π-overlap in the anion. Another is the electron-withdrawing effect of the benzene ring compared to the methyl groups in AA. That this electronic effect must play a significant role in determining the pK_a values is seen by the fact that the pK_a *difference* between ID and AA becomes larger with increasing Me_2SO content, showing the decrease in the attenuation of the electronic effect on the anion in the less solvating medium.

If coplanarity were the reason for the higher k_0 with ID, one would not expect the k_0^{ID}/k_0^{AA} ratio to depend on the solvent. If the electronic effect were the *direct* cause of the difference in k_0, the k_0^{ID}/k_0^{AA} ratio should become larger rather than smaller in the Me_2SO-rich solvents, due to the reduced attenuation of the electronic effect.

(3) A more satisfactory way to explain the enhanced k_0 for ID is that it is related to the electron-withdrawing effect in an *indirect* way. That is, the electronic effect can be viewed as a stabilization of the developing negative charge which in the transition state is to a significant extent localized on carbon. In other words, it is the retarded delocalization of the negative charge into the carbonyl groups (imbalanced transition state) that leads to the substituent effect on k_0. According to this view, the substituent effect on k_0 is a *response* to the imbalance rather than a primary *cause*, as discussed in more detail elsewhere.[24]

The usual manifestation of this imbalance is that the Brønsted α value obtained by varying the pK_a of the carbon acid is larger than the β value obtained by varying pK_a^{BH}. The substituent dependence of k_0 is just a different manifestation of the same phenomenon.[24,28]

That the Brønsted α value indeed exceeds β in our system is seen by defining α as $\log [k_1^B(ID)/k_1^B(AA)]/(pK_a^{AA} - pK_a^{ID})$ for a given base B. For example, with piperidine one obtains $\alpha = 0.55$ in 50% Me_2SO and $\alpha = 0.56$ in 90% Me_2SO. Hence the imbalances are $\alpha - \beta$ = 0.55 − 0.41[29] = 0.14 in 50% and $\alpha - \beta$ = 0.56–0.49[29] = 0.07 in 90% Me_2SO. Note that the reduction in the imbalance in the Me_2SO-rich solvent of course parallels the decrease in the k_0^{ID}/k_0^{AA} ratio.

Conclusions. (1) The intrinsic rate constant for ionization of ID by carboxylate ions increases strongly upon addition of Me_2SO to the solvent. This increase can be attributed to the combination of two effects: one is the late solvation of the carbanion, the other the early desolvation of the carboxylate ion. These effects can be described by eq 16.

(2) The increase in k_0 for the reaction with amines is much smaller because the rate-enhancing contribution of the carboxylate ions is replaced by a rate-*retarding* effect which arises from the late solvation of the protonated amine. This is described by eq 21.

(3) The Brønsted β values increase with Me_2SO content of the solvent. At least part of the reason for this increase may be the larger solvent effect on the stability of the carboxylate ions with increasing pK_a^{COOH} (larger $\log \gamma_{COO^-}$) and a larger solvent effect on the stability of the protonated amines with decreasing $pK_a^{NH^+}$ (more negative $\log \gamma_{NH^+}$), respectively.

(4) With the carboxylate ions, the increase in $\log \gamma_{COO^-}$ levels off at high pK_a^{COOH} which is the probable cause for the downward curvature of the Brønsted plots in the Me_2SO-rich solvents.

(5) The value of $\log \gamma_{OH^-}$ is disproportionately small relative to $pK_a^{H_2O}$ when compared with $\log \gamma_{COO^-}$. This is the probable reason why the OH^- point deviates more negatively from the Brønsted line defined by the carboxylate ions when the Me_2SO content of the solvent increases. In a similar way, the increasingly less negative deviation of the water point from a Brønsted line defined by the amines can be arttributed to a disproportionately small $-(\log\gamma_{H^+})$ value (relative to $pK_a^{H_3O^+}$).

(6) The fact that the identity of the deviating points from the Brønsted plots defined by the primary amines changes with solvent may be attributed to differences in $\log \gamma_{NH^+}$ for certain amines.

(7) The larger k_0 value for the ID compared to the AA system is a consequence of an exalted substituent effect on the transition state which is caused by the lag of charge delocalization behind proton transfer. Another manifestation of the same phenomenon is that $\alpha > \beta$ ("imbalance").

