Tetrahedron Vol. 46, No. 12, pp 4171-4178, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

PHOTOCHEMICAL OXYGENATION OF STYRENE BY PYRIMIDO[5,4-G]PTERIDINE 10-OXIDE AS A CHEMICAL MIMIC OF HEMIN-CATALYSED OXYGENATION

Magoichi Sako*, Seiji Ohara, Kosaku Hirota, and Yoshifumi Maki*
Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 Chome, Gifu 502, Japan

(*Received in Japan* 1 *February* 1990)

Abstract --- Irradiation of styrene (5) in the presence of pyrimido[5,4-g]-pteridine 10-oxide (1) with u.v.-visible light resulted in the formation of styrene oxide (6) and phenylacetaldehyde (7) which are proved as primary metabolites of (5). Chemical and physico-chemical facts showed that the photo-oxygenation of (5) by (1) involves an initial single-electron transfer followed by oxygen-atom transfer, accommodating the oxygenation mode of cytochrome P-450 and other hemoproteins. Comparative experiments with 3-methylpyridazine 2-oxide (3) and pyridine N-oxide (4) demonstrated the simplicity and the mechanistic characteristic of the photooxygenation by (1).

INTRODUCTION

The complex photochemistry of heterocyclic N-oxides is classified as intramolecular rearrangement of the N-oxide function or oxygen-atom transfer (deoxygenation). [1] Although the latter reaction has been considered to be a simple chemical model for the biological oxygenations by hepatic microsomal monooxygenases, e.g., cytochrome P-450, it usually occurs only as a minor process in the photochemical reactions of the N-oxides and its mechanism is not always unequivocal.

Some authors favour the oxygen-atom transfer by a collision mechanism between a photo-excited N-oxide (or its oxaziridine isomer) and the substrates. [2b] Currently a majority of authors tend to believe that an atomic oxygen is generated after photoexcitation of the N-oxides and then intercepted by the substrates. (oxene mechanism) [3-5] It is also generally accepted that a singlet-excited state of the N-oxides is responsible for the intramolecular rearrangement *via* an oxaziridine intermediate, whereas a triplet-excited state of the N-oxides plays a significant role for the generation of the atomic oxygen. [1, 6, 7]

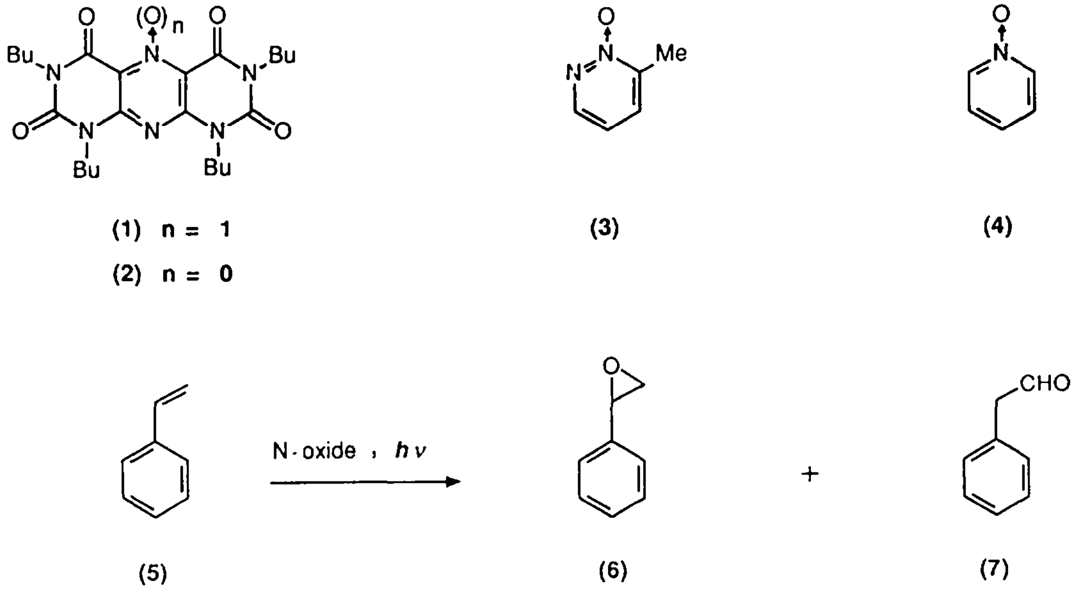
Our previous articles [8] have documented that a novel heterocyclic N-oxide, pyrimido[5,4-g]pteridine 10-oxide (1),[9] transfers its oxygen to the various electron-rich substrates under photochemical conditions most reasonably *via* an initial single-electron transfer (s.e.t.) process without accompanying appreciable intramolecular rearrangements. For example, the photooxygenation of benzene, toluene, and anisole by (1) has been proved to occur cleanly *via* the s.e.t. from the benzene derivatives to a singlet-excited (1). [10]

