(30) (a) An equilibrium constant for the addition of hydrogen cyanide to acetophenone in 96% ethanol at 70 °C is estimated[30b] to be $\sim 10^{-1}$ M^{-1}. The equilibrium constant for the addition of aliphatic amines to aldehydes in water at 20–25 °C is $\sim 10^{-3}$ smaller than that observed for hydrogen cyanide and is independent of amine basicity.[28] Since the ratio of equilibrium constants for the addition of different nucleophiles to aldehydes is independent of electron-withdrawing groups[28] on the aldehyde, the ratio probably applies to acetophenone as well. To the extent that solvent and temperature effects on the ratio are small, the equilibrium constant for the addition of amines to acetophenone to give the neutral carbinolamine will be near 10^{-4} M^{-1}. Whereas the change in solvent might raise the estimated equilibrium constant, the increased steric requirements[30c] and resonance effect[28] would be expected to lower it. (b) Lapworth, A.; Manske, R. *J. Chem. Soc.* **1930,** 1976. Evans, D. P.; Young, J. R. *Ibid.* **1954,** 1310. (c) Prelog, V.; Kobelt, M. *Helv. Chim. Acta* **1949,** 32, 1187.
(31) Miller, D. L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1966,** 1507, 1511, 1514. Miller, D. L.; Ukena, T. *Ibid.* **1969,** *91,* 3050.
(32) DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961,** *83,* 4400. Benkovic, S. J.; Schray, K. J. *Biochemistry* **1968,** *7,* 4090. Haake, P.; Allen, G. *Proc. Natl. Acad. Sci. U.S.A.* **1971,** *68,* 2691; *J. Am. Chem. Soc.* **1973,** *95,* 8080; *Ibid.* **1976,** *98,* 4990.
(33) Kirby, A. J.; Warren, S. G. "The Organic Chemistry of Phosphorus"; Elservier: Amsterdam, 1967; p 281 ff. Bruice, T. C.; Benkovic, S. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; Vol. 2, pp 22–25, 157–159. Benkovic, S. J.; Schray, K. J., *Enzymes* **1973,** *8,* 201. Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969; pp 81–83, 112–115, 151, 160–161, 608. Butcher, W. W.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955,** *77,* 2420. Barnard, P. W. C.; Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Silver, B. L.; Vernon, C. A. *Chem. Ind. (London)* **1955,** 760. Todd, A. R. *Proc. Natl. Acad. Sci.* U.S.A. **1959,** *45,* 1389. Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967,** *89,* 415. Haake, P.; Ossip, P. S. *Ibid.* **1971,** *93,* 6924. Gorenstein, D. G. *Ibid.* **1972,** *94,* 2523. Kluger, R. *J. Org. Chem.* **1973,** *38,* 2721.
(34) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1964,** *86,* 1410. Traylor, P. S.; Westheimer, F. H. *Ibid.* **1965,** *87,* 553. Kirby, A. J.; Jencks, W. P. *Ibid.* **1965,** *87,* 3209. Benkovic, S. J.; Benkovic, P. A. *Ibid.* **1967,** *89,* 4714. Kirby, A. J.; Varvoglis, G. A. *J. Chem. Soc. B.* **1968,** 135. Guthrie, J. P. *J. Am. Chem. Soc.* **1977,** *99,* 3991.
(35) Mildvan, A. *Adv. Enzymol.* **1979,** *49,* 103.
(36) Rose, I. A. *J. Biol. Chem.* **1960,** *235,* 1170. Robinson, J. L.; Rose, I. A. *Ibid.* **1972,** *247,* 1096. Kuo, D. J.; Rose, I. A. *J. Am. Chem. Soc.* **1978,** *100,* 6288. Kuo, D. J.; O'Connell, E. L.; Rose, I. A. *Ibid.* **1979,** *101,* 5025.
(37) Lowe, G.; Sproat, B. S. *J. Chem. Soc., Chem. Comm.* **1978,** 783; *J. Chem. Soc., Perkin Trans. 1* **1978,** 1622.
(38) Rose, I. A. *Adv. Enzymol.* **1979,** *50,* 361. See addendum.
(39) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1978,** *100,* 2558. Blättler, W. A.; Knowles, J. R. *Ibid.* **1979,** *101,* 510; *Biochemistry* **1979,** *18,* 3927.
(40) Warren, W. A. *Biochim. Biophys. Acta* **1968,** *156,* 340. Horiguchi, M.; Rosenberg, H. *Ibid.* **1975,** *404,* 333.
(41) Westheimer, F. H. *Acc. Chem. Res.* **1968,** *1,* 70.
(42) (a) Buchanan, J. *Adv. Enzymol.* **1973,** *39,* 91. Greenberg, D. M.; Wynston, L. K.; Nagabhushanam, A. *Biochemistry* **1965,** *4,* 1872. (b) Lieberman, I. *J. Biol. Chem.* **1956,** *223,* 327. Koshland, D. E., Jr. *Biochemistry* **1971,** *10,* 3365. Markham, G. D.; Reed, G. H. *J. Biol. Chem.* **1978,** *253,* 6184.
(43) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971,** *68,* 1678.
(44) van de Werf, P.; Orlowski, M.; Meister, A. *Proc. Natl. Acad. Sci. U.S.A.* **1971,** *68,* 2982.
(45) Roberts, J. W.; Roberts, C. W.; Craig, N. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978,** *75,* 4714. Bridges, B. A. *Nature (London)* **1979,** 514.
(46) Herskho, A.; Ciechanover, A.; Rose, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **1979,** *76,* 3107.
(47) De Martino, G. N.; Goldberg, A. L. *J. Biol. Chem.* **1979,** *254,* 3712.
(48) Lipmann, F. *Adv. Enzymol.* **1941,** *1,* 99.