Experimental Section

Materials. 1,3-Indandione (Aldrich) was recrystallized from ethanol, mp 131–132 °C (130 °C).[30] The 1,3-indandione anion (ID^-) has a λ_{max} = 421 nm (ϵ 2190) in 10% Me_2SO and of 414 ± 1 nm (ϵ 2400 ± 100) in the other solvent mixtures used in this study. The purification, where needed, of the amines and the carboxylic acids has been described in a previous paper.[1] Phenol was recrystallized from petroleum ether, 3,5-dimethylphenol and 4-chlorophenol from hexanes, and 4-cyanophenol from water, while 3-chloro- and 3,5-dichlorophenol were used without further purification. Me_2SO was stored over 4-Å molecular sieves prior to use.

Kinetic Experiments and pK_a Determinations. The methods used were basically the same as described before.[1] Due to the relatively low extinction coefficient of ID^- at the wavelength used for the kinetic measurements (415–420 nm), the excess of the buffer over the ID or ID^- concentration was sometimes less than 10-fold. When this was the case, a small correction was applied in the calculation of k_{obsd}.

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Singlet Oxygen Production in Superoxide Ion–Halocarbon Systems

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Abstract: A search for singlet oxygen chemiluminescence at 1268 nm was made in a number of reactions of superoxide ion. Carbon tetrachloride and carbon tetrabromide reacted with suspended potassium superoxide to produce 1268-nm emission consistent with singlet oxygen. Chloroform was less reactive but produced 1268-nm emission when the concentration of superoxide ion in the halocarbon phase was increased with 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6). The dismutation of superoxide ion in deuterium oxide was not accompanied by 1268-nm chemiluminescence. Less than 0.02 mol of singlet oxygen was produced per mole of superoxide ion between p^2H 6 and 9.

The suggestion of Khan that some electron-transfer reactions of superoxide ion (O_2^-) produce singlet oxygen ($^1O_2$)[1] has stimulated many investigators to search for $^1O_2$ as a product of the spontaneous dismutation of O_2^- in H_2O. Most studies using chemical traps have failed to demonstrate ${}^1O_2$, particularly when stringent criteria for the identification of ${}^1O_2$ were employed, and no investigator has been able to detect the characteristic dimole emission of ${}^1O_2$.[2-4] Khan, however, has reported the presence of monomole ${}^1O_2$ emission in a system in which a suspension of KO_2 in CCl_4 was made to flow across a wet fritted disk.[5] This study deserves consideration because the method used has been shown to have high sensitivity and high specificity for singlet oxygen even in complicated biochemical systems.[6] Unfortunately, there is an ambiguity in Khan's study.[3] While Khan believed that the CCl_4 functioned as an inert, low dielectric solvent which allowed low concentrations of O_2^- and H_2O to react,[7] Arudi et al. have proposed that the observed ${}^1O_2$ was the result of a reaction sequence initiated by a reaction between O_2^- and CCl_4.[3]

In view of these unresolved questions, I undertook studies designed to (1) identify monomole ${}^1O_2$ emission from enzymatically generated O_2^- in H_2O and (2) to clarify the mechanism of infrared emission in O_2^-–halocarbon–H_2O systems.

Experimental Section

Chemiluminescence Spectrometer. The infrared chemiluminescence spectrometer used and the method of calibration of ${}^1O_2$ yields using the H_2O_2 + HOCl reaction have been described previously.[6,8] Spectral analysis was done by using a series of interference filters.[8]

Chemicals and Reagents. Cytochrome C, Type VI, β-carotene, Type I, deuterium oxide (${}^2H_2O$), 99.8%, and KO_2 were obtained from Sigma Chemical Co. Xanthine oxidase was purchased from Boehringer Mannheim and dialyzed for 24 h against two changes of pH 7.0, 25 mM sodium phosphate buffer containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). It was then dialyzed an additional 2 h against an equivalent buffer made with ${}^2H_2O$. The xanthine oxidase had an activity of 0.7 units/mg.[9] Acetaldehyde was a product of the Eastman Kodak Co. and was distilled on the day of use. Carbon tetrabromide and 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6), 99.5%, were obtained from Aldrich Chemical Co. Reagent grade carbon tetrachloride was a product of Mallinckrodt Co. Chloroform, HPLC grade, was obtained from Fisher Scientific Co. and contained 0.5% ethanol. Other inorganic chemicals, EDTA and ethanol were reagent grade. Oxygen, 99.6%, was obtained from Matheson Gas Products. Water was glass distilled. For buffers made with ${}^2H_2O$, the apparent pH, measured with a glass electrode, was adjusted to a value 0.4 units higher than the desired p^2H.[10]

