Photobehavior of Mixed $n\pi^*/\pi\pi^*$ Triplets: Simultaneous Detection of the Two Transients, Solvent-Dependent Hydrogen Abstraction, and Reequilibration upon Protein Binding

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In the present work, a systematic study on hydrogen abstraction by the excited triplet states of 4-methoxybenzophenone (1) and 4,4'-dimethoxybenzophenone (2) from 1,4-cyclohexadiene (3), 4-methylphenol (4), 1,2,3,4-tetrahydroquinoline (5), and 1-methyl-1,2,3,4-tetrahydroquinoline (6) in different media has been undertaken. Laser flash photolysis (LFP) revealed that in nonpolar solvents, 1 and 2 triplets have a $n\pi^*$ configuration with the typical benzophenone-like T-T absorption spectrum (λ_{max} ca. 525 nm). Conversely, in aqueous solution, transient absorption spectra with maxima at 450 and 680 nm, attributed to the $\pi\pi^*$ triplet, were obtained. Quenching of 1 or 2 triplet by 3 led to ketyl radical formation with rate constants in the range of $10^6-10^8 \text{ M}^{-1} \text{ s}^{-1}$, being one order of magnitude higher in acetonitrile than in aqueous media. The rate constants of quenching by 4 and 5 were similar in both polar and nonpolar solvents; the highest value was found for 6 in acetonitrile ((6.3 to 6.9) × $10^9 \text{ M}^{-1} \text{ s}^{-1}$). For mechanistic insight, LFP of 1 or 2 in the presence of dimethoxybenzene as electron donor was performed. The results showed that in this case, triplet quenching is favored in aqueous solution. In addition, 2 included in human serum albumin (HSA) was submitted to LFP. The decay kinetics, monitored at 430 nm, fitted well with three lifetimes of 0.45, 1.4, and 14.4 μ s assignable to 2 in bulk solution and in site II or in site I of HSA, respectively. This assignment was confirmed by using oleic acid and ibuprofen as selective displacement probes.

INTRODUCTION

One of the most deeply studied photoreactions of aromatic ketones is hydrogen atom abstraction.¹ The reactivity of a carbonyl compound toward a hydrogen donor depends on the electronic configuration of the lowest triplet excited state $(n\pi^* \text{ or } \pi\pi^*)$,² which may change with solvent polarity and aromatic ring substitution. Therefore, acetophenone presents a $n\pi^*$ triplet in nonpolar solvents and a $\pi\pi^*$ triplet in polar solvents; electron-donating substituents may produce an inversion of the electronic configuration of the acetophenone triplet.³ By contrast, the benzophenone triplet is $n\pi^*$ in both polar and nonpolar solvents;⁴ inversion of the configuration from $n\pi^*$ to $\pi\pi^*$ is favored by increasing solvent polarity, as in 4-methoxybenzophenone (1) and 4,4'-dimethoxybenzophenone (2), where the aromatic ring bears electron-donating substituents.⁵ Therefore, laser flash photolysis (LFP) of cyclohexane solutions of 1 shows

the formation of the $n\pi^*$ triplet alone, whereas a $n\pi^*/\pi\pi^*$ mixture is observed in acetonitrile. Similar studies on 2 in acetonitrile solution also reveal the presence of an equilibrated $n\pi^*/\pi\pi^*$ mixture. In aqueous solution, both aromatic ketones show only their $\pi\pi^*$ triplets.^{5,6}

The reactivity of $n\pi^*$ ketone triplets toward aliphatic or benzylic hydrogen donors is higher than that presented by $\pi\pi^*$ triplets. Moreover, it is generally accepted that the reactivity is basically due to $n\pi^*$ triplet excited states and that $\pi\pi^*$ triplets are essentially unreactive. Furthermore, $\pi\pi^*$ ketones are thought to react through the thermally populated $n\pi^*$ triplets.^{4,7,8} Therefore, in the case of a high energy gap between the two states, the

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Chart 1. Chemical Structures of Ketones and Donors



ketone is typically unreactive. However, if an electron transfer step precedes (or is coupled to) hydrogen transfer, then the electronic configuration of the lowest triplet state becomes unimportant.⁹

