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Solvent Effects in Hydrogen Abstraction from Cholesterol by Benzophenone Triplet Excited State

Inmaculada Andreu, Fabrizio Palumbo, Fedele Tilocca, Isabel M. Morera, Francisco Boscá,* and Miguel A. Miranda*

Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia,
Consejo Superior de Investigaciones Científicas, Avda. de Los Naranjos s/n,
46022 Valencia, Spain

fbosca@itq.upv.es; mmiranda@qim.upv.es

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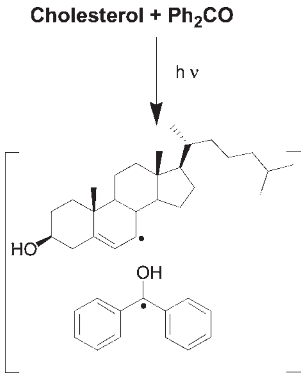
ABSTRACT



Hydrogen abstraction from the C-7 position of cholesterol (Ch) by triplet excited benzophenone (BZP) exhibits remarkable solvent-dependence in product studies. Kinetic measurements on the intramolecular version of the process in dyads containing covalently linked Ch and BZP units reveal important solvent effects and significant stereodifferentiation.

Oxidative activation of cholesterol (Ch) at the C-7 position is a process of considerable chemical and biological interest. Thus, the major pathway for the synthesis of bile acids from Ch is initiated by C-7 hydroxylation, which is achieved by the 7α-hydroxylase (CYP7A1), a member of the P450 family of metabolic enzymes.[1] It has also been claimed that C-7 oxidation is involved in the interception of blood and plasma oxidants by Ch, which thereby may act as an effective *in vivo* antioxidant.[2] In addition, Ch is a major target for oxidative damage.[3,4] This process can occur by a Type I mechanism (*via* free radicals) through abstraction of an allylic hydrogen from Ch by photosensitizing agents upon UVA light activation.[5]

Despite the importance of Type I Ch photooxidation, the key hydrogen abstraction step has not been previously subjected to mechanistic studies. In this context, we wish now to report on the photoreaction between the benzophenone (BZP) triplet excited state and Ch, a paradigmatic process of general interest.

Cholesterol is an essential structural component of cell membranes, where it is required to achieve proper permeability and fluidity. In the body, Ch can also be found in the liver or intestines, as well as in the bloodstream, where it is transported within lipoproteins. In addition, Ch is present

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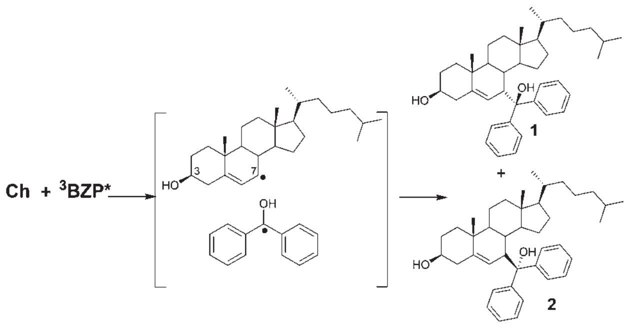
in all foods containing animal fat. Hence, it makes sense to investigate the generation of Ch-derived radicals in lipophilic media of different characteristics. In this connection, a recent discussion has arisen on whether hydrogen abstraction from carbon is subjected to significant solvent effects. Specifically, controversial results have been reported on the solvent-dependence of H-abstraction reactivity from C−H donors such as 1,4-dienes by cumyloxyl radical.[6]

With this background, the present work deals with the solvent effects on abstraction of the Ch C-7 allylic hydrogens by the triplet excited state of BZP. This issue has been addressed by performing both steady-state and time-resolved studies in different organic solvents.

To investigate photoproducts formation, steady-state photolysis of BZP/Ch mixtures (1.5:1 molar ratio) was performed under anaerobic conditions, in dichloromethane and acetonitrile, using a multilamp photoreactor equipped with UVB-lamps (λ_{max} = 300 nm, Gaussian distribution). In dichloromethane, no Ch-derived product was obtained, whereas in acetonitrile two diastereomeric photoproducts **1** and **2** were isolated.

