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## A Laser Flash Photolysis Study of Fenofibric Acid in Aqueous Buffered Media: Unexpected Triplet State Inversion in a Derivative of 4-Alkoxybenzophenone<sup>¶</sup>

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#### ABSTRACT

Laser excitation of aqueous solutions of fenofibric acid (FA) at pH 7.4 show the formation of two reaction intermediates, the triplet state and the hydrated electron. The former is longer lived in water than in acetonitrile; its anionic form decays irreversibly by decarboxylation to give a carbanion that protonates before or after rearrangement. Several spectroscopic and quenching studies suggest that in aqueous media the triplet state of FA has a  $\pi,\pi^*$  character, in comparison with an  $n,\pi^*$  character in organic media. Further, the known chemistry of the triplet, including decarboxylation and hydrogen abstraction, occurs predominantly from the  $n,\pi^*$  state, and as a consequence, activation energies are higher when the lowest triplet has a  $\pi,\pi^*$  character. Photoionization is more important in aqueous than in organic media and involves a biphotonic process. Hydrated electrons are trapped by FA, leading to the corresponding ketyl radical after protonation.

#### INTRODUCTION

The photochemistry of pharmaceutical products has received much attention in the last decade, prompted by reports on photosensitization reactions produced by certain active ingredients in drugs. Those related to the benzophenone chromophore have been studied in great detail, and a recent review has been published on the topic (1).

Fenofibric acid (FA) is a hypolipidemic drug circulating in the blood stream after the metabolism of the commercial prodrug fenofibrate (FB) (2,3). This molecule consists of a substituted benzophenone, its ground state absorption extending over the UVA part of the solar spectrum (4). The

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combined effects of UVA and FA can result in photosensitized DNA damage (5), peroxidation of fatty acids (6) and red blood cell hemolysis (1). However, its phototoxic effect is reportedly lower compared with another benzophenonederived drug, ketoprofen (5). The reactivity of FA and FB in ethanol suggest an  $n,\pi^*$  lowest triplet state, in agreement with calculations (7). No such reactivity has been measured in water.



In this work we present laser flash photolysis (LFP) studies performed in aqueous buffered solutions and compare with acetonitrile:water mixtures. We analyze the reactivity toward decarboxylation and hydrogen abstraction of FA and its ester form, FB. We also report triplet-energy values as well as spectroscopic characteristics of the T-T absorption band for FA and FB, measured over a wide solvent polarity range.

Our studies are aimed at understanding the photophysical and photochemical behavior of FA in water. All the parameters measured indicate that an inversion of the lower triplet state of fenofibric acid  $(n,\pi^* \text{ to } \pi,\pi^*)$  occurs when going from acetonitrile to water; this would account for the lower reactivity of FA and its photoproducts as compared with those of ketoprofen in aqueous media.

Our results also show that biphotonic processes occur under pulsed laser excitation, where solvated electrons, together with ketyl and cyclohexadienyl radicals, are readily observable.

#### MATERIALS AND METHODS

*Materials*. FB (2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid isopropyl ester), tetramethylammonium hydroxide (TMAOH), sodium phosphate (monobasic), sodium hydroxide (semiconductor grade), sorbic acid (2,4-hexadienoic acid), 1-naphthalenemethanol (1-NM) and ethanol were purchased from Sigma-Aldrich Canada

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*Abbreviations:* FA, fenofibric acid; FB, fenofibrate; LFP, laser flash photolysis; 1-NM, 1-naphthalenemethanol; TMAOH, tetrameth-ylammonium hydroxide.

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**Figure 1.** Transient absorption spectrum of  $3.5 \times 10^{-5} M$  FA in 0.01 *M* phosphate buffer solution of pH 7.4; laser at 308 nm; N<sub>2</sub> bubbled. Time windows: ( $\mathbf{V}$ ) 0.04  $\mu$ s; ( $\Delta$ ) 0.40  $\mu$ s; ( $\mathbf{\Phi}$ ) 1.28  $\mu$ s; and ( $\Box$ ) 4.04  $\mu$ s after the laser pulse. Inset: kinetic profile at 340 nm.

(Oakville, Canada). All solvents were high-performance liquid chromatography (HPLC)–grade and purchased from OmniSolv (Gibsstown, NJ) and were used without further purification. Water was purified through a Millipore MilliQ system. The 0.1 *M*, pH 7.4 phosphate buffer was prepared with monobasic sodium phosphate in water, and the solution was brought to pH with NaOH.

