4498 *J. Org. Chem.* **1995,** *60,* 4498−4503

Solvent and Electronic Effects on the Reaction of Guanosine Derivatives with Singlet Oxygen

Chimin Sheu and Christopher S. Foote*

Department of Chemistry and Biochemistry, University of California, Los Angeles,
Los Angeles, California 90024-1569

Received February 23, 1995[⊗]

The total rate constants (sum of chemical reaction and physical quenching, $k_r + k_q$) for singlet oxygen removal by a number of 8-substituted guanosine derivatives in different solvents were measured by time-resolved infrared singlet oxygen luminescence decay. The rate constants for chemical quenching were determined separately by competition experiments. For all of the derivatives except the 8-oxo, quenching is 2 orders of magnitude faster than reaction. For the 8-oxo derivative, the ratio is only around 3. There is a slight increase in both ($k_r + k_q$) and k_r in polar solvents, and the increase is larger for the more reactive substrates. The reactivities of guanosine derivatives in photooxygenation correlate with their ionization potentials and with the Brown σ^+ parameter. The initial interaction with singlet oxygen is discussed in terms of these parameters.

Introduction

Because the roles that singlet oxygen plays in certain biological processes are of great interest, its reactions have been extensively studied for many years.[1−11] Interactions of singlet oxygen with biomolecules may involve chemical reaction and/or physical quenching of the excited oxygen.[12−15] The chemical reaction modes of singlet oxygen are ene, [2 + 2], and [2 + 4] cycloadditions to olefins and dienes and oxidation of low-ionization potential molecules with nonbonding electrons, such as sulfur, nitrogen, and phosphorus.[2,16,17] Physical quenching via energy transfer is limited to molecules with excited triplet energies below the excitation energy of singlet oxygen (22 kcal), such as β-carotene and its analogs.[18] Iodine,[19] naphthalocyanines,[20,21] and some metal complexes[22,23] also quench singlet oxygen predominantly by this mechanism. Most other compounds are believed to quench singlet oxygen by forming exciplexes or charge-transfer complexes.[12−14] Both chemical and physical interactions with singlet oxygen are important, and the ratio between these two reaction channels depends on factors such as solvent and electronic effects in the substrate. The ratio of the rate constants of physical quenching (k_q) to chemical reaction (k_r) of singlet oxygen is connected to the balance between biological protection and biological damage.[24−26]

Nucleic acids are an important target for singlet oxygen damage in living cells.[3,9,11,27] Photosensitized oxidative damage to nucleic acid derivatives occurs almost exclusively at guanine residues,whether as free bases, as nucleosides, or in DNA.[28−32] During the past two decades, several groups have performed extensive studies of photodynamic damage to nucleic acids.[3,9,33−35] However, the extremely low solubility of purine derivatives in most solvents, the instability of the primary products,

---

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1995.

(1) *Singlet Oxygen*; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979.

(2) *Singlet O₂*; Frimer, A. A., Ed.; CRC Press: Boca Raton, FL, 1985; Vol. I−IV.

(3) Cadet, J.; Vigny, P. In *The Photochemistry of Nucleic Acids*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; pp 1−272.

(4) Epe, B. *Chem.-Biol. Interact.* **1991**, *80,* 239−260.

(5) Foote, C. S. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976; pp 85−133.

(6) Foote, C. S. In *Oxygen and Oxy-radicals in Chemistry and Biology*; Rodgers, M. A. J., Powers, E. L., Eds.; Academic Press: New York, 1981; pp 425-440.

(7) Marx, J. L. *Science* **1987**, *235,* 529−531.

(8) Piette, J. *J. Photochem. Photobiol., B* **1990**, *4,* 335−342.

(9) Piette, J. *J. Photochem. Photobiol. B* **1991**, 241−260.

(10) Sies, H. *Angew. Chem., Int. Ed. Engl.* **1986**, *25,* 1058−1072.

(11) Sies, H.; Menck, C. F. M. *Mutat. Res.* **1992**, *275,* 367−375.

(12) Foote, C. S. In *Singlet Oxygen*; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979; pp 139-173.

(13) Gorman, A. A.; Rodgers, M. A. J. In *Handbook of Organic Photochemistry*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, FL, 1989; pp 229−256.

(14) Gorman, A. A. In *Advances in Photochemistry*; Volman, D. H., Hammond, G. S., Neckers, D. C., Eds.; John Wiley and Sons: New York, 1992; pp 217−274.

(15) Lissi, E. A.; Encinas, M. V.; Lemp, E.; Rubio, M. A. *Chem. Rev.* **1993**, *93,* 699−723.

(16) Gollnick, K.; Kuhn, H. J. In *Singlet Oxygen*; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979; pp 287−429.

(17) Schaap, A. P.; Zaklika, K. A. In *Singlet Oxygen*; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979; pp 174−243.

(18) Foote, C. S.; Denny, R. W. *J. Am. Chem. Soc.* **1968**, *90,* 6233−6235.

(19) Wilkinson, F.; Farmilo, A. *J. Photochem.* **1984**, *25,* 153−160.

(20) Krasnovsky, A. A., Jr.; Rodgers, M. A. J.; Galpern, M. G.; Kenney, M. E.; Luk'yanets, E. A. *Bioorg. Khim.* **1990**, *16,* 1413−18.