Dioxygen Transfer from 4a-Hydroperoxyflavin Anion. 2. Oxygen Transfer to the 10 Position of 9-Hydroxyphenanthrene Anions and to 3,5-Di-*tert*-butylcatechol Anion

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Abstract: The reaction of the peroxy anion of N^5-ethyl-4a-hydroperoxy-3-methyllumiflavin (4a-$FlEtO_2^-$) with the anions of 3,5-di-*tert*-butylcatechol (VIII), 10-ethoxy-9-phenanthrol (Ia), and 10-methyl-9-phenanthrol (Ib) has been investigated. All products may be accountable through the transfer of O_2 from the 4a-$FlEtO_2^-$ reactant to the phenolate anions with the production of reduced flavin anion ($FlEt^-$) and a hydroperoxycyclohexadienone. From $VIII^-$(*t*-BuOH) there was obtained 3,5-di-*tert*-butyl-*o*-quinone (IX) and Ib^- yielded (*t*-BuOH or CH_2CN) 10-hydroxy-10-methyl-9,10-dihydro-9-phenanthrone (IIIb), while Ia^- provided both 9,10-phenanthrenequinone (V) and monoethyl 1,1′-diphenate (IVa) (the ratio of V:IVa being solvent dependent). The mechanisms for the decomposition of intermediate peroxide anions to products are discussed. The conversion of Ia^- to IVa by oxygen transfer from 4a-$FlEtO_2^-$ amounts to a catalysis by $FlEt^-$ of the reaction of $^3O_2$ with Ia and serves as a biomimetic reaction of flavoenzyme dioxygenase. The kinetics for the reaction of $VIII^-$ with 4a-$FlEtO_2^-$ require the formation of an intermediate. Since the rate constants for the reaction of both $VIII^-$ and 2,6-di-*tert*-butyl-4-methylphenolate anion with 4a-$FlEtO_2^-$ are identical under saturating conditions by these phenolate ions, it is concluded that the intermediate is formed in a unimolecular reaction from 4a-$FlEtO_2^-$ ($k = 0.36$ s^{-1}) as in eq 19. Dissociation of 4a-$FlEtO_2^-$ to $FlEt^-$ + O_2 and reaction of phenolate ions with O_2 may be discounted since the second-order rate constants for the reaction of phenolate ions with O_2 are less than required for the kinetic competency of this process. Dissociation of 4a-$FlEtO_2^-$ to yield neutral flavin radical ($FlEt\cdot$) + $O_2^{-}\cdot$ followed by reduction of $FlEt\cdot$ by phenolate ion to provide $FlEt^-$ and phenoxy radical with the coupling of the latter with $O_2^{-}\cdot$ is also improbable. Thus, though the second-order rate constants for $1e^-$ reduction of $FlEt\cdot$ by the various phenolate species are sufficiently large to allow the kinetic competency of this step, there exists no evidence that $O_2^{-}\cdot$ can couple with any radical species to provide a hydroperoxide. The oxygen-donating intermediate formed from 4a-$FlEtO_2^-$ is suggested to be the 4a,10-dioxetane (XII) or an oxygen molecule more loosely associated with $FlEt^-$. The equilibrium constant for the formation of such an intermediate may be as small as 10^{-5} if the rate of reaction of phenolate ion with this species approaches a diffusion-controlled process.

Of much present concern are the mechanisms by which the mono- and dioxygenase enzymes combine with triplet molecular oxygen to provide species capable of transferring one or both of the oxygen atoms to a substrate molecule. The only non-metal-requiring oxygenases require flavin molecules as cofactors. This laboratory has been engaged in mechanistic studies of the reaction of oxygen and oxygen species with flavins and the problem of oxygen activation by flavins.[1-8] The transfer of molecular oxygen in toto by metallodioxygenase enzymes was first recognized by Hayaishi.[9] The mechanisms of these reactions are at least partially understood and metal-centered biomimetic systems have been explored.[10]

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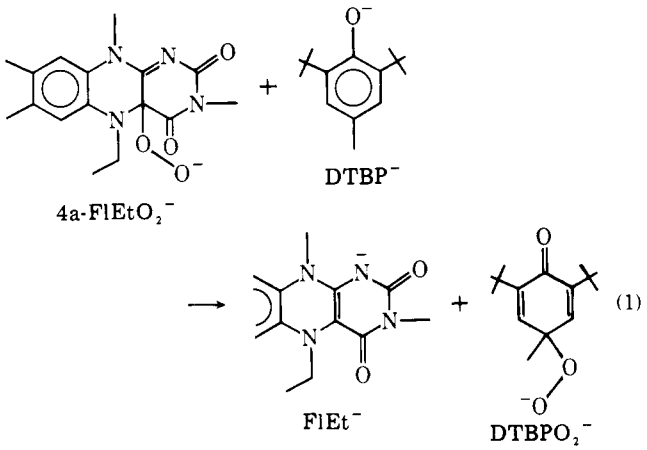
Some understanding of flavin monooxygenases exists. Thus, flavin biomimetic systems for the activation of $^3O_2$ and transfer of a single oxygen atom to a substrate have been reported.[1,2,4,8]

The identity or similarity of the spectrum of 4a-$FlEtO_2H$[2,5] to that of the initial product obtained upon the reaction of molecular oxygen with reduced flavomonooxygenases[11] has established the importance of 4a-hydroperoxyflavins in the mechanisms of these enzymes. It may only be presumed that 4a-hydroperoxyflavins are also involved in flavoenzyme dioxygenase reactions.[12] In a previous publication from this laboratory (part 1 of these studies)[7] there is described the reaction between 3-methyl-5-ethyllumiflavin-4a-peroxy anion (4a-$FlEtO_2^-$) and 2,6-di-*tert*-butyl-4-methylphenolate ion (eq 1). In this reaction (*t*-BuOH solvent) 4a-$FlEt\text{-}O_2^-$ becomes

