(4) Experimental details for these and other compounds will appear elsewhere.[5]
(5) P. S. Engel, R. A. Melaugh, F. D. Rossini, M. Mansson, J. W. Timberlake, and A. W. Garner, *J. Chem. Thermodyn.*, in press.
(6) The author is grateful to Dr. Margret Mansson, University of Lund, Sweden, for carrying out these measurements. See ref 5 for experimental details.
(7) S. W. Benson and H. E. O'Neal, *Natl. Stand. Ref. Data Ser., Natl. Bur. Stand.*, **No. 21**, (1970).
(8) The strain energies reported previously[3] for **5** and **6** were based on a *cis*-diazene model.
(9) For leading references, see E. R. Talaty, A. K. Schwartz, and G. Simons, *J. Am. Chem. Soc.*, **97**, 972 (1975); J. M. Howell and L. J. Kirschenbaum, *J. Am. Chem. Soc.*, **98**, 877 (1976).
(10) T. Mill and R. S. Stringham, *Tetrahedron Lett.*, 1853 (1969).
(11) N. C. Baird, P. deMayo, J. R. Swenson, and M. C. Usselman, *J. Chem. Soc., Chem. Commun.*, 314 (1973).
(12) K. N. Houk, Y. M. Chang, and P. S. Engel, *J. Am. Chem. Soc.*, **97**, 1824 (1975).
(13) Although structural data on cis diazenes are seriously lacking, a bond angle of 120–125° seems reasonable for *cis*-**1**. An earlier estimate of 130–140° is probably too large.
(14) Possible complications introduced by nonplanarity of diazenes are being neglected because theoretical calculations show that even *cis*-di-*tert*-butylethylene is nearly planar[15] and that the potential energy curve for twisting about the N═N bond is similar to that for the same process in olefins.[11]
(15) O. Ermer and S. Lifson, *J. Am. Chem. Soc.*, **95**, 4121 (1973).
(16) P. S. Engel and D. J. Bishop, *J. Am. Chem. Soc.*, **97**, 6754 (1975).
(17) L. D. Fogel and C. Steel, *J. Am. Chem. Soc.*, in press.
(18) For a nice discussion of the trimethylene problem, see R. G. Bergman in "Free Radicals", Vol. 1, J. K. Kochi, Ed., Wiley, New York, N.Y., 1973.
(19) W. R. Roth and M. Martin, *Justus Liebigs Ann. Chem.*, **702**, 1 (1967).
(20) R. J. Crawford and M. Ohno, *Can. J. Chem.*, **52**, 3134 (1974).
(21) L. Kiefer and J. W. Timberlake (University of New Orleans) have recently obtained a value of $\Delta H^{\ddagger}$ for **3** in solution larger than Crawford and Mishra's gas phase figure. Thus the observed differences in E^* for **2**, **3**, and **4** (Table II) are probably nothing more than experimental error.

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Received November 3, 1975

Spectroscopic Evidence for the Generation of Singlet Oxygen in Self-Reaction of *sec*-Peroxy Radicals

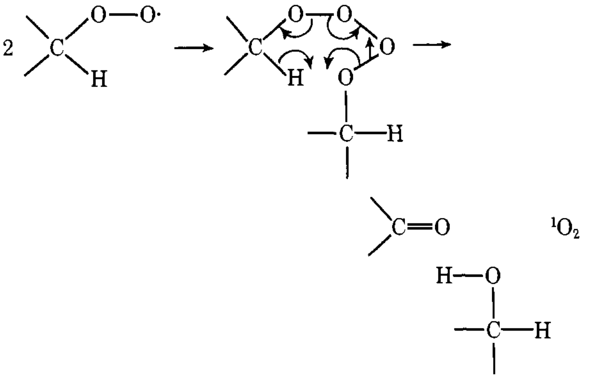
Sir:

The role of singlet molecular oxygen ($^1O_2$) in enzyme-mediated peroxidation reactions in vivo has been an area of considerable interest in recent years. Howes and Steele have demonstrated chemiluminescence in the system containing rat liver microsomes and NADPH, and postulated the generation of $^1O_2$ in the process of lipid peroxidation.[1] We have recently succeeded in the direct spectroscopic determination for the generation of $^1O_2$ in the NADPH-dependent microsomal lipid peroxidation system.[2]