(21) Krasnovsky, A. A., Jr.; Rodgers, M. A. J.; Galpern, M. G.; Richter, B.; Kenny, M. E.; Lukjanetz, E. A. *Photochem. Photobiol.* **1992**, *55,* 691−696.

(22) Carlsson, D. J.; Mendenhall, G. D.; Suprunchuk, T.; Wiles, D. M. *J. Am. Chem. Soc.* **1972**, *94,* 8960−8962.

(23) Rajadurai, S.; Das, P. K. *J. Photochem.* **1987**, *37,* 33−40.

(24) Foote, C. S.; Chang, Y. C.; Denny, R. W. *J. Am. Chem. Soc.* **1970**, *92,* 5216−5218.

(25) Foote, C. S.; Clough, R. L.; Yee, B. G. In *Tocopherol, Oxygen and Biomembranes*; de Duve, C., Hayaishi, O., Eds.; Elsevier: Amsterdam, 1978; pp 13−21.

(26) Di Mascio, P.; Kaiser, S.; Sies, H. *Arch. Biochem. Biophys.* **1989**, *274,* 532−538.

(27) Kochevar, I. E.; Dunn, D. A. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990.

(28) Sussenbach, J. S.; Berends, W. *Biochim. Biophys. Acta.* **1965**, *95,* 184−185.

(29) Simon, M. I.; Van Vunakis, H. *J. Mol. Biol.* **1962**, *4,* 488−499.

(30) Sastry, K. S.; Gordon, M. P. *Biochim. Biophys. Acta.* **1966**, *129,* 42−48.

(31) Rosenthal, I.; Pitts, J. N. J. *Biophys. J.* **1971**, *11,* 963−966.

(32) Rougee, M.; Bensasson, R. V. *C.R. Acad. Sci. Ser. II* **1986**, *302,* 1223−1226.

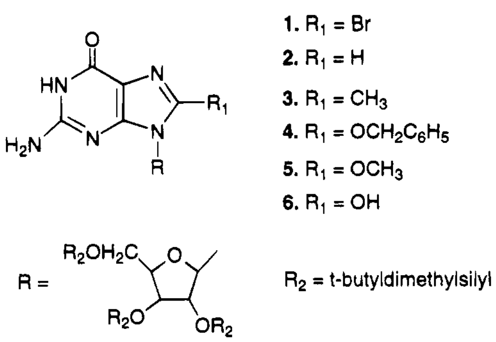
(33) Cadet, J.; Teoule, R. *Photochem. Photobiol.* **1978**, *28,* 661−667.

(34) Cadet, J.; Decarroz, C.; Wang, S. Y.; Midden, W. R. *Israel J. Chem.* **1983**, *23,* 420−429.

(35) Cadet, J.; Berger, M.; Decarroz, C.; Wagner, J. R.; Van Lier, J. E.; Ginot, Y. M.; Vigny, P. *Biochimie* **1986**, *68,* 813−834.

0022-3263/95/1960-4498$09.00/0

Scheme 1



and analytical difficulties in the separation of the relatively polar and unstable photoproducts from guanine and its nucleosides have retarded progress. In previous work, we were able to characterize several unstable primary products by low-temperature NMR studies and solve some of the mechanistic questions by using guanosine derivatives with improved solubility in organic solvents.[36,37] In this paper, solvent and electronic effects on the initial reaction between these guanosine derivatives and singlet oxygen are investigated.

Results

Preparation of 8-Substituted Guanosine Derivatives. 8-Substituted guanosine derivatives **1**−**6** (Scheme 1) were synthesized according to modified literature procedures as described elsewhere.[38,39]

Direct Determination of Rate Constants of Compounds 1−6 with $^1O_2$. Direct measurements of total removal of singlet oxygen by substrates were made by measuring the decay of $^1O_2$ by its luminescence at 1270 nm.[40] The rate constant of $^1O_2$ luminescence decay, k_{obs}, is described by eq 1, where $(k_r + k_q)$ is the total rate constant for $^1O_2$ removal by substrate [S] (the sum of the rate constants for chemical reaction and physical quenching by the substrate) and k_d is the rate constant for radiationless decay of $^1O_2$ in the solvent. A typical result is shown in Figure 1, and the $(k_r + k_q)$ values for the 8-substituted guanosine derivatives (**1** - **6**) (Scheme 1) in different solvents are tabulated in Table 1.

From inspection of Table 1, the total quenching rate constants $(k_r + k_q)$ increase in more polar solvents. Changing the solvent from methylene chloride-d_2 to acetonitrile-d_3 increases reaction rates between 5- and 16.5-fold for all six derivatives. The initial interaction of $^1O_2$ with **1** and with **2**−**6** is very similar with respect to changes in solvent polarity, as shown in Figure 2, in which a plot of $\log(k_r + k_q)$ for compound **1** vs $\log(k_r + k_q)$ for **2** has a slope of 0.99 (r = 0.99). Similar results were obtained for the other compounds (**3**−**5**), with slopes ranging from 0.83 to 1.28; only the 8-oxo/hydroxy derivative **6** gave a larger slope, 1.71.

