophys*. **1997**, *342*, 275; e) G. Condorelli, G. De Guidi, S. Giuffrida, P. Miano, S. Sortino, A. Velardita, *Med. Biol. Environ*. **1996,** *24*, 103.
[4] a) E. Fasani, M. Mella, D. Caccia, S. Tassi, M. Fagnoni, A. Albini, *Chem. Commun*. **1997**, 1329; b) E. Fasani, A. Profumo, A. Albini, *Photochem. Photobiol*. **1998**, *68*, 666; c) E. Fasani, F. F. Barberis Negra, M. Mella, S. Monti, A. Albini, *J. Org. Chem*. **1999**, *64*, 5388; d) E. Fasani, M. Rampi, A. Albini, *J. Chem. Soc., Perkin Trans. 2* **1999**, 1901; e) E. Fasani, M. Mella, S. Monti, A. Albini, *Eur. J. Org. Chem*. **2001**, 391.
[5] a) L. Elrod, C. L. Linton, B. P. Shelat, C. F. Wong, *J. Chromatogr*. **1990**, *519*, 125; b) Y. Yoshida, E. Sato, R. Moroi, *Arzneim.-Forsch*. **1993**, *43*, 601; c) K. Demachi, H. Maeda, Y. Todo, K. Ojima, T. Kokei, H. Imaizumi, M. Tai, Y. Nakajima, H. Narita, *Yakugaku Zasshi* **1995**, *115*, 716; d) N. Mizushima, F. Ohuchi, T. Fujii, *Jpn. J. Hosp. Pharm* **1990**, *16*, 198.
[6] a) T. Morimura, Y. Nobuhara, H. Matsukura, *Chem. Pharm. Bull*. **1997**, *45*, 373; b) T. Morimura, T. Ohno, H. Matsukara, Y. Nobuhara, *Chem. Pharm. Bull*. **1995**, *43*, 1000; c) T. Morimura, K. Kohno, Y. Nobuhara, H. Matsuhura, *Chem. Pharm. Bull.* **1997**, *45*, 1828.
[7] a) L. J. Martinez, G. Li, C. F. Chignell, *Photochem. Photobiol*. **1997**, *65*, 599; b) M. Engler, G. Ruesing, F. Soergel, U. Holzgrabe, *Antimicrob. Agent Chemother*. **1998**, *42*, 1151; c) S. Sortino, G. De Guidi, S. Giuffrida, S. Monti, A. Velardita, *Photochem. Photobiol*. **1998**, *67*, 167.
[8] G. Condorelli, G. De Guidi, S. Giuffrida, S. Sortino, R. Chillemi, S. Sciuto, *Photochem. Photobiol*. **1999**, *70*, 280.
[9] S. Monti, S. Sortino, E. Fasani, A. Albini, *Chem. – Eur. J.* **2001**, *7*, 2501.
[10] S. Navaratnam, J. Claridge, *Photochem. Photobiol*. **2000**, *72*, 283.
[11] J. Ferguson, G. Phillips, J. McEwan, T. Moreland, B. E. Johnson, *Brit. J. Dermatol*. **1988**, *4*, 119; M. E. Hidalgo, C. Pesoa, E. Fernandez, A. M. Cardenas, *J. Photochem. Photobiol. A: Chem*. **1993**, *73*, 135.
[12] E. M. Tiefenbacher, E. Haen, B. Przybilla, H. Kurz, *J. Pharm. Sci*. **1994**, *83*, 463.
[13] a) S. Tammilehto, H. Salomies, K. Torniainen, *J. Planar Chromatogr*. **1994**, *7*, 368; b) K. Torniainen, S. Tammilehto, V. Ulvi, *Int. J. Pharm*. **1996**, *132*, 53; c) K. Torniainen, J. Mattinen, C. P. Askolin, S. Tammilehto, *J. Pharm. Biomed. Anal*. **1997**, *15*, 887; d) K. Torniainen, C. P. Askolin, J. Mattinen, *J. Pharm. Biomed. Anal*. **1997**, *16*, 439.
[14] J. P. Sanchez, J. M. Domagala, S. E. Hagen, C. L. Heifetz, M. P. Hutt, J. B. Nichols, A. K. Trehan, *J. Med. Chem*. **1988**, *31*, 983; A. M. Hay, S. Hobbs-Dewitt, A. A. MacDonald, R. Ramage, *Synthesis* **1999**, 1979.
[15] J. Cornelisse, in 'CRC Handbook of Organic Photochemistry and Photobiology', Eds. W. H. Horspool and P. S. Song, CRC Press, Boca Raton, 1995, p. 2650; A. M. J. Van Eijk, A. M. Huizer, C. A. G. O. Varma, J. Marquet, *J. Am. Chem. Soc*. **1988**, *111*, 88.
[16] P. Bilski, L. J. Martinez, E. B. Koker, C. F. Chignell, *Photochem. Photobiol*. **1996**, *64*, 496.
[17] R. E. Huie, P. Neta, *J. Phys. Chem*. **1984**, *88*, 5665.
[18] D. Stanbury, *Adv. Inorg. Chem*. **1989**, *35*, 69; P. Maruthamuthu, P. J. Neta, *J. Phys. Chem*. **1978**, *82*, 710.
[19] A. Profumo, E. Fasani, A. Albini, *Heterocycles* **1999**, *51*, 1499.
[20] S. Steenken, L. Goldbayarana, *J. Am. Chem. Soc*. **1998**, *120*, 3928; M. Nakashima, E. Hayon, *J. Phys. Chem*. **1970**, *74*, 3290.
[21] S. Sortino, G. Marconi, S. Giuffrida, G. De Guidi, S. Monti, *Photochem. Photobiol*. **1999**, *70*, 280.
[22] D. L. Ross, C. M. Riley, *Int. J. Pharm*. **1992**, *83*, 267.
[23] A. H. Parola, S. G. Cohen, *J. Photochem*. **1980**, *12*, 41; D. Doepp, J. Heufer, *Tetrahedron Lett*. **1982**, *23*, 1553; M. Takani, T. Matsuura, I. Saito, *Tetrahedron Lett*. **1974**, 661; T. W. Bentley, D. J. Richards, M. G. Hutchings, *Tetrahedron Lett*. **1986**, *27*, 5261; E. Fasani, T. Soldi, A. Albini, S. Pietra, *Gazz. Chim. Ital*. **1990**, *120*, 109.
[24] 'Drugs. Photochemistry and Photostability', Eds. A. Albini and E. Fasani, Royal Soc. Chem., Cambridge, 1998; 'Photostability of Drugs and Drug Formulations', Ed. H. Tønnesen, Taylor & Francis, London, 1996.
[25] V. A. Kuzmin, A. K. Chibisov, *Dokl. Akad. Nauk SSSR* **1973**, *212*, 1146.
[26] T. D. Gootz, K. E. Brighty, *Med. Res. Rev*. **1996**, 433; H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, *J. Med. Chem*. **1980**, *23*, 1358.
[27] M. Renmann, M. A. Eisenstat, J. D. Weaver, *Tetrahedron Lett*. **1994**, *35*, 8303.

Received May 23, 2001