It has been suggested that dismutation of superoxide anions produces $^1O_2$ (eq 1).[3,4] Superoxide dismutase inhibits the generation of $^1O_2$ in eq 1 by catalyzing the dismutation of $O_2\cdot^-$ to give ground state molecular oxygen.[5]

$$2O_2\cdot^- \xrightarrow{2H^+} {}^1O_2 + H_2O_2 \qquad (1)$$

The addition of the dismutase to the microsomal lipid peroxidation system, however, did not inhibit the light emission originated from bimol collision of $^1O_2$, indicating the $^1O_2$ generation in this system does not involve eq 1.[2] Thus, we believe that $^1O_2$ is generated by the interaction of lipid peroxide intermediates (radicals) which might be produced during phospholipid peroxidation. This is supported by the finding that $^1O_2$ is generated in the self-reaction of *sec*-butyl peroxy radicals (eq 2).[6]


(2)

We have now identified $^1O_2$ spectroscopically in self-reactions of linoleic acid peroxy radicals (possible intermediates in NADPH-dependent microsomal lipid peroxidation) identical with the case of *sec*-butylperoxy radicals, and have proved the generation of two types of $^1O_2$, both $^1\Sigma_g{}^+$ and $^1\Delta_g$, during these reactions even in aqueous system.

Peroxy radicals were produced from *sec*-butyl hydroperoxide[7] or linoleic acid hydroperoxide[8] by oxidation with ceric ion. Either linoleic acid hydroperoxide (0.127 mmol) or *sec*-butyl hydroperoxide (0.15 mmol) in 15 ml of 0.1 M NH_4OH-NH_4Cl buffer (pH 9.0) was transferred to a quartz cell in a new type of spectrometer[9] and, in the dark, excess ceric ammonium nitrate (1.27 mmol in 2 ml of water) was slowly added to the solution through a capillary tube to maintain a constant generation of $^1O_2$ without vigorous bubbling. The solution was not stirred during spectral analysis. The ceric oxidation of *sec*-butyl hydroperoxide (0.15 mmol) in 15 ml of benzene, a well-known $^1O_2$ generating system, was also carried out according to a modified method of Howard and Ingold.[6]

The data obtained from the spectral analysis of chemiluminescence in the aqueous systems (system 1 and 2 contained *sec*-butyl hydroperoxide and linoleic acid hydroperoxide as the source of *sec*-peroxy radicals, respectively) are summarized in Table I, and the typical emission spectrum

Table I. Emission Spectra Observed during Ceric Oxidation of Hydroperoxides

Vibrational transition of $^1O_2$	I_{max} obsd,[f] nm			I_{max}, nm ref
	System 1	System 2	System 3	
$(^1\Sigma_g{}^+)\,(^1\Sigma_g{}^+)\,(0, 0) \rightarrow 2(^3\Sigma_g{}^-)\,(0, 0)$	None	None	None	400[a]
$(^1\Sigma_g{}^+)\,(^1\Delta_g)\,(0, 0) \rightarrow 2(^3\Sigma_g{}^-)\,(0, 0)$	480 (0.45)	470 (0.26)	None	478[b] 480[a]
$(^1\Delta_g)\,(^1\Delta_g)\,(2, 0) \rightarrow 2(^3\Sigma_g{}^-)\,(0, 0)$	520–530 (1.00)	520–530 (0.43)	512–520 (0.30)	520[a]
$(^1\Delta_g)\,(^1\Delta_g)\,(1, 0) \rightarrow 2(^3\Sigma_g{}^-)\,(0, 0)$	570 (0.62)	570 (0.90)	595 (0.85)	578[c] 580[a]
$(^1\Delta_g)\,(^1\Delta_g)\,(0, 0) \rightarrow 2(^3\Sigma_g{}^-)\,(0, 0)$	620–640 (0.66)	640 (0.64)	630 (1.00)	633[c,d]
Unspecified transition	**670** (0.67)	670 (1.00)	680 (0.80)	670[e]
$(^1\Delta_g)\,(^1\Delta_g)\,(0, 1) \rightarrow 2(^3\Sigma_g{}^-)\,(0, 0)$	—	—	—	703[d]

[a] K. Furukawa, E. W. Gray, and E. A. Ogryzlo, *Ann. N.Y. Acad. Sci.*, 171, 175 (1970). [b] A. U. Khan and M. Kasha, *J. Am. Chem. Soc.*, 88, 1574 (1966). [c] H. H. Seliger, *J. Chem. Phys.*, 40, 3133 (1964); R. J. Brown and E. A. Ogryzlo, *Proc. Chem. Soc. London*, 117 (1964). [d] A. U. Khan and M. Kasha, *J. Am. Chem. Soc.*, 92, 3293 (1970). [e] U. Sander and J. Stauff, *An. Soc. Quim. Argent.*, 59, 149 (1971). [f] Number in parentheses represents the relative intensity in mean value which was obtained by three different runs.