FA (2-[4-(4-chlorobenzoyI)phenoxy]-2-methylpropanoic acid) was obtained after hydrolysis of the ester form, FB, with NaOH (2.7 M) in 50:50 H<sub>2</sub>O-methanol under reflux for 5 h. After methanol evaporation, the aqueous mixture was subsequently extracted with ether (three washes) to remove any nonreacted FB. The aqueous phase was then brought to pH 5 with a 1:10 HCl-H<sub>2</sub>O mixture. Ethyl acetate was then used for another series of extractions (three washes); the organic phase containing the FA was dried with NaHSO<sub>4</sub> and filtered and the solvent evaporated under low pressure. The product was a white powder that was confirmed to be FA by comparing the <sup>1</sup>H-NMR (CDCl<sub>3</sub>) spectra taken before and after the saponification, showing the disappearance of the peaks corresponding to the isopropyl group ( $\delta$  1.2 [d, 6H, J1 = 6 Hz], 5.1 [m, 1H]).

Nanosecond LFP. The LFP system has been previously described (8,9). Samples were excited either with a Lumonics EX-530 laser with a Xe–HCl–Ne mixture generating pulses of 6 ns at 308 nm with  $\leq$  100 mJ output at the source or with the third harmonic of a Surelite Nd:YAG laser generating pulses of 8 ns duration at 355 nm with 20 mJ output. The signals from the monochromator–photo-multiplier system were initially captured by a Tektronix 2440 digitizer and transferred to a PowerMacintosh computer that controlled the experiment with software developed in the LabVIEW 4.1 environment from National Instruments (Austin, TX).

All transient spectra and kinetics were recorded employing a flow system with a 7 × 7 mm, 2 mL capacity fused silica cuvette from Luzchem Research (Morin Heights, Canada). Samples were purged in a storage tank for 30 min either with N<sub>2</sub>, O<sub>2</sub> or N<sub>2</sub>O. The quenching rate constants were obtained with static samples contained in 7 × 7 mm fused silica cuvettes. A fresh solution was employed to obtain each decay rate; two to four laser shots were delivered to the sample and the solution was shaken between shots to avoid any interference caused by the accumulation of photoproducts. Each solution was purged for 15 min with N<sub>2</sub>, O<sub>2</sub> or N<sub>2</sub>O before data acquisition.

FA concentration was *ca*. 0.035 mM for irradiation at 308 nm. When the excitation was carried out at 355 nm, the sample concentration was *ca*. 1 mM. These values ensured an absorbance of 0.3 to 0.5 in the laser cell at the excitation wavelength.

Aqueous solutions of FA were prepared in pH 7.4 buffers, 10 and 100 m*M* in phosphate, for irradiation at 308 and 355 nm, respectively. The solutions prepared in methanol or acetonitrile:water had 10 m*M* TMAOH to ensure that FA was present in its dissociated form.

The 355 nm pulses from a Nd:YAG laser were used to determine quenching rate constants with 1-NM, in order to avoid absorption by the quencher.



**Figure 2.** Transient absorption spectrum of  $3.5 \times 10^{-5} M$  FA in 0.01 *M* phosphate buffer solution of pH 7.4; laser at 308 nm; N<sub>2</sub> bubbled. Time windows: ( $\bigcirc$ ) 8  $\mu$ s; ( $\square$ ) 139  $\mu$ s and ( $\blacklozenge$ ) 1740  $\mu$ s after the laser pulse. Also shown is an enlarged section of the 8  $\mu$ s time window ( $\Delta$ ).

*Steady state measurements.* Phosphorescence spectroscopy was performed using a Perkin Elmer LST 50 luminescence spectrometer. The spectra were recorded at 77 K in different glasses. Absorption spectra were recorded using a Varian CARY 1E spectrophotometer.

*Product studies.* Photolysis of FA in phosphate buffer aqueous solutions was done with 308 and 355 nm lasers as the excitation sources. The samples received 100 laser shots and were then extracted with dichloromethane. The solvent was removed under vacuum, and the resulting mixture was analyzed with a Fisons GC/MS 8060/8000 Series equipped with a J&W DB5 30 m  $\times$  0.32 mm column.

HPLC studies of the irradiated solutions were carried out on a Varian instrument using a reverse phase Zorbax SB-C18 column; the mobile phase used was 15:85:1 water:methanol:acetic acid. The detection used a Varian 9065 Polychrom diode array detector. The absorption spectra of the products were compared with those reported previously (6).

