Photochemistry of Kynurenine, a Tryptophan Metabolite: Properties of the Triplet State

Yuri P. Tsentalovich,*,† Olga A. Snytnikova,† Peter S. Sherin,†,‡ and Malcolm D. E. Forbes§

International Tomography Center, Institutskaya 3a, Novosibirsk, Russia 630090, Novosibirsk State University, 630090 Novosibirsk, Russia, and Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599

Received: October 25, 2004; In Final Form: January 25, 2005

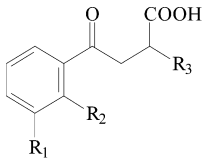
Photolysis of aqueous kynurenine (KN) solutions results in the formation of triplet kynurenine ^{T}KN*. In low pH solutions, triplet formation occurs with almost 100% efficiency, while in neutral solutions the triplet quantum yield is $\Phi_T = 0.018 \pm 0.004$. The dissociation constant of ^{T}KN*, which is attributed to deprotonation of the anilino group, has a pK_a value of 4.7. Similar triplet absorption spectra were obtained under direct and acetone-sensitized photolysis. The large difference in quantum yields as a function of pH is attributed to excited-state properties of the first excited singlet state of KN. The rate constant quenching for ^{T}KN* by oxygen is $k_q = 2 \times 10^9$ M^{-1} s^{-1}.

Introduction

The eye lenses of mammals contain molecules that are believed to play the role of ultraviolet filters. These compounds are typically metabolic products of the amino acid tryptophan, and include the structures illustrated in Chart 1: kynurenine (KN), *N*-formylkynurenine (NFK), 3-hydroxykynurenine (3OHKN), 3-hydroxykynurenine glucoside (3OHKG), and 4-2-amino-3-hydroxyphenyl-4-oxobutanoic acid glucoside (AHBG).[1−5] These compounds show strong absorbance between 300 and 400 nm, protecting the lens and retina from light-induced tissue damage. In the human aging process, the lens proteins undergo chemical modifications that result in the eventual accumulation of colored compounds and an increase in fluorescence of the lens tissue.[6−9] It has been suggested that modification of the lens crystallins occurs through photosensitized reactions involving these UV filter compounds, and this chemistry may lead to damage in the form of cataracts or discoloration.[8,10−16] Another source of the crystallin modification might be the thermal decomposition of the UV filters.[17,18]

To date in the chemical literature there have been few reports on the fundamental photochemical reactions of kynurenines. The early consensus seemed to be that KN and most of its derivatives were photochemically inert, with the only exception being *N*-formylkynurenine, NFK. For this compound it has been reported that photochemistry occurs from the first excited triplet state, which either directly reacts with a substrate or alternatively produces highly reactive singlet oxygen.[19−21] To the best of our knowledge, literature data regarding the properties of the KN triplet state (^{T}KN*) do not exist. For KN and some of the other derivatives in Chart 1, it has been found that the compounds can exhibit very short fluorescence lifetimes.[8] It has also been reported that the photolysis of KN and 3OHKN in air-saturated solutions yields very little singlet oxygen and/or superoxide.[14] These observations led researchers to conclude that, in mammalian eyes, kynurenines convert light energy to benign reactivity channels. However, some interesting photochemical reactivity of KN has been observed recently. For example, it has been reported that kynurenines can photoreduce oxygen and nitromethane, and are able to photooxidize cysteine, NADH, and other biologically important molecules.[15] The mechanisms of these reactions are not yet fully understood. Biological studies have shown that the concentration of kynurenines in lenses decreases with age, while the amount of kynurenine-modified proteins increases.[22,23]

CHART 1



	R_1	R_2	R_3
1. KN	H	NH_2	NH_2
2. NFK	H	NHCHO	NH_2
3. 3OHKN	OH	NH_2	NH_2
4. 3OHKG	OGlu	NH_2	NH_2
5. AHBG	OGlu	NH_2	H

We suggest that reactions between kynurenines and crystallins in mammalian lenses might be a cause of cataractogenesis or other eye diseases, and therefore a detailed study of the photochemistry of KN and its derivatives is of interest. We report here results from our preliminary studies on kynurenine photophysics and photochemistry. The goal of the present work is to establish that photoinduced formation of ^{T}KN* does indeed take place with UV light excitation, and to provide characterization data on ^{T}KN* as a function of pH.

