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How Drug Photodegradation Studies Led to the Promise of New Therapies and Some Fundamental Carbanion Reaction Dynamics along the Way

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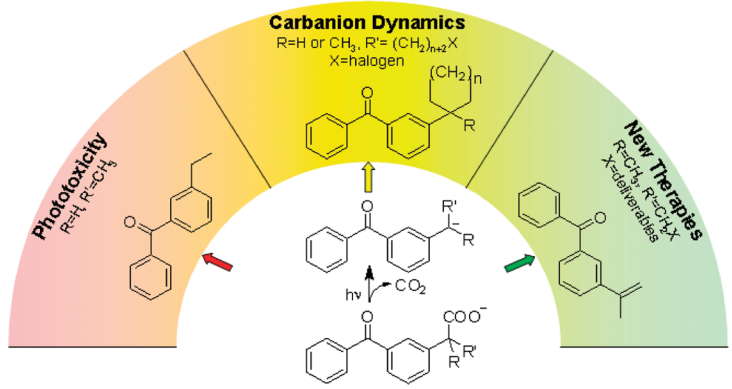

The photodegradation of nonsteroidal anti-inflammatory drugs (NSAIDs), a class of medications that includes aspirin and ibuprofen, has generated considerable interest since the 1990s, largely because of the phototoxic and photoallergic effects that frequently accompany their therapeutic use. Among NSAIDs, ketoprofen, which contains a benzophenone chromophore, has been extensively studied, reflecting both its notorious adverse effects and the fascination that photochemists have with benzophenone. The photochemistry of ketoprofen involves the intermediacy of an easily detectable carbanion with a remarkable lifetime of 200 ns in water; its life expectancy can in fact be extended to minutes under carefully controlled anhydrous conditions.

Over the past decade, we have used some key properties of the ketoprofen carbanion to conduct mechanistic studies on carbanions under various conditions. In particular, its ease of photogeneration provides the temporal control required for kinetic studies, which, combined with its long lifetime and readily detectable visible absorption, have enabled extensive laser flash photolysis work. These studies have led to an intimate understanding of the reaction dynamics for carbanions in solution, including the determination of absolute rate constants for protonation, S_N2, and elimination reactions. Together they provide excellent exemplars of reactivity patterns that today are part of all introductory curricula in organic chemistry and illustrate the fundamentals of nucleophilic substitution paradigms.

More recently, we have begun to exploit the photochemistry of ketoprofenate and have developed the ketoprofenate photocage, a valuable tool for the photocontrolled cleavage of protecting groups and concomitant drug release. The photorelease has been illustrated with ibuprofen, among many other molecules. These photocages have been further improved with the use of the xanthone chromophore; the goal is the release of antiviral agents taking advantage of the improved UVA absorption of xanthone (xanthonate photocages).

In this Account, we survey our work of the past few years on the photochemistry of ketoprofen and related chromophores. Beginning with studies on the phototoxicity of ketoprofen, we have made the journey to new prodrug candidates, unraveling mechanistic elements of aroyl-substituted benzyl carbanions along the way.

1. Introduction

Understanding the photostability and photodegradation of medicinal drugs leads to decisions concerning formulation, packaging, and shelf life of many pharmaceuticals, largely based on ICH compliance.[1] Further, warnings about possible adverse effects resulting from exposure to sun-

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light reflect information derived from drug photostability studies. This interest has been reflected in numerous studies published during the last two decades.[2−5] Particular attention has been paid to NSAIDs (nonsteroidal anti-inflammatory drugs), frequently containing chromophores that absorb UVA light.[2] Among these, ketoprofen and related compounds have received special attention, partly because of reports relating to adverse effects and to some extent reflecting the fascination that photochemists have had with the benzophenone chromophore for over a century.[6] The singlet-mediated photodegradation of ketoprofen conjugate base (Scheme 1, **1**) is simple yet uncommon chemistry for a substituted benzophenone.[7]

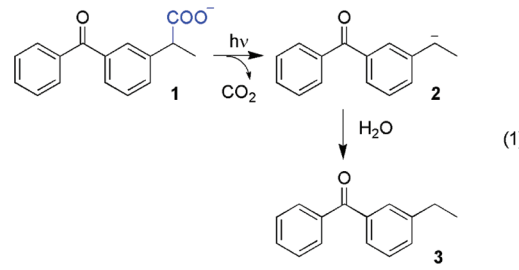
Carbanion **2** has been characterized spectroscopically by laser flash photolysis (LFP, typically 266, 308, or 355 nm excitation and 6−10 ns pulses) and in water absorbs at ca. 600 nm with a surprisingly long lifetime of 200 ns (see Figure 1). This Account tells the story of the mechanism of reaction 1 (Scheme 1) and related processes. Our interest in understanding the reactivity of ketoprofen has stimulated work on carbanions and led to the invention of photocages that hold promise for novel antiviral therapies.

2. Why Is Ketoprofen Phototoxic?

2.1. Photodegradation Mechanism, Intermediates, and (Phototoxic) Products. Triplet benzophenone has been extensively studied. Indeed, triplet state processes were responsible for the photochemistry of benzophenone in isopropanol that Ciamician studied on the rooftops of the Bologna laboratories that today are named after him.[6] Further, almost all photoreactions of benzophenone proceed from its triplet state. Not so for ketoprofen conjugate base (ketoprofenate, **1**), which provides an unusual (and sometimes controversial) example of singlet photochemistry. Reaction 1 occurs with a quantum yield of 0.75, and the carbanion decays with first-order kinetics, Figure 1. Although reaction 1 may be counterintuitive in the context of benzophenone photochemistry, it has the appeal of producing a ground-state carbanion in a spin-allowed process.