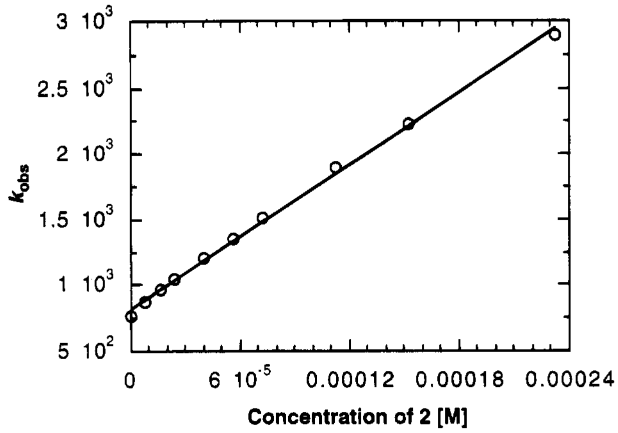


Figure 1. Luminescence quenching of singlet oxygen by compound **2** in acetonitrile-d_3; k_{obs} is the observed rate constant for the decay of singlet oxygen in the presence of the substrate. The plot of k_{obs} vs [**2**] has a slope of $(k_r + k_q)$ and an intercept of k_d.

Table 1. Rate Constants[a] for the Reactions of Singlet Oxygen with 8-Substituted Guanosine Derivatives in Different Solvents

	$(k_r + k_q)$ (M^{-1} s^{-1}) × 10^{-6} (relative to 2, conditions A) at various conditions			
compd	A[b]	B[c]	C[d]	D[e]
1	0.93 (0.65)	1.03 (0.73)	4.47 (3.15)	5.31 (3.74)
2	1.42 (1.00)	1.75 (1.23)	6.33[f] (4.46)	9.15 (6.44)
3	2.38 (1.68)	3.16 (2.23)	8.58 (6.04)	12.10 (8.52)
4	3.42 (2.41)	4.41 (3.11)	23.40 (16.48)	27.30 (19.23)
5	3.60 (2.54)	5.07 (3.57)	36.80 (25.92)	38.10 (26.83)
6	3.95 (2.78)	3.46 (2.44)	55.40[f] (39.01)	65.20 (45.92)

[a] Sensitizing dyes were excited with either the second (532 nm) or third (355 nm) harmonic of a Quanta Ray DCR-2 Nd:YAG laser. [b] A: methylene chloride-d_2, TPP. [c] B: benzene-d_6, acridine. [d] C: acetone-d_6, TPP. [e] D: acetonitrile-d_3, acridine. [f] Reference 38.

The singlet oxygen quenching rate constants depend not only on the solvent polarity but also on the substituents at the C-8 position. Changing the substituents from bromo to hydroxyl[41] increases the reaction rate by a factor of about 4 in methylene chloride-d_2 and about 12 in acetonitrile-d_3. The sensitivity to solvent polarity is greater for the more electron-rich substituents. The effect of changing from methylene chloride-d_2 to acetonitrile-d_3 increases the rate by a factor of about 6 for **1**−**3** (Br, H, CH_3) but by 10−15 for **4**−**6** (OR or OH substituents).

The chemical quenching rate constants (k_r) for the guanosine derivatives in acetone-d_6 were determined separately by competition experiments. The k_r values for compounds **2** and **6** in acetone-d_6 were determined by competition with 2-methyl-2-pentene (2M2P) and tetramethylethylene (TME), respectively. Both 2M2P and TME are known to quench $^1O_2$ only chemically, so that $(k_r + k_q) \simeq k_r$. The $(k_r + k_q)$ values of the alkenes were determined in acetone by singlet oxygen luminescence quenching as previously described.[40,42] The disappearance of the substrates in a photooxidized mixture was monitored by NMR spectroscopy, and the results were fit to the equation of Higgins et al.[43] The k_r values for

---

(36) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 10446−10447.

(37) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 474−477.

(38) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.*, in press.

(39) Sheu, C.; Foote, C. S. In preparation.

(40) Ogilby, P. R.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 3423−3430.

(41) 8-Hydroxyguanosine exists predominantly as the 6,8-dioxo tautomeric form in aqueous solution (see ref 38 and references cited therein) and therefore should be called 7,8-dihydro-8-oxoguanosine. The tautomeric equilibrium in organic solvents is unknown. For simplicity, we use the name 8-hydroxyguanosine to represent compound **6**.

(42) Wilkinson, F.; Brummer, J. G. *J. Phys. Chem. Ref. Data* **1981**, *10*, 809−1000.

(43) Higgins, R.; Foote, C. S.; Cheng, H. *ACS Adv. Chem. Ser.* **1968**, *77*, 102.

