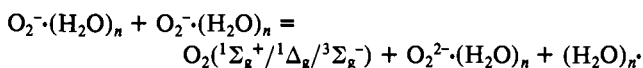# Direct Spectral Evidence of the Generation of Singlet Molecular Oxygen ($^1\Delta_g$) in the Reaction of Potassium Superoxide with Water[†]

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The chemistry of molecular oxygen has experienced a tremendous resurgence of interest in the last 15 years due to evidence of facile generation and ubiquitous presence of two species of activated oxygen, singlet molecular oxygen and superoxide anion, in chemical and biological systems, and for the last 10 years we have been trying to prove that these two activated oxygen species are interconvertible under appropriate conditions.[1,2] In 1970 we suggested that singlet oxygen is generated in electron-transfer reactions of O_2^-, on the basis of the sensitized luminescence of a number of chromophores when water is added to a solution of potassium superoxide in dry Me_2SO and the formation of trace amounts of peroxidic product when the singlet oxygen scavenger 2,5-dimethylfuran is added to the KO_2 water reaction.[2a] The use of chemiluminescence and chemical scavenging as general techniques for demonstrating the presence of singlet oxygen is being subject to increasing criticism in the literature as it is now apparent that singlet oxygen is only one of several small oxygen-containing species which may be involved in these reactions; e.g., O_2^-, OOH, OH·.[1e] Several laboratories have pursued the problem of conversion of superoxide to singlet oxygen with, however, only negative,[3] partially positive,[4] or inconclusive[5] results. We report here the use of a recently developed highly sensitive spectrometer in the 1.0–1.6-μm region to obtain a $O_2(^1\Delta_g)$ emission spectrum from the reaction of water with KO_2 particles suspended in CCl_4, providing incontrovertible evidence of the generation of singlet oxygen in the reaction

$$O_2^-\cdot(H_2O)_n + O_2^-\cdot(H_2O)_n =$$
$$O_2(^1\Sigma_g^+/^1\Delta_g/^3\Sigma_g^-) + O_2^{2-}\cdot(H_2O)_n + (H_2O)_n\cdot$$

(1) The following volumes are devoted to the current phase of molecular oxygen research: (a) "Oxidation of Organic Compounds, Volume III. Ozone Chemistry, Photo and Singlet Oxygen and Biochemical Oxidations": R. F. Gould, Ed., *Adv. Chem. Ser.*, No. 77 (1969). (b) "International Conference on Singlet Molecular Oxygen and Its Role in Environmental Sciences": A. M. Trozzolo, Ed., *Ann. N.Y. Acad. Sci.*, **171**, article 1 (1970). (c) "Singlet Molecular Oxygen": Proceedings of a Symposium February 10–13, 1975, Bhaba Atomic Research Centre, Bombay, M. S. Chada, Ed., INSDOC Delhi, India, 1976. (d) "Singlet Molecular Oxygen, Benchmark Papers in Organic Chemistry", Vol. 5, A. P. Schaap, Ed., Dowden, Hutchinson and Ross, Stroudsburg, PA, 1976. (e) "Singlet Oxygen and Related Species in Chemistry and Biology": Ajit Singh and Abram Petkau, Guest Eds., *Photochem. Photobiol.*, **28**, No. 4/5 (1978). (f) A. M. Michelson, J. M. McCord, and I. Fridovich, Eds., "Superoxide and Superoxide Dismutases", Academic Press, New York, 1977. (g) B. Ranby and J. F. Rabek, Eds., "Singlet Oxygen, Reactions with Organic Compounds and Polymers", Wiley, New York, 1978. (h) "Oxygen Free Radicals and Tissue Damage", Ciba Foundation Symposium 65 (New Series), Excerpta Medica, Amsterdam, 1979. (i) H. H. Wasserman and R. W. Murray, Eds., "Singlet Oxygen", Academic Press, New York, 1979.
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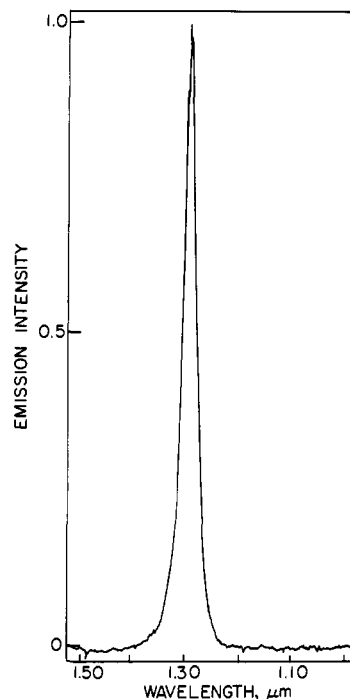**Figure 1.** Chemiluminescence spectrum in the 1.0–1.5-μm region of the reaction of 8% H_2O_2 and 7% NaOCl aqueous solution at room temperature. The emission peak at 1.29 μm is the (0,0) $^1\Delta_g \rightarrow ^3\Sigma_g^-$ transition of molecular oxygen shifted from the true position at 1.268 μm due to the combined monochromator–detector spectral sensitivity.
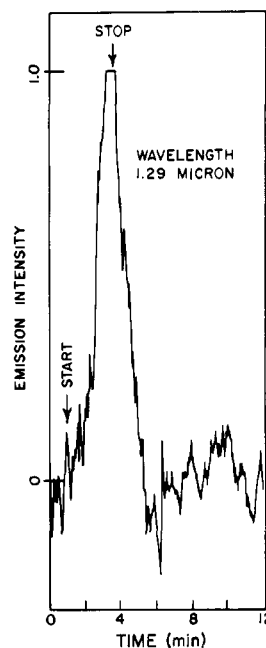

Figure 2. Time vs. emission intensity at 1.29 μm from the reaction of finely divided suspension of KO_2 in CCl_4 with water.