In addition, the X–H bond energy and the oxidation potential of the donor have a strong influence on formal hydrogen abstraction. Therefore, as a function of the above parameters, the process can occur by a variety of mechanisms, ranging from pure hydrogen atom abstraction (hydrocarbons or alcohols)^{3,10} to electron transfer, followed by proton transfer from the initially formed radical cation to the carbonyl radical anion (amines).^{11–14} Between these two extreme situations, an intermediate behavior can be found in benzylic or phenolic derivatives. In the former, the involvement of a charge-transfer complex has been reported,^{9,15} whereas for the latter, coupled electron–proton transfer within hydrogen bonded exciplexes has been suggested.^{16–20}

In this context, the aim of the present work is to perform a systematic study on the triplet reactivity of 4-methoxybenzophenone (1) and 4,4'-dimethoxybenzophenone (2) in the presence of different types of hydrogen donors (Chart 1) using the LFP technique. Our primary interest is (i) to establish the conditions for simultaneous detection of the two types of triplets, (ii) to determine whether for the same donor the change in the medium polarity may have an influence on the reactivity through triplet inversion, and (iii) to compare for each type of ketone triplet the reactivity changes as a function of the hydrogen donor nature.

In addition, as a paradigm to study the influence of constrained microenvironment on the nature and behavior of the triplet excited state, aromatic ketone **2** has been included in human serum albumin (HSA). This protein contains different binding sites with distinct properties; its two high affinity sites for small heterocyclic or aromatic compounds are known as site I and site II. In the former, hydrophobic interactions predominate, whereas in the latter, binding is governed by hydrogen bonding and electrostatic interactions.²¹ The properties of triplet excited states are very sensitive to the intraprotein microenvironment.²² Therefore, analysis of the triplet decay can provide information about substrate distribution among the bulk solution and the protein binding sites, whereas changes in the spectral shape are expected to report on the possible reequilibration between $n\pi^*$ and $\pi\pi^*$ triplets.

EXPERIMENTAL SECTION

Materials and Solvents. 4-Methoxybenzophenone (1), 4,4'dimethoxybenzophenone (2), 1,4-cyclohexadiene (3), 4-methylphenol (4), 1,2,3,4-tetrahydroquinoline (5), 1,4-dimethoxybenzene (7), 1,3-cyclohexadiene, biphenyl, fluorene, dibenzofuran, toluene, 2-propanol, and HSA were purchased from Aldrich.

1-Methyl-1,2,3,4-tetrahydroquinoline (6) was synthesized by treatment of an acetone solution of **5** with methyl iodide in the

presence of potassium carbonate.²³ The purity of all products was checked by HPLC and ¹H NMR. Reagent-grade solvent acetonitrile was purchased from Scharlau and used without further purification. The neutral buffer PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) was purchased from Sigma.

Absorption Spectra. Optical spectra in different solvents were measured on a Perkin-Elmer Lambda 35 UV/vis spectro-photometer.

Laser Flash Photolysis Experiments. The LFP experiments were carried out by using a Q-switched Nd/YAG laser (Quantel Brilliant, 355 nm, 14 mJ per pulse, 5 ns fwhm) coupled to an mLFP-111 Luzchem miniaturized equipment. This transient absorption spectrometer includes a ceramic xenon light source, 125 mm monochromator, Tektronix 9-bit digitizer TDS-3000 series with 300 MHz bandwidth, compact photomultiplier and power supply, cell holder and fiber optic connectors, fiber optic sensor for laser-sensing pretrigger signal, computer interfaces, and a software package developed in the LabVIEW environment from National Instruments. The LFP equipment supplies 5 V trigger pulses with programmable frequency and delay. The rise time of the detector/digitizer is \sim 3 ns (2.5 GHz sampling). The laser pulse is probed by a fiber that synchronizes the LFP system with the digitizer operating in the pretrigger mode. All transient spectra were recorded using $10 \times 10 \text{ mm}^2$ quartz cells with 4 mL of capacity, and all were bubbled during 20 min with N2. Absorbance of the samples was kept between 0.2 and 0.3 at the laser wavelength. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Dynamic studies on the photoreaction of 1 and 2 with 3-7 were performed in cyclohexane, acetonitrile, and mixtures of acetonitrile and buffered aqueous solution using 355 nm laser excitation (Nd/YAG).