While mechanistic experiments were largely conducted in water, additional work in methanol and acetonitrile−water mixtures under basic conditions allowed us to unequivocally assign a subnanosecond lifetime for the decarboxylation reaction of ketoprofenate (Scheme 1). We note that our emphasis has largely been on the carboxylate form that is relevant under physiological conditions. Temperature studies of the carbanion gave Arrhenius preexponential values of ca. 1×10^9 s^{-1} for the pseudo-first-order reaction of protonation, consistent with a bimolecular reaction. The protonation took place with surprisingly low activation energy values of ca. 10 kJ/mol, a value expected for an energy barrier arising solely from solvent viscosity.[8] Additionally, formation of the carbanion in D_2O instead of H_2O lead to a small increase in the carbanion lifetime. The kinetic isotope effect (KIE) measured ($k_H/k_D = 1.2$), albeit small, indicates O−H bond breaking in the transition state for the reaction.[7]

SCHEME 1. Photodegradation of Ketoprofen Conjugate Base (Ketoprofenate, **1**) via Formation of a Carbanion Intermediate (**2**) To Yield 3-Ethylbenzophenone (**3**)



(1)

The possible involvement of the triplet state of ketoprofenate in Reaction 1 has been an issue of much interest. In an attempt to study the triplet state, we examined **1** in various water−acetonitrile mixtures. When the mole fraction of water is 0.13 and in the presence of 8 mM base, a small amount of detectable triplet is formed with a lifetime of 3 μs; this triplet, expected to be in its carboxylate form, does not yield carbanion **2**. Thus, the carbanion precursor must be the singlet state. Further, the quantum yield of 0.75 in pure water (*vide supra*) is likely to reflect some inefficiency in the singlet decarboxylation, where the unaccounted quantum yield of 0.25 (difference between 1.0 and 0.75) most probably reflects unproductive singlet pathways (arguably not yielding triplet **1**, which would be expected to be readily detectable in water).

While we have been able to demonstrate that the photodecarboxylation of ketoprofenate occurs from the singlet state in water-acetonitrile mixtures,[8] it has been more difficult to confirm the multiplicity in pure water, largely because benzophenones are not fluorescent in water, which is a limitation since fluorescence spectroscopy is our best tool for studying singlet excited states.

We then decided to look at similar compounds where the benzophenone chromophore was replaced with the closely related xanthone chromophore, which shows fluorescence in water,[10,11] thus facilitating the study of singlet photochemistry.[12] We prepared three ketoprofen analogues, the xanthone acetic acid isomers **4**−**6** (Scheme 2). Surprisingly, we found that while **4** and **6** underwent photodecarboxylation in neu-

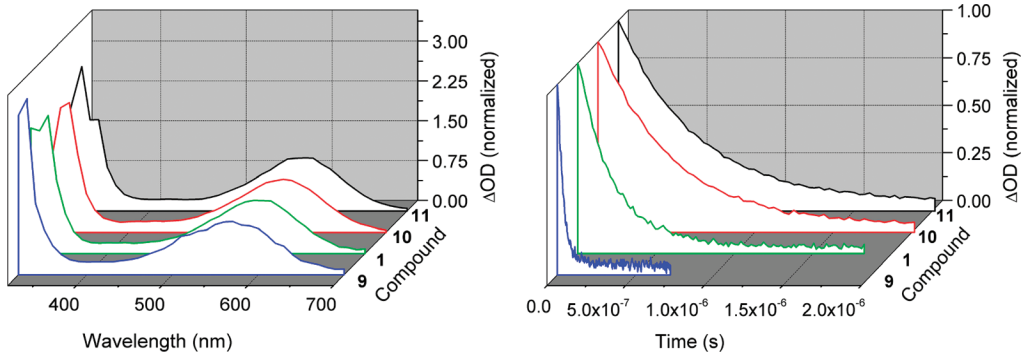
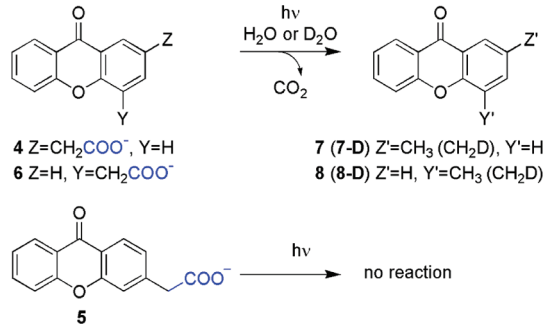


FIGURE 1. Transient absorption spectra (left) generated on laser flash photolysis (LFP) of ketoprofenate (**1**) and compounds **9**, **10**, and **11** (see Scheme 3 and discussion below). Traces were recorded immediately after laser excitation in a 1% v/v methanol 0.1 M KOH aqueous solution under N_2O (λ_{ex} = 308 nm). Transient absorption decays (right) monitored at 600 nm for the same compounds. Adapted from refs 7 and 9.

SCHEME 2. Photodecarboxylation of Xanthones **4** and **6**[a]



[a] Note that **5** is unreactive.

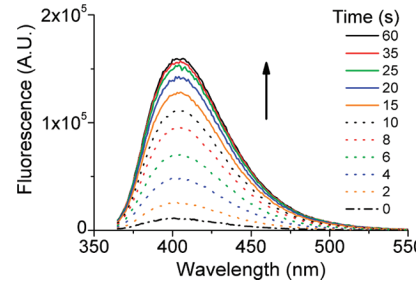


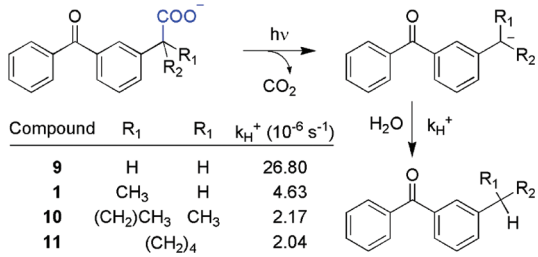
FIGURE 2. Fluorescence increase with irradiation of **4** (0.02 mM) in pH 7.4 phosphate buffer. Spectra at various times after irradiation. Adapted from ref 12.