Experimental Section

A detailed description of our laser flash photolysis experiments has been published earlier.[24,25] Solutions in a rectangular cell, inner dimensions 10 mm × 10 mm, were irradiated with a Lambda Physik EMG 101 excimer laser, 308 nm, pulse energy up to 100 mJ, pulse duration 15−20 ns. The dimensions of the laser beam at the front of the cell were 3 mm × 8 mm. The monitoring system includes a DKSh-150 xenon short-arc lamp connected to a high current pulser, a homemade monochromator, an Electron Tubes Ltd. 9794B photomultiplier, and a LeCroy 9310A digitizer. The monitoring light, concentrated in a

* Corresponding author. E-mail: yura@tomo.nsc.ru.
† International Tomography Center.
‡ Novosibirsk State University.
§ University of North Carolina.

10.1021/jp045142k CCC: $30.25
Published on Web 04/06/2005

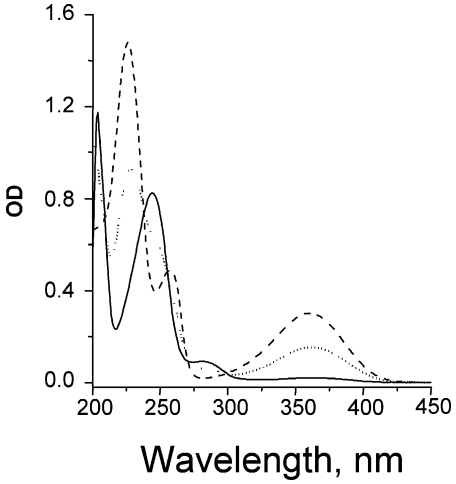


Figure 1. UV−visible spectra of 7.1×10^{-5} M aqueous solution of kynurenine: dashed line, at pH 7.0; dotted line, at pH 1.6; solid line, at pH 0.5.

TABLE 1: Main Spectroscopic Features of the Neutral and Protonated Forms of Kynurenine

pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}
7.0	226	2.2×10^4
	258	7.2×10^3
	360	4.5×10^3
0.1	204	1.6×10^4
	245	1.3×10^4
	280	1.4×10^3

rectangular beam of 3 mm height and 1 mm width, passed through the cell along the front laser-irradiated window. Thus, in all experiments the excitation optical length was 1 mm, and the monitoring optical length was 8 mm. All solutions were bubbled with argon for 15 min prior to, and during, irradiation. Actinometry was performed using naphthalene in cyclohexane. The incident laser energy was determined by triplet naphthalene absorption at 414 nm (absorption coefficient 2.45×10^4 M^{-1} cm^{-1}, triplet quantum yield 0.75).[26,27]

D,L-Kynurenine from Sigma/Aldrich was used as received. Solutions were prepared with the use of phosphate buffers. Below pH 1, the acidity of solutions was adjusted by addition of hydrochloric acid. In all cases, the pH values of solutions were measured by a glass electrode prior to and after irradiation.

Results and Discussion

A. General Absorption Spectrum Features and pH Dependence. The first step in our investigation was to measure the pK_a values of kynurenine in aqueous solution and to determine how the protonation at different sites in the molecule can alter its optical spectrum. Several absorption spectra of kynurenine in neutral and acidic solutions are presented in Figure 1; the main spectroscopic features of the neutral and protonated forms of kynurenine are summarized in Table 1. They are in good agreement with data previously published by Atherton et al.[28] Significant changes in the absorption spectra occur at pH values between 0 and 3, while above pH 4.0 no noticeable changes in the spectrum are observed. Examining the structure of KN, it is clear that there exists more than one protonation site, and therefore a titration study at one particular wavelength will provide more information on the acid−base chemistry of the molecule.