Scheme 1. Formation of Photoproducts **1** and **2** upon Photolysis of Ch and BZP in Acetonitrile



As shown in Scheme 1, formation of **1** and **2** has to be explained by intermolecular hydrogen abstraction from the C-7 position of Ch, followed by C−C coupling of the generated radical pair. The structures of the photoproducts were unambiguously assigned on the basis of their NMR spectroscopic data (^{1}H, ^{13}C). The stereochemistry of the new chiral center was determined by NOE experiments; the most significant interactions were observed between the phenyl groups and the protons of the convex β-face in photoproduct **2**. The details are given in the Supporting Information.

The reaction kinetics was studied by laser flash photolysis experiments at 355 nm. Thus, when the reciprocal triplet lifetime was plotted against Ch concentration a linear relationship was found in dichloromethane as solvent (see Figure 1, insert). The quenching rate constant was found to be 2.2 × 10^8 M^{-1} s^{-1}. However, under these conditions extensive H-abstraction from the solvent was observed. This is clear from the comparison of the long-lived component in the kinetic traces at 545 nm (ascribed to the ketyl radical formed by hydrogen abstraction from the solvent) obtained in the presence and in the absence of Ch (Figure 1). In acetonitrile quenching was faster, but the kinetic parameters could not be accurately determined due to solubility limitations; nonetheless, the rate constant, estimated from the initial slope of the nonlinear plot (not shown), was *ca.* 1 order of magnitude higher. Similar solubility problems were found in other organic solvents such as methanol or dioxane, which in addition are better hydrogen donors than acetonitrile. Thus, the major difficulties in obtaining reliable data for the intermolecular photoreaction between Ch and BZP are related to the low solubility of Ch and the possibility of hydrogen abstraction from the solvent by triplet BZP. To circumvent these problems, a possible strategy could be to examine the intramolecular version of the process, using covalently linked Ch and BZP units. This would prevent the need for high quencher concentrations and should result in a dramatic decrase of the triplet lifetimes, minimizing hydrogen abstraction from the solvent. Moreover, the covalent link between the Ch and BZP units would completely block radical cage escape, favoring C−C coupling.

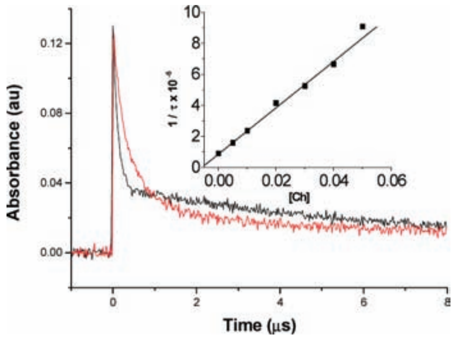


Figure 1. Trace obtained at 545 nm upon laser excitation of BZP in dichloromethane solutions in the absence (red) and in the presence of Ch (black, 0.02 M). Inset: Stern−Volmer plot showing the effect of Ch addition on the rates of BZP triplet decay at 610 nm.

Following this idea, diasteromeric dyads, **3a** and **3b** (Scheme 2), were submitted to laser flash photolysis (λ_{exc} = 355 nm) under a variety of conditions, in order to determine the kinetic parameters and the quantum yields of the relevant processes.

We have previously demonstrated that the transient absorption spectra of **3a** and **3b** in dichloromethane do not correspond to the triplet excited states but instead to the corresponding biradicals, generated by intramolecular hydrogen abstraction. Subsequent cyclization gives rise to C−C coupling photoproducts in a diastereoselective

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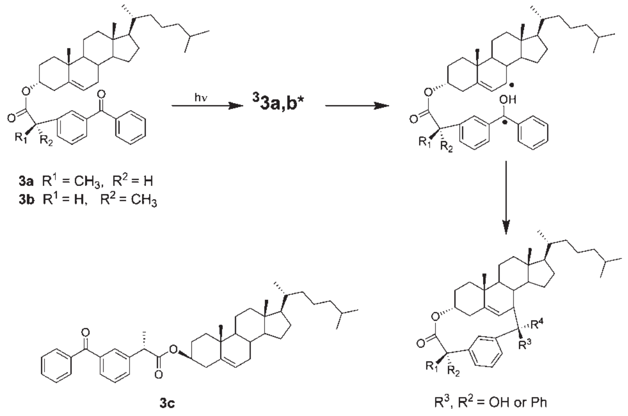
manner.[5] Here, the transient absorption spectra of **3a,b** were recorded in different solvents (dichloromethane, acetonitrile, dioxane, methanol, and octanol) 50 ns after the 355 nm laser pulse. In all cases, the transients displayed similar spectral features.