Xanthine Oxidase System. The ${}^2H_2O$ buffers used were first saturated with oxygen at 25 °C. Xanthine oxidase in 1.5 mL of buffer was placed in the chemiluminescence spectrometer. The reaction was then initiated by the rapid injection of an additional 1.5 mL of buffer containing acetaldehyde. The light emission at 1268 nm was then integrated for the next minute. Superoxide production in this system was measured in a separate experiment by adding cytochrome C, 2 mg/mL, and measuring the increase in optical density at 550 nm using an absorbance coefficient of 21.1 $mM^{-1}\ cm^{-1}$.[11]

Table I. Superoxide Ion and Singlet Oxygen Production in the Xanthine Oxidase–Acetaldehyde System[a]

	p^2H			
	6	7	8	9
O_2^-/min, μM[b]	42 ± 6	134 ± 7	109 ± 4	127 ± 2
$[O_2^-]$, M[c]	6.5×10^{-7}	3.7×10^{-6}	1.1×10^{-5}	3.6×10^{-5}
I/I_0[d]	0.94	0.75	0.51	0.24
${}^1O_2$/min, μM[e]	−0.3 ± 0.1	−0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.2

[a] 0.2 mg/mL of xanthine oxidase; 50 mM acetaldehyde; 50 mM buffers, p^2H 6–8, sodium phosphate, p^2H 9, sodium borate, deuterium oxide solvent. [b] Measured by cytochrome C reduction at 550 nm. [c] Estimated steady-state concentration of O_2^-. [d] Ratio of 1268-nm emission expected in the presence of the steady-state concentration of O_2^- to that expected without quenching by O_2^-. [e] Calculated from 1268-nm emission and corrected for quenching by O_2^-.

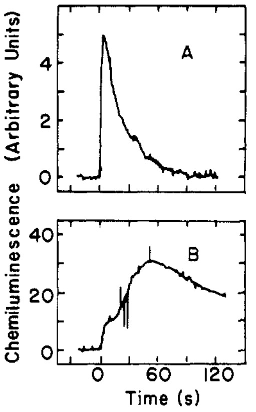

Figure 1. Chemiluminescence at 1268 nm from the reaction of KO_2 with halocarbons. (A) CCl_4, 2 mL, injected into 50 mg of finely ground KO_2. (B) $CHCl_3$, 2 mL, with 10 mM 18-crown-6 injected into 5 mg of finely ground KO_2. Both systems were mixed by a flow of O_2 bubbled through the halocarbon–KO_2 suspension.

Potassium Superoxide–Halocarbon Systems. For experiments without 18-crown-6, 50 mg of finely ground KO_2 was placed in the bottom of a test tube in the chemiluminescence spectrometer. The reaction was initiated by the injection of 2 mL of halocarbon. A small flow of O_2 via a 16-gauge Teflon tube continuously agitated the KO_2 particles. The light emission was integrated for 2 min. Experiments containing 18-crown-6 were done in a similar manner except only 5 mg of KO_2 was used.

Potassium Superoxide–Carbon Tetrachloride–Deuterium Oxide System. Finely ground KO_2, 50 mg, was placed in a 12 × 75 mm test tube and placed in the spectrometer. A 5 mm diameter glass tube ending in an 8 mm long fritted cylinder was dipped in ${}^2H_2O$ and placed above the KO_2. The reaction was then initiated by injecting 2 mL of CCl_4 into the test tube. A flow of oxygen through a 16-gauge Teflon tube formed small bubbles carrying the particles of KO_2 to contact the wet fritted cylinder. The light emission was then integrated for the next 2 min. Control experiments were done in which a dry fritted cylinder was used.