Figure 2 shows the pH dependence of the optical density of a 7.1×10^{-5} M kynurenine solution in water at 245 nm. The pK_a values were obtained by fitting the calculated titration curves

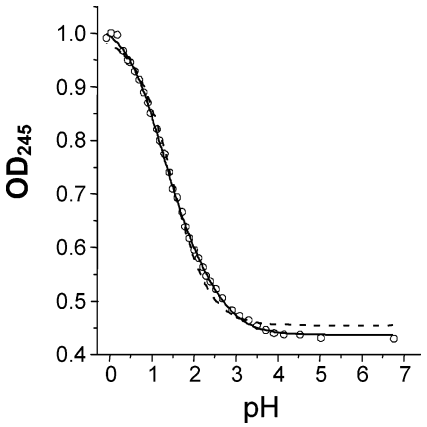


Figure 2. pH dependence of KN absorbance at 245 nm. Dashed line: calculated titration curve with one pK_a value, pK_a = 1.5. Solid line: calculated titration curve with two pK_a values, pK_{a1} = 1.2 and pK_{a2} = 2.4.

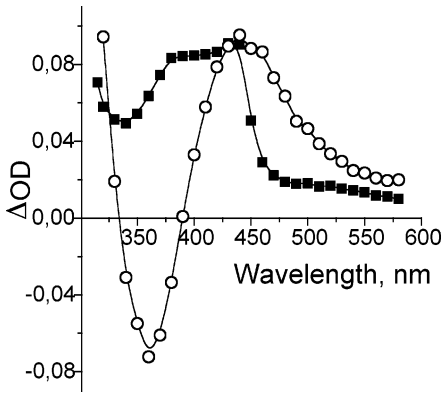


Figure 3. Transient absorption spectra, obtained in acetone-sensitized photolysis of KN at pH 7.0 (circles) and pH 0.1 (squares) taken 3 μs after the laser flash. The solutions are 1×10^{-4} M KN in a 1:10 acetone: water mixture.

to the experimental data using Matlab software. Simulation of the titration curve with two pK_a values (solid line in Figure 2), pK_{a1} = 1.2 and pK_{a2} = 2.4, results in a much better fit than with only one value (dashed line), pK_a = 1.5. The largest changes in these spectra occur at low pH values. We have assigned the pK_{a1} value of 1.2 to the anilinium group and the pK_{a2} value of 2.4 to the carboxyl proton. The 2.4 pK_a value is typical for the −COOH group in the majority of amino acids. For most protonated anilines the typical pK_a value is between 4 and 5.[29] However, pK_a = 2.04 was reported for ortho-carboxylic acid substituted aniline. Thus, the unexpectedly low pK_a value for the aniline moiety in kynurenine can be attributed to the presence of the ortho-carbonyl group, which decreases the basicity of the aniline group through inductive and resonance effects. In the work of Atherton et al.,[28] where similar experimental conditions were used, the pK_a values for kynurenine were reported as 1.9, 5.1, and 8.5. These were assigned to the −COOH group, the anilino group, and the NH_2 group, respectively. We did not observe any spectral changes near pH 5. Moreover, these authors[28] noted that the changes in the absorption spectrum in the pH range from 1.0 to 3.5 are much stronger than any observed changes at higher pH values. It is more likely, in our opinion, that the strong spectral changes seen between pH 1.0 and 3.5 should be attributed to the protonation of the anilino group rather than the carboxyl group.

B. Acetone-Sensitized Photolysis of KN. Transient absorption spectra of photoexcited KN are presented in Figure 3. These spectra were obtained during the photolysis of 1×10^{-4} M

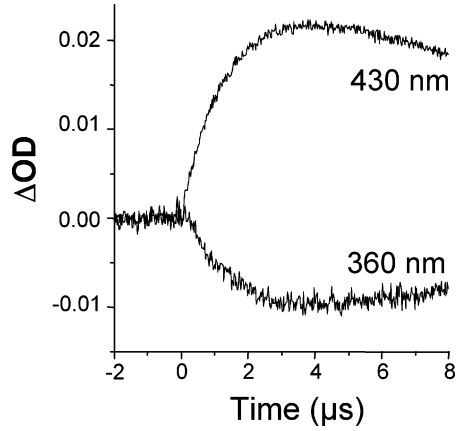


Figure 4. Transient absorption kinetics recorded at 360 and 430 nm during acetone-sensitized photolysis of KN in neutral solution.