Scheme 2. Structures of **3a**–**c** and Products Resulting from Irradiation of Dyads **3a** and **3b**



As an example, Figure 2 shows the spectrum of dyad **3a** in methanol (top), which corresponds essentially to the biradical, with a lifetime in the submicrosecond time scale. For comparison, the results obtained for **3c**, which also contains the Ch and BZP units but cannot adopt the folded conformation due to its 3β configuration, are shown in Figure 2 (bottom). In the latter, the typical BZP triplet–triplet absorption was initially observed; the ketyl radical formed by intermolecular H-abstraction from methanol was found to develop at a later stage and did not decay within several microseconds.

As a consequence of the spectral overlap of triplets and biradicals and because of the short triplet lifetimes, it was not possible to achieve direct observation of the T–T absorption of dyads **3a** and **3b** in different media for reliable kinetic measurements. Hence, further experiments were performed to obtain accurate values for the triplet lifetimes (τ_T) by the well-established energy transfer method, using naphthalene as the acceptor (see Supporting Information).[7,8] From these data the intramolecular quenching rate constants (k_{iq}) were determined; the values (ranging between 2.3×10^7 and 1.0×10^8 M^{-1} s^{-1}) are given in Table 1.

Direct kinetic analysis of the decay traces at 545 nm led to determination of biradical lifetimes (τ_{BR}). The quantum yields of biradical formation (ϕ_{BR}) were estimated by the comparative method,[9] from the short-lived component of the transient absorbance at 545 nm immediately after the laser pulse. This was achieved using BZP as standard and assuming the same molar absorption coefficient for the biradicals and for the longer-lived BZP ketyl radical formed by intermolecular hydrogen abstraction from the solvent. It is interesting to note that, even in good hydrogen-donating solvents (dioxane, methanol), the intramolecular process (ϕ_{BR} = 0.65–0.73) was by far more efficient than its intermolecular counterpart (ϕ_{KR} = 0.05–0.14). As expected, all radicals arising from hydrogen abstraction reactions were quenched by oxygen with rate constants in the range of (1.5–5.0) × 10^9 M^{-1} s^{-1}.

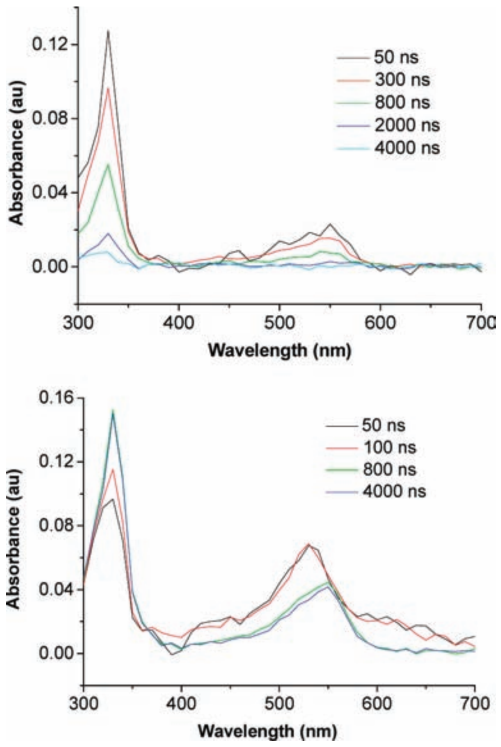


Figure 2. Transient absorption spectrum of dyad **3a** (top) and **3c** (bottom) under N$_2$ in methanol at different times after the laser pulse (λ_{exc} = 355 nm).

After establishing the values of k_{iq} and ϕ_{BR}, the rate constants for intramolecular hydrogen abstraction (k_H) and physical quenching (k_π) were determined in all solvents taking into account that $k_H = k_{iq} \times \phi_{BR}$ and $k_{iq} = k_H + k_\pi$. The obtained results are summarized in Table 1. Although deriving clear-cut correlations between the k_H values and the solvent properties was not straightforward, important solvent effects and significant stereodifferentiation were indeed noticed. The observed trends serve as examples for the presently debated existence of solvent effects on CH hydrogen abstractions. Thus, a much faster process (*ca.* 4×) occurred in dichloromethane than in dioxane or methanol, whereas an intermediate situation was found for acetonitrile. In most solvents, a significant stereodifferentiation was also observed, with higher k_H values for **3b** than for **3a**.