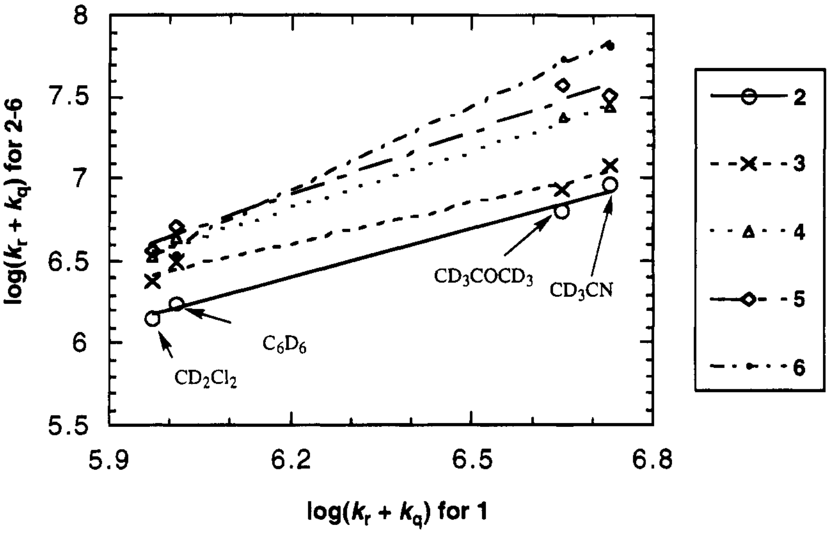


Figure 2. Plot of $\log(k_r + k_q)$ for compound **1** vs $\log(k_r + k_q)$ for compounds **2–6** in various solvents.

Table 2. Total Quenching ($k_r + k_q$) and Chemical Quenching (k_r) Rate Constants for Reactions of Various 8-Substituted Guanosine Derivatives with Singlet Oxygen in Acetone-d_6 and Their Oxidation Potentials ($E_{p/2}$)

subst	$(k_r + k_q)^a$ $(M^{-1}\,s^{-1}) \times 10^{-6}$	k_r $(M^{-1}\,s^{-1}) \times 10^{-5}$	$E_{p/2}$ (V vs. AgCl)
1	4.47	1.05	1.34
2	6.33^b	1.36^b	1.28^b
3	8.58	2.82	1.16
4	23.4	6.46	1.02
5	36.8	11.56	0.98
6	55.4^b	192.0^b	0.85^b

[a] Repeated from Table 1 for comparison. [b] Reference 38.

other compounds were determined similarly by competition experiments between two nucleosides in acetone. These results are summarized in Table 2. Oxidation potentials for compounds **1–6** were determined by cyclic voltammetry. The scans were irreversible in all cases, and the results are expressed as "half-peak" potentials, $E_{p/2}$. These results are also summarized in Table 2.

As shown in Table 2, $(k_r + k_q)$ for compounds **1–6** are significantly larger than those for product formation (k_r) alone. The values of $k_r/(k_r + k_q)$ for compounds **1–5** range from 2.1% to 3.3%; thus most of the singlet oxygen is scavenged by physical quenching. This situation is very similar to that with many other biomolecules, such as α-tocopherols,[44,45] which quench singlet oxygen more rapidly than they react with it. However, the ratio for compound **6** is 34.7%, about an order of magnitude larger than the average of compounds **1–5**, and is discussed in a later section.

A linear correlation between the log of the total rate constant of singlet oxygen removal and the oxidation potential of the corresponding derivative was observed for compounds **1–6** in methylene chloride-d_2 and acetonitrile-d_3 (Figure 3). The slopes are −1.29 (correlation coefficient 0.954) and −2.16 (correlation coefficient 0.992), respectively, in the two solvents. The larger slope in acetonitrile-d_3 indicates that the polar transition state is stabilized more by the more polar solvent. The plot of $\log k_r$ vs half-peak potential ($E_{p/2}$) for compounds **1–5** in acetone-d_6 is also linear over the whole range (Figure 4) with a slope of $-2.79\ V^{-1}$ (correlation coefficient 0.992). Since the value of k_r for **6** is an order of magnitude larger than the values for **1–5**, this point is omitted from the graph. The reasons for deviation of **6** are unclear and are discussed in a later section.

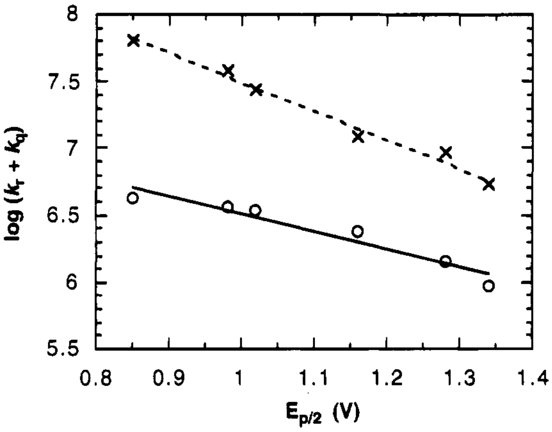


Figure 3. Correlation of $\log(k_r + k_q)$ vs $E_{p/2}$ for 8-substituted guanosine derivatives in methylene chloride-d_2 (o) and in acetonitrile-d_3 (x).

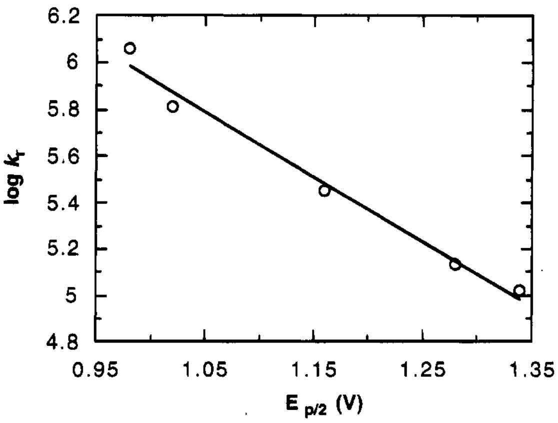


Figure 4. Correlation of $\log k_r$ (chemical quenching) vs $E_{p/2}$ (half-peak oxidation potential) for 8-substituted guanosine derivatives in acetone-d_6.

