Aqueous Photochemistry of Methyl-Benzoquinone

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Chemical trapping studies combined with optical and electron paramagnetic resonance measurements were employed to examine the mechanisms of the aqueous photochemistry of methyl-benzoquinone (mBQ) at both low and high quinone concentrations. At low [mBQ], dimethylsulfoxide (DMSO) reacted with a photogenerated intermediate to form a methyl radical, but methane did not, thereby unequivocally excluding the hydroxyl radical. DMSO at concentrations between 50 mM and 2 M completely suppressed the formation of the hydroxylated quinone, while only slowing the formation of the hydroquinone, suggesting reaction with either the triplet state or an intermediate arising from the triplet. Addition of Cl^-, a putative physical quencher of the triplet, inhibited the DMSO reaction both noncompetitively and competitively in a fashion similar to that observed previously with nitrite, formate, and salicylic acid, thus providing further evidence for a reactive intermediate distinct from the triplet. This intermediate is attributed to a water−quinone exciplex. The relative yield of the methyl radical from the DMSO reaction decreased with increasing [mBQ], suggesting that at high concentrations, a bimolecular reaction of the triplet with the ground-state quinone outcompetes the formation of the quinone−water exciplex.

Introduction

Although the aqueous photochemistry of 1,4-benzoquinones has been studied for decades,[1−6] this topic remains an active area of investigation.[7−10] Irradiation of benzoquinone[1,11−13] and other substituted 1,4-benzoquinones[3,14] in water is known to produce such products as semiquinone, benzene-1,2,4-triol, hydroquinone, and hydroxylated quinone,[11,15,16] but the mechanism(s) by which these products is formed is still a matter of debate.

The principal controversy lies in the nature of the reactive intermediate(s) that are produced. On the basis of spin trapping studies, Ononye et al.[17,18] and later Alegría et al.[19,20] suggested that the hydroxyl radical (OH) was produced through hydrogen atom abstraction from water by the excited triplet state of the 1,4-benzoquinone with concomitant formation of a semiquinone radical (or radical anion at alkaline pH). OH was then suggested to react with quinone to produce a hydroxylated semiquinone intermediate, with disproportionation subsequently producing the hydroquinone and hydroxylated quinone (Scheme 1).

This mechanism was disputed by Pochon et al.[9] based on evidence obtained for methyl-benzoquinone from chemical trapping studies employing dimethylsulfoxide (DMSO) and from laser flash photolysis experiments. Although DMSO was found to react with an intermediate to generate a methyl radical, consistent with the presence of OH, kinetic competition studies showed that this intermediate exhibited a profile of reactivity that differed substantially from OH. The noncompetitive inhibition of the DMSO reaction in the presence of nitrite, salicylate, and formate at low quinone concentrations provided further evidence that this intermediate was distinct from the triplet state. On the basis of these and other observations, this intermediate was suggested to be a quinone−water exciplex with considerable charge-transfer character. The possible fate of this intermediate includes relaxation to the ground state, collapse to form a photohydrate, or reaction in the presence of suitable substrates such as DMSO (Scheme 2).

Although agreeing with Pochon et al.[9] that the OH radical was not produced and that DMSO reacted with an intermediate to produce radicals, Görner disputed the existence of a transient intermediate other than the excited triplet state in aqueous solution.[15] Surprisingly, a later study claimed that DMSO acted exclusively as a physical quencher of the triplet state and that no chemical reaction occurred between DMSO and any transient intermediate in aqueous solution.[16] In both of these studies[15,16] as well as an earlier study,[21] a concerted reaction of water with the triplet to form a photohydrate was suggested at low quinone concentrations; enolization of this photohydrate then produced a tri-hydroxybenzene, which was subsequently oxidized by the parent quinone (Scheme 3).

At high quinone concentrations, direct reaction of the triplet quinone with ground-state quinone was suggested,[15,16,22,23] leading ultimately to the same products (Scheme 4).

Here, we employed additional chemical trapping studies[24,25] (Scheme 5) combined with optical absorption and electron paramagnetic resonance (EPR) measurements to examine further the possible intermediates involved in the aqueous photochemistry of methyl-benzoquinone (mBQ). A stable nitroxide (3ap) was employed to trap the methyl radical arising from the reaction of DMSO with oxidizing intermediates; the alkoxyamine adduct (I) was then derivatized with fluorescamine to form III and subsequently separated by HPLC and quantified fluorometrically.[9,25−30] To discriminate between the formation of OH and other (oxidizing) intermediates, CH_4 (Scheme 5) was also employed as a reaction probe. At low mBQ concentrations, we show that DMSO reacts with an intermediate to produce a methyl radical but that CH_4 does not, thereby unequivocally excluding OH.

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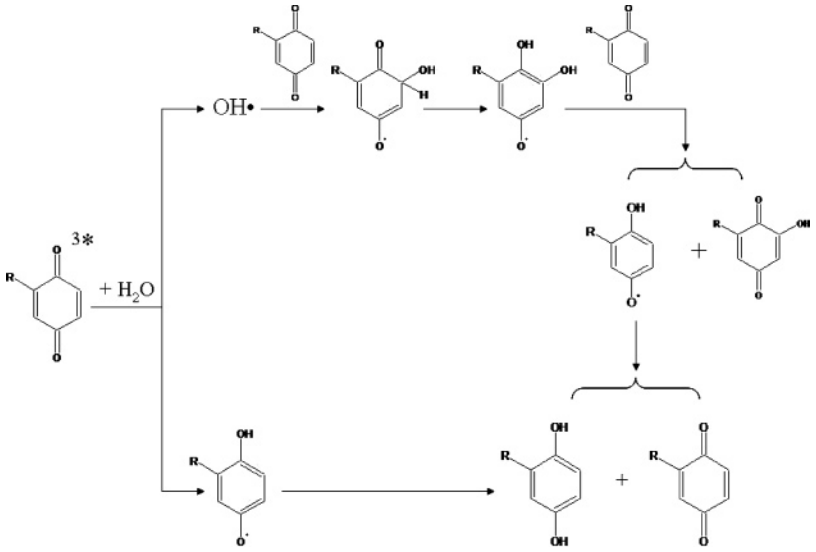