In cyclohexane solution, LFP of 1 afforded a transient with a spectrum similar to that displayed by benzophenone (Figure 1A). However, in deareated acetonitrile solution, LFP of 1 led to transients absorbing in the 300–700 nm range. The spectrum showed a broad band centered at 520 nm and a shoulder at 450 nm, assigned to the $n\pi^*$ and $\pi\pi^*$ triplet, respectively (Figure 1B).⁵ Under the same conditions, **2** presented two bands centered at 540 and 425 nm. The former matched with the previously reported $n\pi^*$ triplet, ¹⁹ whereas the latter (more important in acetonitrile) can be assigned to the $\pi\pi^*$ triplet (Figure 1A,B).

When LFP of 1 and 2 was performed in a mixture of PBS and acetonitrile (3:1 v/v), the obtained spectra, with maximum at 450 nm and significant absorption in the 600–700 nm region, corresponded to the $\pi\pi^*$ triplet of both aromatic ketones (Figure 1C). In the case of 1, the triplet energy in water was previously estimated to be ca. 13 kJ mol⁻¹ lower than that in cyclohexane (288 kJ mol⁻¹).⁵ However, for 2, it was assumed in previous studies that the lowest triplet is basically $n\pi^{*,3,9}$ and its energy was estimated from the phosphorescence 0,0 band at 77 K in methyltetrahydrofuran as 290 kJ mol^{-1.9}. This triplet energy value has also been assumed in acetonitrile solution.^{19b} To determine in a systematic way the energy of the two closely lying triplet states of 2 in solution at room temperature, quenching experiments with several potential energy acceptors were undertaken. Therefore, it was expected that the most



Figure 1. Normalized transient absorption spectra obtained by 355 nm laser photolysis of 4-methoxybenzophenone (\bullet , red) and 4,4'-dimethoxybenzophenone (\blacksquare) 0.12 μ s after the pulse in (A) cyclohexane, (B) MeCN, and (C) PBS/MeCN (3:1, v/v).

stable triplet in toluene is $n\pi^*$, whereas in polar medium (water–MeCN mixtures), the $\pi\pi^*$ triplet should be lower in energy. Therefore, 1,3-cyclohexadiene,²⁴ biphenyl,²⁴ fluorene,²⁴ and dibenzofuran²⁵ were selected as triplet quenchers.

The rate constant of triplet-triplet energy transfer $(k_{\rm ET})$ depends on the triplet energy gap $(\Delta E_{\rm T})$ between the donor and the acceptor, as given by Sandros' eq 1²⁶

$$k_{\rm ET} = (k_{\rm max} \exp(-\Delta E_{\rm T}/RT))/(\exp(-\Delta E_{\rm T}/RT) + 1) \qquad (1)$$

where k_{max} is the optimum rate constant for the system.

Table 1. Rate Constants for Energy Transfer from 4,4'-Dimethoxybenzophenone to 1,3-Cyclohexadiene, Biphenyl, Fluorene, and Dibenzofuran, Together with the Estimated Values of the Triplet Excited State Energies

	$k_{ m ET} ({ m M}^{-1} { m s}^{-1})^a$		
	$E_{\rm T}$ (kJ mol ⁻¹)	PBS/acetonitrile	toluene
1,3-cyclohexadiene	219^{b}	$4.0 imes 10^9$	4.3×10^9
biphenyl	271^{b}	$4.9 imes 10^9$	4.6×10^9
fluorene	282^b	$4.6 imes 10^9$	3.1×10^9
dibenzofuran ^{c,d}	289 ^e	2.8×10^8	8.9×10^8

^{*a*} Obtained using 2 × 10⁻⁵ to 1.5 × 10⁻³ M concentrations of the quenchers under N₂. ^{*b*} Value in nonpolar solvent.^{24 c} $\Delta E_{\rm T}$ = 7 kJ mol⁻¹ in PBS/acetonitrile. ^{*d*} $\Delta E_{\rm T}$ = 3 kJ mol⁻¹ in toluene. ^{*e*} Value in ethyl acetate.²⁵

Therefore, LFP of 2 in PBS/MeCN was performed at 355 nm in the presence of increasing amounts of quenchers, and the decay of the T-T band was analyzed at 430 nm to determine the quenching rate constants. When the reciprocal triplet lifetimes of 2 were plotted against the quencher concentrations, different straight lines were obtained (not shown). The slopes (intermolecular $k_{\rm ET}$ values) are given in Table 1, together with the triplet energies of the acceptors, required for application of the Sandros' equation and assuming that the optimum rate constant for the system (k_{max}) is $4.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, the average of the first two entries. Therefore, a reasonable estimation for the $\pi\pi^*$ triplet energy of 2 is 282 kJ mol⁻¹. In a similar way, quenching experiments in toluene solution ($\lambda_{max} = 355$ nm), where k_{max} was taken as 4.4×10^9 M⁻¹ s⁻¹, led to a $n\pi^*$ triplet energy of 286 kJ mol $^{-1}$ (Scheme 1). A simplified energy diagram for the triplet excited states of 1 and 2 in different solvents is shown in Scheme 1.