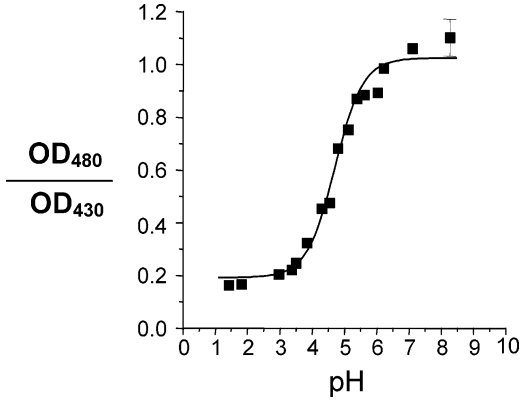


Figure 5. pH dependence of ratio of ^{T}KN* absorption at 480 nm to that at 430 nm, measured 2 μs after the laser pulse. The solutions are 1×10^{-4} M KN in a 1:10 acetone:water mixture.

kynurenine in a 1:10 acetone:water mixture. With such concentrations of each chromophore, the absorption of acetone at 308 nm ($\epsilon_{308} = 0.4$ M^{-1} cm^{-1}) is by an order of magnitude stronger than that of kynurenine ($\epsilon_{308} = 870$ M^{-1} cm^{-1} in the neutral solution and $\epsilon_{308} = 260$ M^{-1} cm^{-1} at low pH). The circles in Figure 3 represent the spectrum obtained in a neutral solution (pH 7.0), while the squares correspond to the spectrum obtained in the presence of 1 M HCl (pH 0.1).

Figure 4 shows typical kinetic traces recorded at 360 and 430 nm for the neutral solution. The negative absorption observed at 360 nm during photolysis in neutral solution corresponds to the depletion of the starting material. The growing signal at 430 nm is attributed to the formation of triplet kynurenine.

$$\mathrm{Ac} \xrightarrow{h\nu,\mathrm{ISC}} {}^{\mathrm{T}}\mathrm{Ac} \quad (1)$$

$$2\,{}^{\mathrm{T}}\mathrm{Ac} \xrightarrow{k_1} \mathrm{Ac} + {}^{1}\mathrm{Ac}^* \quad (2)$$

$${}^{\mathrm{T}}\mathrm{Ac} + \mathrm{KN}\ (\mathrm{KNH_2}^{2+}) \xrightarrow{k_2} \mathrm{Ac} + {}^{\mathrm{T}}\mathrm{KN}^*\ ({}^{\mathrm{T}}\mathrm{KNH_2}^{2+}*) \quad (3)$$

$$2\,{}^{\mathrm{T}}\mathrm{KN}^*\ ({}^{\mathrm{T}}\mathrm{KNH_2}^{2+}*) \xrightarrow{k_{\mathrm{T-T}}} 2\mathrm{KN}\ (\mathrm{KNH_2}^{2+}) \quad (4)$$

Structures in parentheses in eqs 3−4 correspond to species observed in acidic conditions.

The absorption of triplet acetone at wavelengths above 300 nm is small[30] and can be neglected. Since in acidic solution KN does not absorb above 300 nm, the spectrum, obtained at pH 0.1 (Figure 3, squares), should be attributed to the absorption of the $^{T}KNH_2^{2+}$* only. The spectrum obtained under neutral conditions is a superposition of the triplet absorption spectrum (^{T}KN*) and the depletion spectrum of the starting compound (KN).