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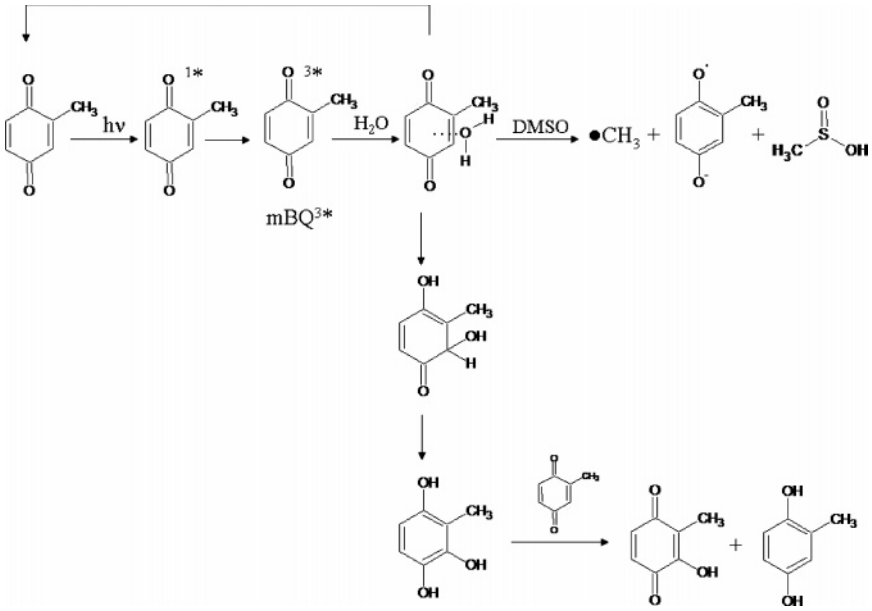

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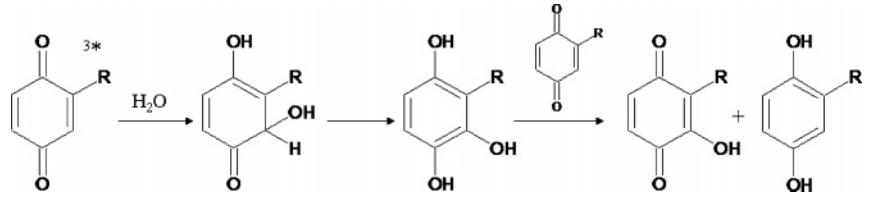
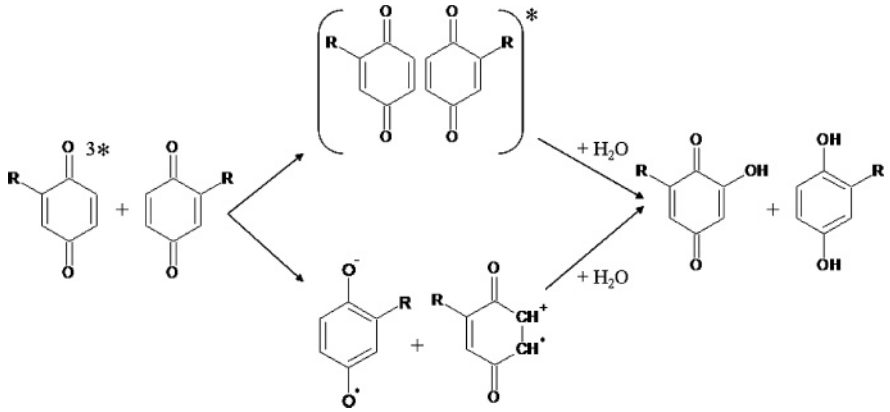


On the basis of the noncompetitive inhibition of the DMSO reaction in the presence of Cl^-, we provide further evidence that this intermediate is distinct from the triplet state and instead attribute it to a quinone−water exciplex (Scheme 2). At increasing mBQ concentrations, a decrease in the yield of I in the presence of DMSO indicates a change of mechanism. On the basis of these results, reaction mechanisms at low and high mBQ concentrations are discussed.

Experimental Procedures

mBQ 98%, HPLC grade DMSO 99%, HPLC grade acetonitrile 99.93+%, *o*-phosphoric acid 85 wt % (99.999%), boric acid 99.999%, sodium dihydrogen phosphate 99.999%, sodium hydrogen phosphate 99.995%, sodium hydroxide 99.98%, fluorescamine (>97% TLC powder), 3-carbamoyl-proxyl free radical 97%, potassium chloride, iron(II) sulfate, sodium formate 99%, sodium nitrite 99.9%, and ethylenediaminetetraacetic acid (EDTA) were obtained from Aldrich. 3-Amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (3ap) 99% was purchased from Acros. Reagent grade sodium nitrate, HPLC grade methanol, 2-propanol, and chloroform were obtained from Fisher. A Millipore Milli-Q system provided water for all the experiments. Methylbenzoquinone was purified by sublimation and stored at 4 °C in amber glass.

SCHEME 4



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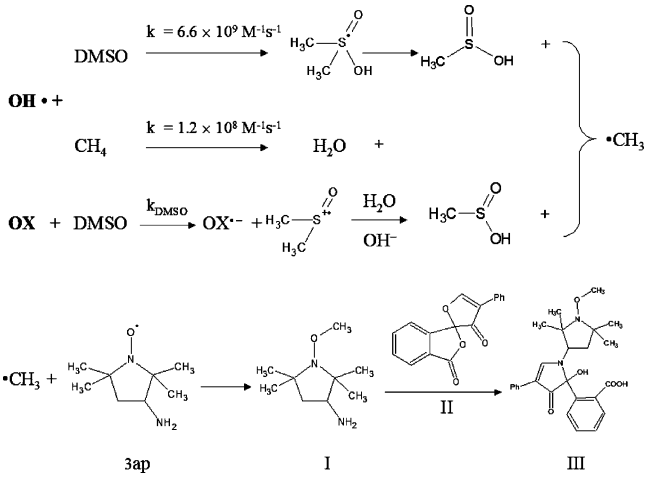


Optical studies were performed in 10 mM, pH 7.0 phosphate buffer under both anaerobic and aerobic conditions. Stock solutions of 400 μM mBQ, 40 mM, pH 7.0 phosphate buffer, 1 mM 3-ap, and 200 mM DMSO in water were used to prepare different concentrations of mBQ in the absence and presence of varying concentrations of 3ap and DMSO. Samples were irradiated in a 1 cm quartz cuvette with a Spectral Energy PS 300-1 Xenon arc lamp. The light was first passed through a Spectral Energy GM252 monochromator set to 320 nm with a bandpass of 10 nm. The light intensity was 9.60×10^{-4} W/cm^2 at the surface of the cell for all the studies as determined with an International Light 1700 radiometer. Under aerobic conditions, the samples were purged with air in the headspace during the irradiation. For anaerobic studies, the sample solution was bubbled with ultrapure N_2 for 15 min prior to irradiation, and the headspace was continuously purged with N_2 during irradiation. Absorption spectra were obtained with a Hewlett-Packard 8452A diode array spectrophotometer.