The detector is a liquid-nitrogen-cooled photoconductive intrinsic germanium diode, Model 403 L infrared spectrometer, Applied Detector Corporation (Fresno, CA), signal amplified through a FET preamplifier cooled in liquid nitrogen. Further signal amplification is through a PAR CR-4 low-noise amplifier. The amplified signal is fed into a PAR Model 160 boxcar integrator in an experimental setup similar to the one in ref 6.

In these experiments, finely divided particles of KO_2 (Alfa Products, Danvers, MA), highly insoluble in CCl_4 (Analytical

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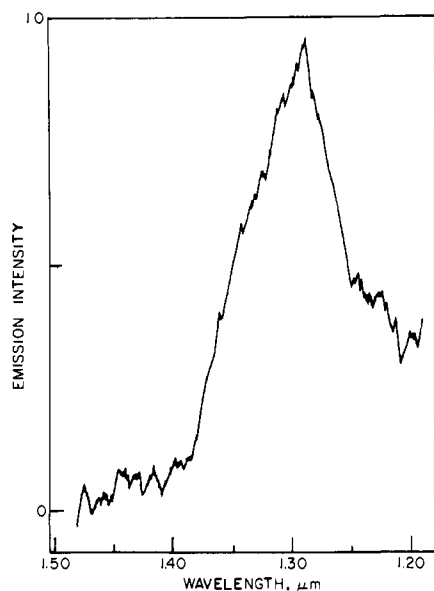
Figure 3. Emission spectrum of (0,0) $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ of molecular oxygen originating from the reaction of finely divided suspension of KO_2 in CCl_4 with water. The apparent peak position at 1.284 μm is a shift from the true position of 1.268 μm due to the combined monochromator–detector spectral sensitivity.

Reagent, Mallinckrodt, Paris, KY), were suspended by constant stirring. The reaction cell was designed such that the suspension of KO_2 in CCl_4 flows on one side of a fritted disk, the side facing the monochromator. Water flows continuously on the other side of the disk. The whole assembly is placed inside a closed wooden box lined with black paper, and the experiment takes place in a darkened room.

Figure 1 is the chemiluminescence spectrum of the H_2O_2/OCl^- reaction. The emission peak at 1.29 μm is the (0,0) $^1\Delta_g \rightarrow {}^3\Sigma_g$ transition, shifted from the true position of 1.268 μm due to the combined monochromator–detector spectral sensitivity. Figure 1 depicts the wavelength calibrator for the present experimental setup.

Figure 2 is a recording of time vs. intensity of emission at 1.29 μm. The emission appears when the suspended KO_2 particles in CCl_4 begins to flow over the wet fritted disk. The electronics were set at the shortest possible time constant with poor but acceptable signal-to-noise ratio so that the rise and fall of the emission with the start and stop of the CCl_4 suspension of KO_2 over the wet disk is clearly displayed.

Figure 3 is a spectrum of the emission scanned from 1.40 to 1.20 μm showing a peak at 1.284 μm, corresponding to the O_2 (0,0) transition $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ in this experimental setup. The heterogeneity of the reaction presents difficulties in maintaining steady-state reaction conditions. A higher integration time constant for the electronics was used to improve the signal-to-noise ratio in obtaining this spectrum than in the time–intensity curve.

In a previous theoretical appraisal of the disproportionation reaction of the superoxide anion, several factors became evident:[2c] (i) The reaction is thermodynamically favored both by free energy and by entropy considerations. (ii) The nature of the product, i.e., whether the molecular oxygen is electronically excited or in the ground state depends largely on the dielectric of the solvent medium. (iii) However, as the yield of excited product increases with increasing solvent dielectric constant, the generation of the short-lived and easily quenched $^1\Sigma_g^+$ singlet oxygen becomes predominant. (iv) Moreover, superoxide anion quenching of $^1O_2$ by electron-transfer reaction is four orders of magnitude more efficient than solvent quenching for O_2 ($^1\Delta_g$). (v) Furthermore, unlike solvent quenching of O_2 ($^1\Sigma_g^+$) which is theoretically predicted to proceed to O_2 ($^1\Delta_g$) excited state,[7] electron-transfer

quenching by O_2^- is directly to the ground state. We plan to extend these investigations to other electron-transfer reactions of the superoxide anion, particularly those involving metal ions, and to evaluate the efficiency of singlet oxygen generation in these electron-transfer reactions.

A large number of reports have appeared postulating the involvement of singlet oxygen and/or superoxide anion in biological processes, such as the biosynthesis of prostaglandins,[8] microsomal lipid oxidase reactions,[9,10] xanthine oxidase[11-13] and phagocytosing polymorphonuclear leukocytes,[14] and the fertilization of sea urchin eggs.[15] These reports are currently based on indirect evidence of chemical or luminescence studies. We believe that with the new spectrometer and improved data-processing accessories it will be possible to provide unequivocal evidence of singlet oxygen in such systems by monitoring directly the singlet oxygen ($^1\Delta_g$) emission.

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Structures of Eleven Zervamicin and Two Emerimicin Peptide Antibiotics Studied by Fast Atom Bombardment Mass Spectrometry[1,2]

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We have recently revised the previously assigned[3] structures of alamethicins I and II to **1** and **2**[4] and have assigned the structures of the peptaibophol antibiotics emerimicins III and IV[5] and antiamoebins I[6] and II[7] as **3–6**, respectively. These structures

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(2) Paper 7 in the series Peptaibophol Antibiotics. Paper 6 ref 9.

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