tral aqueous solution to give **7** and **8**, respectively, with quantum yields close to that of ketoprofenate ($\Phi > 0.6$), **5** was unreactive. The stark contrast in the reactivity of these isomers is governed by the *"meta* effect", first reported by Zimmerman after he found that functional groups tend to influence photoreactions on aromatic rings much more strongly when they are at the *meta* position rather than *para* (the reverse of the ground-state ordering).[13] In both **4** and **6**, the electron-withdrawing carbonyl group is *meta* to the carbanion that is formed, while in **5**, the carbonyl is *para*. We found reactive isomers **4** and **6** to be weakly fluorescent in neutral aqueous solution ($\Phi_F < 1\%$); however, brief irradiation with a hand-held TLC lamp caused the fluorescence emission to increase by a factor of 15−30 due to formation of the highly fluorescent methylxanthone photoproducts (Figure 2).[12] The weak fluorescence of **4** and **6** relative to their photoproducts (and their much shorter singlet lifetime) is consistent with a singlet state decarboxylation process. The most compelling evidence for the singlet state decarboxylation of **4** and **6** came from LFP experiments. We were able to directly observe the triplet states of **4**−**6** and quench them with a variety of triplet quenchers including oxygen, naphthalene−methanol, and sorbate. Using high concentrations of sorbate (50 mM), we were able to entirely quench the triplet absorption for **4**−**6**, while this same concentration of sorbate had no effect on photoproduct yields. The most reasonable explanation of this observation is that decarboxylation proceeds exclusively via the singlet excited state.

2.2. Studies in Live Cells. The question of the origin of ketoprofen phototoxicity has many features in common with similar concerns relating to other NSAIDs. Our approach reflects our interest in molecules, their chemical changes, and the mechanisms responsible for these processes.

Studies of ketoprofen photodegradation and its adverse effects on cells lead to a surprising, yet chemically meaningful, conclusion, that is, that ketoprofenate, the dominant species at pH 7.4, is not phototoxic but rather that adverse effects should be attributed to 3-ethylbenzophenone (**3**), the carbanion protonation product. In fact, very early in the exposure, **1** is, in a strange way, a UV protector.[14] Indeed, excited ketoprofenate decays mainly via "instantaneous"decarboxylation (<6 ns, typical laser pulse duration); therefore, competing pathways such as hydrogen abstraction or energy transfer to molecular oxygen are unlikely. Such deleterious photoreactions require the subsequent excitation of 3-ethylbenzophenone, the major photoproduct.

SCHEME 3. Protonation Rate Constants for Carbanions from Ketoprofen and Its α-Alkyl-Substituted Derivatives



Compound	R_1	R_1	k_H^+ (10^{-6} s^{-1})
9	H	H	26.80
1	CH_3	H	4.63
10	$(CH_2)CH_3$	CH_3	2.17
11	$(CH_2)_4$		2.04

3. Exploring Carbanion Reactivity

3.1. Effects of Substitution on Protonation Rates. In order to gain further insights into the factors governing carbanion protonation, we directed our efforts to studying the effect of carbanion substitution.[9] Accordingly, we prepared the series of ketoprofen derivatives **9**−**11** and studied them together with ketoprofen (**1**). Upon photolysis of their conjugate bases, structures **1**, **9**, and **10** and **11** gave secondary, primary, and tertiary benzylic carbanions, respectively. In all cases, the carbanions were formed with similar quantum yields, ranging from 0.66 to 0.76, and gave rise to almost identical absorption spectra (Figure 1).

We observed a large effect due to alkyl substitution where the primary carbanion reacted faster than the secondary carbanion, which in turn reacted faster than the tertiary carbanion. A 5-fold increase in carbanion lifetime was observed upon introduction of the first methyl group with lifetimes being ca. 40 and 200 ns for primary and secondary carbanions, respectively. Addition of a second alkyl group led to a modest 2-fold increase in the tertiary carbanion lifetime (Figure 1).

The comparison of primary, secondary, and tertiary carbanions is a key part of our introduction to organic chemistry, where we learn that substitution by electron-donating alkyl groups reduces the carbanion stability, leading to the well-established stability order tertiary < secondary < primary < methyl. We were therefore initially surprised to observe that the carbanion reactivity measured in water was contrary to expectations based on charge stabilization. We reasoned that under our experimental conditions, protonation is controlled by entropic rather than enthalpic factors. Electron withdrawal by the benzoylphenyl group, common to all the carbanions studied herein, accounted for most of the stabilization whereas steric hindrance by bulky groups controlled the reactivity toward protonation.

3.2. Nucleophilic Substitution Reactions: Intra-S_N2 and a Powerful Method for Mechanistic Studies. We discussed above changes in protonation rates with varying substitution pattern. Given the well-established notorious effects of even traces of water on reactive carbanions, we were surprised at the rather slow protonation of the ketoprofen carbanion (Figure 1). While a 200 ns lifetime may initially appear short, given the presence of 55 M water, this lifetime reflects rather modest protonation kinetics.

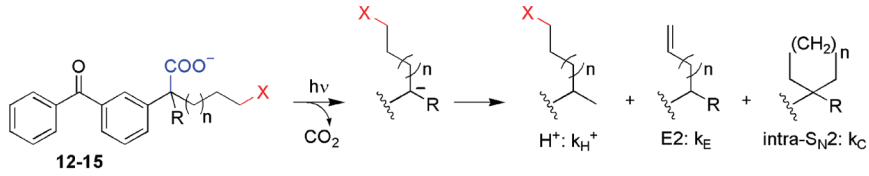
We were curious to see whether we could take advantage of the "slow" protonation kinetics by coaxing the ketoprofen carbanion into undergoing reactions other than simple protonation. One reaction that initially interested us was nucleophilic substitution in which the photogenerated carbanion acts as the nucleophile. We reasoned that for this reaction to have the best chance of being kinetically competitive with protonation, the electrophilic center (i.e., the leaving group) should be tethered directly to the carbanion structure in order to achieve high effective concentrations. Such reactions are also of interest because they can result in interesting cyclizations; additionally, they provide a very powerful method to perform mechanistic studies and learn about carbanion reactivity.