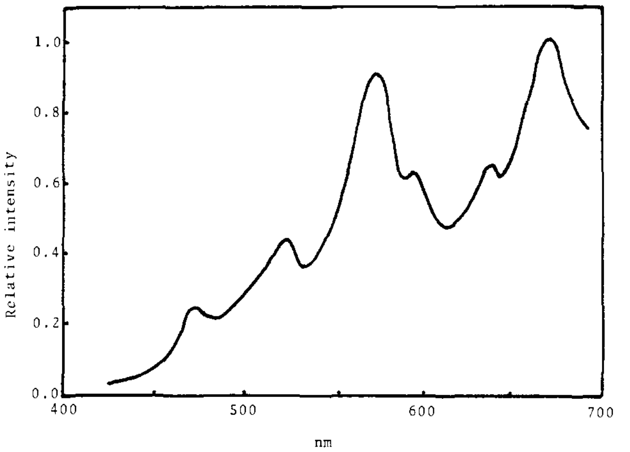


Figure 1. Emission spectrum observed in the reaction of linoleic acid hydroperoxide with ceric ion.

in the reaction of linoleic acid hydroperoxide with ceric ion is shown in Figure 1. The peaks of light emission in the microsomal lipid peroxidation system (between 400 and 680 nm), which have been reported previously, are also presented in the same table (system 3). In the present systems, five characteristic peaks around 470–480, 510–530, 560–580, 630–640, and 670 nm are distinguishable (i.e., quite similar to those of the emission in the microsomal lipid peroxidation system), although the relative intensities of the peaks vary in the different runs. The latter effect is probably caused by the inconstency of the light source. The relative light intensities listed in Table I resemble those from the emission in the reaction of alkali metals in hexamethylphosphoramide with H_2O and O_2 (a heterogeneous system),[10] but differ from those obtained from aqueous H_2O_2-OCl^- systems (a homogeneous system).[12] As five peaks are typical for all the spectra taken with aqueous systems and independent of the *sec*-hydroperoxides, it seems reasonable to assign the emission to transition of $(^1\Sigma_g{}^+)(^1\Delta_g)$ and $(^1\Delta_g)(^1\Delta_g)$ dimers, with the exception of the species peaking near 670 nm.[13] The emitter would not be the excited carbonyl compounds which could be generated in the decomposition of the cyclic intermediate (eq 2).[14] Only one prominent peak around 465-500 nm corresponding to $^1\Sigma_g{}^+ + {}^1\Delta_g \rightarrow 2(^3\Sigma_g{}^-)$, in contrast, was observed during ceric oxidation of *sec*-butyl hydroperoxide in benzene. This discrepancy may be attributable to a different lifetime of the $^1\Sigma_g{}^+$ state in the solvent used. It is well documented that this species is sensitive to water quenching,[3] a result which is consistent with the findings reported here.

We observed D_2O effects on light emission from the aqueous systems.[15] One-tenth milliliter of 7.5×10^{-3} M linoleic acid hydroperoxide (or *sec*-butyl hydroperoxide) in 0.1 M NH_4OH-NH_4Cl buffer (pH 9.0) was mixed with 2.8 ml of D_2O or H_2O and the reaction was initiated by a rapid addition of 0.1 ml of 7.5×10^{-3} M ceric ammonium nitrate. Total light output in the visible region was then determined for the initial 15 min with a photon counter.[16] Under these conditions, the total light output (initial 15 min) of the D_2O system increased 1.5–3.0-fold over that of H_2O system.

In mammalian cells, the membrane and microsome are abundantly rich in unsaturated fatty acids, including linoleic acid. It is expected that $^1O_2$ would be generated by self-reactions of *sec*-peroxy radicals of unsaturated fatty acids produced in the process of peroxidative cleavage of phospholipids, and this expectation is strongly supported by the observed chemiluminescence of the NADPH-dependent microsomal lipoxygenation system[1,2] and microbicidal system of polynuclear leucocytes.[17]