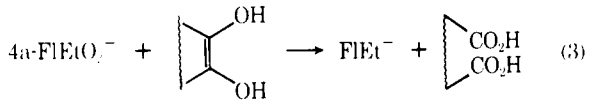


(1)

reduced to the 1,5-dihydroflavin and the phenolate ion is converted into the corresponding dienone hydroperoxide. The reaction is very facile (stopped flow) and the products are obtained in ~95% yield. Since reduced N^5-ethyllumiflavin reacts with molecular oxygen to yield 4a-$FlEtO_2^-$ the reaction of eq 1 represents one-half of the catalytic cycle of eq 2. We

$$\begin{aligned} FlEt_2^- + O_2 &\rightarrow 4a\text{-}FlEtO_2^- \\ 4a\text{-}FlEtO_2^- + DTBP^- &\rightarrow FlEt^- + DTBPO_2^- \end{aligned} \quad (2)$$

report herein extensions of the 4a-$FlEtO_2^-$ dioxygenation reaction to other substrates. It is our purpose both to gain an understanding of the mechanism of this reaction and to provide examples wherein dioxygenation by 4a-$FlEtO_2^-$ will lead to a dioxygenase reaction (eq 3).



(3)

Experimental Section

General. All melting points were measured on a Thomas Model 40 micro hot stage apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian T60 spectrometer using tetramethylsilane (Me_4Si) as internal reference. Chemical shifts were expressed as δ values (parts per million downfield from Me_4Si). All spectrophotometric determinations were performed on a Cary 118 spectrophotometer thermostated at 30.0 ± 0.2 °C. Rapid spectral changes were followed with a Durham stopped-flow spectrophotometer under an oxygen-free N_2 atmosphere.

Materials, 9,10-Phenanthrenequinone (V), 3,5-di-*tert*-butylcatechol (VIII), and 3,5-di-*tert*-butyl-*o*-quinone (IX) were obtained from Aldrich Chemical Co. *tert*-Butyl alcohol was distilled under nitrogen. Dioxane was refluxed over benzophenone and Na for 3 h and distilled under nitrogen. Tetrahydrofuran was first distilled from Na and then from $LiAlH_4$ under nitrogen. Acetonitrile was refluxed over P_2O_5 for 6 h and distilled under nitrogen. All solvents were kept under a dry nitrogen atmosphere. The preparation of all solutions, weighing of solids, etc., were carried out in a dry nitrogen atmosphere.

N^5-Ethyl-4a-hydroperoxy-3-methyllumiflavin (4a-FlEtOOH). 4a-FlEtOOH has been synthesized in this laboratory in 85 to >99% purity,[1,2] λ_{max} 370 nm (ϵ_{370}(*t*-BuOH) 8000 M^{-1} cm^{-1}).

10-Ethoxy-9-phenanthral (Ia). Ia was synthesized according to a literature procedure.[13] The product was isolated by silica gel column chromatography (chloroform–*n*-hexane (1:1)) and its purity determined by thin layer chromatography. Recrystallization from *n*-hexane under N_2 and drying over P_2O_2 at 64 °C for 6 h in vacuo provided Ia free of ethanol: mp 74–76 °C (lit.[13] 73–76 °C); ^{1}H NMR ($CDCl_3$) δ 1.65 (t, 3 H), 4.27 (q, 2 H), 6.30 (s, 1 H), 7.50–8.87 (m, 8 H).

10-Methyl-9-phenanthrol (Ib). According to the method of Eistert and El-Chahawi,[14] Ib was obtained by the deethylation of 10-methyl-9-ethoxyphenanthrene, which in turn was obtained by the reaction of 9-fluorenone with excess diazoethane: mp 122–123 °C (lit.[14] 122–123 °C); NMR ($CDCl_3$) δ 2.68 (s, 3 H), 5.32 (br s, 1 H), 7.50–8.8 (m, 8 H).

Monoethyl 1,1′-Diphenate (IVa). Diphenic anhydride was first prepared by treatment of 1,1′-diphenic acid (0.51 g, 0.0021 mol) with excess acetic anhydride according to the method reported by Roberts et al.[15] The anhydride so obtained was employed in the syntheses of IVa without further purification.

The anhydride (0.38 g, 0.0017 mol) was added to a solution which was previously prepared by dissolving 1 g of Na in 10 mL of EtOH. The solution was poured into a mixture of 100 mL of 2 N HCl and 200 mL of dichloromethane after stirring for 10 min at room temperature. The organic layer was separated, washed with water twice, and dried over Na_2SO_4 overnight. Evaporation of the solvent gave a light yellow solid which was purified by column chromatography (silica gel) using $CHCl_3$–ether (1:1) as an eluent. The product eluted before impurities. Recrystallization from *n*-hexane twice afforded monoethyl 1,1′-diphenate (0.2 g): yield 34% based on diphenic acid used: mp 99–100 °C; NMR ($CDCl_3$) δ 0.95 (t, 3 H), 4.06 (q, 2 H), 7.10–7.60 (m, 6 H), 7.95–8.20 (m, 2 H), and 10.28 (br s, 1 H); UV (*t*-BuOH) λ_{max} 283 nm (ϵ_{283} 3090 M^{-1} cm^{-1}).

2-(2′-Acetylphenyl)benzoic acid (IVb) was prepared after a method of Reid and Conte.[16] IVb was synthesized by reaction of 95% formic acid with 7-oxo-5-[bis(ethoxycarbonyl)methylene]-5,7-dihydrodibenz[*c*,*e*]oxepin, which was obtained by treatment of 1,1′-diphenic anhydride with diethyl malonate in the presence of triethylamine and acetic anhydride: mp 121 °C (lit.[16] 120–121 °C); UV (*t*-BuOH) λ_{max} 288 nm (ϵ_{288} 2920 M^{-1} cm^{-1}); NMR ($CDCl_3$) δ 2.22 (s, 3 H), 7.00–8.10 (m, 8 H), 10.37 (s, 1 H).