Chemical trapping experiments employing 3ap and DMSO (Scheme 5) are described elsewhere.[9] Samples containing mBQ, 3ap, and DMSO, with or without competitors, were irradiated in a 1 cm cuvette employing a monochromatic irradiation system under the same conditions as those in the optical study. In experiments where CH_4 was used in place of DMSO, the sample solution was first degassed with N_2 for 3 min and then saturated with CH_4 by bubbling for 5 min ([CH_4] was 1.5 mM).[31]

The effect of the quinone concentration was first assessed by examining the relative initial rates of III production for mBQ solutions ranging from 10 μM to 10 mM under conditions in which III production was shown to be independent of the DMSO and 3ap concentrations, namely, over the range of 50−200 mM for DMSO and 10−500 μM for 3ap. Relative yields (Φ_R) of III were calculated with respect to that obtained for 10 μM mBQ through the following equation:

$$\Phi_R = \frac{\Phi_{[mBQ]}}{\Phi_{10\mu M}} = \frac{R_{[mBQ]}(1 - 10^{-A_{10\mu M}})}{R_{10\mu M}(1 - 10^{-A_{[mBQ]}})} \quad (1)$$

where $R_{[mBQ]}$ and $R_{10\mu M}$ are the initial rates of III formation, and $A_{[mBQ]}$ and $A_{10\mu M}$ are the absorbances (1 cm path length) for a given [mBQ] and for 10 μM mBQ, respectively.

The loss of 3ap and its dependence on the quinone concentration was measured using a Bruker/IBM ER 200D-SRC EPR spectrometer using the following standard instrument settings: frequency 9.7 GHz; power 10 mW; modulation amplitude 1.0 G; and time constant 80 ms. Samples were irradiated with a polychromatic system until no further loss of mBQ and 3ap was observed, with the relative 3ap consumption ratio (reacted [3ap]/reacted [mBQ]) then obtained.

Control experiments employing identical mBQ concentrations but varying 3ap and DMSO concentrations showed that 3ap spin loss was independent of DMSO and 3ap concentrations over the range of 50−200 mM and 10−500 μM, respectively, under anaerobic conditions for all concentrations of mBQ. The 3ap concentrations employed in the experiments were 50 μM for 10−50 μM mBQ, 250 μM for 100−300 μM mBQ, and 500 μM for 500 μM to 10 mM mBQ.

The quantum yield for III production was determined using 100 μM mBQ with 140 mM DMSO and 50−200 μM 3ap under anaerobic conditions in pH 7, 10 mM phosphate buffer. Samples were irradiated at 430 nm using the monochromatic irradiation system described previously. Apparent quantum yields were calculated using the equation

$$\Phi = \frac{RP}{0.588 I_0(1 - 10^{-A})} \quad (2)$$

where R is the initial rate of III production in molecules cm^{-3} s^{-1}, P is the path length of the cell (1 cm), I_0 is the lamp intensity at the surface of the cell in photons cm^{-2} s^{-1} acquired from the radiometer reading, A is the initial absorbance of the solutions at the irradiation wavelength, and the factor 0.588 accounts for the difference between the radiometric and actinometric measurements.[25]

Results and Discussion

Photoreaction of Low Concentrations of mBQ with DMSO in Aqueous Solution. Although contradicting the prior

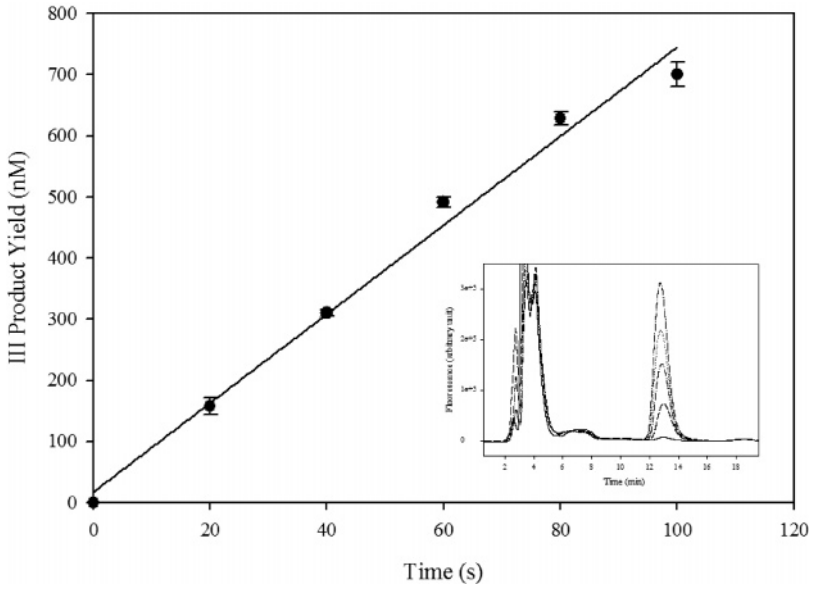


Figure 1. Dependence of the yield of III on irradiation time under anaerobic conditions. Sample contained 20 μM mBQ, 50 μM 3ap, and 50 mM DMSO, irradiated at 320 nm with a lamp intensity of 7.1×10^{-4} W cm^{-2}. Inset: chromatograms obtained at consecutive irradiation times showing the increase of III (peak at 13 min). The blank contained 20 μM mBQ and 50 μM 3ap and was irradiated for 100 s under the same conditions. Error bars represent ± 1 SD about the mean of three independent determinations.

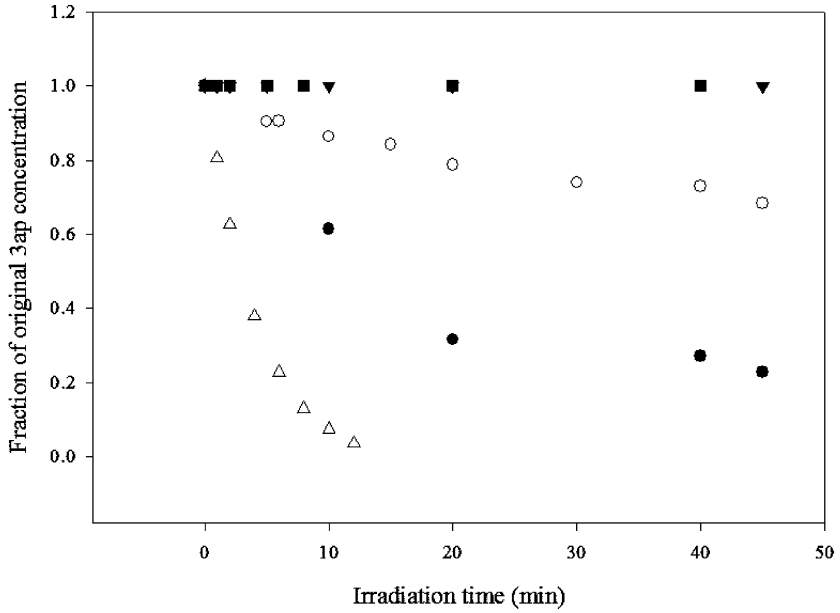


Figure 2. Fractional loss of 3ap with irradiation time as measured by EPR. Samples containing 20 μM mBQ + 50 μM 3ap (▼), 50 μM 3ap + 50 mM DMSO (■), 20 μM mBQ + 50 μM 3ap + 50 mM DMSO (○), 500 μM mBQ + 100 μM 3ap + 50 mM DMSO (●), and 500 μM mBQ + 50 μM 3ap + 50 mM DMSO (△) were irradiated at 320 nm with a light intensity of 5×10^{-4} W cm^{-2}.