Quantum yield determination. Steady state photolysis was carried out in a photoreactor, with only one 254 nm 8 W mercury lamp and for the periods described in Fig. 10. Ketoprofen was employed as a standard (the quantum yield of photodecarboxylation is 0.75 at pH 7.4) (10). The solutions were 1 mM in the substrate and 80 mM in TMAOH, to ensure that all the photons would be absorbed under these conditions and that FA and ketoprofen would be in their anionic forms. To quantitate the starting material as well as the photoproducts produced, a calibration curve was made with FA at 254 nm and employed equally for FA and for photoproduct 1, which has the same chromophore structure. In the case of photoproduct 2, the concentration was corrected for the difference in molar absorptivity existing between FA and this compound at 254, as reported in the literature (6). A separate curve was prepared for ketoprofen.

#### RESULTS

In this work, we have examined the photochemistry of FA in aqueous media. FA is the biologically active drug produced by hydrolysis of FB. Most of the work was carried out at pH 7.4 to approximate physiological conditions.

Laser excitation of FA at pH 7.4 under nitrogen leads to the spectrum of Fig. 1. The early spectrum corresponds to a mixture of two species, as revealed by a number of confirmatory experiments (*vide infra*). The bands at 360 and 460 nm are assigned to the triplet state of FA, which at this pH is present as the anion, whereas the absorption at  $\lambda > 700$ nm is probably dominated by the hydrated electron, with contributions from triplet absorption. At 340 nm, we also observe the formation of another species after triplet decay (Figs. 1 and 2). The fact that the time profile for the change



**Figure 3.** Electron absorption spectrum obtained from the difference between the spectrum of  $3.5 \times 10^{-5} M$  FA in 0.01 *M* phosphate buffer of pH 7.4; laser 308 nm, N<sub>2</sub> bubbled; and the spectrum of a solution under the same conditions but equilibrated with N<sub>2</sub>O. The observed time windows are those registered 0.16  $\mu$ s after the laser pulse. The spectra were normalized to the same value at 380 nm before subtraction. (**●**) N<sub>2</sub>O; (**□**) N<sub>2</sub>; (+) solvated electron. Inset: transient absorption spectrum of  $3.5 \times 10^{-5} M$  FA in 0.01 *M* phosphate buffer solution of pH 7.4; laser 308 nm, N<sub>2</sub>O bubbled. Time windows: (**□**) 8  $\mu$ s and (**♦**) 344  $\mu$ s after the laser pulse.

in absorbance ( $\Delta$ OD) shows a valley at 340 nm implies that the triplet state cannot be the immediate precursor of this species (see the inset of Fig. 1).

In order to verify the assignment of the long wavelength signals to the hydrated electron, we carried out experiments in N<sub>2</sub>O-saturated solutions. This is a common diagnostic test for hydrated electrons, which are efficiently scavenged by N<sub>2</sub>O (11). The results are shown in Fig. 3, which includes a difference spectrum obtained by subtracting the spectrum under N<sub>2</sub>O (where only triplet states are observed) from that recorded under N<sub>2</sub> (where both triplet and hydrated electrons are monitored). The difference spectrum is in full agreement with that expected for the solvated electron (12).

The triplet state assignment is not as straightforward as that for the hydrated electron; *i.e.* 360 and 460 nm are not the regions for the typical absorption of substituted benzophenones. In fact, for FA we have reported  $\lambda_{max}$  values of 340 and 520 nm for its triplet state in methanol (13). To test the hypothesis that the 360 and 460 nm signals are caused by the triplet state, we carried out quenching experiments with two selective triplet quenchers, sorbic acid and 1-NM, and obtained quenching rate constants of  $1.9 \times 10^9$  and  $2.9 \times 10^9 M^{-1} s^{-1}$ , respectively. Further, in the case of 1-NM, the decay of the FA triplet (monitored at 480 nm) occurs concurrently with the growth of the 420 nm signal characteristic of the triplet state of 1-NM (see Fig. 4).

Consistent with our triplet state and hydrated electron assignments, both signals are quenched by oxygen. The triplet signal at 340 nm now decays with a rate constant of  $5.9 \times 10^6 \text{ s}^{-1}$ , compared with *ca.*  $2 \times 10^6 \text{ s}^{-1}$  under nitrogen or N<sub>2</sub>O. From the difference, we estimate the quenching rate constant for oxygen at  $4 \times 10^9 M^{-1} \text{ s}^{-1}$ , a value in the range expected for these reactions.