References and Notes

(1) R. M. Howes and R. H. Steele, *Res. Commun. Chem. Pathol. Pharmacol.*, **3**, 349 (1972).
(2) M. Nakano, T. Noguchi, K. Sugioka, H. Fukuyama, M. Sato, Y. Shimizu, Y. Tsuji, and H. Inaba, *J. Biol. Chem.*, **250**, 2404 (1975).
(3) A. U. Khan, *Science*, **168**, 476 (1970).
(4) R. H. Arneson, *Arch. Biochem. Biophys.*, **136**, 352 (1970). Recently Nilsson and Kearns pointed out that dismutation of superoxide anions is not necessarily the generating system of $^1O_2$: R. Nilsson and D. R. Kearns, *J. Phys. Chem.*, **78**, 1681 (1974).
(5) J. M. McCord and I. Fridovich, *J. Biol. Chem.*, **244**, 6047 (1969).
(6) J. A. Howard and K. U. Ingold, *J. Am. Chem. Soc.*, **90**, 1056 (1968).
(7) H. R. Williams and H. S. Mosher, *J. Am. Chem. Soc.*, **76**, 2978 (1954).
(8) Linoleic acid hydroperoxide believed to be mostly in the form of C_{13}-OOH was prepared by enzymatic hydroperoxidation of linoleic acid (100 mg) in 100 ml of 0.04 M NH_4OH–NH_4Cl buffer (pH 9.0) with 15 mg of soya-bean lipoxidase (Type 1, Sigma Chemical Co.) at 0° under O_2 flushing and purified by thin layer chromatography (Silica Gel G) in *n*-hexane–ether–acetic acid (100:100:1); G. J. Garssen, J. E. G. Vliegenthart, and J. Boldingh, *Biochem. J.*, **122**, 327 (1972). The hydroperoxide concentration was determined spectrophotometrically by using ϵ_{234}, 25 000 l. mol^{-1} cm^{-1}; A. E. Johnston, K. T. Zilch, E. Selke, and H. J. Dutton, *J. Am. Oil Chem. Soc.*, **38**, 367 (1961).
(9) H. Inaba, Y. Shimizu, and Y. Tsuji, *Jpn. J. Appl. Phys., Suppl.*, **14**, 23 (1975).
(10) The chemiluminescence in the reaction yields three characteristic peaks around 580–590, 630–640, and 670–680 nm[11] which are typical for all the spectra and independent of alkaline metal (K, Li, or Na). The relative light intensities of these peaks are in the range between 0.7 and 1.0; U. Sander and J. Stauff, *An. Soc. Quim. Argent.*, **59**, 149 (1971).
(11) The authors assign the peak around 670–680 nm to be $(^1\Delta_g)(^1\Delta_g)(0,0) \rightarrow 2(^3\Sigma_g)(1,0)$ which is far from the properly identified peaks in NaOCl–H_2O_2 system at 633 and 703 nm.
(12) The chemiluminescence in this system yields two principal bands at 633 and 703 nm, as well as two other faint bands (at 478 and 578 nm) and an extremely weak band at 670 nm; H. H. Seliger, *J. Chem. Phys.*, **40**, 3133 (1964); A. U. Khan and M. Kasha, *J. Am. Chem. Soc.*, **92**, 3293 (1970).
(13) A. U. Khan and M. Kasha, *J. Am. Chem. Soc.*, **92**, 3293 (1970).
(14) The organic materials undergoing autoxidation emit very weakly with a maximum emission generally in the range between 420 and 450 nm. The emitter of this bluish luminescence appears to be an electrically excited carbonyl compound ($n\pi^*$); R. F. Vasilev, *Prog. React. Kinet.*, **4**, 305 (1967).
(15) Both intensity and lifetime of chemiluminescence in a NaOCl–H_2O_2 aqueous system have been reported to be enhanced nearly ten times by solvent deuterium effect; T. Kajiwara and D. R. Kearns, J. *Am. Chem. Soc.*, **95**, 5886 (1973).
(16) Y. Shimizu, H. Inaba, K. Kumaki, K. Mizuno, S. Hata, and S. Tomioka, *IEEE Trans. Instrum. Meas.*, **Im-22**, 153 (1973).
(17) R. C. Allen, S. J. Yevich, R. W. Orth, and R. H. Steele, *Biochem. Biophys. Res. Commun.*, **60**, 909 (1974).

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Received September 2, 1975

Rearrangement of Optically Active Ethyl (*E*)-3-Methyl-3-phenylglycidate. Evidence for Concerted Carbethoxy Migration

Sir:

The mechanism of the acid-catalyzed rearrangement of acyl groups to adjacent positive centers still remains a subject of controversy.[1] The 1,2-migration of the carbonyl group in α,β-epoxy carbonyl compounds has received particular attention in both mechanistic[1] and synthetic[1b,2] studies (1). Carbonyl migration, catalyzed by boron trifluoride, has been observed with epoxy ketones,[3] esters,[4] and thiol esters.[5] The reaction has been established to be intramolecular by "crossed product" studies.[6] House has also