Thus, the *N*-oxide (1) appears to possess characteristic photochemical reactivities among the heterocyclic *N*-oxides so far investigated. It is noticeable that the photooxygenation by (1) formally parallels the biological oxygenations by cytochrome *P*-450, which can be unified in most cases by the involvement of the initial s.e.t. from substrates to the oxidising species symbolised by $[Fe^{IV}=O]^{+\bullet}$.[11, 12]

Metabolic activation of styrene (5) has been extensively studied in view of its carcinogenicity, mutagenicity, neurotoxicity, and cytotoxicity.[13] Oxygenation of (5) by hemoproteins such as cytochrome *P*-450, hemoglobin, and chloroperoxidase to give styrene oxide (6) and phenylacetaldehyde (7) has been well documented and its mechanism also has been discussed.[12, 14]

The photooxygenations of (5) by 3-methylpyridazine 2-oxide (3)[3b] and pyridine *N*-oxide (4)[2a] in methylene chloride have been reported to give (6) and acetophenone in low yields, respectively.

In the continuation of previous works, we called attention to evaluate the photochemical reaction of (5) with (1) as a chemical mimic of hemin-catalysed oxygenation. Comparative experiments using (3) and (4), which oxygenate the substrates *via* the oxene mechanism, well demonstrated the simplicity and the mechanistic characteristic of the photooxygenation by (1) as a chemical mimic for the biometabolism of (5) leading to (6) and (7).



Scheme 1

RESULTS AND DISCUSSION

A solution of (5)[500 mmol] in acetonitrile containing (1)[λmax: 370 (ε= $2.2\text{x}10^4$)nm] [5 mmol] was irradiated with u.v.-visible light through a $BiCl_3$ solution filter (>355 nm) at ambient temperature under argon atmosphere for 10 min. During this period, 31 % of (1) was deoxygenated to give pyrimido[5,4-g]pteridine (2) [9] almost quantitatively without the formation of detectable other products originated from (1). Styrene oxide (6) and phenylacetaldehyde (7) were obtained as major oxygenated products in 49 % and 12 % yields (based on the consumed 1), respectively. The yields and structures of these products were determined by g.c. and g.c.-mass analyses and spectral comparison with authentic samples. The yields of (6) and (7) were not optimised and elongation of the irradiation time caused the decrease of the conversion yields, suggesting the occurrence of further photooxygenation of (6) or (7) by (1). [15] No formation of a detectable amount of acetophenone isomeric with (7) was confirmed by careful g.c.-mass analysis.

Addition of an excess amount of $H_2{}^{18}O$ to the photoreaction medium resulted in no ^{18}O incorporation to the oxygenated products (6) and (7), indicating that the oxygen-atom on the side-chain of the products (6) and (7) originates from the N-oxide (1) but not from a small amount of water containing in the medium. No conversion of (6) into (7) was observed under the photochemical conditions employed and the concurrent formation of (6) and (7) was also realised by product distributions in the irradiation-time course.