10-Hydroxy-10-methyl-9,10-dihydro-9-phenanthrone (IIIb). Rapid bubbling of O_2 through a solution of Ib^- (0.209 g, 0.001 mol) in 5 mL of ethanol containing KOH (0.1 g in 5 mL of H_2O) for 10 min yielded (after pouring into ice-water, collecting, and recrystallization from *n*-hexane) a 60% yield of IIIb: mp 86 °C (lit.[17] 87 °C); UV (*t*-BuOH) λ_{max} 326 nm (ϵ 3050 M^{-1} cm^{-1}), 278 (10 100), and 270 (9500); NMR ($CDCl_3$) δ 1.54 (s, 3 H), 3.97 (s, 1 H), 7.18–8.10 (m, 8 H).

1-Hydroperoxy-1,2,3,4-tetrahydronaphthalene (VI). VI was prepared by autoxidation of tetrahydronaphthalene initiated by AIBN at 60 °C for 48 h. Purity of the hydroperoxide was determined by conventional iodometric titration (97%).

2,6-Di-*tert*-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadien-1-one (VII). VII was synthesized by the method of Kharasch and Joshi.[18] Iodometric titration showed that the hydroperoxide was 85% pure.

Product Analysis. In a typical experiment, solutions of Ia or Ib containing the appropriate concentrations of *t*-BuO^-K^+ were prepared in a glovebox under dry N_2. Reactions were initiated by the addition of an aliquot (5 mL) of the basic solution of Ia or Ib to a weighed portion of solid 4a-FlEtOOH. Typical concentrations were as follows: [Ia] = 7.8×10^{-3} M, [*t*-BuO^-K^+] = 7.7×10^{-3} M, and [4a-FlEtOOH] = 3.3×10^{-4} M. After the mixture was stirred for several minutes, 0.05 mL of glacial acetic acid was added. In order to determine the yield of $FlEt^-$ a portion of the acidified solution was transferred to a Thunberg cuvette and mixed with a solution of the nitroxide radical (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxy) which is known to convert FlEtH to FlEt·.[6] The concentration of FlEt· (=[FlEtH]) was determined by its absorbance at 640 nm (ϵ_{640} 5000 M^{-1} cm^{-1}).[7] λ_{max} and extinction coefficients of products follow: IX, 398 nm (ϵ 1890 M^{-1} cm^{-1}); V, 414 nm (ϵ 1620 M^{-1} cm^{-1}); IVa, 283 nm (ϵ 3090 M^{-1} cm^{-1}); IVb, 288 nm (ϵ 2920 M^{-1} cm^{-1}); IIIb, 278 nm (ϵ 10 100 M^{-1} cm^{-1}).

The yield of products from the substrates was determined by LC.

Table I. Reaction of 4a-FlEtOOH with 10-Methyl-9-phenanthrol in the Presence of *tert*-Butoxide

4a-FlEtOOH,[a] ×10⁴ M	Ib, ×10³ M	*t*-BuOK, ×10³ M	solvent	reaction time, min	products, %[b] FlEtH	IIIb
3.14	7.00	6.82	*t*-BuOH	5	69	35
3.14	7.00	6.82	*t*-BuOH	10	53	42
2.77	7.98	7.93	*t*-BuOH	10	57	59
2.72	8.98	6.91	*t*-BuOH	10	46	65
3.86	8.55	11.6	*t*-BuOH	10	57	28
1.79	7.85	7.61	*t*-BuOH	10	40	89
3.63	8.02	7.37	CH_3CN[c]	10	13	52

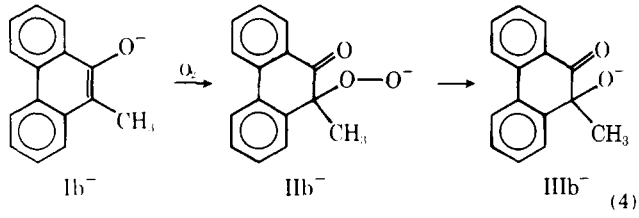
[a] The purity of 4a-FlEtOOH used was 85–93%. [b] The yields of FlEtH and IIIb were based on 4a-FlEtOOH employed. [c] No formation of IVb was observed by LC analysis.

The LC analysis was carried out with a Du Pont Instrument reverse phase column (Lichrosorb 5RP-8, 25 cm, 4.6 mm), using acetonitrile–acetic acid–water, 25:25:50 (v/v), and acetonitrile–water, 40:60 (v/v), as solvents for Ia and Ib, respectively, at a flow rate of 1.2 mL/min. The products were monitored at 283 nm (=λ_{max} of IVa and IVb). The retention times (using CH_3CN–AcOH–H_2O) of Ia, V, and IVa, were 26.5, 6.6, and 9.5 min, respectively, and the retention times (using CH_3CN–H_2O) of Ib, IVb, IIb, and IIIb were 35.0, 6.8, 11.3, and 12.3 min, respectively.

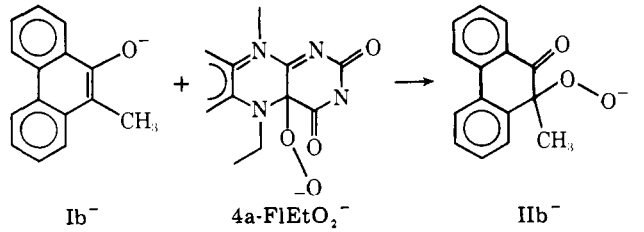
Results

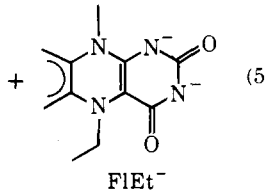
The kinetics for the reaction of 4a-$FlEtO_2^-$ with the phenanthrene substrates of this study could not be followed spectrophotometrically. This is due to the fact that the absorbances of substrate anions under the condition of [substrates$^-$] ≫ [4a-FlO_2^-] exceeded greatly the absorbance of 4a-$FlEtO_2^-$. For this reason, the oxygen-transfer reactions were allowed to proceed for a set time period (generally 10 min), the reaction was quenched, and products were determined. Kinetic analyses of partial reactions were feasible and have been carried out. A means was designed to follow dioxygen transfer from 4a-$FlEtO_2^-$ to 3,5-di-*tert*-butylcatechol anion. All reactions were investigated at 30 °C under strictly anaerobic conditions and unless stated to the contrary in absolute *t*-BuOH.