We now turn our attention to the growth observed at 340 nm. As already indicated, the presence of a valley in the time profile requires that either the growth has a different precursor (*i.e.* other than the triplet state) or that one or more intermediates are involved in the reaction path. Further,



**Figure 4.** Transient absorption spectra of  $8.9 \times 10^{-4} M$  FA in 0.1 M phosphate buffer solution of pH 7.4. The quencher used was  $2 \times 10^{-3} M$  1-NM; laser 355 nm; N<sub>2</sub> bubbled. Time windows: (•) 29 ns; (□) 200 ns; (+) 425 ns; and ( $\Delta$ ) 680 ns after the laser pulse. Inset: kinetic traces obtained at (•) 480 nm and at (□) 420 nm.

these intermediates need to be transparent or at least less absorbing than both the triplet state and the species responsible for the signal growth. In our case, the simplest hypothesis is that the 340 nm signal is derived from the hydrated electron. This is confirmed by the fact that electron scavenging with N<sub>2</sub>O eliminates the growth at 340 nm. The 340 nm transient, which also shows a signal at 540 nm (see Fig. 2), is assigned to the ketyl radical formed by reduction of ground state FA after trapping of the hydrated electron. Its absorption agrees well with the spectrum of the acid form of FA reported in methanol 5  $\mu$ s after the laser pulse, when hydrogen abstraction from the solvent occurs readily (13). The ketyl radicals have a lifetime of *ca*. 140  $\mu$ s under our conditions and decay by mixed first–second order kinetics.

The trace in the inset of Fig. 1 is analyzed with the sum of two exponentials. The fitting gives rate constants of 2.05  $\times$  10<sup>6</sup> s<sup>-1</sup> (triplet decay) and 8.9  $\times$  10<sup>5</sup> s<sup>-1</sup> (ketyl growth). From the latter number and the concentration of FA (3.5  $\times$  $10^{-5}$  M), we estimate that the hydrated electron reacts with FA with a rate constant of 2.6  $\times$  10<sup>10</sup>  $M^{-1}$  s<sup>-1</sup>, a value in line with others for aromatic ketones (14–18). Interestingly, the presence of a negative charge (i.e. the carboxylate) does not cause any significant slowdown in the electron capture reaction. We note that the actual value may be slightly lower than our estimate if the electron decays by other pathways in addition to trapping by FA. We also observe that in addition to the transients discussed previously, there is a considerable bleaching at 300 nm (see inset of Fig. 3). This indicates that the processes taking place (including triplet decay) lead to irreversible chemical changes.

In order to determine the origin of the various transients discussed, we also carried out power dependence studies. These measurements are normally used to differentiate monophotonic and multiphotonic processes. In a qualitative experiment, we used 355 nm excitation; the laser available for these experiments has considerably lower peak power than the excimer laser used for 308 nm excitation. Under these conditions, the experiment under nitrogen gave a spectrum virtually identical to that obtained with 308 nm under  $N_2O$ , clearly showing that hydrated electrons were not produced under these conditions (data not shown).

In a separate experiment, we determined the power dependence at 308 nm for three separate transients: the electron



**Figure 5.** Power dependence of  $3.5 \times 10^{-5} M$  FA in 0.01 *M* phosphate buffer solution of pH 7.4; laser at 308 nm; N<sub>2</sub> bubbled. The full dose corresponds to *ca.* 25 mJ per pulse. Taken at ( $\bullet$ ) 340 nm, 3.5 µs after the laser pulse; ( $\Box$ ) 460 nm, immediately after the laser pulse; and ( $\Delta$ ) 720 nm, immediately after the laser pulse and corrected for triplet contribution.

(720 nm) and the triplet state (460 nm) immediately after the laser pulse and the ketyl radical (340 nm) after its growth was complete (see inset of Fig. 1). The plot shown in Fig. 5 clearly shows a quadratic dependence for the electron and the ketyl radical (fitted with a parabola) and a linear dependence, as expected, for the triplet state. This shows that electron ejection (and as a consequence ketyl radical formation) is dominantly (and probably exclusively) the result of twophoton ionization.

We have attributed the triplet signals to triplet FA. However, two questions remain. Conceivably, the triplet state observed could be that of the carbanion produced after FA photodecarboxylation; further, the lifetime of the triplet (0.5  $\mu$ s) is rather short for a substituted benzophenone in water (19), suggesting that reactive pathways play a key role in determining the triplet lifetime.