work of both Pochon et al.[9] and Görner,[15] von Sonntag et al.[16] claimed that DMSO does not undergo a chemical reaction with intermediate(s) produced during the irradiation of 1,4-benzoquinones but instead acts as a purely physical quencher of the excited triplet state. Although later work[32] also clearly contradicts this conclusion, additional studies were performed here to clarify this issue further.

Employing the method outlined in Scheme 5, the irradiation of low concentrations of mBQ (<30 μM) in the presence of 50 μM 3ap and 50 mM DMSO under anaerobic conditions produced a product that increased linearly with irradiation time (initial rate conditions, Figure 1). This product was not generated in the absence of either DMSO or 3ap and was significantly reduced in the presence of air, consistent with a competition between 3ap and O_2 for a carbon-centered radical such as the methyl radical (see Supporting Information Figure S-1).

This product exhibited chromatographic retention characteristics identical to III (Supporting Information Figure S-2), formed either through the Fenton reaction[24] or through nitrite photolysis.[25] The hydroxyl radical produced from these sources is known to react rapidly with DMSO to produce the methyl radical,[33] which will then react with 3ap, ultimately to yield III following derivatization with fluorescamine (Scheme 5). The product generated from the Fenton reaction was previously identified as III by high-resolution mass spectrometry.[24,25]

Consistent with the chromatographic measurements, EPR experiments showed that the loss of 3ap was observed only in the presence of both mBQ and DMSO; 3ap spin loss was not

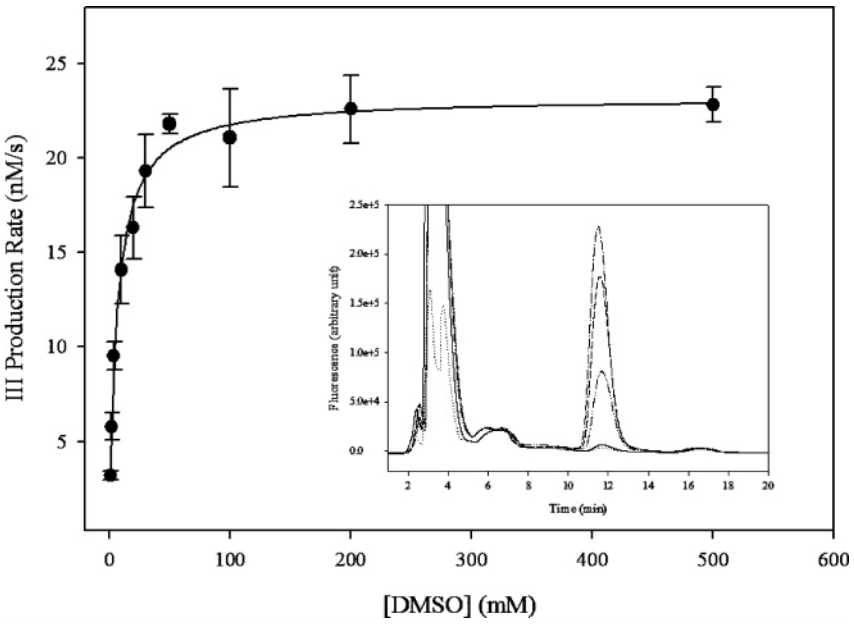


Figure 3. Dependence of the initial rate of III production on DMSO concentration. Samples containing 20 μM mBQ, 50 μM 3ap, and varying concentrations of DMSO were irradiated at 320 nm anaerobically for 1 min employing a lamp intensity of 6.0×10^{-4} W cm^{-2}. Inset: peak at 11.8 min is the fluorescent product III. 50 μM 3ap + 50 mM DMSO (•••), 20 μM mBQ + 50 μM 3ap (—), 20 μM mBQ + 50 μM 3ap + 5 mM DMSO (—••—), 20 μM mBQ + 50 μM 3ap + 20 mM DMSO (— —), and 20 μM mBQ + 50 μM 3ap + 50 mM DMSO (—•—•—•). Error bars represent ±1 SD about the mean of three or more independent determinations.

observed for irradiated samples containing either mBQ and 3ap or DMSO and 3ap alone (Figure 2). The relative 3ap consumption ratio (reacted [3ap]/reacted [mBQ] following extended irradiation) decreased with increasing concentrations of mBQ, although the absolute 3ap consumption increased with increasing mBQ concentrations over the range from 10 μM to 5 mM (see Effect of Quinone Concentration).

In the presence of mBQ and DMSO, the yield of III as determined chromatographically was linearly related to the loss of 3ap as measured by EPR (Supporting Information Figure S-3). Although the slope of this relationship was 0.77, we could find no chromatographic evidence for the formation of other products. Further, the addition of micromolar quantities of Cu^{2+} to re-aerated EPR samples did not increase 3ap spin levels as one would expect if significant amounts of the hydroxylamine were formed.[34] Thus, our evidence suggests that the deviation of the slope from one is a result of a small systematic bias in either the LC calibration or the spin measurements and thus that III is most likely formed exclusively.

Under anaerobic conditions, the initial rate of III formation exhibited a hyperbolic dependence on DMSO concentration (Figure 3), with the rate becoming independent of DMSO concentration over the range of ~50 mM to 2 M DMSO. The DMSO concentration at which the rate reached half of its maximal value, $[DMSO]_{1/2}$, was 5.7 mM in 10 mM phosphate buffer, identical to the value obtained in water alone at the same pH value. This value is slightly lower than the one previously reported[9] (8.7 mM) based on fewer data. The quantum yield for the formation of III at lower mBQ concentrations (≤100 μM) in the presence of saturating [DMSO] was 0.37 ± 0.04. At [DMSO] > 2 M, the rate of III formation decreased with increasing [DMSO] (Supporting Information Figure S-4), reaching a value in the presence of 13.4 M DMSO (95% DMSO/5% H_2O, v/v) that was ~20% of the maximum. This result implies that water is required for the formation of the reactive intermediate and that DMSO by itself is not reactive chemically with the mBQ triplet.