Analogous irradiation of (5)[500 mmol] in the presence of the N-oxide (3)[λ_{max}: 323 (ε= $1.4\text{x}10^4$)nm][5 mmol] with 323 nm light for 10 min. resulted in the complete consumption of (3) and the formation of the oxygenated products (6)(40 %) and (7)(11 %). In this reaction, the deoxygenated pyridazine was obtained only in 52 % yield and the formation of other products derived from (3) was observed by g.c. analysis of the reaction mixture, suggesting the concurrent occurrence of the intramolecular rearrangements of (3) reported previously. [3, 4] Upon employment of pyridine N-oxide (4)[λ_{max}: 274 (ε= $2.0\text{x}10^4$)nm] [2a] as an oxidant, only trace amounts (<1 % yields) of (6) and (7) were obtained after irradiation with 274 nm light for 10 min.

Previous studies [2a, 3b] on the photooxygenations of (5) by (3) and (4) in methylene chloride have documented the formation of acetophenone instead of (7) as a minor product, respectively. However, the reinvestigation of the photoreactions of (5) with (3) and (4) in methylene chloride showed the formation of (7) rather than acetophenone.

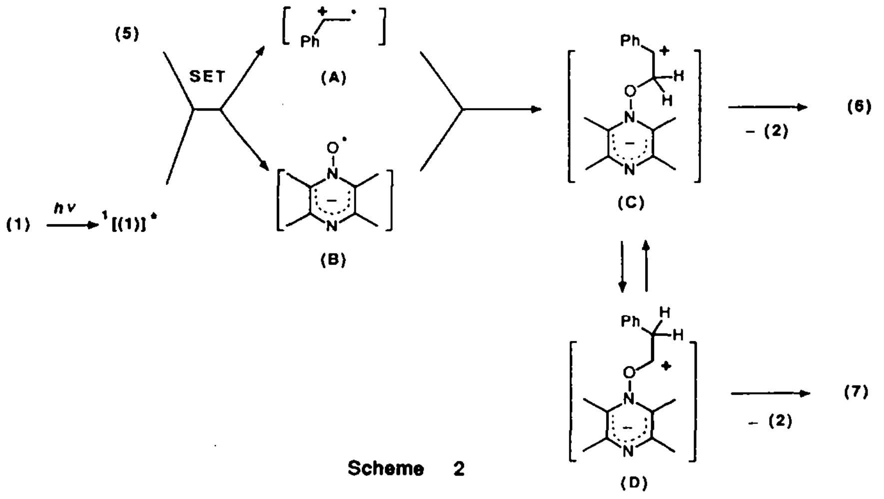
Thus, it is evident that the photooxygenation of (5) by the N-oxide (1) cleanly occurs in comparison with the cases of the N-oxides (3) and (4).

In the photooxygenation of cyclohexene by (3) in methylene chloride, [4a] the consumption rate of (3) has been found not to be affected by the presence of cyclohexene in the reaction medium, which supports the oxene mechanism via the direct liberation of the atomic oxygen from the excited (3). The analogous phenomenon was observed in the photoreaction of (5) with (3) in acetonitrile. In sharp contrast, (1) in acetonitrile was very stable to u.v.-lights and addition of (5) to the solution resulted in the smooth consumption of (1) with a concentration-dependence under irradiation. This discrepancy suggests that the photooxygenation of (5) by (1) involves a reaction mode different from the oxene mechanism proposed for the photooxygenation by (3) and that is requisite of an appreciable interaction between (1) and (5). No formation of a charge-transfer complex between (1) and (5) in acetonitrile, however, was observed in the difference spectrum of a mixture of (1) and (5) vs (1).

Experiments for the wavelength-dependence showed that the photooxygenation of (5) by (1) occurs most efficiently by irradiation at around 365 nm which is near the longest u.v.-absorption band of (1). Quantum yields for the formation of (6) and (7) were 0.02 and 0.005, respectively, under irradiation at 365 nm. The fluorescence of (1)(λ_{max} : 394 nm; τ_s= 2.95 ns) [16] in acetonitrile was effectively quenched by the addition of (5) to the medium. Stern-Volmer plots for the fluorescence quenching of (1) by (5) were linear ($k_q\tau_s$= 3.5 M^{-1}) and the estimated rate-constant (k_q= ca. $1.2x10^9 M^{-1}s^{-1}$) was near the diffusion-controlled limit. These facts indicate that a singlet-excited (1) is responsible for the present photooxygenation.