Data Analysis. Each experiment was done 3 times and was reported as the mean plus or minus the standard error.

Results and Discussion

Xanthine Oxidase System. As shown in Table I, the dismutation of superoxide ion in ${}^2H_2O$ did not produce 1268-nm chemiluminescence in amounts detectable with the current instrumentation. Any singlet oxygen produced did not exceed 0.02 mol per mole of superoxide ion. This result was consistent with most prior studies.[2-4] The failure to detect ${}^1O_2$ was not due to the quenching of ${}^1O_2$ by O_2^-. Table I shows the estimated concentration of O_2^- at each p^2H and the factor by which the concentration of O_2^- will reduce the ${}^1O_2$ emission. In these calculations, the steady-state assumption was made for O_2^-, and a ${}^1O_2$ half-life in ${}^2H_2O$ of 56 μs was used.[12] Values of 4.9 for the pK of ${}^2HO_2\cdot$, 2.06×10^7 $M^{-1}\ s^{-1}$ for $K_{{}^2HO_2+O_2^-}$, and 1.6×10^9 $M^{-1}\ s^{-1}$ for the quenching of ${}^1O_2$ by O_2^- were used.[13,14]

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Table II. Spectral Analysis of Near-Infrared Emission in Potassium Superoxide–Halocarbon Systems

filter, nm[a]	H_2O_2 + HOCl[b]	KO_2–CCl_4[c]	KO_2–CCl_4–CBr_4[d]	KO_2–$CHCl_3$–(18-crown-6)[e]
1070	0.000 ± 0.002	0.06 ± 0.02	−0.01 ± 0.01	0.0001 ± 0.0005
1170	0.003 ± 0.003	0.00 ± 0.03	0.007 ± 0.004	0.001 ± 0.0002
1268	1.00 ± 0.02	1.0 ± 0.16	1.0 ± 0.1	1.0 ± 0.02
1375	0.59 ± 0.01	0.28 ± 0.02	0.44 ± 0.11	0.32 ± 0.03
1475	0.14 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.04 ± 0.004
1580	0.03 ± 0.004	0.03 ± 0.02	0.04 ± 0.01	0.02 ± 0.001
1680	0.014 ± 0.002	−0.1 ± 0.03	0.026 ± 0.005	0.001 ± 0.002

[a] The emission intensity in each system is normalized so that the peak emission is given a value of 1.0. [b] ^2H_2O, p^2H 5, 100 mM sodium acetate, 100 mM NaCl, 0.5 mM H_2O_2, and 0.5 mM HOCl. [c] 50 mg of KO_2 in 2 mL of CCl_4. [d] 50 mg of KO_2 in 2 mL of CCl_4 with 90 mM CBr_4. [e] 5 mg of KO_2 in 2 mL of $CHCl_3$ with 10 mM 18-crown-6.

Table III. Effect of Halocarbon Reactant and β-Carotene on the 1268-nm Chemiluminescence in Potassium Superoxide–Halocarbon Systems

system	rel emission at 1268 nm[a]
control, no additions[a]	1.0 ± 0.05
control + 10 μM β-carotene	0.06 ± 0.01
control + 90 mM CBr_4	5 ± 1
$CHCl_3$ replacing CCl_4	0.04 ± 0.02

[a] Emission from the KO_2–CCl_4 system, a suspension of 50 mg of KO_2 in 2 mL of CCl_4 agitated with a flow of O_2, is given a value of 1.0.

Table IV. Effect of β-Carotene and Benzoyl Peroxide on the 1268-nm Chemiluminescence in the Potassium Superoxide–(18-Crown-6)–Chloroform System

system	rel emission
control, no additions[a]	1.0 ± 0.08
control + 1 mM β-carotene	0.12 ± 0.04
control + 30 mM benzoyl peroxide	15 ± 2

[a] 2 mL of $CHCl_3$ with 10 mM 18-crown-6 injected into 5 mg of finely ground O_2. The suspension is agitated with a flow of O_2.

The data of Nagano and Fridovich using a similar xanthine oxidase system also suggested that the quenching by O_2^- was not a major factor at p^2H 8.1.[4] The limits for $^1O_2$ production shown in Table I include the correction for quenching by O_2^-.