10-Methyl-9-phenanthrol anion (Ib$^-$) reacts with molecular oxygen in strongly basic ethanol to yield 10-hydroxy-10-methyl-9,10-dihydro-9-phenanthrone (IIIb). The intermediate peroxide anion (IIb$^-$) is too unstable under basic conditions for its isolation (eq 4). The experimental results obtained for the reaction of Ib$^-$ with 4a-$FlEtO_2^-$ are tabulated in Table I.

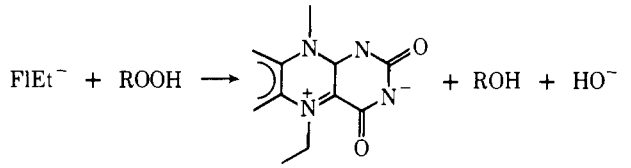


(4)

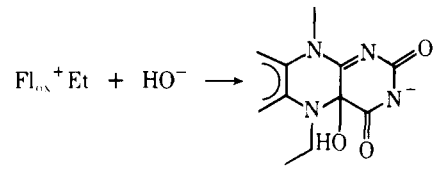




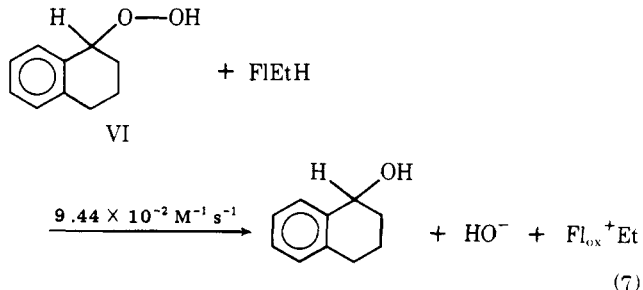
(5)



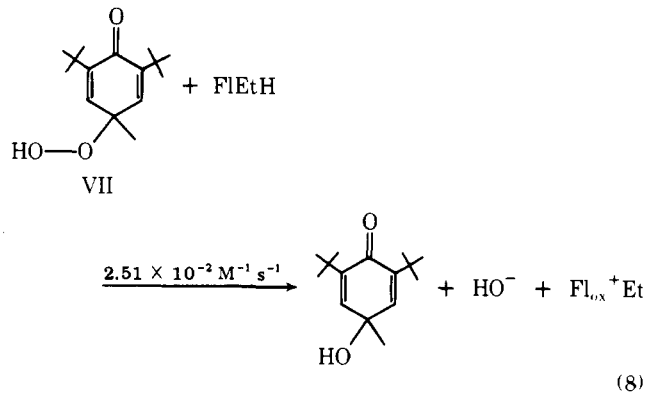
$$FlEt^- + Fl_{ox}{}^+Et \longrightarrow 2FlEt\cdot \quad (6)$$



$$Fl_{ox}{}^+Et + RO^- \longrightarrow \text{4a-FlEtOR}$$



$$FlEtH + Fl_{ox}{}^+Et \longrightarrow 2FlEt\cdot \quad (7)$$



$$FlEtH + Fl_{ox}{}^+Et \longrightarrow 2FlEt\cdot \quad (8)$$

The products of reaction were found to be FlEt$^-$ and IIIb. No evidence for the presence of the 10-hydroperoxy compound IIb could be obtained by LC and comparison to authentic IIb. The inability to detect IIb is likely due to its instability in the basic reaction solution and possibly also to its reduction by FlEt$^-$. The fact that FlEt$^-$ is not consumed in the reaction (Table I) may be explained by the finding that both FlEt· and $Fl_{ox}{}^+Et$ are reduced to FlEt$^-$ by Ib$^-$ which is present in excess (vide

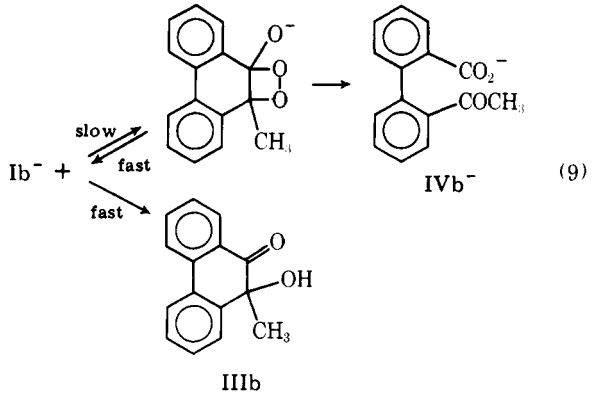
Table II. Reaction of 4a-FlEtOOH with 10-Ethoxy-9-phenanthrol in Various Solvents

4a-FlEtOOH,[a] ×10⁴ M	Ia, ×10³ M	*t*-BuOK, ×10³ M	solvent	reaction time, min	products, %[b] FlEt	V	IVa
a. 5.70	6.43	6.03	CH_3CN	10	FlEt· 32	53	43
b. 3.73	7.86	7.47	CH_3CN	10	FlEt· 20	45	29
c. 2.95	6.50	6.79	CH_3CN	10	FlEt· 22	36	32
d. 3.98	5.77	5.99	*t*-BuOH	20	58	66	[c]
e. 3.25	7.70	8.04	*t*-BuOH	20	54	92	[c]
f. 3.33	7.98	7.86	*t*-BuOH	30	50	86	0.7
g. 3.33	7.89	7.86	*t*-BuOH	150	28	94	0.9
h. 4.52	6.06	5.88	THF	10	no data	51	[c]
i. 4.52	6.06	5.88	THF	120	no data	98	[c]
j. 5.04	8.00	7.96	dioxane	30	no data	78	10

[a] 4a-FlEtOOH used was 85–92% pure. [b] The yields were based on 4a-FlEtOOH employed. [c] No formation of IVa was detected by LC.

infra). The reactions of the hydroperoxides VI and DTBP-O_2H with $FlEt^-$ were investigated in *t*-BuOH under anaerobic conditions with [organic peroxide] = 3.6×10^{-3} M > [$FlEt^-$] = 7.8×10^{-4} M. The products in each case were determined to be FlEt· (λ_{max} 640 nm) and the corresponding alcohol (LC comparison to authentic alcohol).