Further evidence for the reaction of DMSO with a photogenerated intermediate was provided by optical absorption measurements (Figure 4). In the absence of DMSO, irradiation of mBQ at 320 nm under anaerobic conditions produced a loss of mBQ (decrease in absorption at 250 nm, Figure 4A), concomitant with the formation of a hydroxy-benzoquinone (increase in absorption at 520 nm)[15] and the hydroquinone (increase in absorption at 280 nm).[16] Under our experimental conditions, the loss of mBQ and the formation of the hydroquinone and hydroxy-benzoquinone followed first-order kinetics with a rate coefficient of 0.24 min^{-1} (Figure 5). The rate of mBQ loss and of hydroquinone and hydroxy-benzoquinone formation was unaffected by the presence of O_2 (air), an increase in the mBQ concentration from 20 to 50 μM, or the presence of 75 μM 3ap. In contrast, under anaerobic conditions, the addition of 100 mM DMSO, a concentration known to fully scavenge the intermediate completely suppressed the formation of the hydroxy-benzoquinone (Figure 4B), while only slowing the loss of mBQ and the formation of the hydroquinone by a factor of 4—5 (0.05 min^{-1}, Figure 5). Importantly, increasing the concentration of DMSO to 200 mM did not affect this pattern or the kinetics (Figure 5), indicating that the intermediate was quantitatively scavenged at [DMSO] ≥ 100 mM, as well as unequivocally demonstrating that DMSO cannot be acting as a physical quencher of the triplet state in this concentration regime.[16] Under aerobic conditions, the rate of loss of mBQ and the formation of hydroquinone was further slowed (0.020 min^{-1}, Figure 5).

These results can be understood with reference to Schemes 2 and 3. In the absence of a reactive substrate such as DMSO, the reaction of water with mBQ, either through a concerted reaction with the triplet state (Scheme 3) or through the collapse of a water—quinone exciplex (Scheme 2), will lead to the consumption of 2 mol of mBQ for each mole of intermediate that goes on to react (see Schemes 2 and 3). In contrast, the reaction of DMSO with the intermediate (either triplet or exciplex) circumvents the formation of the water addition

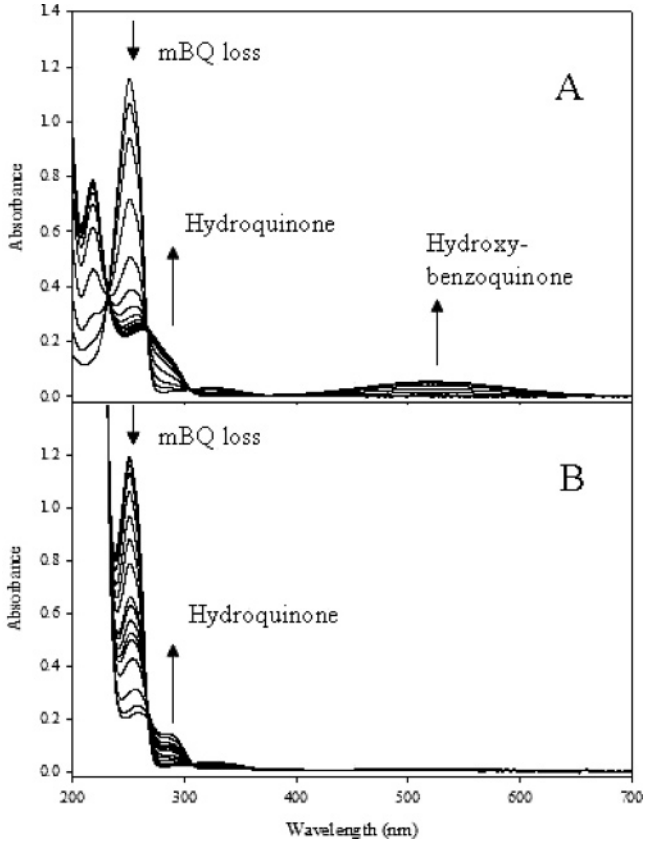


Figure 4. Changes in the absorption spectrum of mBQ upon irradiation in the absence (A) and presence (B) of DMSO. (A) Sample contained 50 μM mBQ in 10 mM, pH 7.0 phosphate buffer and (B) sample contained 50 μM mBQ and 100 mM DMSO in 10 mM, pH 7.0 phosphate buffer. Samples were irradiated at 320 nm with a light intensity of 9.6×10^{-4} W cm^{-2}.

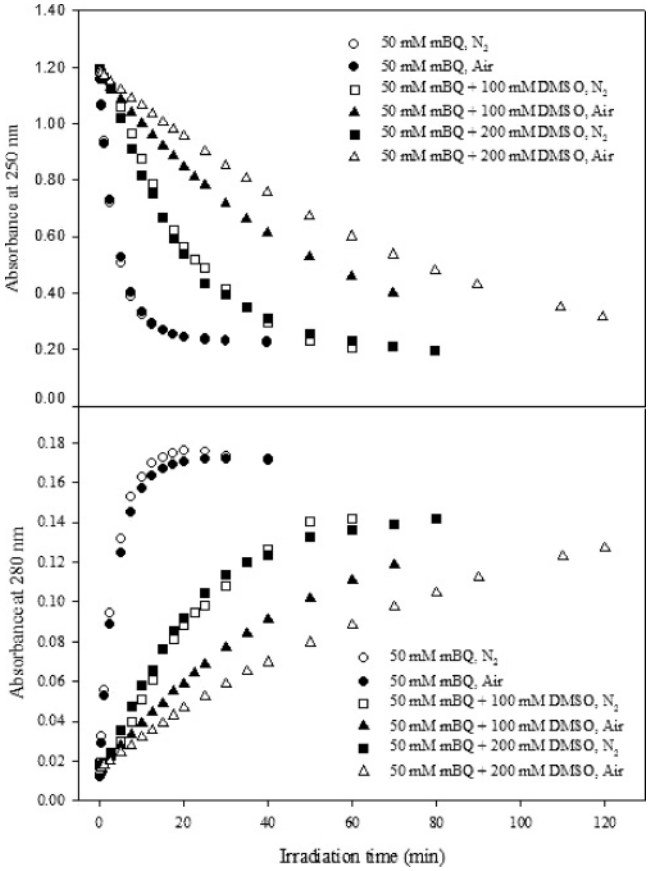


Figure 5. Time dependence of mBQ loss (absorbance at 250 nm) and hydroquinone formation (absorbance at 280 nm) in the absence and presence of DMSO upon irradiation at 320 nm with a light intensity of 9.6×10^{-4} W cm^{-2} under anaerobic and aerobic conditions. Samples were prepared in 10 mM, pH 7.0 sodium phosphate buffer.