The correlation of electronic effects with k_r for the 8-substituted guanosine derivatives was also shown by

---

(44) Fahrenholtz, S. R.; Doleiden, F. H.; Trozzolo, A. M.; Lamola, A. A. *Photochem. Photobiol.* **1974**, *20*, 505–509.

(45) Foote, C. S.; Ching, T.-Y.; Geller, G. G. *Photochem. Photobiol.* **1974**, *20*, 511–514.

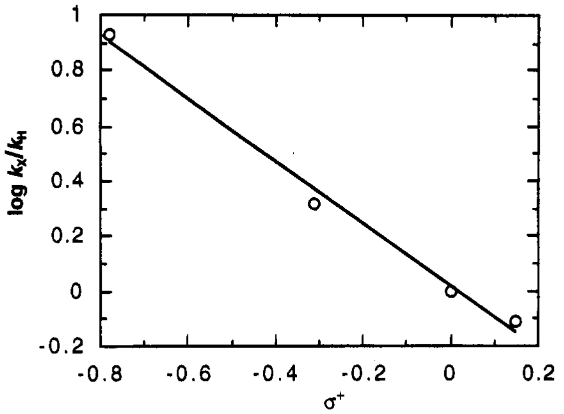


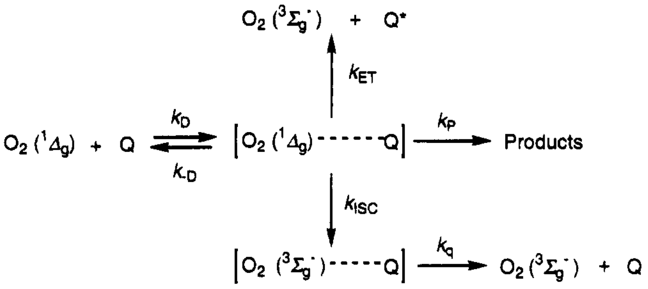
Figure 5. Hammett plot of chemical quenching (log k_X/k_H in acetone-d_6) vs σ^+; slope = −1.1; correlation coefficient = 0.996.

a Hammett plot with the Brown σ^+ parameters[46] (Figure 5; ϱ (slope) = −1.1; correlation coefficient 0.991). The correlation is significantly lower for plots against Hammett σ_{para} or σ_{meta} values (data not shown). This excellent correlation with σ^+ indicates that the reaction involves a resonance-stabilized transition state.[47] Similar results were obtained by plotting the log of the relative total quenching rate; $\log[(k_r + k_q)_X/(k_r + k_q)_H]$ vs σ^+; $\varrho = -0.97$; correlation coefficient 0.979 (not shown). This behavior is similar to that observed by Foote and Denny for *p*-substituted trimethylstyrenes,[48] where a ϱ of −0.92 (however, with σ, not σ^+) was observed. Larger negative ϱ values were also observed by Foote and co-workers[49,50] in singlet oxygen reactions with phenols and sulfides and by Young et al. in photooxygenation of a series of substituted dimethylanilines.[51] Because of its deviation from the line, the point for compound **6** was not plotted in Figure 5.

Discussion

Deactivation of singlet oxygen by substrates can proceed by physical quenching (k_q) or by chemical reaction (k_r) or a mixture. A general mechanism[14] for singlet oxygen processes is depicted in Scheme 2, where k_D is the forward rate constant of formation of the intermediate complex, k_{-D} is the reverse, k_{ET} is the deactivation rate constant from energy transfer, k_{ISC} is the rate constant for a spin−orbit/coupling-induced intersystem crossing within the complex, and k_P is the rate constant for product formation. The involvement of a rapidly and reversibly formed intermediate as indicated in Scheme 2 is now generally accepted.[49,51−53] Depending on the excitation energy of the quencher, Q, and its ionization potential, this intermediate can be described as a covalent adduct (a perepoxide) or as an exciplex or a charge-transfer complex. This adduct can collapse to products (k_p), transfer energy (k_{ET}), giving an excited quencher (Q*), or decay to ground state molecules without excitation of the quencher (k_{isc}).

Scheme 2



Arrhenius plots for the quenching of singlet oxygen by amines such as 1,4-diazabicyclo[2.2.2]octane (DABCO) and strychnine[54] show that there are two distinct regions of slope, one corresponding to a positive and the other to a negative activation enthalpy. The two limits correspond to diffusion-limited ($k_{-D} \ll k_{ISC}$) and pre-equilibrium ($k_{-D} \gg k_{ISC}$) conditions, respectively. Experimental results indicate that at room temperature most amine quenching occurs under preequilibrium conditions; therefore the effects of the electron-donating ability of amines can both help stabilize the intermediate complex (i.e., lower k_{-D}) and promote the spin−orbit coupling or chemical reaction (i.e. increase k_{ISC} or k_P). A similar analysis has also been applied to other systems, such as phenols and α-tocopherol.[49,53]

The reaction of singlet oxygen with 1,3-dienes has also been well studied. Two processes have been proposed for this reaction. Endoperoxide formation is favorable if the diene unit is s-*cis* and essentially planar. When such an arrangement is not possible, the 1,2-cycloaddition reaction often takes over. In our cases, endoperoxide formation, similar to that from cyclopentadiene and furans, has been observed by low-temperature NMR spectroscopy in the case of compound **3**.[36] The fact that the endoperoxide regenerates starting material upon warming suggests a pathway for the quenching process. Although 1,2-cycloaddition may also be possible, there is no evidence for this path. Although endoperoxides from compounds **1**, **2**, and **5** have not been directly observed, it is likely that their reactions resemble that of **3**. The similar values of $k_r/(k_r + k_q)$ for compounds **1**−**5** in Table 2 suggest that their reactions are similar and that endoperoxide formation is the primary reaction for all.