Potassium Superoxide–Halocarbon Systems. The suggestion of Arudi et al. that the $^1O_2$ seen by Khan was the result of a reaction between O_2^- and CCl_4 as well as the work of Roberts and Sawyer showing a high reactivity of O_2^- with CCl_4 and $CHCl_3$ prompted me to look for $^1O_2$ in KO_2–halocarbon systems.[3,15] A suspension of KO_2 in CCl_4 agitated by a flow of O_2 produced near-infrared emission. The spectral distribution of the chemiluminescence shown in Table II as well as the quenching of the emission by β-carotene shown in Table III was consistent with $^1O_2$. The quenching by β-carotene has to be regarded as weak evidence in favor of $^1O_2$, since β-carotene as well as a number of other $^1O_2$ traps react rapidly with haloperoxy radicals which are likely intermediates in this system.[16]

The presence of O_2^- in the system, an efficient quencher of $^1O_2$, makes a quantitative estimate of the $^1O_2$ yield difficult, but the amount of $^1O_2$ is likely to be very small. Possible mechanisms for the formation of $^1O_2$ include both electron-transfer reactions, such as reaction 3, and the recombination of $CCl_3O_2\cdot$ via a process analagous to the Russell mechanism, as shown in reaction 2.[17]

$$O_2^-\cdot + CCl_4 \rightarrow CCl_3O_2\cdot + Cl^- \quad (1)$$

$$2CCl_3O_2\cdot \rightarrow {}^1O_2 + \text{products} \quad (2)$$

$$O_2^-\cdot + CCl_3O_2\cdot \rightarrow CCl_3O_2^- + {}^1O_2 \quad (3)$$

The addition to the system of the brominated halocarbon, CBr_4, significantly increased the intensity of the emission. This is correlated with the increased reactivity of brominated halocarbons with O_2^- relative to chlorinated halocarbons.[18] No 1268-nm emission was seen when $CHCl_3$ replaced CCl_4. However, when the concentration of O_2^- in $CHCl_3$ was increased with 18-crown-6, strong chemiluminescence at 1268 nm was generated. The very high concentration of O_2^- explains the poor quenching effect of β-carotene in this system. Danen and Arudi have presented evidence for the efficient production of $^1O_2$ when KO_2 reacts with diacyl peroxides in benzene.[19] When benzoyl peroxide was added to the KO_2–(18-crown-6)–$CHCl_3$ system, the infrared emission intensity increased by a factor of 15.

The assignment of $^1O_2$ as the infrared emitting species rested principally on the spectral distribution. The evidence from studies with added β-carotene was consistent with a competition between O_2^- and β-carotene for the quenching of $^1O_2$, but as discussed earlier, other explanations are possible. While emission from $^1O_2$ can rationalize all of the experimental results, the data are not sufficient to completely rule out other sources of emission. Past studies in this laboratory have used the deuterium isotope effect on the half-life of $^1O_2$ to provide additional evidence for the assignment of near-infrared emission to $^1O_2$.[6] Unfortunately, this cannot be done for the systems in the current study.

Potassium Superoxide–Carbon Tetrachloride–Deuterium Oxide System. I have been unable to demonstrate 1268-nm emission that can be attributed to the reaction of KO_2 and ^2H_2O. The relative chemiluminescence in a KO_2–CCl_4 system containing a wet fritted cylinder was 1.1 ± 0.2 when compared to a similar KO_2–CCl_4 system containing a dry fritted cylinder. Thus, the emission previously observed by Khan must have been due to the reaction of KO_2 and CCl_4.

Conclusions

The halocarbons, CBr_4, $CHCl_3$, and CCl_4 were shown to react with KO_2 to produce near-infrared emission consistent with $^1O_2$. The dismutation of O_2^- in ^2H_2O was not accompanied by 1268-nm emission, demonstrating that less than 0.02 mol of $^1O_2$ was produced per mole of O_2^-.

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Registry No. O_2, 7782-44-7; $O_2^{\cdot -}$, 11062-77-4; KO_2, 12030-88-5; CCl_4, 56-23-5; CBr_4, 558-13-4; $CHCl_3$, 67-66-3; ^2H_2O, 7789-20-0; 18-crown-6, 17455-13-9.

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