The photoreaction of (5) with (1) was significantly suppressed with a concentration-dependence by the addition of a strong electron acceptor, tetracyanoethylene or tetracyanoquinodimethane, to the reaction medium. [17] The free-energy change (ΔG_{et}= -9.60 kcal mol^{-1}) [18] roughly calculated for the s.e.t. from the electron donor (5)($E^{ox}_{1/2}$= 1.91 V vs SCE in acetonitrile) [19] to the singlet-excited (1)[$E^{red}_{1/2}$(1) = -0.97 V vs SCE in acetonitrile] suggests that the s.e.t. process is exothermic. These facts accommodate the involvement of the s.e.t. from (5) to the singlet-excited (1) in the initial stage of the photoreaction.

Taking into consideration the above experimental facts and their mechanistic implications, a most conceivable reaction sequence for the photooxygenation of (5) by (1) is as shown in Scheme 2.



Scheme 2

Excitation of (1) followed by the s.e.t. from (5) to the singlet-excited (1) generates styrene cation-radical (**A**) and N-oxide anion-radical (**B**). Coupling of the radical ions (**A**) and (**B**) results in the formation of a zwitterionic intermediate (**C**). High spin density on the methylene carbon atom in (**A**) [20] and the coupling of photochemically generated radical-ion pair have been documented. [21] Fragmentation of (**C**) could give rise to the products (2) and (6). 1,2-Hydrogen shift in (**C**) leads to a transient intermediate (**D**) which collapse to give (2) and (7).

The present mechanism is alternative to the oxene mechanism for the photochemical oxygenation by the heterocyclic N-oxides such as (3) and (4) and formally parallels that proposed for the oxygenation of (5) catalysed by cytochrome P-450 and other hemoproteins, [12, 14] in that it involves the initial s.e.t. process.

CONCLUSION

As mentioned above, the N-oxide (1) oxygenates (5) more slowly than the case of the N-oxide (3) under the photochemical conditions to give the epoxide (6) and aldehyde (7) which are primary metabolites of (5). [14] The quantitative deoxygenation of (1) to (2), however, indicates that the photooxygenation by (1) occurs with the simplicity without accompaning the photochemical intramolecular rearrangements. Although the oxidised products (6) and (7) are in agreement with the cases of (3) and (4), the photooxygenation by (1) mimics well the biometabolism of (5) in the mechanistic point of view rather than the cases of (3) and (4).

Studies on the construction of other heterocyclic N-oxides with such prominent functions are now in progress.

EXPERIMENTAL

Irradiation was carried out by using a 400 W high-pressure mercury arc lamp (Riko Kagaku Sangyo) through a $BiCl_3$ solution filter (>355 nm) at ambient temperature under argon atmosphere. A grating monochrometer (JASCO CRM-FA spectroirradiator) with 2 KW Xe lamp and 4 nm band width was used for the photoreaction of (5) with (3) or (4), for the wavelength-dependence experiments, and for the measurements of quantum yields. The spectroscopic measurements were performed with the following instruments; u.v. absorption spectra with Shimadzu-260 spectrophotometer; Mass spectra with a JEOL JMS-D 300 machine operating at 70 eV; Fluorescence spectra with a Hitachi 650-60 spectrometer. G.l.c. analyses were performed with a Shimadzu GC-8APF instrument with 1.2 m column (PEG 5 % on Uniport HP). T.l.c. analyses were performed on Silica gel plates (Silicagel $70F_{254}$ Plate-Wako) and by employment of the mixed solvent (benzene-ethyl acetate = 5:2).

T.l.c scanning was carried out with a Shimadzu CS-9000 Dual Wavelength Flying-spot Scanner (detection: 370 nm for 1 and 2).

Photochemical Oxygenation of Styrene (5) by 1,3,6,8-Tetrabutyl-2,4,5,7(1H,3H,6H,8H)-pyrimido[5,4-g]pteridinetetrone 10-Oxide (1), 3-Methylpyridazine 2-Oxide (3), or Pyridine N-Oxide (4).