After characterization of the two triplets of 2, experiments on hydrogen abstraction were undertaken. The results are summarized in Table 2. When 1,4-cyclohexadiene (3) was used as donor, the ratio between the quenching rate constants measured in acetonitrile and PBS/MeCN (3:1 v/v) resulted to be ca. 30 and 13 for 1 and 2, respectively. In a previous study on the photophysical properties of 1, it was shown that an increase in the polarity of the medium produces a decrease in $\pi\pi^*$ triplet energy, without affecting the $n\pi^*$ triplet.⁵ LFP of 5.0 \times 10⁻⁴ M solutions of 1 or 2 in different mixtures of PBS and acetonitrile in the presence of 3 (2.5 \times 10⁻³ to 5 \times 10⁻² M) were carried out to analyze the influence of solvent polarity on the effectiveness of hydrogen abstraction by the excited ketones. Therefore, Figure 2 shows the dependence of the rate constant on the molar fraction of acetonitrile, where it can be clearly seen that the kinetics become faster in enriched acetonitrile solutions.

As mentioned above, carbonyl compounds with lowest-lying $\pi\pi^*$ triplets abstract H atoms from hydrocarbons much more slowly than their analogs having similar excitation energies but with the lowest $n\pi^*$ triplets. In addition, it has been postulated that $\pi\pi^*$ triplets abstract hydrogen atoms predominantly via their thermally populated, higher energy $n\pi^*$ states. In this context, temperature changes should modify the reactivity. To check this point, we performed LFP experiments with **2** in toluene and PBS/MeCN solutions in the presence of 2-propanol as hydrogen donor, at temperatures ranging between 5 and 65 C° and using 550 or 430 nm, respectively, as the monitoring

Scheme 1. Energetic Diagram for the Lowest Triplet States of 1 (-, blue) and 2 (-, red) in Solvents of Different Polarity



 Table 2. Rate Constants for Quenching of 1 and 2 Excited

 Triplet States by Donors

	$k_{\rm q} ({\rm M}^{-1} {\rm s}^{-1})$ for (1)		$k_{ m q} ({ m M}^{-1} { m s}^{-1})$ for (2)			
	PBS/ MeCN	MeCN	PBS/ MeCN	M	MeCN	
quencher	450 nm (ππ*)	520 nm (nπ*)	440 nm (ππ*)	430 nm (ππ*)	550 nm (nπ*)	
3	$2.8 imes 10^6$	$7.5 imes 10^7$	3.8×10^{6}	5.2×10^7	4.8×10^{7}	
4	3.1×10^{9}	1.2×10^{9}	3.9×10^9	1.3×10^{9}	1.0×10^{9}	
5	$5.5 imes 10^9$	$1.5 imes 10^{10}$	$7.1 imes 10^9$	$1.4 imes 10^{10}$	$1.5 imes 10^{10}$	
6	3.9×10^9	6.3×10^9	6.3×10^9	6.4×10^9	6.9×10^9	
7	9.5×10^8	7.2×10^7	3.7×10^9	6.9×10^7	6.9×10^7	

wavelength. The donor selection was based on its low volatility compared with 1,4-cyclohexadiene, which should minimize concentration changes at higher temperatures. The obtained data actually showed that the quenching constant increases with temperature, and those changes were more pronounced in polar solvent. The Arrhenius plot (Figure 3) provided the activation energy E_a and the pre-exponential factor for **2** in the two solvents. Therefore, E_a in toluene resulted to be ca. 8 kJ mol⁻¹, whereas in PBS/MeCN, it was estimated at 21 kJ mol⁻¹. Hence, it is possible to correlate the activation energy for hydrogen abstraction with the energy gap between the two states, provided that the intrinsic activation energy for the reaction of the $n\pi^*$ triplets is also taken into account.