The significantly higher value of $k_r/(k_r + k_q)$ for compound **6** suggests that it is reacting by a somewhat different pathway than the other derivatives. The fact that the 8-oxo compound exists as two tautomers complicates the analysis. While the keto tautomer predominates in aqueous solution,[37,55−57] it is not known which tautomer is favored in the aprotic solvents used in this study. In any case, the 8-hydroxy (**6a**) and 8-oxo (**6b**)

---

(46) Ritchie, C. D.; Sager, W. F. *Prog. Phys. Org. Chem.* **1964**, *2*, 323.

(47) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry,* 3rd ed.; Happer & Row: New York, 1987; pp 143−159.

(48) Foote, C. S.; Denny, R. W. *J. Am. Chem. Soc.* **1971**, *93*, 5162−5167.

(49) Thomas, M. J.; Foote, C. S. *Photochem. Photobiol.* **1978**, *27*, 683−693.

(50) Kacher, M. L.; Foote, C. S. *Photochem. Photobiol.* **1979**, *29*, 765−769.

(51) Young, R. H.; Martin, R. L.; Feriozi, D.; Brewer, D.; Kayser, R. *Photochem. Photobiol.* **1973**, *17*, 233−244.

(52) Firey, P. A.; Ford, W. E.; Sounik, J. R.; Kenny, M. E.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1988**, *110*, 7626−7630.

(53) Gorman, A. A.; Gould, I. R.; Hamblett, I.; Standen, M. C. *J. Am. Chem. Soc.* **1984**, *106*, 6956−6959.

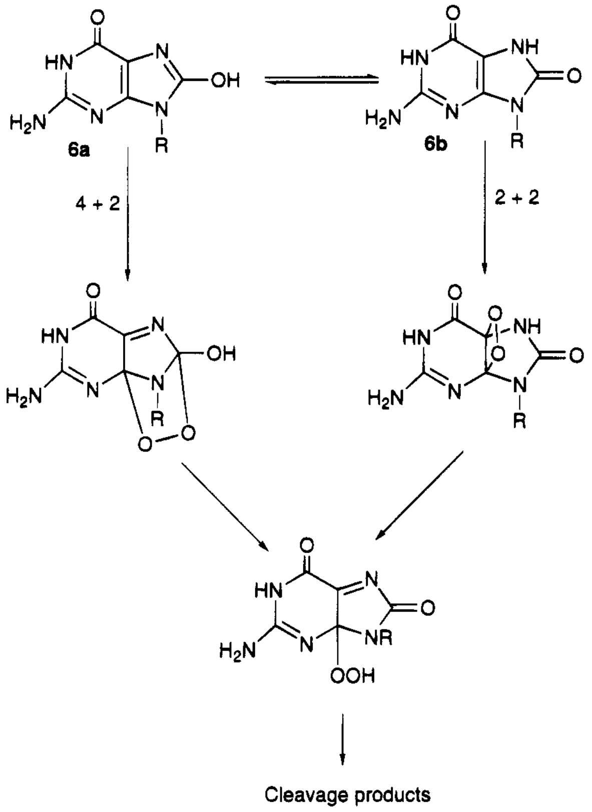
(54) Gorman, A. A.; Hamblett, I.; Lambert, C.; Spencer, B.; Standen, M. C. *J. Am. Chem. Soc.* **1988**, *110*, 8053−8059.

(55) Holmes, R. E.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 1772−1776.

(56) Oda, Y.; Uesugi, S.; Ikehara, M.; Nishimura, S.; Kawase, Y.; Ishikawa, H.; Inoue, H.; Ohtsuka, E. *Nucleic Acids Res.* **1991**, *19*, 1407−1412.

(57) Uesugi, S.; Ikehara, M. *J. Am. Chem. Soc.* **1977**, *99*, 3250−3253.

Scheme 3. Reaction of Tautomers of 6



tautomers probably differ only slightly in energy, and the compound may in fact react with singlet oxygen via the 8-hydroxy tautomer although [2 + 2] reaction with the 8-oxo tautomer is also possible. It is clear that the initial endoperoxide from **6**, if it is formed at all, is much less stable than in the other cases (as mentioned above, the endoperoxide from **3** could be characterized at low temperatures, but not that from **6**). Possible reaction modes for the two tautomers are shown in Scheme 3. Any reaction via the 8-oxo tautomer or ring opening of the endoperoxide could lead to an irreversibility not present in the other tautomers.