(a) Photoreaction of (5) with N-Oxide (1). --- A mixture of (5)(0.29 ml, 2.5×10^{-3} mol) in acetonitrile (5 ml) containing (1)(12.2 mg, 2.5×10^{-5} mol) was irradiated externally for 10 min. T.l.c. and g.c. analyses of the reaction mixture showed 31 % consumption of (1)(by t.l.c.-densitometry, R_F 0.27) and the formation of pyrimido[5,4-g]pteridine (2)(R_F 0.37, almost quantitative yield), styrene oxide (6)(49 %), and phenylacetaldehyde (7)(12 %). The yields are based on the consumed (1). Retention time of (7) in g.c. was very close to that of acetophenone. The formation of (7), however, was distinguished clearly from that of acetophenone by comparison with fragmentation pattern in the mass spectra of authentic samples.

Elongation of the irradiation time resulted in the decrease of the conversion yields of the products. The yields of the products (6) and (7) were as follows. Conversion yield (irradiation time): 32 % (20 min) and 25 % (30 min) for (6); 7 % (20 min) and 6 % (30 min) for (7). The oxygenation of (5) did not proceed in the dark (e.g. reflux for 1 hr) or in the absence of (1).

b) Photoreaction of (5) with the N-Oxide (3) or (4). --- Under the conditions analogous to the case of (1), a solution of (5)[500 mmol] in acetonitrile containing (3)[5 mmol] was irradiated with 323 nm light for 10 min. T.l.c. and g.c. analyses of the reaction mixture showed the complete consumption of (3) and the formation of 3-methylpyridazine (52 %, by g.c.), (6)(40 %), and (7)(11 %). The formation of other products originated from (3) was detected by g.c. analysis of the reaction mixture.

Analogously, irradiation of a solution of (5) and (4) in acetonitrile with 274 nm light resulted in the formation of (6)(1 %) and (7)(0.3 %) together with other products.

Photooxygenation of (5) by (1) in the presence of $H_2{}^{18}O$. --- A solution of (5)(0.29 ml, 2.5×10^{-3} mol) and (1)(12.2 mg, 2.5×10^{-5} mol) in acetonitrile (5 ml) containing $H_2{}^{18}O$ (^{18}O content= 99 %)(50 μl) was irradiated for 10 min under the conditions described above. T.l.c. and g.c. analyses of the reaction mixture showed 36 % consumption of (1) and the formation of (6) and (7) in 35 % and 10 % yields based on the consumed (1), respectively. No ^{18}O incorporation into these products was confirmed by g.c.-mass analysis.

Wavelength-Dependence Experiment for the Photochemical Formation of (6) from (5) by (1). --- A solution of (5)[500 mmol] and (1)[5 mmol] in dry acetonitrile was degassed carefully and irradiated at various wavelengths (339-392 nm, ca. 9×10^7 erg·cm^{-2}) for 10 min. The yields of (6) in these photoreactions were determined by g.c. analysis. The results were as follows. Yields (wavelength, nm): 12.5 % (339), 14.5 % (365), 11.5 % (381), and 9.5 % (392). A charge-transfer band was not observed in the difference spectrum of a mixture of (1)[5 mmol] and (5)[2.0 mol] vs (1)[5 mmol] in dry acetonitrile.

Quantum Yields for the Formation of (6) and (7) in the photooxygenation of (5) by (1). --- The quantum yield measurement was carried out at 20°C by using potassium ferrioxalate actinometry at 365 nm excitation. A solution of (5)[500 mmol] and (1)[5 mmol] in dry acetonitrile was purged well with argon. Under the conditions employed, the mixed solution absorbed more than 95 % of the incident light. After irradiation for 7 min, the formation of (6) and (7) was assayed by g.c. analysis. The quantum yields were estimated to be 0.02 for the formation of (6) and 0.005 for (7).