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Table 1. Photophysical and Photochemical Parameters of Triplet Excited States of Dyads **3a** and **3b** in Different Solvents[a]

		τ_T (μs)	$k_{iq}/10^6$ (s^{-1})[c]	$k_H/10^6$ (s^{-1})[d]	$k_\pi/10^6$ (s^{-1})[d]	ϕ_{BR}[e]	τ_{BR}[d] (μs)	ϕ_{KR}[e]	ϕ_{PR}[f]
3a	CH_2Cl_2[b]	0.010	100	80	20	0.80	0.28	0.00	0.30
3a	CH_3CN	0.025	33	26	7	0.80	0.60	0.00	0.70
3a	Dioxane	0.030	23	19	4	0.70	0.66	0.14	0.76
3a	CH_3OH	0.030	28	20	8	0.69	0.80	0.05	0.76
3b	CH_2Cl_2[b]	0.010	100	80	20	0.80	0.22	0.00	0.47
3b	CH_3CN	0.020	50	37	13	0.75	0.56	0.00	0.70
3b	Dioxane	0.020	39	33	6	0.73	0.62	0.13	0.76
3b	CH_3OH	0.020	45	31	14	0.65	1.10	0.05	0.64

[a] Relative errors lower than 5% of the stated values. [b] Data reported in part in ref 5. [c] The intramolecular quenching rate constants were obtained by using the equation $k_{iq} = 1/\tau_t - 1/\tau_0$, where τ_i are the triplet lifetimes of compounds **3a** and **3b** and τ_0 is the BZP triplet lifetime in the different solvents. [d] The rate constants for hydrogen abstraction and physical quenching by the π system were obtained using the following equations: $k_H = k_{iq} \times \phi_{BR}$ and $k_{iq} = k_H + k_\pi$. [e] The biradical quantum yields were determined by the comparative method, using BZP as standard and assuming the same molar absorption for the biradicals and the BZP ketyl radical.[9] [f] Photodegradation quantum yield was determined using *N*-methyldiphenylamine as actinometer.[10]

To investigate the influence of solvents on the overall photoreduction process, solutions of dyads **3a** and **3b** in dichloromethane, acetonitrile, dioxane, and methanol were photolyzed under identical conditions, and the disappearance of the carbonyl chromophore with irradiation time was monitored by the progressive decrease of the 254 nm absorption band. Some representative results are shown in Figure 3. The photoreduction quantum yields were determined using *N*-methyldiphenylamine as actinometer[10] (see Table 1). Again, important solvent effects and significant stereodifferentiation were observed. Noteworthy, the less efficient photoreaction was found for dichloromethane, in spite of the fact that the highest k_{iq}, ϕ_{BR}, and k_H values were measured in this solvent. Actually, biradical formation does not necessarily lead to the C–C coupling products. This is because biradicals can also follow alternative decay pathways, for instance back oxygen-to-carbon hydrogen transfer to regenerate the starting materials, a process that would be retarded by stabilization of the 1-hydroxy-biradicals *via* hydrogen bonding to the solvents.

In summary, hydrogen abstraction from the C-7 position of Ch by triplet excited BZP is a process of considerable chemical and biological interest, which exhibits a remarkable solvent-dependence. In the intermolecular version, a striking difference is observed in product studies between dichloromethane and acetonitrile, although kinetic effects are difficult to evaluate due to solubility limitations. By contrast, covalent linking of the Ch and BZP units in dyads **3a** and **3b** has made it possible to obtain a complete set of kinetic measurements under different conditions. This has revealed important solvent effects and significant stereodifferentiation in most of the involved processes and particularly in the rate constant of intramolecular hydrogen abstraction.

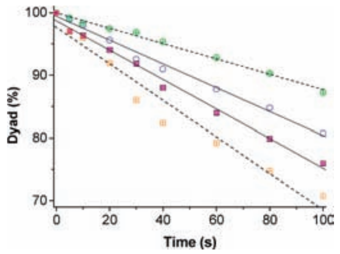

Figure 3. Photoreduction of dyads **3a** and **3b** in different deaerated media as a function of the irradiation time. The percentage of unreacted compound was monitored by UV-absorption spectroscopy at 254 nm. Green: **3a** in CH_2Cl_2. Blue: **3b** in CH_2Cl_2. Orange: **3a** in MeOH. Magenta: **3b** in MeOH.

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Supporting Information Available. Spectroscopic data and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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