In the reaction of 4a-$FlEtO_2^-$ with Ib^- special attention was given to the presence of 2-(2′-acetylphenyl)benzoic acid (IVb) in the course of LC analysis in which instance authentic IVb was employed. No trace of this compound could be detected. This negative result finds ready interpretation in the reactions of eq 9. Thus, the decomposition of $IIb^- \rightarrow IIIb^-$ is a rapid reaction and dioxetane formation, with its accompanying 40 kcal mol^{-1} thermodynamic barrier,[19] should be slow when compared to the rate of opening of the dioxetane ring by elimination to provide IIb^-.

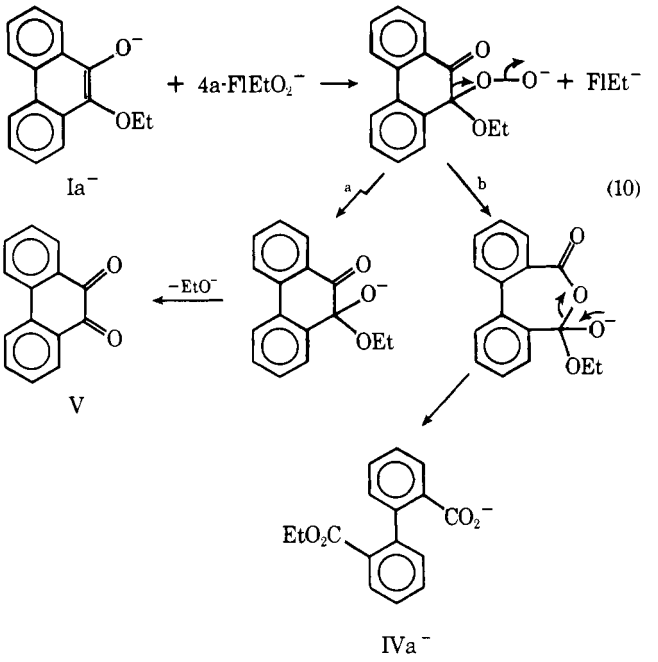


(9)

10-Ethoxy-9-phenanthrol anion (Ia^-) reacts with 4a-$FlEtO_2^-$ to provide 9,10-phenanthroquinone (V) and monoethyl 1,1′-diphenate (IVa). The ratio of V:IVa is dependent upon the polarity of the solvent (Table II). In acetonitrile an almost equal yield of both components is obtained but little or no IVa is obtained in dioxane, THF, or *t*-BuOH. Formation of products from the substrate Ia^- is accountable through the intermediacy of a common peroxy anion. Yields of $FlEt^-$ (nitroxide assay, see Experimental Section) of about 60% based on $[4a\text{-}FlEtO_2H]_{initial}$ were obtained in *t*-BuOH and THF. Longer reaction times were advantageous to the production of V but resulted in lower yields of $FlEt^-$ (~30%).

For the reaction carried out in acetonitrile solvent, the radical FlEt· appeared on acidification with acetic acid without addition of nitroxide. In run a the yield of FlEt· amounted to 35%. A like appearance of FlEt· on acidification of the spent solution resulting from the reaction of $VIII^-$ with 4a-$FlEtO_2^-$ was also observed. The explanation afforded (vide infra) for this observation is applicable to the present case.

3,5-Di-*tert*-butylcatechol anion ($VIII^-$) at 4×10^{-3} M reacts with 4a-$FlEtO_2^-$ ([4a-$FlEtO_2H$] = 1.2×10^{-4} M;

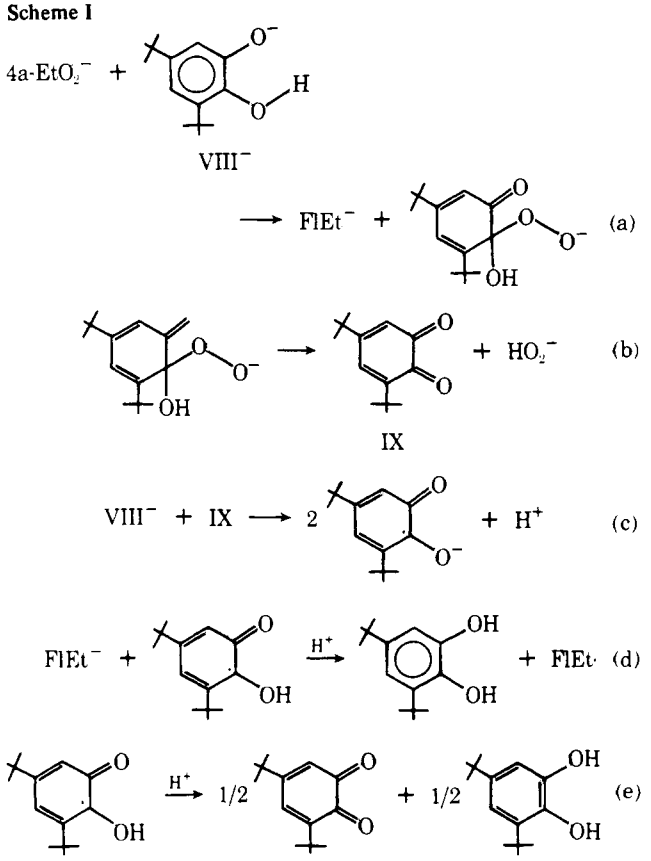


(10)