We prepared structures **12**−**15** and examined the ability of their resultant carbanions to cyclize in aqueous solution via intramolecular nucleophilic attack of the carbanion to the electrophilic centers (what we named intra-S_N2 reaction to emphasize the parallels with S_N2 reactions).[15,16] We recognized that protonation and possibly elimination might be competing carbanion reactions (Scheme 4).

Irradiation of compounds **12a**−**15c** in basic aqueous solutions gave predominantly the protonated products. Within a given family, that is, **12a**−**12c**, there was little variation in the observed carbanion decay rate constant, albeit in all cases the protonation was faster for the alkyliodide-substituted carbanion. Consistent with the results from the previous section, protonation rate constants were influenced by steric effects; we observed a ca. 2-fold reduction in the protonation rate constant going from secondary (i.e., **12** or **14**) to tertiary (i.e., **13** or **15**) carbanions. The alkyl chain length played no major role in the protonation rates; we observed decay lifetime values in the range of 130−170 ns for carbanions generated upon irradiation of compounds in families **12** and **14** and in the range of 310−390 ns for those from compounds in families **13** and **15**.

Whereas we observed no elimination products in basic aqueous solution for any of **12**−**15**, we were able to detect small amounts of cyclization products following irradiation of **14c** and **15c**. From the observed decay rate constant (k_{obs}) for the intermediate carbanions resulting from **14c** and **15c** obtained by LFP and from the fraction of cyclized products in the photolysate (F_C), from HPLC studies, we were able to establish aqueous cyclization rate constants (k_C) of 4.7×10^5 and

SCHEME 4. Reaction of Photogenerated Aroyl-Substituted Benzyl Carbanions



12-15

12a R=H, X=Cl, n=2 **13a** R=CH_3, X=Cl, n=2 **14a** R=H, X=Cl, n=3 **15a** R=CH_3, X=Cl, n=3
12b R=H, X=Br, n=2 **13b** R=CH_3, X=Br, n=2 **14b** R=H, X=Br, n=3 **15b** R=CH_3, X=Br, n=3
12c R=H, X=I, n=2 **13c** R=CH_3, X=I, n=2 **14c** R=H, X=I, n=3 **15c** R=CH_3, X=I, n=3

TABLE 1. Rate Constants for Cyclization (k_C) of Photogenerated Carbanions from **12**−**15** in DMSO[15,16]

compound	k_C (s^{-1})
12a	2.8×10^5
12b	3.6×10^7
12c	$>5.0 \times 10^7$
13a	4.0×10^5
13b	$>5.0 \times 10^7$
13c	$>5.0 \times 10^7$
14a	$<6.0 \times 10^4$
14b	6.1×10^5
14c	4.9×10^6
15a	$<1.1 \times 10^4$
15b	3.1×10^5
15c	1.9×10^6

7.7×10^4 s^{-1} for **14c** and **15c**, respectively, according to eq 1.

$$k_C = F_C k_{obs} \quad (1)$$

In an attempt to favor the cyclization pathway, we tried to reduce the amount of water available to the photogenerated carbanions and thus slow down the protonation pathway. We decided to examine the photochemistry of **12**−**15** in dry DMSO containing NaH (added as a basic media and to act as sacrificial drying agent for traces of moisture), and in all cases, the major product obtained was that resulting from cyclization through the expected intra-S_N2 reaction. We also observed some protonation products, which presumably resulted from reaction of the carbanion with traces of water. We observed no elimination products by HPLC.[15,16]

From the observed decay rate constants and product ratio studies in DMSO and water, we determined absolute rate constants for intramolecular S_N2 reactions of aroyl-substituted benzyl carbanions (Table 1). These data provided detailed information on how effects such as the nature of the leaving group, steric crowding, structure of the carbon skeleton, and the nature of the solvent will influence the rates of the intra-S_N2 reaction. Our findings reinforce concepts that are normally taught in introductory organic chemistry courses, although with our system, we can illustrate them using absolute rate constants.

We observed that rates for the intra-S_N2 reactions of the alkylchlorides were 2 orders of magnitude slower than those for the corresponding alkylbromides in both **12** and **13** (five-membered ring, secondary and tertiary carbanions, respectively), and the reaction for both alkyliodides was too fast for our LFP system. These results reinforce the expected dependence on leaving group ability, that is, $k_{Cl^-} < k_{Br^-} < k_{I^-}$. Interestingly, addition of an extra carbon in the alkyl chain reduced the reactivity gap between the various alkylhalides, we observed differences of only 1 order of magnitude for **14** or **15** (six-membered ring) in going from chloride to bromide to iodide.

From our data, we further determined that cyclization to form five-membered rings (**12** and **13**) is roughly 2 orders of magnitude faster than that for six-membered rings (**14** and **15**). This 2 orders of magnitude difference is very frequently observed in five- vs six-membered ring cyclizations and arises mostly from entropic rather than enthalpic factors.[17] Indeed, six-membered rings are usually less strained than five-membered rings. The difference in reactivity can be largely attributed to the activation entropy ($\Delta S^{\ddagger}$) for cyclization, where formation of a six-center transition state requires additional rotational restrictions leading to a lower entropy of activation for the six-center over the five-center ring.