The ester, FB, is a good control compound because it does not undergo the decarboxylation-rearrangement that characterizes the photolysis of the FA anion (6,20). In order to ensure solvent compatibility, it was necessary to carry out the experiments in acetonitrile:water mixtures. Figure 6 shows the spectra for FB in various solvent mixtures; base has been added to match the conditions used with FA in organic solvents (vide infra). Clearly, FB undergoes the same shifts as FA in water, thus supporting the assignment of the early transient of Fig. 1 to triplet FA. However, the triplet lifetime of FB is much longer than that of FA. For example, in 8:2 acetonitrile:water, the FB triplet lives 3.80 µs, thus supporting a reactive decay pathway for FA. The FB triplet lifetime does not change significantly in different acetonitrile:water mixtures. In the case of FA in the presence of base (TMAOH), the lifetime decreases as the fraction of organic solvent in the mixture increases and is 0.025 µs in 93:7 acetonitrile:water, about 20 times shorter than in water at pH 7.4. Studies on the quantum yield of photodegradation of FA (vide infra) clearly show that the excited state decarboxylation is responsible for the fast triplet decay in the case of the FA anion.

Given the large difference between the FA triplet lifetimes in aqueous systems at pH 7.4 and in basic 93:7 acetonitrile: water, we decided to explore the reactivity of the triplet with 1,4-cyclohexadiene, a good hydrogen donor. In order to ensure solvent compatibility, water was replaced by a 2:8 ace-



**Figure 6.** Normalized transient absorption spectrum of 0.035 m*M* FB under N<sub>2</sub> atmosphere in different acetonitrile:water mixtures with 10 m*M* NaOH; only half of the experimental points are shown for clarity. Laser wavelength 308 nm. ( $\blacksquare$ ) 2:8 acetonitrile:water, 320 ns after the laser pulse; ( $\bigcirc$ ) 4:6 acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 6.5:3.5 acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 8:2 acetonitrile:water, 320 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 320 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; ( $\triangle$ ) 9 pure acetonitrile:water, 640 ns after the laser pulse; 640 ns

tonitrile:water mixture, and the longer-lived FB triplet was used as a model.

Figure 7 shows quenching plots in both solvents. The second order rate constant (anticipated to be owing to hydrogen abstraction) corresponds to the slope of these plots. The rate constant is  $1.3 \times 10^8 M^{-1} s^{-1}$  in the acetonitrile-rich solvent, which compares well with the value of  $2.9 \times 10^8$  for benzophenone itself in acetonitrile (21). In contrast, in the water-rich solvent we see no quenching, yielding a conservative upper limit of *ca*.  $1 \times 10^7 M^{-1} s^{-1}$  for the quenching rate constant. The observation is consistent with a change in the nature of the excited triplet from  $n,\pi^*$  (acetonitrile) to  $\pi,\pi^*$  (water).

The temperature dependence of the decay of triplet FA was also examined in 93:7 acetonitrile:water in the presence of TMAOH and in water at pH 7.4. These plots are shown in Fig. 8 and yield  $\log(A/s^{-1})$  values of 12.7 and 11.9 for the aqueous and acetonitrile rich solutions; the corresponding activation energies are 36.9 and 23.4 kJ/mol, respectively.

The triplet energy for FA was determined in a glass at 77 K from the maximum of the lowest energy band of the phos-



**Figure 7.** Rate of decay of the triplet state of FB measured at 700 nm in the presence of different amounts of 1,4-cyclohexadiene. ( $\bullet$ ) 0.06 mM FB, 93:7 acetonitrile:water, N<sub>2</sub> bubbled and ( $\Delta$ ) saturated solution of FB in 2:8 acetonitrile:water, N<sub>2</sub> bubbled. Laser wavelength 308 nm.



**Figure 8.** Arrhenius plot for FA. ( $\bullet$ ) Determined in 93:7 acetonitrile:water, under N<sub>2</sub> atmosphere, 0.08 mM FA, 3.4 mM TMAOH. ( $\bigcirc$ ) Determined in 0.1 *M* buffer of pH 7.4, under N<sub>2</sub>O atmosphere, 0.07 mM FA. Laser wavelength 308 nm.

phorescence spectra (*i.e.* the 0-0 band) shown in Fig. 9. The data are summarized in Table 1 and clearly show that a decrease in triplet energy accompanies the increase in water content, for a total triplet energy change of 13 kJ/mol. These changes in triplet energy were also accompanied by changes in the phosphorescence lifetime (longer in higher polarity glasses), also consistent with a switch toward a  $\pi,\pi^*$  character (22). We note this evolution may be more complete in solution at room temperature, where solvation may be more extensive and where the species is the carboxylate anion, as opposed to the acid at 77 K in the experiments of Table 1.