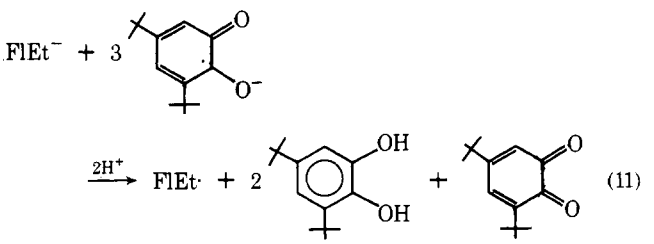
[*t*-BuO^-K^+] = 4×10^{-3} M) in *t*-BuOH under anaerobic conditions. Acidification of the reaction solution by a small amount of deoxygenated acetic acid provided FlEt· and 3,5-di-*tert*-butyl-*o*-quinone (IX) in yields of ~70 and 60%, respectively. The yields of product were not sensitive to the alteration in [*t*-BuO^-K^+] of fourfold employed. Product analysis (LC) indicated the absence of such known oxidation products of VIII as γ-lactone, 3,5-di-*tert*-butyl-5-(carbomethyl)-2-furanone, and 3,5-di-*tert*-butyl-5-(carboxyhydroxymethyl)-2-furanone.[20]

On completion of the reaction of $VIII^-$ with 4a-$FlEtO_2^-$, and prior to acidification, the reaction solution possessed a pronounced blue color. The visible spectrum of the reaction solution beyond 390 nm proved to be identical (λ_{max} 730 nm) with that of 3,5-di-*tert*-butyl-*o*-benzosemiquinone (which was generated independently from VIII by disproportionation with an equal concentration of the quinone IX). In addition, the blue solution exhibited no absorbance at 490 and 398 nm, indicating the absence of FlEt· and the quinone IX. On acidification of the reaction solution the absorbance due to the di-*tert*-butyl-*o*-benzosemiquinone disappeared and in its place there was found to be absorbance by FlEt·. These observations may be explained by the reactions of Scheme I. Reaction c, Scheme I, was studied in a separate experiment by reacting $VIII^-$ (4×10^{-3} M), IX (4×10^{-4} M), and *t*-BuO^-K^+ (8×10^{-3} M), which provided the semiquinone (λ_{max} 730 nm, ϵ 680 cm^{-1} M^{-1}) in 100% yield (no IX remained). By use of the extinction coefficient of the semiquinone it was then calculated that this species was found in 96% yield in the reaction between 4a-

Scheme I

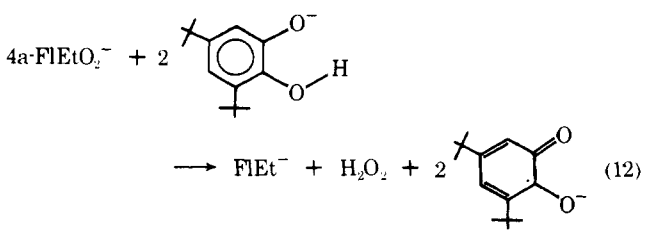


$FlEtO_2^-$ and $VIII^-$. Reaction e of Scheme I was also established in a separate experiment in which a solution of the semiquinone (8.22×10^{-4} M) in *t*-BuOH containing *t*-BuO^-K^+ was acidified with acetic acid to provide a 48% yield of IX. On comproportionation the semiquinone should produce a 50% yield of IX. Reaction d of Scheme I could also be established in a separate experiment. When a *t*-BuOH solution of $FlEt^-$ (1.93×10^{-4} M) containing semiquinone (4.24×10^{-4} M) and *t*-BuO^-K^+ (8.52×10^{-3} M) was acidified with acetic acid, there were immediately formed FlEt· (1.83×10^{-4} M) in 95% yield and IX (1.03×10^{-4} M) in 43% yield. Since the reactions of eq d and e would both be in operation under the concentrations used (i.e., eq 11) the yield of quinone IX is



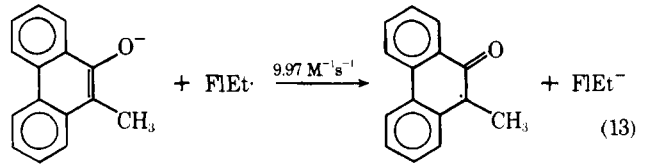
86% of theory. From Scheme I, the theoretical yield of IX is 50% and that of FlEt· is 100%. These expectations may be compared to the determined product yields of 60% for IX and 70% for FlEt

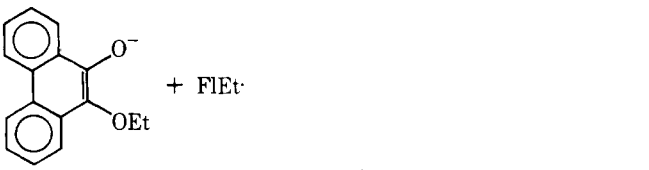
Summing reactions a, b, and c of Scheme I provides eq 12.