product and instead produces a single semiquinone radical; because of disproportionation, the formation of 4 mol of intermediate is thus required for the consumption of 2 mol of mBQ (forming 2 mol of hydroquinone). Because of this difference in the reaction stoichiometry, the rate coefficient for mBQ loss in the presence of (saturating) [DMSO] is expected to be 4-fold lower, as observed (Figure 5), so long as two conditions are met: (1) The fraction of intermediate that goes on to react is equivalent in the absence and presence of (saturating) DMSO and (2) recycling of the hydroquinone to quinone through reaction with the products of the DMSO/quinone reaction does not take place. The observation that the quantum yield reported by Görner[15] for mBQ loss in water (0.8) is approximately 2-fold higher than the quantum yield we measure for the production of the methyl radical at low mBQ concentration (0.37 ± 0.04) is consistent with the first condition; in the absence of DMSO, 2 mol of mBQ is consumed for each mol of intermediate (Scheme 2), whereas in the presence of DMSO, only 1 mol of methyl radical is produced. The significant further reduction in the rate coefficient for mBQ loss in the presence of DMSO and air (Figure 5) can be reasonably attributed to the partial reoxidation of either the semiquinone or the hydroquinone to the quinone through reaction with the methylperoxy radical formed by O_2 addition to the methyl radical or through the partial oxidation of the semiquinone by O_2.[10]

Tests of the Identity of the Reactive Intermediate. Hydroxyl Radical. As previously reported,[9] the dependence of the initial rate of III formation on [DMSO] is incompatible with OH as the intermediate (Figure 3); the value of $[DMSO]_{1/2}$ (5.7 mM) is far larger than that predicted based on competitive reactions with other solution constituents ($[DMSO]_{1/2} = \sim 52$ μM). However, replacing DMSO with CH_4 provides an additional and particularly stringent test for the presence of OH. Because the C−H bond strength in methane is very high, only exceedingly strong oxidants such as OH are capable of reacting with it ($k_{OH} = 1.2 \times 10^8$ M^{-1} s^{-1}, Scheme 5). III was not detected upon irradiation of 20 μM mBQ solutions saturated with methane (1.5 mM)[31] and containing 30 μM 3ap, unequivocally excluding OH as an intermediate.

Triplet State. If DMSO was reacting only with the triplet, then the addition of physical quenchers of the triplet would give rise to purely competitive inhibition kinetics for the formation of III; $[DMSO]_{1/2}$ would increase with increasing competitor concentration, but the maximal initial rate under saturating [DMSO] (R_{max}) would remain unchanged. However, the addition of Cl^-, a compound believed to act as a purely physical quencher of quinone triplet states,[15] clearly does not produce this behavior (Figure 6). Instead, the formation of III is inhibited both noncompetitively and competitively in the presence of increasing [Cl^-], consistent with previous work where this same behavior was observed in the presence of formate, nitrite, and salicylate.[9]

The simplest mechanism that is consistent with these data is provided in Scheme 6, where Cl^- quenches both the (triplet) precursor to the DMSO reactive intermediate as well as the intermediate itself, thereby producing noncompetitive and competitive inhibition, respectively. Within this scheme, the

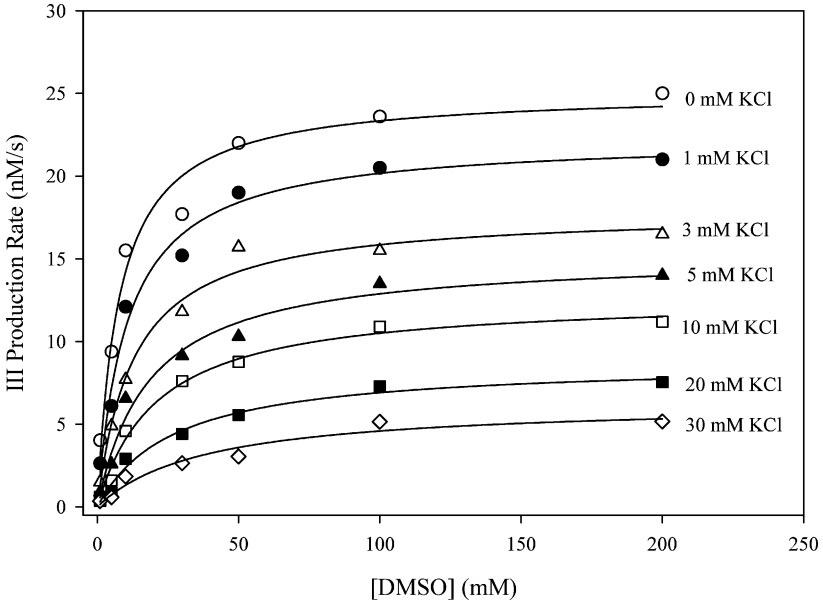


Figure 6. Competitive and noncompetitive effects of Cl^- on the initial rates of III production. Samples containing 20 μM mBQ, 50 μM 3ap, DMSO, and increasing concentrations of KCl (0.0 mM KCl (○), 1.0 mM KCl (●), 3.0 mM KCl (△), 5.0 mM KCl (▲), 10.0 mM KCl (□), 20.0 mM KCl (■), and 30.0 mM KCl (◇)) were irradiated for 30 s with filtered polychromatic light (350 nm cutoff filter) under anaerobic conditions.

noncompetitive and competitive inhibition by Cl^- can be parametrized

$$R_{DMSO} = \left\{\frac{k_f k_{H_2O}[H_2O]}{k_{H_2O}[H_2O] + {}^0k_T + k_{Cl}[Cl]}\right\} \left\{\frac{k_{DMSO}[DMSO]}{k_{DMSO}[DMSO] + k_d + k'_{Cl}[Cl]}\right\} = \frac{C_1[DMSO]}{C_2 + [DMSO]} \quad (3)$$

$$C_1 = \frac{k_f k_{H_2O}[H_2O]}{k_{H_2O}[H_2O] + {}^0k_T + k_{Cl}[Cl]} \quad (4)$$

$$C_2 = \frac{k_d + k'_{Cl}[Cl]}{k_{DMSO}} \quad (5)$$

$${}^0C_1 = \frac{k_f k_{H_2O}[H_2O]}{k_{H_2O}[H_2O] + {}^0k_T} \quad (6)$$

$${}^0C_2 = \frac{k_d}{k_{DMSO}} \quad (7)$$

$$\frac{{}^0C_1}{C_1} = 1 + \frac{k_{Cl}[Cl^-]}{k_{H_2O}[H_2O] + {}^0k_T} \quad (8)$$