When 4-methylphenol (4) was used as hydrogen donor, the quenching rate constants for 1 and 2 in acetonitrile were found to be $(1.2 \text{ and } 1.0) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, respectively, more than one order of magnitude higher that those obtained for 3 ((7.5 and $4.8) \times 10^7 \text{ M}^{-1} \text{s}^{-1}$) under the same conditions (Table 2). In aqueous medium, these differences were even more pronounced (ca. three orders of magnitude). The marked substrate and solvent dependence of the reaction rate strongly suggest a change in the reaction mechanism, associated with the involvement of charge separation.

Similar behavior was observed for 1,2,3,4-tetrahydroquinoline (5) and 1-methyl-1,2,3,4-tetrahydroquinoline (6). It is well known that amines are good electron donors and that in their photoreaction with ketones electron transfer precedes proton transfer.²⁷



Figure 2. Plot of the reaction rate constants of 1,4-cyclohexadiene with 1 (\bullet , red) and 2 (\blacksquare) versus molar fraction of MeCN in MeCN/PBS mixtures.



Figure 3. Arrhenius plots for the reaction of triplet excited 2 with 2-propanol in toluene (\blacksquare) and PBS/MeCN (3:1 v/v) (\bullet , red).

Table 3. Free-Energy Changes for the Intermolecular Electron Transfer and Exciplex Formation in Acetonitrile Using the Rehm-Weller Relationships^a

			ketone 1		ketone 2	
_	quencher	$E_{\mathrm{ox}}\left(\mathbf{V}\right)$	$\Delta G_{\mathrm{et}} \left(\mathrm{eV} \right)$	$\Delta G_{\mathrm{ex}} \left(\mathrm{eV} \right)$	$\Delta G_{\mathrm{et}} \left(\mathrm{eV} \right)$	$\Delta G_{\mathrm{ex}} \left(\mathrm{eV} \right)$
	4	1.54 ^{19b}	0.44	0.79	0.52	0.87
	5	0.60 ³¹	-0.49	-0.15	-0.42	-0.07
	6	0.62^{31}	-0.48	-0.13	-0.40	-0.05
$^{a}E_{red}(1): -1.95$ V and $E_{red}(2): -2.016$ V versus SCE in MeCN. ^{19b}						

The rate constants for quenching of 1 and 2 by the same donor in a given solvent were similar. In addition, it is remarkable that the rate constants for quenching of the $n\pi^*$ or $\pi\pi^*$ triplets of 2 in MeCN by a selected hydrogen donor were comparable. This is in



Figure 4. (A) Normalized transient absorption spectrum of 2 in PBS in the absence and in the presence of HSA (1.25×10^{-3} M). (B) Laser flash photolysis ($\lambda_{exc} = 355$ nm) of 2 (-) and 2/HSA at different molar ratios 1:0.25 (-, red) and 1:2.5 (-, blue). Normalized decays were monitored at 430 nm.

agreement with the fact that the two triplet levels of **2** are strongly mixed, as expected for a triplet energy gap <8 kJ mol^{-1.4,28}.

By contrast, when the quencher used was 1,4-dimethoxybenzene, which can only play the role of an electron donor, the rate constants for both ketones were more than one order of magnitude higher in aqueous medium than in organic solvent. This can be attributed to stabilization of the resulting radical ion pair by hydration.

The rate constants for reaction of 1 and 2 with 4, 5, and 6 were high compared with those obtained with 3. The difference can be explained by facilitation of hydrogen abstraction through exciplex formation or photoinduced electron transfer. In general, exciplex formation is favored in nonpolar solvents, whereas photoinduced electron transfer dominates in polar media.²⁹ The energetics of radical ion pair formation resulting from electron transfer (et) can be calculated with eq 2^{30}

$$\Delta G_{\rm et} = E_{\rm ox} - E_{\rm red} - E^* + (2.6/\varepsilon - 0.13) \,\,{\rm eV} \tag{2}$$

where $E_{\rm ox}$ and $E_{\rm red}$ are the redox potentials (V) of donor and acceptor, respectively, E^* is the excitation energy (eV) of the involved excited state, and ε is the dielectric constant of the solvent (37.5 for acetonitrile). Thus, using the values given in Table 3 for the above-mentioned parameters,^{19b,31} an exergonic thermodynamics for electron transfer quenching of the triplet state of 1 or 2 can be established for 5 and 6, in acetonitrile. By contrast, the process was found to be endergonic for phenol.