Product studies were performed using as the excitation source a 355 and a 308 nm laser. A total of 100 shots was delivered on each 2 mL sample, and 10 samples were collected. The samples exposed to a given wavelength were then combined and extracted with dichloromethane. After solvent evaporation, the samples were injected on a GC/MS instrument. The same procedure was followed with a nonirradiated sample for comparison. From the analysis of the mass spectra corresponding to each of the peaks in the chromatogram, we were able to identify 4-chloro-4'-isopropoxy benzophenone (1) and 4-chloro-4'-(1-hydroxy-1-methylethyl) benzophenone (2) (see Scheme 1), in agreement with the results previously obtained in methanol solutions (13). No difference in product distribution was found in using different lasers as the excitation source.

The analysis of photoproducts was also done in an HPLC provided with a reverse phase column. The same product ratio was obtained for compounds 1 and 2 when FA was irradiated either at 355 or 308 nm in buffer solution. Minor



**Figure 9.** Normalized phosphorescence spectra of FA in ( $\Box$ ---) water, saturated solution,  $\lambda_{exc}$  310 nm; ( $\bigcirc$ --) 2:1 ethylene glycol-water, 0.2 m*M*,  $\lambda_{exc}$  314 nm; and ( $\blacklozenge$ ---) 4:1 ethanol-methanol, 0.08 m*M*,  $\lambda_{exc}$  305 nm. Also shown is ( $\Delta$ ---) the spectrum of FB in 0.1 m*M* methylcyclohexane,  $\lambda_{exc}$  288 nm.

amounts of FA dimers originating from the FA ketyl radical were also detected after irradiation with the 308 nm laser.

The quantum yield ( $\Phi$ ) for FA photodegradation was determined after steady state irradiation of FA in 93:7 acetonitrile:water and water under basic conditions and using ketoprofen as a standard; the results are presented in Fig. 10. In both cases, photoproducts **1** and **2** were the only species formed. The ratio of compound **1** to compound **2** in aqueous solutions is 69:31 (slightly different from that previously reported, *i.e.* 78:22 [23]) and that in 93:7 acetonitrile:water is 1:1. The product **2** is rationalized by a Wittig rearrangement of the carbanion, which is disfavored in more polar solvents; this results in the observed change in product ratio with solvent composition, in agreement with previous results in phosphate buffer solution, acetone and methanol (23).

The preceding results lead us to propose a reaction scheme for FA similar to that encountered in methanol solutions. The general mechanism shown in Scheme 1 seems to apply to the FA anion photochemistry in all the conditions employed, although with different efficiencies for each solvent system.

### DISCUSSION

There are several differences between the photobehavior of FA in aqueous solutions and in organic polar solvents such as methanol, where its photochemistry is already well understood (13). One of these differences relates to the occur-

Table 1. Solvent effects on the triplet energy of FA at 77 K. Also shown are the triplet decay rate constants and their respective weights

| Solvent                   | 0,0 Band (nm) | Triplet<br>energy<br>(kJ/mol) | $k_1$ (s <sup>-1</sup> ) | $A_1$ | $k_2$ (s <sup>-1</sup> ) | $A_2$ |  |
|---------------------------|---------------|-------------------------------|--------------------------|-------|--------------------------|-------|--|
| Methylcyclohexane*        | 418           | 287                           | 144                      | 1     |                          |       |  |
| 4:1 Methanol:ethanol      | 417           | 287                           | 103                      | 1     |                          |       |  |
| 2:1 Ethylene glycol:water | 423           | 283                           | 29                       | 0.47  | 11                       | 0.53  |  |
| Water                     | 437           | 274                           | 51                       | 0.54  | 10                       | 0.46  |  |

\*Solution of fenofibrate was employed. Excitation wavelengths employed were 288 nm for FB in methylcylcohexane, 305 nm for FA in methanol:ethanol, 310 nm for FA in water and 314 nm for FA in ethylene glycol:water.



Scheme 1. Photodegration mechanism for FA.

rence of photoionization processes; it is not unusual for these processes to be enhanced in aqueous media. Further, in the case of FA at pH 7.4, where it is present as the carboxylate, photoionization does not need to overcome electrostatic attractions in order to eject the electron (as is the case for neutral species). Similar processes have been reported for benzophenone and ketoprofen (15,24–26).

Hydrated electrons decay predominantly by addition to the ground state of FA with a rate constant value of around 2.6  $\times 10^{10} M^{-1} \text{ s}^{-1}$ . Interestingly, the rate is just what it would be if no negative charge were present in the substrate. Similar observations were made in the case of the 3,3',4,4'-benzophenonetetracarboxylate ion (16).

We note that the spectrum observed after electron trapping by FA is clearly that of the ketyl radical, rather than that of the benzophenone radical anion. This is not unexpected because the  $pK_a$  of the ketyl radical from benzophenone is 9.25 (19), and thus, rapid protonation should occur at pH 7.4.