$$C_2 - {}^0C_2 = \frac{k'_{Cl}[Cl^-]}{k_{DMSO}} \quad (9)$$

Here, R_{DMSO} is the initial formation rate of III, k_f is the formation rate of the triplet in units of M s^{-1} ($k_f = I_a\Phi_{isc}$, where I_a is the rate of light absorption in the reaction volume of the cell, and Φ_{isc} is the quantum yield for intersystem crossing to the triplet), and ${}^0k_T$ is the natural triplet decay rate of mBQ. C_1 and ${}^0C_1$ are the values of maximal R_{DMSO} (R_{max}) in the presence and absence of the quencher (e.g., Cl^-), while C_2 and ${}^0C_2$ provide the values of $[DMSO]_{1/2}$ in the presence and absence of the quencher, respectively. Values of these parameters were obtained by nonlinear least-squares fits of the data in Figure 6 to eq 3.

The dependence of ${}^0C_1/C_1$ on $[Cl^-]$ is predicted to be linear (eq 8 and Figure 7), with the reciprocal of the slope equaling the concentration of Cl^- at which R_{max} decreases to 50% of the maximal value ($[Cl]_{1/2}$ = 8.0 mM, Table 1). This value, which within our model provides information only on the quenching of the triplet state by Cl^-, is more than 3 times larger than the value obtained by Görner[15] for the inhibition of mBQ decomposition by Cl^- ($[Cl^-]_{1/2}$ = 2.3 mM). Because our data indicate that the inhibition of mBQ decomposition results not only from quenching of the triplet but also from the quenching of an intermediate arising from the triplet (Scheme 6 and Figure 6), this difference is readily understandable. Importantly, this difference also strongly implies that the intermediate is not easily distinguished from the triplet by time-resolved absorption measurements as was claimed previously.[15]

The slope of the dependence of $C_2 - {}^0C_2$ on the Cl^- concentration provides information on the rate of the reaction of the inhibitor with the intermediate relative to DMSO (eq 9). This parameter, along with previous values obtained for other inhibitors, is provided in Table 1. These values were employed to place bounds on the rate constants within this model.

Effect of Quinone Concentration. The relative yield of III (Φ_R) decreased with increasing mBQ concentration in a biphasic manner, with greater decreases at [mBQ] < 2 mM and much smaller decreases thereafter (Figure 8). This same pattern was observed in the ratio of reacted 3ap to reacted mBQ measured by EPR, obtained when the loss of mBQ was complete. Interestingly, and somewhat surprisingly, increasing [mBQ] did not observably affect $[DMSO]_{1/2}$, indicating that mBQ acts as a purely noncompetitive inhibitor of the DMSO reactive intermediate. These results thus suggest that with increasing mBQ concentrations, ground-state mBQ reacts directly with ^{3}mBQ in competition with its reaction with water (Scheme 6), consistent with prior work.[15,16] Although this bimolecular reaction appears ultimately to give rise to the same products,[15,16] the intermediate(s) generated must be either unreactive with 3ap

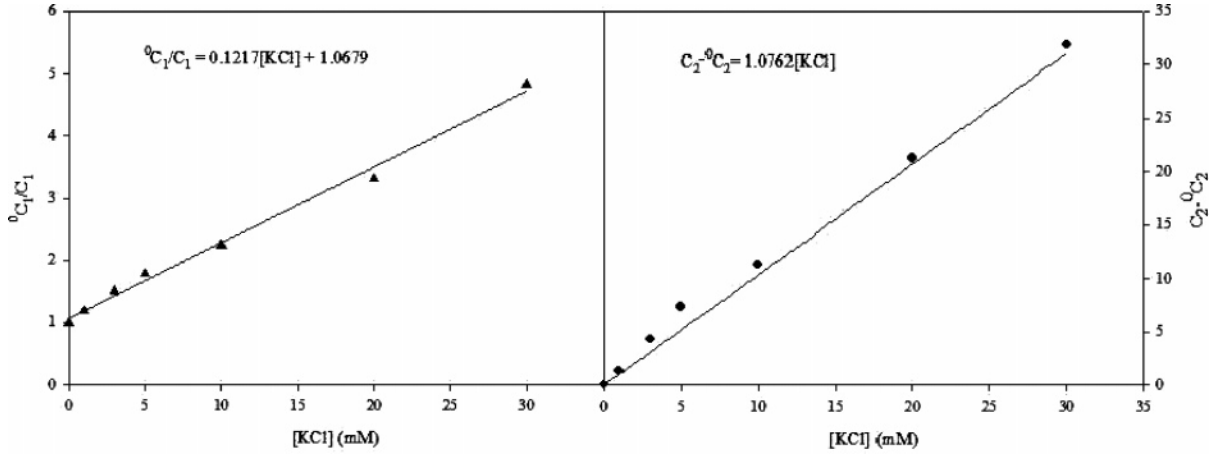


Figure 7. Effect of KCl concentration on maximal III formation rates ($^0C_1/C_1$) and $[DMSO]_{1/2}$ ($C_2 - {}^0C_2$) values.

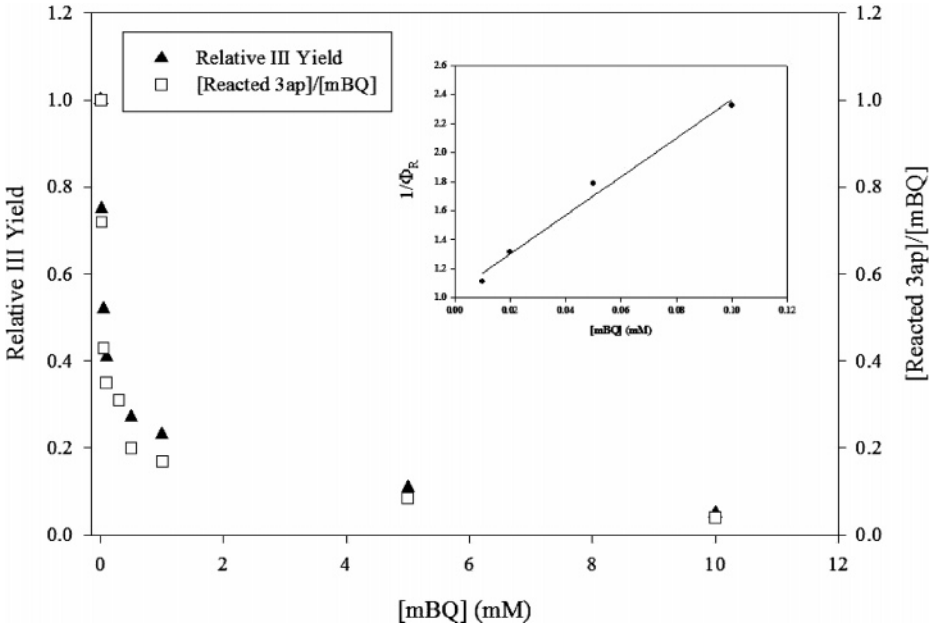


Figure 8. Dependence on mBQ concentration of relative III yield obtained chromatographically and of [reacted 3ap]/[mBQ] ratio obtained from EPR. Inset: linear relationship between the reciprocal of relative III yield ($1/\Phi_R$) on [mBQ] at low mBQ concentrations.