Quenching Experiment of the Fluorescence of (1). --- Three samples of (1)[2 $\times 10^{-6}$ mol] in dry acetonitrile containing (5)[2.13×10^{-1} mol], [4.16×10^{-1} mol], and [6.09×10^{-1} mol] were prepared. Fluorescence intensities of these samples obtained by the excitation at 365 nm were measured at the emission wavelength of (1)(394 nm) and compared with the control. Stern-Volmer plots for the fluorescence-quenching of (1) by (5) were linear and the quenching-rate constant (k_q) was estimated to be ca. 1.2×10^9 $M^{-1}s^{-1}$ (at room temperature) from the slope ($k_q\tau_s$= 3.5 M^{-1}) and the measured fluorescence life-time of the N-oxide (1)(τ_s = 2.95 ns).

Inhibitory Effect of Electron-Transfer Quenchers on the Photooxygenation of (5) by (1). --- To a solution of (5)[500 mmol] and (1)[5 mmol] in acetonitrile, tetracyanoethylene (TCNE) or tetracyanoquinodimethane (TCNQ)[0.05 or 0.25 mmol] was added and the mixture was irradiated for 10 min. In each run, the assay of the photoproduct (6) was carried out by g.c. analysis. In the control run, a solution of (1) and (5) without TCNE or TCNQ was irradiated under the identical conditions. The inhibitory effect of TCNE and TCNQ on the formation of (6) in the photoreaction of (5) with (1) were as follows. The relative conversion yield of (6)(mole ratio of TCNE or TCNQ to 1): 0.76 (0.1) and 0.68 (0.5) for TCNE; 0.5 (0.1) and 0.31 (0.5) for TCNQ.

ACKNOWLEDGEMENT

We thank Professor Noboru Mataga of Osaka University for the measurement of the fluorescence lifetime of the N-oxide (1). This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture in Japan.

REFERENCES AND NOTES

1. For recent reviews on the photochemistry of the heterocyclic N-oxides, see G. G. Spence, E. C. Taylor, and O. Buchardt, Chem. Rev., **1970**, 70, 231; A. Albini and M. Alpegiani, ibid., **1984**, 84, 43.
2. a) D. M. Jerina, D. R. Boyd, and J. W. Daly, Tetrahedron Lett., **1970**, 457. b) M. N.

Akhtar, D. R. Boyd, J. D. Neill, and D. M. Jerina, J. Chem. Soc., Perkin I, **1980**, 1693 and references cited therein.