Our experimental conditions are optimized as follows: kynurenine concentrations are above 2×10^{-4} M; laser energies are below 4 mJ/pulse, which corresponds to a light flux of about 1.6×10^{-2} J cm^{-2} and to the initial triplet acetone concentration of about 6×10^{-5} M. Under such conditions the triplet−triplet annihilation reaction (eq 2) can be neglected, and therefore the only channel for triplet acetone decay is energy transfer to kynurenine, i.e., the reaction shown in eq 3. This allows for the precise determination of both the rate constant k_2 in eq 3 and the extinction coefficient for triplet kynurenine ϵ^{T}. The rate constant k_2 was measured in neutral solution by monitoring the signal decay at 360 nm and the signal growth at 430 nm. In this experiment the laser energy was varied between 1 and 4 mJ, and the kynurenine concentration was varied from 1×10^{-4} to 5×10^{-4} M. The observed pseudo-first-order rate constants for the signal decay at 360 nm and growth at 430 nm were identical and increased linearly with the kynurenine concentration, i.e., $k_{obs} = k_2$[KN], and our linear fit yielded $k_2 = (4.5 \pm 0.4) \times 10^9$ M^{-1} s^{-1}.

The extinction coefficient for triplet kynurenine, ϵ^{T}, was determined under similar experimental conditions. For our analysis we assume that, at low laser intensities, energy transfer from triplet acetone to kynurenine occurs with 100% efficiency. For protonated and neutral KN triplet states, the extinction coefficients were determined by monitoring the triplet absorption at 430 nm and were calculated according to the equation

$$\epsilon^{\mathrm{T}}{}_{430} = \frac{\Delta\mathrm{OD}_{430}}{T_0 L} \quad (5)$$

where ΔOD_{430} is the triplet absorption monitored 2 μs after the laser pulse at 430 nm, T_0 is the initial triplet acetone concentration, calculated using the measured laser energy and solution optical density at 308 nm, and $L = 0.8$ cm is the optical length. For the neutral triplet state $\epsilon^{T}{}_{430}$ was determined to be 3700 M^{-1} cm^{-1}, while for the protonated triplet state $\epsilon^{T}{}_{430}$ was found to be 3500 M^{-1} cm^{-1}.

Transient absorption spectra obtained for triplet kynurenine can be compared with the spectra of related compounds published in the literature, notably those of tryptophan and *N*-formylkynurenine, NFK. The spectrum of triplet NFK is very similar to the spectrum of triplet kynurenine presented in this work. It has a maximum at 440 nm and $\epsilon^{T}{}_{430} = 7000$ M^{-1} cm^{-1}.[19,21] The triplet state of tryptophan can exist in neutral and protonated states with p$K_a = 3.2$.[31] Both forms have optical spectra of very similar shapes, with maxima at 450 and 400 nm for the neutral and protonated species, respectively.[31−34] We have observed a similar blue shift after protonation in the present work for ^{T}KN*.

From the result above and from Figure 3, we can conclude that the absorption coefficients of neutral and protonated triplets are similar at 430 nm, but differ significantly at longer wavelengths. This difference was used for the determination of the pK_a value of triplet kynurenine. Figure 5 shows the pH dependence of the optical density (OD) of triplet kynurenine, measured at a delay time of 2 μs after the laser pulse at 480 nm. For accuracy, these data are normalized to the absorption measured at 430 nm. The titration curve we obtained was simulated according to the equation

$$\mathrm{OD} = \frac{\mathrm{OD_a}[\mathrm{H^+}] + \mathrm{OD_n}K_a}{[\mathrm{H^+}] + K_a} \quad (6)$$

where OD_a and OD_n are the triplet absorptions in extremely acidic and neutral solutions, respectively, and K_a is the dissociation constant. The best fit, represented in Figure 5 by a solid line, gives a pK_a value of 4.7. Significant changes in the triplet absorption spectra, obtained in neutral and acidic solutions, indicate that the pK_a value of 4.7 should be attributed to deprotonation of the anilino group of ^{T}KN*.

C. Direct Kynurenine Photolysis. Direct photolysis experiments were performed with 1.1 mM aqueous kynurenine solutions in neutral pH 7.0 and acidic pH 0.1 aqueous solutions. In both cases, the observed transient spectra are very similar to the ones obtained in acetone-sensitized photolysis. However, the intensities of the signals in neutral solution are much weaker than those measured under acidic conditions, which is opposite to that observed in the acetone-sensitized case. The quantum yields for triplet state formation Φ_T were determined by the initial triplet absorption at 430 nm, using the previously determined value of ϵ^T_{430} (see above). In acidic solution the quantum yield for formation of protonated ^{T}KN* is close to unity, while in neutral solution Φ_T for this structure was determined to be 0.018 ± 0.004. This large difference in the quantum yields is by itself a very interesting observation. Apparently, this phenomenon should be attributed to the different properties of the protonated and deprotonated singlet excited states of kynurenine ^{S}KN*. As follows from our results, the main channel of the protonated ^{S}KNH_2^{2+}* decay is the intersystem crossing into the triplet state, while for the deprotonated species ^{S}KN* other channels (such as fluorescence and radiationless transition) play a major role. The origin and generality of this phenomenon will be explored in future experiments in our laboratories.