In comparing the cyclization in DMSO and in water, we were able to examine solvent effects in the intra-S_N2 reactivity. Whereas water is a polar protic solvent capable of strongly stabilizing the carbanion via hydrogen bonding, DMSO is a polar aprotic solvent and gives a "naked" carbanion, expected to be much more reactive. Consistent with our expectation, cyclization of **14c** and **15c** was more than an order of magnitude faster in DMSO than in water. This again enables us to illustrate fundamental organic chemistry concepts with absolute rate constants.

Our results also teach us something about the reactivity of "water-in-water" compared with the reactivity of water when present in small amounts in organic solvents. We found that protonation of the carbanions from **12**−**15** with even traces

of water in DMSO can still compete with the cyclization route, indicating that these water traces are very reactive. To more closely evaluate the effect of water on the carbanion lifetime in DMSO, we performed quenching studies of the ketoprofen carbanion in DMSO containing either H_2O or D_2O. We obtained bimolecular rate constants of 5.1×10^7 and 1.1×10^7 M^{-1} s^{-1} for the quenching by H_2O and D_2O, respectively, leading to a primary KIE effect value of 5. While initially we were surprised that the value for the primary KIE is so much larger than that observed in pure water (*vide supra*), a careful analysis of pK_a values in DMSO (32 for water[18] and 26.8 for *p*-aroyl-toluene[19]) indicates that the proton transfer reaction in DMSO is closer to thermoneutrality compared with that in water, which should in turn result in a larger magnitude for the KIE in DMSO vs water.[20,21]

Extrapolation of the water quenching to a "pure water in DMSO" situation, that is, 55 M water that is as reactive as are small amounts of water in DMSO, predicts a value for $k_{protonation}$ of 2.8×10^9 s^{-1} ($\tau = 0.36$ ns); if this was the case, the carbanion would not be detectable in aqueous solutions using nanosecond LFP. We conclude therefore that water is 560-fold more reactive in DMSO than in aqueous solutions! A similar trend involving larger $k_{protonation}$ in polar nonprotic solvents was observed in acetonitrile water mixtures where the carbanion rapidly decayed with lifetime values of 100 and 25−30 ns for water mole fractions of 0.7 and 0.13, respectively.[8]

3.3. The Life Expectancy of Carbanions: Attempts to Study Intermolecular Nucleophilic Reactions. The realization that we could adjust widely the carbanion lifetimes by modification of the solvent environment led us to consider that we may be able to initiate *inter*molecular carbanion (S_N2) reactions photochemically. The "photo-Grignard" chemistry, as we referred to it in our laboratories, stimulated much work in Ottawa and a collaboration with colleagues in Valencia. We reasoned that lengthening the carbanion lifetime (by reducing the availability of water) would have the obvious appeal of allowing intermolecular reactions to take place in competition with protonation. We could thus foresee a spatial and temporal control of carbanion chemistry to take place for a carefully designed photochemical experiment.

Of the many approaches we tried, three emerged as successful: (1) using dry DMSO (as previously discussed), (2) encapsulation in a zeolite framework,[22] and (3) work in rigorously dried THF.[23] In fact, this last approach was so successful that the photograph of the blue carbanion solution (with multiminute lifetime) became the table of contents graphic when we reported these experiments (Figure 3).

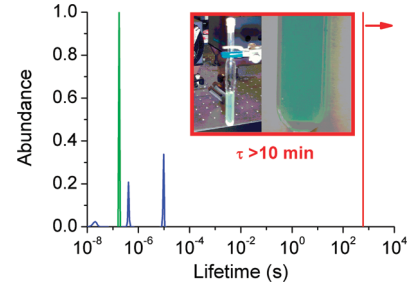
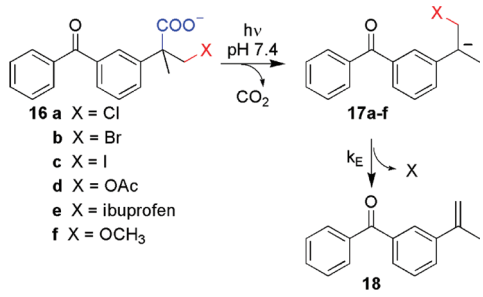


FIGURE 3. Lifetime distribution analysis for ketoprofen carbanion with abundances shown in colors: (in blue) compiled from kinetic data acquired at 600 nm at various times after 266 nm laser excitation of ketoprofenate in zeolite NaY under vacuum;[22] (in green) obtained from kinetic data acquired at 600 nm following ketoprofenate excitation at 308 nm in aqueous pH 7.4 solution under N_2O;[7] (in red) estimated from the visible carbanion absorption, following ketoprofenate excitation at 355 nm in dry THF under argon. Inset shows the photograph of the blue carbanion, adapted from the table of contents graphic of ref 23.

The encapsulation of ketoprofen sodium salt within the cages of zeolite NaY followed by exhaustive drying under vacuum created a suitable environment for increasing the photogenerated carbanion life expectancy. The encapsulated carbanion decayed with complex kinetics; a lifetime distribution study indicated a 50-fold enhancement ($\tau \approx 10$ μs) of the carbanion lifetime compared with that in aqueous solution. A second component with a 400 ns lifetime was also observed, suggesting that the encapsulated carbanion experienced more than one environment within the zeolite cavities. In fact, the shorter lifetime might correspond to carbanions generated in cavities containing traces of water that we were unable to remove.