Although rate constants for 1,4-cycloadditions often do not correlate well with substrate ionization potentials, as pointed out by Monroe,[14,58] a good Hammett linear free-energy relationship for the addition of singlet oxygen to symmetrical (σ^+) or unsymmetrical (σ) furans was observed by Clennan and Mehrsheikh-Mohammadi, who proposed an intermediate exciplex.[59,60] The reactions of all the guanosine derivatives with $^1O_2$ are affected similarly by changes in solvent polarity, suggesting that they go through an initial intermediate or transition state with polar character. The oxygenation rate constants (k_r) are also mildly electrophilic (Figure 5, $\varrho = -1.13$), comparable to most singlet oxygen reactions, for example, as shown by the rate-increasing effect of methyl groups on the photooxygenation of olefins.[61] Correlation with σ^+ instead of σ_{para} or σ_{meta} indicates that some resonance electron demand is made on the substituents at the transition state. This behavior is very similar to the photooxygenation of phenols, which also shows a best fit with σ^+ with a slope = −1.72.[49] The inefficiency of product formation during singlet oxygen oxidation of guanosine derivatives suggests that either $k_{ISC} \gg k_p$ or a considerable amount of k_{-D} is involved in the quenching step. Quenching via energy transfer can be neglected, since guanosine derivatives have much too high a triplet energy. The smaller value of ϱ for guanosine derivatives than for phenols indicates that there is somewhat less charge development at the quenching step.

The energetics of electron transfer from the guanine moiety to singlet oxygen at the transition state can be evaluated by using the Weller equation[62] $\Delta G_{et} = 23.1(E_{ox} - E_{red}) - E_{0\text{-}0}$; where E_{ox} and E_{red} are the half-peak potentials for oxidation of guanine and reduction of the oxygen and $E_{0\text{-}0}$ is the excitation energy for singlet oxygen. Since the reduction potential of O_2 in CH_3CN is −0.87 V vs SCE and $E_{0\text{-}0}$ ($^1O_2$) = 1.0 V,[63-65] complete electron transfer from guanosine (E_{ox} = 1.23 V vs SCE) to singlet oxygen would be substantially endothermic. Rehm and Weller showed that endothermic complete electron transfer should give a slope of $-16.4\ V^{-1}$ for plots of log k_r vs $E_{p/2}$. This compares with slopes of −3.5, −3.2, and $-3.1\ V^{-1}$ for the reaction of singlet oxygen with alkyl amines,[51] methoxybenzenes,[66,67] and phenols,[49] respectively, indicating that all these processes involve only partial electron transfer. The slope for the reaction of singlet oxygen with guanosine derivatives ($-2.8\ V^{-1}$) is even smaller, indicating a similar but slightly smaller charge transfer at the transition state.

Although a correlation between log ($k_r + k_q$) vs Kirkwood-Laidler-Eyring[68] $(\epsilon - 1)/(2\epsilon + 1)$ solvent polarity scales for singlet oxygen cycloaddition reactions was obtained by Gollnick,[69] only a weak trend and no real correlation was obtained in this study (data not shown). The observations of faster reaction rates in more polar solvents in this study are similar to results obtained by Thomas and Foote[49] in the photooxygenation of phenols but in contrast to studies using amines,[70] which show a significant decrease in quenching rates in more polar solvents. As pointed out by Clennan,[70] this discrepancy may be caused by the fact that hydrogen bonding dramatically decreases the nucleophilicity of amines. The heterocyclic nucleus of the guanosine derivatives apparently plays an electron-donating role similar to that of phenols in these reactions.

Conclusion

Three lines of evidence indicate that there is partial charge transfer involved in the reaction of $^1O_2$ with guanosine derivatives. First, the overall rates of singlet oxygen deactivation ($k_r + k_q$) increase in more polar solvents, indicating that the transition state for reaction is quite polar. Second, a plot of log ($k_r + k_q$) vs halfpeak

---

(58) Monroe, B. M. *J. Am. Chem. Soc.* **1981**, *103*, 7253–7256.

(59) Clennan, E. L.; Mehrsheikh-Mohammadi, M. E. *J. Org. Chem.* **1984**, *49*, 1321–1322.

(60) Clennan, E. L.; Mehrsheikh-Mohammad, M. E. *J. Am. Chem. Soc.* **1984**, *106*, 7112–7118.

(61) Foote, C. S. *Acc. Chem. Res.* **1968**, *1*, 104–110.

(62) Rehm, D.; Weller, A. *Ber. Bunsenges.* **1969**, *73*, 834–839.

(63) Ryan, M. D.; Swanson, D. D.; Glass, R. S.; Wilson, G. S. *J. Phy. Chem.* **1981**, *85*, 1069–1075.

(64) Sawyer, D. T. In *Oxygen Chemistry*; Sawyer, D. T., Ed.; Oxford University Press: New York, 1991.

(65) Manring, L. E.; Foote, C. S. *J. Phys. Chem.* **1982**, *86*, 1257–1259.

(66) Saito, I.; Matsuura, T. *Tetrahedron Lett.* **1970**, *57*, 4987–4990.

(67) Saito, I.; Imuta, M.; Matsuura, T. *Tetrahedron* **1972**, *28*, 5307–5311.

(68) Frost, A. A.; Pearson, R. G. *Kinetics and Mechanism*; John Wiley: New York, 1961.

(69) Gollnick, K.; Griesbeck, A. *Tetrahedron Lett.* **1984**, *25*, 725–728.