In regards to the thermodynamics of exciplex formation, calculations according to eq 3,³⁰ where the μ^2/ρ^3 value is 0.31 in acetonitrile (where μ the viscosity and ρ is the polarity solvent used), indicate that the process is disfavored for all ketone-quencher pairs. (See Table 3.)

$$\Delta G_{\rm ex} = E_{\rm ox} - E_{\rm red} - E^* - \mu^2 / \rho^3 [(\varepsilon - 1/2\varepsilon + 1) - 0.19] + 0.38 \text{ eV}$$
(3)

Although all required redox potentials in aqueous medium were not available from the literature, it can be assumed that electron transfer is favored and exciplex formation is disfavored in comparison with acetonitrile.



Figure 5. Contribution (%) of the different lifetime components of the T-T signal of 4,4'-dimethoxybenzophenone (5×10^{-4} M) in the different microenvironments: free (gray) and within site I (blue) or site II (purple) of HSA (1.25×10^{-3} M), obtained from the A_1 , A_2 , and A_3 values of the decay fitting at 430 nm in the presence of different concentrations of oleic acid.

To study the influence of microheterogeneous media on the nature and behavior of the triplet excited state, we included ketone **2** in HSA. Aqueous solutions containing **2** and HSA (molar ratio from 1:0 to 1:5) were prepared in neutral buffer PBS and submitted to LFP. The transient absorption spectra obtained after laser excitation ($\lambda_{exc} = 355 \text{ nm}$) in the presence of HSA were similar to that obtained for **2** in acetonitrile. This is shown in Figure 4A, where the transient spectrum in PBS solution is also included for comparison. Clearly, a modification of the spectral shape is observed upon the addition of HSA, pointing to a marked reequilibration from $\pi\pi^*$ to $\pi\pi^*$ triplets within the more lipophilic protein cavities. Moreover, HSA complexation led to clear changes in the decay traces up to 1:2.5 molar ratios (Figure 4B).

The decay kinetics, monitored at 430 nm in air-equilibrated solutions, fitted well with three lifetimes of 0.45, 1.4, and 14.4 μ s. Regression analysis of the decay curves for several **2**/HSA ratios was performed to obtain the preexponential factors A_1 , A_2 , and A_3 corresponding to the different lifetimes. The shortest-living

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Figure 6. Relationship between site I (blue) and site II (black) contribution in the presence of (A) oleic acid and (B) ibuprofen.

component was associated with the presence of free 2 in the bulk solution, already determined in PBS (see above). However, the other two longer lifetimes can be correlated with the existence of two HSA-bound species.

To support further this assignment, LFP experiments were performed in the presence of oleic acid, a well-established displacing probe for site I ligands.³² The addition of this probe resulted in a reduced contribution of the longest lifetime component. Thus, at 1:2.5:2.5 2/HSA/oleic acid molar ratio, this value dropped to ca. 23% (Figure 5), as compared with 50% in the absence of oleic acid.

Thus, the longest lifetime (14.4 μ s) component is confirmed as corresponding to 2 within site I, whereas the 1.4 μ s component can be safely assigned to site II-bound 2. Further support for this assignment was obtained upon the addition of ibuprofen, a site II displacer.³³ As expected, the results (Figure 6B) showed the opposite trend to that shown by oleic acid, revealing a diminished contribution of 2 in site II with increasing amounts of ibuprofen, accompanied by a slight increase in 2 in site I.

CONCLUSIONS

Using the 4-methoxy and 4,4-dimethoxy derivatives of benzophenone (1, 2), the two types of triplets ($n\pi^*$ and $\pi\pi^*$) can be simultaneously detected in the same medium by transient absorption spectroscopy. Their relative contributions are strongly dependent on the polarity of the solvent; the $\pi\pi^*$ band (430–450 nm) is predominating in aqueous medium, whereas the $n\pi^*$ absorption (525–550 nm) is enhanced in nonpolar solvents.

The reactivity toward different types of hydrogen donors has been compared through the quenching rate constants. Interestingly, when both $n\pi^*$ and $\pi\pi^*$ are present in a given medium, their kinetic behavior is very similar, even when the reaction mechanism proceeds through pure hydrogen abstraction. Finally, upon addition of protein (HSA) in aqueous medium, a marked reequilibration from $\pi\pi^*$ to $n\pi^*$ is observed through modification in the spectral shape; this is explained by the enhanced lipophilicity of protein binding sites.

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