As a result of our success in lengthening the carbanion lifetime, we tried to add electrophiles to see whether the carbanion was capable of engaging in intermolecular nucleophilic substitution or addition reactions. We were greatly pleased to observe high yields (~82%) of the product resulting from addition of the carbanion to acetaldehyde. Our attempts to induce substitution reactions, performed by addition of bromoethane, lead mostly to formation of 3-ethylbenzophenone, with the substitution product obtained as a minor component. In retrospect, the highly basic nature of the ketoprofen carbanion might have instead promoted the E2 elimination of bromoethane to give 3-ethylbenzophenone (as observed), bromide, and ethene. Our experiments represent the first example of lifetime enhancement of carbanions upon encapsulation within zeolites. Carbanions can now be added to the list of reactive intermediates that see a lifetime enhancement when encapsulated in zeolites, joining cations, free radicals, radical ions, and excited species such as triplet states.[24]

SCHEME 5. Mechanism of Photorelease of Leaving Groups from **16**



Our attempts to photogenerate ketoprofen-derived carbanions in THF led to the most dramatic lifetime enhancements. To dried THF (distilled over Na), we added small amounts of NaH to both deprotonate ketoprofen and act as a sacrificial drying agent for traces of moisture. Laser irradiation of ketoprofenate in this solvent gave a blue solution visible to the naked eye (Figure 3). The absorption spectrum of this solution showed spectral features consistent with our expectations for the carbanion of ketoprofen, and the solution retained its color for tens of minutes. Initial attempts to harness this lifetime enhancement to promote intermolecular substitution reactions (S_N2) were successful: addition of methyl iodide to the solution led to its rapid decoloration and the detection of the substitution product (3-isopropylbenzophenone) by GC-MS analysis. We believe that our experimental conditions will allow us to study the reaction of this carbanion with a variety of electrophiles and enable us to measure their absolute reaction rate constants.

4. Exploiting Carbanion Reactivity

4.1. Elimination Reactions: The Invention of Ketoprofenate Photocages. Following our work on substitution reactions, it seemed natural to explore whether elimination reactions could be triggered photochemically by introduction of a suitable leaving group. We prepared ketoprofen derivatives **16**, in which the leaving group X was bromide, iodide, and chloride, with the thought that the photogenerated carbanion **17** might be able to eliminate the halide to form alkene **18** (Scheme 5). We of course recognized that the carbanion might still react with water to compete with the elimination, and we had a good idea how fast the reaction of the carbanion with water might be ($k > 10^6$ s^{-1}). To our delight, irradiation of **16a**–**c** gave **18** as the only photoproduct, indicating that elimination of these halides is very favorable. Having established that elimination reactions could be initiated for photogenerated carbanions, it occurred to us that the ketoprofenate moiety could be the basis for a new family of photocages with potential applications in biology and the health sciences. The natural questions were whether this new cage could release molecules of interest and how the new cage would compare with well-established photocages, such as the widely studied *o*-nitrobenzyl system (*o*NB). Wirz has reported a detailed analysis of the desirable properties of a good photocage, and the extent to which *o*-nitrobenzyl compounds meet these criteria.[25]

At this point, we realized that our elimination reaction (see Scheme 5) showed several features that are highly sought after in photocage applications and in many respects were superior to the widely used *o*NB group.[25] First, the elimination is remarkably clean and efficient, with quantitative release of anionic leaving groups, no radical formation, and quantum yields approximating those for ketoprofenate decarboxylation ($\phi \approx 0.75$). Second, the reaction byproduct **18** is not highly absorbing or reactive (unlike the byproducts from the *o*NB group). Third, the carboxylate group imparts excellent aqueous solubility, which is ideal for using such a photocage in biological applications. Of course, before we could get too excited about the potential biological applications of this reaction, we had to actually demonstrate that we can release molecules that are somehow biologically active, beyond simple halide ions.

Our first attempt to eliminate biologically relevant molecules from **16** focused on the release of carboxylic acids, since these are widespread in biological and medicinal chemistry. We were able to prepare and irradiate a number of "caged" carboxylic acids, and we were pleased to see the same result for these that we saw for halides: protonation of the intermediate carbanion was unable to compete with elimination. We demonstrated this with the release of ibuprofen (another NSAID) from our ketoprofenate photocage: irradiation of **16e** in water (pH > pK_a) gave "free" ibuprofen and **18** as the only photoproducts with a quantum yield greater than 0.7,[26] thus demonstrating that the "ketoprofenate" photocage has great potential for biological applications.

4.2. How Fast Are Ketoprofenate Photocages? In cases such as release of bromide, chloride, phosphate, or carboxylic acids, ketoprofenate cages are so reactive that they cannot be monitored by LFP, since carbanion lifetimes of 10 ns or shorter characterize these cages.[26] We know how fast protonation of the intermediate carbanion by water should be (i.e., $10^6 < k < 10^8$ s^{-1}, see section 3). The elimination rate must be much faster than this for us to see only the elimination pathway.

The elimination of methoxide, a much poorer leaving group, provided an interesting system for several reasons: first, the release of alcohols opens the door to delivering many biologically interesting molecules; second, in this system, the carbanion faces a competition between release and protonation, allowing us to apply the toolkit of physical organic chemistry to determine rate constants for the elementary steps involved.

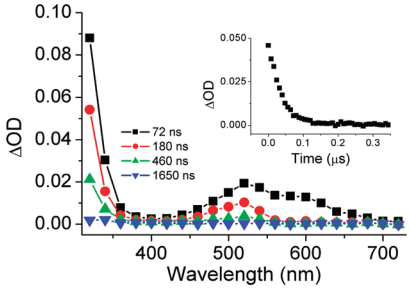


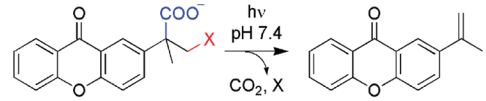
FIGURE 4. Transient absorptions observed on LFP of **16f** in oxygen-purged buffer solution (pH 7.4). The legend indicates the delay times for each trace after the laser pulse. The inset shows signal decay at 600 nm (points), which when fitted with monoexponential kinetics yields $k_{obs} = 2.7 \times 10^7\ s^{-1}$. Adapted from ref 26.