(70) Clennan, E. L.; Noe, L. J.; Wen, T.; Szneler, E. *J. Org. Chem.* **1989**, *54*, 3581–3584.

potentials ($E_{p/2}$) is linear; a similar result was obtained for a plot of log k_r vs $E_{p/2}$. Third, a Hammett plot shows that most of the substituents fit on a straight line with σ^+, with $\varrho = -1.13$. Thus, a charge-transfer intermediate that can lead to either quenching or product formation would be consistent with all the experimental data.

Experimental Section

Materials. Compounds **1**–**6** were synthesized by modified literature procedures as previously described.[38,39] All compounds gave satisfactory spectral and physical data. *N,N*-Dimethylformamide (DMF) was dried by stirring overnight at room temperature with calcium hydride and then distilled under reduced pressure. Deuterated solvents were from Cambridge Isotope Laboratory and were dried over 4 Å molecular sieves before use. Other commercial solvents were reagent grade from Fisher AR and used without further purification. 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine (TPP) was from Aldrich and used as received. Acridine (Aldrich) was recrystallized from toluene. Tetraethylammonium perchlorate was recrystallized from water and dried under reduced pressure.

Determination of Oxidation Potentials. Oxidation potentials for compounds **1**–**6** were determined by cyclic voltammetry (Model CV-1B, Bioanalytical Systems Inc.), using a platinum button as electrode and a Ag/AgCl electrode as reference. The solvent was dry dimethylformamide with 0.10 M tetraethylammonium perchlorate as electrolyte; the solution was deoxygenated by purging with helium. In all determinations, the substrate concentration was 5 mM. The anodic and cathodic limits were 1.9 and 1.0 V, respectively, and the scan rate was 300 mV/s. The scans were irreversible in all cases, and thus the results are expressed as "half peak" potentials, $E_{p/2}$.

General Photolysis Procedure. Photolyses were carried out in 5 mm NMR tubes with 0.01–0.005 M substrate in 0.5 mL of deuterated solvents. A Cermax 300 W Xenon lamp was the light source. A 1% potassium dichromate filter solution was used to cut off wavelengths below 500 nm. The NMR tubes were immersed in a water bath at room temperature with an 18 cm running water IR filter. TPP (5×10^{-5}) was the sensitizer in acetone-d_6. Oxygen was continuously bubbled through the solution during irradiation.

Direct Determination of Singlet Oxygen Quenching Rate Constant ($k_r + k_q$) by Substrates. The rate constants of interaction ($k_r + k_q$) of compounds **1**–**6** were determined by time-resolved studies of singlet oxygen luminescence at 1270 nm. The apparatus was a modification of the one previously described.[40] Sensitizing dyes were excited with either the second (532 nm) or third (355 nm) harmonic of a Quanta-Ray (DCR-2) Nd:YAG laser. The laser pulse was filtered to remove any fundamental from the laser using a 355/532 nm pass/1060 nm reflecting mirror, followed with a Schott KG-3 infrared absorbing filter which removed any residual fundamental radiation. The 355 nm pulse was also filtered with a 355 nm pass/532 nm reflecting mirror. The near infrared emission from $^1O_2$ was monitored at a right angle to the laser beam and filtered with a Schott RG-850 and Silicon 1100 nm (Infrared Optics) cutoff filters. The detector was a liquid nitrogen-cooled germanium photodiode (Model EO-817P, North Coast Scientific Corp.). The signal was averaged (10–12 shots) in a transient digitizer (LeCroy 9410) and then transferred to a Macintosh IIci computer using Labview software. The data were analyzed by fitting with a first-order exponential fit, using Igor graphics software and using a macro written by Dr. R. Kanner.

Determination of the Chemical Reaction Rate (k_r) of the Substrates with Singlet Oxygen. The chemical reaction rate constant, k_r, for compound **6** was determined independently by competition studies against well-defined $^1O_2$ acceptors tetramethylethylene (TME) and for compound **2** by competition with 2-methyl-2-pentene (2M2P). In both cases, the relative rates were determined by measuring the relative product formation or the disappearance of the starting material at low conversion (<20%). The appearance of the products or the disappearance of the starting material was monitored by NMR spectroscopy. A zero order and a first-order baseline correction were applied before the peak integrations, and a small amount of DMF was used as internal integration reference.

Because olefins do not quench $^1O_2$, both ($k_r + k_q$) and k_r values are often used as measures of relative olefin reactivity.[71] By assuming $k_q \ll k_r$ for TME and 2M2P, $(k_r + k_q) \simeq k_r$ values of TME and 2M2P were determined by direct measurements with the laser apparatus. Reaction rates (k_r) of compounds **2** and **6** were determined by competition experiments with the olefin of comparable reactivity. The k_r values for compounds **1**, **3**, and **4** were determined by competition with compound **2**, and a k_r value of compound **5** was determined by competition with compound **4**. Since the photosensitized products of guanosine derivatives are complicated, the relative rates were determined by measuring the relative disappearance of the starting material at low conversion. The relative ratios of k_r values of each pair were calculated as the averages of three independent runs. The calculated k_r values for compounds **1**–**6** are listed in Table 1, and the errors are within 10%.

Acknowledgment. Supported by NIH Grant GM-20080.

JO950343T

---

(71) Manring, L. E.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 4710–4717.