Power dependence studies show that electron photoejection and the resulting ketyl radical formation are predominantly (and presumably exclusively) two-photon processes. The implication of this is that from a biological point of view these processes are not important because they will not occur under conditions of ambient (solar or artificial) illumination (4). Small transient absorption extending from 330 to 420 nm is observed at very long time scales. This may be caused by either a cyclohexadienyl radical proposed as a product from the benzophenone cation after photoionization (27) or by the light absorbing transients that participate in the rearrangement of FA to product 2 in Scheme 1 (13). A detailed interpretation of these minor signals is not essential for understanding the details of the mechanism.

The other key transient observed in this work is the triplet state of FA, which, not surprisingly, results from a monophotonic process. This transient will be important under conditions of laser or ambient illumination. Product studies show that the fast triplet decay reflects decarboxylation, ultimately leading to products 1 and 2.

Several aspects of triplet state spectroscopy and lifetimes (see Tables 1 and 2) need to be considered together: (1) the triplet lifetime of the FA anion is much shorter than for FB; (2) the triplet lifetime of the anion form of FA is 10 and 20 times shorter in methanol and acetonitrile-rich solvents, respectively, than in water; (3) the quantum yield of FA photodegradation is ca. 12 times larger in acetonitrile-rich solutions than in water; (4) the triplet-triplet absorption of FA in water no longer resembles the typical benzophenone triplet structure observed in methanol (13), i.e. the visible maximum is at 460 nm rather than at 525 nm; (5) the triplet state of FB (a model for the FA triplet state) reacts readily with hydrogen donors in organic solvents but is at least 20 times less reactive in water; and (6) the phosphorescence spectra and lifetimes at 77 K suggest a change in the lower triplet excited state from  $n,\pi^*$  in organic solvents to  $\pi,\pi^*$  in water.

The six issues raised in the previous paragraph can be easily rationalized by invoking a  $\pi,\pi^*$  triplet in water for FA. Such state inversion is common for acetophenone and xanthone derivatives but less so for benzophenones, which are usually the archetypes for  $n,\pi^*$  behavior, although singlet photochemistry has been reported recently for ketoprofen, another substituted benzophenone (24,25).

The spectral shift observed for triplet FA is in line with those observed for xanthone and azaxanthone (9,28). The band in the T-T absorption spectrum of FB or FA is 34 kJ/ mol higher in energy in water (460 nm) than in acetonitrile (525 nm).

An interesting observation from this work is that decarboxylation, which in the case of FA involves the triplet state, is much faster for the  $n,\pi^*$  than for the  $\pi,\pi^*$  triplet, and

| Table 2. | Photophysical and | photochemical | parameters | obtained | for FA | and FB | in | different | acetonitrile:wa | ter mixtures |
|----------|-------------------|---------------|------------|----------|--------|--------|----|-----------|-----------------|--------------|
|----------|-------------------|---------------|------------|----------|--------|--------|----|-----------|-----------------|--------------|

|   |          | Ratio acetonitrile:water |                      |                      |                      |                      |  |  |  |
|---|----------|--------------------------|----------------------|----------------------|----------------------|----------------------|--|--|--|
|   | Compound | 93:7                     | 8:2                  | 4:6                  | 2:8                  | 0:1                  |  |  |  |
| Φ Photodegradation*                               | FA       | 0.70                     |                      |                      |                      | 0.06                 |  |  |  |
| $k_{\rm dec} (s^{-1})^{\dagger}$                  | FA       | $3.9 \times 10^{7}$      | $2.4 \times 10^{7}$  | $5.3 \times 10^{6}$  |                      | $2.05 \times 10^{6}$ |  |  |  |
| $k_{\rm dec}  ({\rm s}^{-1}) \ddagger$            | FB       | $2.71 \times 10^{5}$     | $2.64 \times 10^{5}$ | $3.10 \times 10^{5}$ | $2.54 \times 10^{5}$ |                      |  |  |  |
| T-T absorption (nm)                               | FB       | 530                      |                      |                      | 460                  |                      |  |  |  |
| $k_q$ 1,4-CHD (M <sup>-1</sup> s <sup>-1</sup> )§ | FB       | $3.0 \times 10^{8}$      |                      |                      | $\leq 1 \times 10^7$ |                      |  |  |  |

\*Values obtained with 80 mM TMAOH.

†The solution was prepared with 8 mM TMAOH; the rate constants were measured at 650 nm.

<sup>‡</sup>The rate constants were measured at 450 nm.

§The rate constants were measured at 700 nm.