Irradiation of "caged" methoxide **16f** in neutral or basic aqueous solution gives *both* the elimination (i.e., **18**) and protonation products in a 4:1 molar ratio, our first case where the photoreaction does not exclusively follow the elimination route. In this system, we were actually able to detect the carbanion intermediate using LFP (Figure 4). We were able to measure the decay of the intermediate carbanion, which reflects the combination of the rates of elimination and protonation ($k_{obs} = k_E + k_H{}^+$). Using the 4:1 product ratio, we determined the rate of elimination of methoxide to be $5 \times 10^6\ s^{-1}$, or about 7 orders of magnitude faster than that from the *o*NB photocage![27,28]

4.3. Improving the Photocages. With all the nice properties that ketoprofenate cages offer, they actually have two characteristics that "need improvement", even if these are comparable to those for some widely used photocages. Specifically, the benzophenone chromophore has only an n,π^* transition in the UVA region with extinction coefficients around $10^2\ M^{-1}\ cm^{-1}$. While adequate for mechanistic and even some *in vitro* studies, this might be too low for *in vivo* applications. While not a requirement, our wish list included the possibility of molecules that would "report" on the occurrence of release conveniently by fluorescence emission.

We recognized that a photocage based on the photodecarboxylation of xanthone acetic acid might provide both properties, since xanthone has an extinction coefficient about 100 times higher than benzophenone in the UVA region and is measurably fluorescent in water.[10,11] While synthetically more challenging than the ketoprofenate photocages, we have been able to make a number of xanthone-based photocages that operate via the same mechanism as the ketoprofenate cages (Scheme 5).[12] Thus, xanthone photocages are able to eliminate good leaving groups rapidly and quantitatively with no competitive carbanion protonation. An example releasing aniline via the carbamate is shown in Scheme 6, with no other

SCHEME 6. Photorelease of Acetate and Aniline from Xanthonate Photocages **19**



19a X = OAc
b X = OC(O)NHPh

20

photoproducts formed other than alkene **20**. The xanthone photocage retains all of the positive characteristics of the ketoprofenate photocage, including high quantum yield, solubility, and the formation of noninterfering photoproducts. In fact, the photoproduct generated (alkene **20**) has a lower absorption coefficient than the starting material at 337 nm, which is the output wavelength of the nitrogen laser frequently used in biological applications.

5. Closing the Loop

Over a decade ago our interest in ketoprofen was motivated by its biomedical applications. The carbanion and its chemistry can clearly be the cause of adverse reactions, yet as we look forward, we see potential beneficial applications of this chemistry such as in the release of antivirals.

The demonstration that xanthone-derived molecules can release caged molecules efficiently and have improved optical properties opens the door for new applications in the health sciences. In an application of this concept, we aim at releasing antiviral activity, in the form of acyclovir, used to fight herpes simplex, type I, responsible for many cases of blindness and the leading reason for human corneal transplant failure.[29] The structure below (Figure 5) shows a potential target for incorporation into artificial corneas.

6. Final Remarks

An unexplored area in ketoprofen and related materials relates to the switch between a hydrophilic molecule and a hydrophobic one that the chemistry of reaction 1 involves. This change can be applied as a solubility switch or to change the nature of a surface, that is, from hydrophilic to hydrophobic. Eventually these aspects will also enrich the photochemistry of these molecules.

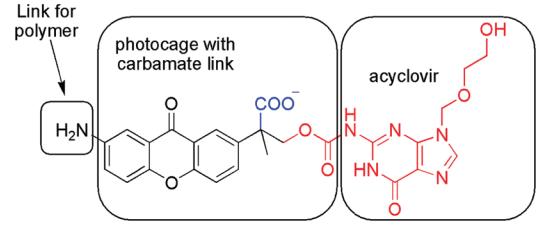


FIGURE 5. Caged antiviral acyclovir.

One of us has frequently pointed out that photochemists are truly passionate about just three molecules and their derivatives and that their efforts have frequently concentrated on them, finding a surprisingly rich and varied spectrum of behaviors. These molecules are pyrene, benzophenone, and ruthenium trisbipyridyl. Judging from the studies that we and other groups have carried out on ketoprofen and related NSAIDs, it is clear that the fascination with at least benzophenone remains alive and well.

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Gonzalo Cosa worked with Prof. Scaiano, and received his Ph.D. in 2002 from the University of Ottawa. He did postdoctoral work in single-molecule biophysics with Prof. Paul Barbara (University of Texas at Austin). He then joined the Faculty of McGill University in 2005. His research centers on designing, preparing, and utilizing smart fluorescent probes for cell imaging and on applying state-of-the-art single-molecule fluorescence methodologies to study protein/DNA/lipid interactions.

Matthew Lukeman received his doctoral degree from the University of Victoria in 2003, where he worked with Prof. Peter Wan, after which he joined the group of Prof. Scaiano as an NSERC postdoctoral fellow. He joined the faculty of Acadia University in 2005, and his current research interests concern the development of new photocages.

J. C. (Tito) Scaiano holds the Canada Research Chair in Applied Photochemistry at the University of Ottawa. Scaiano is also a past Editor-in-Chief of Photochemistry and Photobiology, and a founder of Luzchem Research, an Ottawa instrument manufacturer. His research interests center on organic photochemistry and physical organic chemistry and on the application of these principles to nanochemistry, chemical sensors, and the health sciences field.