3. a) H. Igeta, T. Tsuchiya, M. Yamada, and H. Arai, Chem. Pharm. Bull., **1968**, 16, 767. b) T. Tsuchiya, H. Arai, and H. Igeta, Tetrahedron Lett., **1969**, 2747. c) Idem., ibid., **1970**, 2213.
4. a) Y. Ogawa, S. Iwasaki, and S. Okuda, Tetrahedron Lett., **1981**, 22, 2277. b) Idem., ibid., **1981**, 22, 3637.
5. H. Strub, C. Strehler, and J. Streith, Chem. Ber., **1987**, 120, 355.
6. A. Albini, E. Fasani, and V. Frattini, J. Photochem., **1987**, 37, 355.
7. Recently, it has been reported that acridine N-oxides efficiently oxygenate cyclohexene to give cyclohexenol under the photochemical conditions (>420 nm light), which is suggested to involve a radical mechanism as evidenced by e.s.r. study. (cf. H. J. Xu, S. Q. Lin, S. Y. Shen, and L. Li, J. Photochem. Photobiol., **1989**, 48, 53.)
8. M. Sako, K. Shimada, K. Hirota, and Y. Maki, Tetrahedron Lett., **1985**, 26, 6493 and **1986**, 27, 3877; Idem., J. Am. Chem. Soc., **1986**, 108, 6039; Idem., J. Chem. Soc., Chem. Commun., **1986**, 1704; K. Shimada, M. Sako, K. Hirota, and Y. Maki, Tetrahedron Lett., **1987**, 28, 207; Y. Maki, M. Sako, K. Shimada, T. Murase, and K. Hirota, "The Role of Oxygen in Chemistry and Biochemistry", Ed., W. Ando and Y. Morooka, Elsevier, Amsterdam, 1988, p. 465; Y. Maki, K. Shimada, M. Sako, Y. Kitade, and K. Hirota, Chem. Pharm. Bull., **1988**, 37, 1714; Y. Maki, K. Shimada, M. Sako, and K. Hirota, Tetrahedron, **1988**, 44, 3187.
9. Y. Maki, M. Sako, and E. C. Taylor, Tetrahedron Lett., **1971**, 4271; E. C. Taylor, Y. Maki, and A. Mckillop, J. Org. Chem., **1972**, 37, 1601.
10 M. Sako, S. Ohara, K. Shimada, K. Hirota, and Y. Maki, J. Chem. Soc., Perkin I, in press.
11. For pertinent reviews on the mechanism for the cytochrome P-450 catalysed oxidations, see F. P. Guengerich and T. L. Macdonald, Acc. Chem. Res., **1984**, 17, 9; P. R. Ortiz de Montellano, "Cytochrome P-450: Structure, Mechanism, and Biochemistry", Plenum Press, New York, 1986.
12. T. G. Traylor and F. Xu, J. Am. Chem. Soc., **1988**, 110, 1953 and references cited therein; D. Ostovic and T. C. Bruice, J. Am. Chem. Soc., **1989**, 111, 6511.
13. For example, H. Vainio, H. Norppa, and G. Belvedere, Prog. Clin. Biol. Res., **1984**, 141, 215; R. Male, J. R. Lillehaug, R. Djurhuus, and I. F. Pryme, Carcinogenesis, **1985**, 6, 1367; J. P. Collman, P. D. Hampton, and J. I. Brauman, J. Am. Chem. Soc., **1986**, 108, 7861; P. Vodicka and K. Hemminki, Carcinogenesis, **1988**, 9, 1657; G. A. S. Ansari, J. C. Gan, and B. K. Barton, Arch. Environ. Contam. Toxicol., **1988**, 17, 537; D. V. Parke, C. Ioannides, and D. F. V. Lewis, Hum. Toxicol., **1988**, 7, 397.
14. D. Mansuy, J. Leclaire, M. Fontecave, and M. Momenteau, Biochem. Biophys. Res. Commun., **1984**, 119, 319; P. R. Ortiz de Montellano and C. E. Catalano, J. Biol. Chem., **1985**, 260, 9265; P. R. Ortiz de Montellano, Y. S. Choe, G. DePillis, and C. E. Catalano, J. Biol. Chem., **1987**, 262, 11641.
15 A detailed study on the photooxygenation of the epoxide (**6**) and the aldehyde (**7**) by the N-oxide (**1**) is in progress.
16 The lifetime of fluorescence of (**1**)(τ_S= 2.95 ns) was measured by a single-photon counting method.
17 Electron-transfer quenchers are frequently used to obtain experimental support for the involvement of the s.e.t. process. (cf. K. A. Brown-Wensley, S. L. Mattes, and S. Farid, J. Am. Chem. Soc., **1978**, 100, 4162; S. Ito, I. Saito, and T. Matsuura, ibid., **1980**, 102, 7535.
18. Free-energy change for the s.e.t. was calculated according to the Rehm-Weller relationship. cf. D. Rehm and A. Weller, Isr. J. Chem., **1970**, 8, 259.
19. M. Kojima, H. Sakuragi, and K. Tokumaru, Bull. Chem. Soc. Jpn., **1985**, 58, 521.
20. D. N. R. Rao and M. C. R. Symons, J. Chem. Soc., Perkin II, **1985**, 991.
21. J. L. Stavinoha and P. S. Mariano, J. Am. Chem. Soc., **1981**, 103, 3136; P. H. Mazzocchi and L. Klingler, J. Am. Chem. Soc., **1984**, 106, 7567; Y. Kubo, M. Suto, T. Araki, P. H. Mazzocchi, L. Klingler, D. Shook, and C. Somich, J. Org. Chem., **1986**, 51, 4404.