In oxygen-free solution, the main channel for ^{T}KN* decay is triplet−triplet annihilation. The decay of the 430 nm transient signal is characterized by a quantity $k_{T-T}/\epsilon^T = (1.1 \pm 0.2) \times 10^6$ cm s^{-1}. Using the previously determined value $\epsilon^T_{430} = 3700$ M^{-1} cm^{-1}, we calculate the rate constant for triplet−triplet annihilation k_{T-T} to be $(4.1 \pm 0.8) \times 10^9$ M^{-1} s^{-1}; i.e., it is nearly diffusion controlled. In the presence of oxygen, the triplet decay rate significantly accelerates and becomes monoexponential. In this case the observed pseudo-first-order rate constant is proportional to the oxygen concentration. Assuming that the solubility of oxygen in water at room temperature is about 1.4 mM, the rate constant for the quenching of ^{T}KN* by oxygen, derived from the rate of the triplet decay, is estimated to be about $k_q = 2 \times 10^9$ M^{-1} s^{-1}.

Conclusions

Our studies show that 308 nm photolysis of aqueous KN solutions results in the rapid formation of ^{T}KN*. The following findings support our conclusion that the observed species is, indeed, the kynurenine triplet state: (1) identical spectra were obtained under direct and acetone-sensitized photolysis; (2) the optical spectrum we report is similar to the spectrum of a related species, ^{T}NFK*;[19,21] (3) the observed intermediate is readily quenched by oxygen with a rate constant of $k_q = 2 \times 10^9$ M^{-1} s^{-1}, close to the diffusion limit. Under physiological conditions and neutral pH the yield of ^{T}KN* is rather small, $\Phi_T = 0.018 \pm 0.004$. However, in mammalian eye lenses this route may very well be the primary step in a sequence of reactions such as singlet oxygen production, formation of photochemically active products, and chemical modification of the lens proteins. The reactivity of ^{T}KN* toward biologically important molecules, as well as the identity and photochemical properties of the products formed under KN photolysis, are currently under study in our laboratories.

Acknowledgment. This work was supported by the Russian Foundation for Basic Research Projects No. 05-03-32281 and No. 02-03-32166, by Ministry of Education RF Scientific School 2298.2003.3, and by SB RAS Grant 148 and Presidium Program RAS "Origin and Evolution of Biosphere". M.D.E.F. thanks the National Science Foundation for their support of our program through Grant CHE-0213516.