FOOTNOTES

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REFERENCES

1 International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use. The US FDA in the Federal Register published the "Guidelines for the Photostability Testing of New Drug Substances and Products" on May 16, 1997 (p 27116).
2 Bosca, F.; Miranda, M. A. Photosensitizing drugs containing the benzophenone chromophore. *J. Photochem. Photobiol., B* **1998**, *43*, 1–26.
3 Budac, D.; Wan, P. Photodecarboxylation: mechanism and synthetic utility. *J. Photochem. Photobiol., A* **1992**, *67*, 135–166.
4 Lhiaubet-Vallet, V.; Miranda, M. A. Drug-biomolecule interactions in the excited states. *Pure Appl. Chem.* **2006**, *78*, 2277–2286.
5 Bosca, F.; Marin, M. L.; Miranda, M. A. Photoreactivity of the nonsteroidal anti-inflammatory 2-arylpropionic acids with photosensitizing side effects. *Photochem. Photobiol.* **2001**, *74*, 637–655.
6 Ciamician, G.; Silber, P. Chemische lichtwirkungen. *Ber. Dtsch. Chem. Ges.* **1900**, *33*, 2911–2913.
7 Martinez, L. J.; Scaiano, J. C. Transient intermediates in the laser flash photolysis of ketoprofen in aqueous solutions: Unusual photochemistry for the benzophenone chromophore. *J. Am. Chem. Soc.* **1997**, *119*, 11066–11070.
8 Cosa, G.; Martinez, L. J.; Scaiano, J. C. Influence of solvent polarity and base concentration on the photochemistry of ketoprofen: Independent singlet and triplet pathways. *Phys. Chem. Chem. Phys.* **1999**, *1*, 3533–3537.
9 Cosa, G.; Llauger, L.; Scaiano, J. C.; Miranda, M. A. Absolute rate constants for water protonation of 1-(3-benzoylphenyl)alkyl carbanions. *Org. Lett.* **2002**, *4*, 3083–3085.
10 Heinz, B.; Schmidt, B.; Root, C.; Satzger, H.; Milota, F.; Fierz, B.; Kiefhaber, T.; Zinth, W.; Gilch, P. On the unusual fluorescence properties of xanthone in water. *Phys. Chem. Chem. Phys.* **2006**, *8*, 3432–3439.
11 Mohtat, N.; Cozens, F. L.; Scaiano, J. C. Multistage exit of excited xanthone from micelles. *J. Phys. Chem. B* **1998**, *102*, 7557–7562.
12 Blake, J. A.; Gagnon, E.; Lukeman, M.; Scaiano, J. C. Photodecarboxylation of xanthone acetic acids: C−C bond heterolysis from the singlet excited state. *Org. Lett.* **2006**, *8*, 1057–1060.
13 Zimmerman, H. E. The meta effect in organic photochemistry: Mechanistic and exploratory organic photochemistry. *J. Am. Chem. Soc.* **1995**, *117*, 8988–8991.
14 Vinette, A. L.; McNamee, J. P.; Bellier, P. V.; McLean, J. R. N.; Scaiano, J. C. Prompt and delayed nonsteroidal anti-inflammatory drug-photoinduced DNA damage in peripheral blood mononuclear cells measured with the comet assay. *Photochem. Photobiol.* **2003**, *77*, 390–396.
15 Llauger, L.; Miranda, M. A.; Cosa, G.; Scaiano, J. C. Comparative study of the reactivities of substituted 3-(benzoyl)benzyl carbanions in water and in DMSO. *J. Org. Chem.* **2004**, *69*, 7066–7071.
16 Llauger, L.; Cosa, G.; Scaiano, J. C. First determination of absolute rate constants for the reaction of aroyl-substituted benzyl carbanions in water and DMSO. *J. Am. Chem. Soc.* **2002**, *124*, 15308–15312.
17 Anslyn, E. V.; Dougherty, D. A. *Modern Physical Organic Chemistry*; University Science Books: Sausalito, CA, 2006.
18 Bordwell, F. G. Equilibrium acidities in dimethyl sulfoxide solution. *Acc. Chem. Res.* **1988**, *21*, 456–463.
19 Bordwell, F. G.; Algrim, D.; Vanier, N. R. Acidities of anilines and toluenes. *J. Org. Chem.* **1977**, *42*, 1817–1819.
20 Dixon, J. E.; Bruice, T. C. Dependence of the primary isotope effect (kH/kD) on base strength for the primary amine catalyzed ionization of nitroethane. *J. Am. Chem. Soc.* **1970**, *92*, 905–909.
21 Bockrath, B.; Dorfman, L. M. Submicrosecond formation and observation of reactive carbanions. *J. Am. Chem. Soc.* **1974**, *96*, 5708–5715.
22 Chretien, M. N.; Cosa, G.; Garcia, H.; Scaiano, J. C. Increasing the life expectancy of carbanions by zeolite inclusion. *Chem. Commun.* **2002**, 2154–2155.
23 Laferriere, M.; Sanrame, C. N.; Scaiano, J. C. A remarkably long-lived benzyl carbanion. *Org. Lett.* **2004**, *6*, 873–875.
24 Scaiano, J. C.; Garcia, H. Intrazeolite photochemistry: Toward supramolecular control of molecular photochemistry. *Acc. Chem. Res.* **1999**, *32*, 783–793.
25 Pelliccioli Anna, P.; Wirz, J. Photoremovable protecting groups: Reaction mechanisms and applications. *Photochem. Photobiol. Sci.* **2002**, *1*, 441–458.
26 Lukeman, M.; Scaiano, J. C. Carbanion-mediated photocages: Rapid and efficient photorelease with aqueous compatibility. *J. Am. Chem. Soc.* **2005**, *127*, 7698–7699.
27 Corrie, J. E. T.; Barth, A.; Munasinghe, V. R. N.; Trentham, D. R.; Hutter, M. C. Photolytic cleavage of 1-(2-nitrophenyl)ethyl ethers involves two parallel pathways and product release is rate-limited by decomposition of a common hemiacetal intermediate. *J. Am. Chem. Soc.* **2003**, *125*, 8546–8554.
28 Il'ichev, Y. V.; Schworer, M. A.; Wirz, J. Photochemical reaction mechanisms of 2-nitrobenzyl compounds: Methyl ethers and caged ATP. *J. Am. Chem. Soc.* **2004**, *126*, 4581–4595.
29 This is a joint project with Professor May Griffith of the Ottawa Eye Institute, who has stimulated our interest in therapeutic applications of these photocages.