**Figure 10.** A: Concentration of FA after different irradiation durations with a 254 nm Hg lamp under N<sub>2</sub> atmosphere, 0.08 *M* TMAOH in ( $\blacksquare$ ) water and ( $\square$ ) 93:7 acetonitrile:water; also shown is ( $\blacklozenge$ ) the evolution of ketoprofen concentration in water. B: Product formation over time, following FA irradiation. ( $\blacktriangle$ ) photoproduct 1, water; ( $\blacklozenge$ ) photoproduct 2, water; ( $\triangle$ ) photoproduct 1, 93:7 acetonitrile:water; ( $\bigcirc$ ) photoproduct 2, 93:7 acetonitrile:water. The concentrations were measured by HPLC.

thus, triplet lifetimes are longer in water than in organic solvents (similar results have been obtained for tiaprofenic acid) (29). At least part of this slowdown is reflected in the activation energy. Note for example in Fig. 8 that the lower line clearly has the greater slope, even if differences in preexponential factors are probably within experimental error, given the limited temperature range available.

If decarboxylation occurs from the triplet state of FA, then it is reasonable to assume that at least the nascent carbanion must be in the triplet state. In Scheme 1 we have written the carbanion and the biradical as resonant structures. Whereas singlet and triplet states should be essentially isoenergetic in the biradical case, large differences in energy are anticipated for the carbanion. Thus, the carbanion should be the preferred resonance structure for the singlet, whereas in the triplet manifold the biradical will be dominant. For the biradical to be the nascent species, an electron transfer (carboxylate to carbonyl) may precede decarboxylation.

Phosphorescence studies support the foregoing analysis, *i.e.* increase of aqueous content leads to a decrease in triplet energy and an increase in triplet lifetime. However, the switch to a  $\pi,\pi^*$  state with aqueous content is probably more complete at room temperature than in a glass at 77 K because in the latter the phosphorescence spectrum shows some vibrational resolution in water, whereas at room temperature the unreactivity of the triplet toward hydrogen transfer (see Fig. 7) suggests a complete inversion of states.

In conclusion, in this work we have presented results on the photochemistry of FA both at high and low laser power



**Figure 11.** Energy level diagram for the lowest triplet state  $(T_1)$  of FA in water and acetonitrile, the second lowest triplet state  $(T_2)$  of FA in water and the higher triplet state level giving rise to the  $T_1$ - $T_n$  absorption band, where A is probably in the 5 to 15 kJ/mol range and B (based on the spectral shift for T-T absorption) is likely to be somewhat higher.

(i.e. 308 and 355 nm excitation wavelength) in aqueous buffer solutions. In its dissociated form, FA decarboxylates from the triplet manifold after excitation, in a way similar to that previously reported in alcohol mixtures in the presence of base (13). This deactivation mode is inactive when FA is in its ester form (FB). In the latter case, the triplet state decays with a similar rate, independent of the solvent media; however, in the presence of good hydrogen donors hydrogen abstraction is faster in lower polarity acetonitrile:water mixtures, compared with pure water. This is in parallel with a higher reactivity toward decarboxylation for the FA anion in acetonitrile-rich mixtures, compared with water. This in turn correlates with a higher triplet energy in ethanol vs water and is also accompanied with a hypsochromic shift in the visible T-T absorption band, compared with that in acetonitrile, similar to the behavior of xanthone (9) or azaxanthone (28). These results are explained in terms of FA presenting a  $\pi,\pi^*$  nature lowest triplet state in water, whereas in low polarity acetonitrile:water mixtures the lowest triplet is higher in energy and has an  $n,\pi^*$  character (see Fig. 11). Both decarboxylation and hydrogen abstraction are processes arising from the  $n,\pi^*$  state.

From a biological point of view, these results are of significance. We can now explain the lower reactivity exhibited by FA and its photoproducts when compared with ketoprofen and its respective photoproducts. In the latter case it has been established that the deleterious effects to cell membranes arise from lipid peroxidation. This is a radical-mediated mechanism involving the action of an  $n,\pi^*$  triplet ketone acting as an initiator (30–32). Photoproduct 1, whose structure and excited state spectroscopic behavior after LFP is similar to that of FB (data not shown), should also present a  $\pi,\pi^*$  lower triplet state in water, therefore being less reactive toward lipid peroxidation at physiological pH values. A similar conclusion can be used to explain the lower reactivity of FA, compared with ketoprofen, toward DNA cleavage. As has been shown, this is a process inhibited by radical quenchers and is therefore most probably initiated following hydrogen abstraction by the arylketones (5), those having  $n,\pi^*$  lowest triplet states being more reactive than those having  $\pi,\pi^*$  states.

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