References and Notes

(1) van Heyningen, R. *Nature* **1971**, *230*, 339.
(2) Wood, A. M.; Truscott, R. J. W. *Exp. Eye Res.* **1993**, *56*, 317.
(3) Truscott, R. J. W.; Wood, A. M.; Carver, J. A.; Sheil, M. M.; Stutchbury, G. M.; Zhu, J.; Kilby, G. W. *FEBS Lett.* **1994**, *384*, 173.
(4) Wood, A. M.; Truscott, R. J. W. *Vis. Res.* **1994**, *34*, 1369.
(5) Taylor, L. M.; Aquilina, J. A.; Willis, R. H.; Jamie, J. F.; Truscott, R. J. W. *FEBS Lett.* **2001**, *509*, 6.
(6) Lerman, S.; Borkman, R. *Ophthalmic Res.* **1976**, *8*, 335.
(7) Yu, N.-T.; Barron, B. C.; Kuck, J. F. R. *Exp. Eye Res.* **1989**, *49*, 189.
(8) Dillon, J.; Atherton, S. J. *Photochem. Photobiol.* **1990**, *51*, 465.
(9) Dillon, J.; Wang, R.-H.; Atherton, S. J. *Photochem. Photobiol.* **1990**, *52*, 849.
(10) Bando, M.; Mikuni, I.; Obazawa, H. *Exp. Eye Res.* **1982**, *34*, 953−960.
(11) Dillon, J. *Lens Res.* **1983**, *1*, 133.
(12) Dillon, J. *Curr. Eye Res.* **1984**, *3*, 145.
(13) Tomoda, A.; Yoneyama, Y.; Yamagushi, T.; Shirao E.; Kawasaki, K. *Ophthalmic Res.* **1990**, *22*, 152.
(14) Krishna, C. M.; Uppuluri, S.; Riesz, P.; Zigler, J. S., Jr.; Balasubramanian, D. *Photochem. Photobiol.* **1991**, *54*, 51.
(15) Reszka, K. J.; Bilski, P.; Chignell, C. F.; Dillon, J. *Free Radical Biol. Med.* **1996**, *20*, 23.
(16) Chiarugi, A.; Rapizzi, E.; Moroni, Fu.; Moroni, Fl. *FEBS Lett.* **1999**, *453*, 197.
(17) Taylor, L. M.; Aquilina, J. A.; Jamie, J. F.; Truscott, R. J. W. *Exp. Eye Res.* **2002**, *75*, 165.
(18) Taylor, L. M.; Aquilina, J. A.; Jamie, J. F.; Truscott, R. J. W. *Exp. Eye Res.* **2002**, *75*, 503.
(19) Walrant, P.; Santus, R.; Grossweiner, L. I. *Photochem. Photobiol.* **1975**, *22*, 63.
(20) Pileni, M. P.; Walrant, P.; Santus, R. *Chem. Phys. Lett.* **1976**, *42*, 89.
(21) Pileni, M. P.; Santus, R.; Land, E. J. *Photochem. Photobiol.* **1978**, *28*, 525.
(22) Garner, B.; Shaw, D. C.; Lindner, R. A.; Carver, J. A.; Truscott, R. J. W. *Biochim. Biophys. Acta* **2000**, *1476*, 265.
(23) Bova, L. M.; Sweeney, M. J. H.; Jamie, J. F.; Truscott, R. J. W. *J. Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 200.
(24) Molokov, I. F.; Tsentalovich, Yu. P.; Yurkovskaya, A. V.; Sagdeev, R. Z. *J. Photochem. Photobiol. A: Chem.* **1997**, *110*, 159.
(25) Tsentalovich, Yu. P.; Kulik, L. V.; Gritsan, N. P.; Yurkovskaya, A. V. *J. Phys. Chem. A* **1998**, *102*, 7975.
(26) Bensasson, R. V.; Land, E. J. *Trans. Faraday Soc.* **1971**, *67*, 1904.
(27) Amand, B.; Bensasson, R. V. *Chem. Phys. Lett.* **1975**, *34*, 44.
(28) Atherton, S. J.; Dillon, J.; Gaillard, E. R. *Biochim. Biophys. Acta* **1993**, *1158*, 75.
(29) Braude, E. A.; Nachod, F. C. *Determination of Organic Structures by Physical Methods*; Academic Press: New York, 1955.
(30) Porter, G.; Dogra, S. K.; Loutfy, R. O., Sugamori, S. E.; Yip, R. W. *J. Chem. Soc., Faraday Trans. 1* **1973**, *69*, 1462.
(31) Tsentalovich, Yu. P.; Snytnikova, O. A.; Sagdeev, R. Z. *J. Photochem. Photobiol. A: Chem.* **2003**, *162*, 371.
(32) Bent, D. V.; Hayon, E. *J. Am. Chem. Soc.* **1975**, *97*, 2612.
(33) Volkert, W. A.; Kuntz, R. R.; Ghiron, C. A.; Evans, R. F. *Photochem. Photobiol.* **1977**, *26*, 3.
(34) Stevenson, K. L.; Papadantonakis, G. A.; LeBreton, P. R. *J. Photochem. Photobiol. A: Chem.* **2000**, *133*, 159.