TABLE 1: Effect of Competing Compounds

compound	slope (M^{-1}) of $^0C_1/C_1$ vs [compound]	slope of $C_2 - {}^0C_2$ vs [compound]	rate constant for triplet quenching[a] ($M^{-1}\,s^{-1}$)	rate constant for triplet quenching[b] ($M^{-1}\,s^{-1}$)
mBQ	$\sim 2 \times 10^4$		$\sim 1.2 \times 10^{11}$	$\sim 2 \times 10^{10}$
chloride	120	1.1	6.0×10^8	1.3×10^8
nitrite	420	9.2	2.1×10^9	4.4×10^8
formate	380	0.46	1.9×10^9	4.0×10^8
salicylic acid	3300	8.0	1.6×10^{10}	3.5×10^9

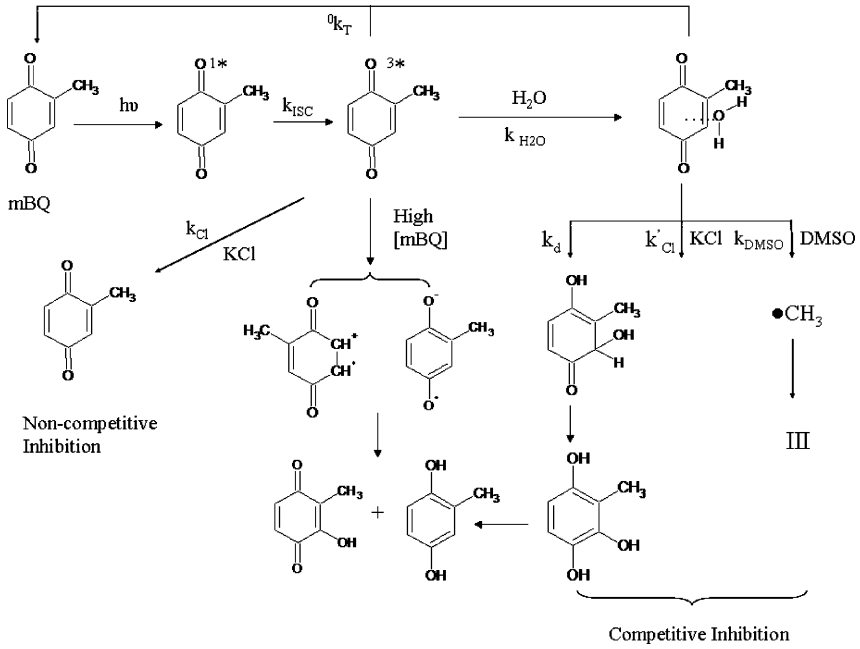
[a] On the basis of a natural triplet lifetime of 1 μs and a triplet lifetime in water of 0.2 μs[15] ($k_{H_2O} = 7.3 \times 10^4\ M^{-1}\,s^{-1}$). [b] On the basis of a natural triplet lifetime of 2 μs and a triplet lifetime in water of 0.95 μs ($k_{H_2O} = 10^4\ M^{-1}\,s^{-1}$)[15].

(and DMSO) or, more likely, are sufficiently short-lived that reaction with 3ap (or DMSO) does not occur. The origin of the slower decrease in Φ_R at [mBQ] > 2 mM is currently unclear but could be due to the equilibrium formation of unreactive ground-state dimers (Scheme 6).

Mechanism and Kinetic Analysis. At low [mBQ], our results indicated that water reacts with ^{3}mBQ to form an intermediate that we attribute to a quinone−water exciplex (Scheme 6). In the absence of sufficiently high concentrations of reactive compounds, this intermediate collapses to form a photohydrate in competition with its relaxation to the ground state (Scheme 3). Only when the quinone is an exceedingly good electron acceptor, such as is the case for tetrachloro-*p*-benzoquinone, has evidence been obtained for the complete transfer of an electron from water to the quinone to form the hydroxyl radical and the semiquinone.[35]

Assuming the triplet lifetimes reported by Görner[15] for low concentrations of mBQ in acetonitrile (1 μs), a relatively inert solvent, and in H_2O (0.2 μs), the second-order rate constant for the triplet−water reaction (k_{H_2O}) can be estimated as 7.3×10^4

SCHEME 6



M^{-1} s^{-1}. Using these values and the values reported in Table 1, the rate constants for triplet quenching by the compounds reported in Table 1 can be calculated using eq 8. Values for these rate constants are reasonable with the lone exception of mBQ itself, having a calculated value above the diffusion limit. There are two possible origins for this discrepancy. First, even at lower [mBQ], a small degree of ground-state dimerization of mBQ to form unreactive pairs could also be enhancing the concentration-dependent inhibition of III formation (and 3ap consumption, Figure 8). Second, and more likely, the values for the triplet lifetimes in water and acetonitrile are not well-constrained at low [mBQ]. Using a slightly larger value for the natural triplet lifetime (2 μs) and the value estimated by Görner[15] for the triplet benzoquinone−water reaction (10^4 M^{-1} s^{-1}), far more reasonable estimates of the rate constants for triplet quenching are obtained (Table 1). Consistent with prior work, our results also suggest that with increasing [mBQ], the competitive reaction of ^{3}mBQ with ground-state mBQ lowers the yield of the quinone−water exciplex and that the route to the products is altered (Scheme 6), although the products ultimately are the same.[11,16]

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Supporting Information Available: Figure S-1: competition between 3ap and dioxygen for the methyl radical; Figure S-2: HPLC chromatogram showing coelution of III synthesized from mBQ photolysis, Fenton reaction, and nitrite photolysis; Figure S-3: relationship between the yield of III determined chromatographically and loss of 3ap determined by EPR on identical samples; and Figure S-4: dependence of the formation rate of III on DMSO concentration. This information is available free of charge via the Internet at http://pubs.acs.org.

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