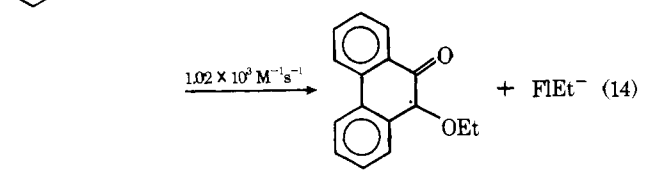


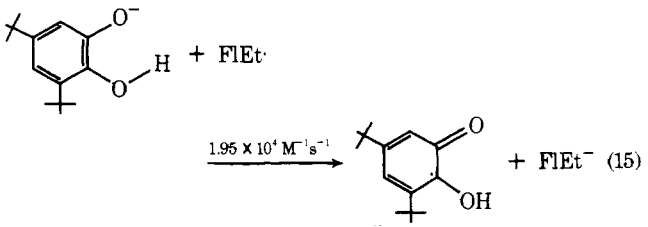
Since reactions b and c are very rapid the transfer of dioxygen from 4a-$FlEtO_2^-$ to $VIII^-$ (reaction a, Scheme I) could be followed by monitoring the appearance of semiquinone with time at 730 nm. In performing the stopped-flow kinetic experiments one drive syringe contained a *t*-BuOH solution of VIII and *t*-BuO^-K^+ at equal concentrations and the other syringe contained a *t*-BuOH solution of 4a-$FlEtO_2^-$ at 1.45×10^{-4} M. At $[VIII^-] \gg [4a\text{-}FlEtO_2^-]$ the appearance of semiquinone followed the first-order (k_{obsd}) rate law. The kinetic study was repeated by mixing on the stopped-flow bench a solution of 4a-$FlEtO_2^-$ (1.45×10^{-4} M) and solutions of VIII (0.324 to 6.48×10^{-3} M) plus *t*-BuO^-K^+ at a concentration of 6.64×10^{-3} M. Plots of k_{obsd} (determined by either procedure) vs. $[VIII^-]$ exhibit saturation in $[VIII^-]$ and a plot of $1/k_{obsd}$ vs. $1/[VIII^-]$ is linear (Figure 1). This observation establishes the presence of an intermediate formed from 4a-$FlEtO_2^-$ or a complex or compound formed from 4a-$FlEtO_2^-$ plus $VIII^-$ (see Discussion).

Reaction of phenolate ions with N^5-ethyl-3-methyllumiflavin radical (FlEt·). were investigated by stopped-flow spectrophotometry under an inert atmosphere using *t*-BuOH as solvent. All reactions were carried out by mixing a solution of phenolate ion (phenol plus equivalent *t*-BuO^-K^+) with one containing FlEt· prepared by mixing equimolar solutions of Fl_{ox}^+Et and FlEtH at 2×10^{-4} M, Phenolate ion was employed in excess of FlEt· and under these conditions the disappearance of FlEt· (640 nm) followed good pseudo-first-order kinetics and plots of k_{obsd} vs. [phenolate anion] were found to be linear. From the slopes of the plots of Figure 2 there were calculated the second-order rate constants of eq 13-15.









Reactions of phenolate ions with O_2 were allowed to proceed in absolute *t*-BuOH containing *t*-BuO^-K^+ and continually saturated with air (constant $[O_2] \simeq 3.15 \times 10^{-3}$ M) at 30 °C for a set period of time. The reactions were then quenched by the addition of acetic acid to slight acidity and product analysis was performed. Results are presented in Table III. The percentage yields reported in Table III are based upon the initial $[O_2]$ and must be considered maximal since $[O_2]$ is constant.

The kinetics for the reaction of O_2 with $VIII^-$ were investigated by monitoring the appearance of semiquinone at 730 nm (eq 16). Reactions were initiated by rapid mixing a solution

Table III. Reaction of Phenolate Ions with Oxygen (30 °C, *t*-BuOH)[a]

	substrate,		[*t*-BuO$^-$]	reaction	product % yields[b]			
		×10^3 M	×10^3 M	time, min	IX	V	IVa	IIIb
1	Ia	(8.05)	7.99	10		<12	0	
2	Ia	(8.04)	7.85	30		<33	trace	
3	Ia	(7.03)	6.93	10		<8.9 × 10^{-4} M	<3.4 × 10^{-4} M	
4	Ib	(7.98)	7.70	10		0		<9
5	VIII	(4.01)	3.81	10	<47			
6	VIII	(4.45)	7.83	10	<46			

[a] The solvent for entry 3 is acetonitrile. [b] Percent yield based on initial [$^3O_2$].

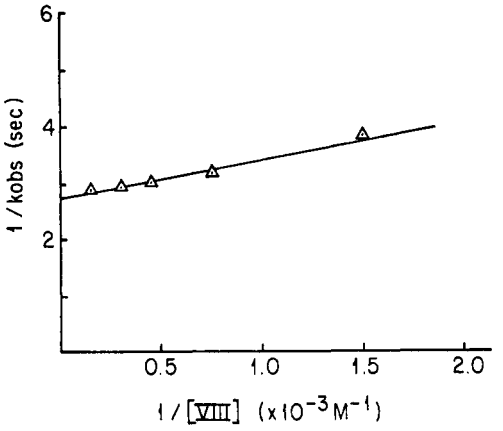


Figure 1. Plot of the reciprocal of the pseudo-first-order rate constants ($1/k_{obsd}$) vs. the reciprocal of the concentration of 3,5-di-*tert*-butylcatechol (VIII) for the reaction of VIII$^-$ with 4a-FlEtO$_2^-$ (1.45 × 10^{-4} M). Reaction was carried out in absolute *t*-BuOH under inert atmosphere with [*t*-BuOK] = 6.64 × 10^{-3} M at 30 °C.

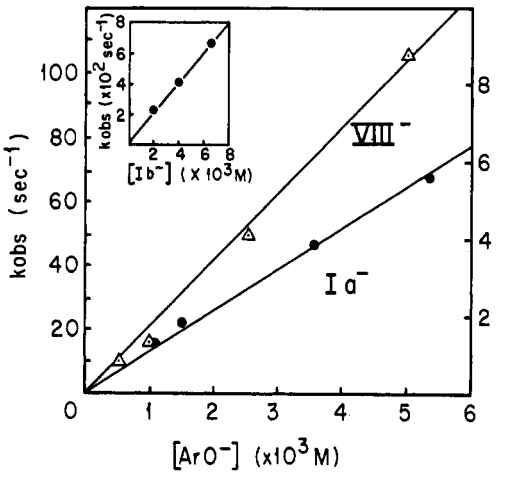
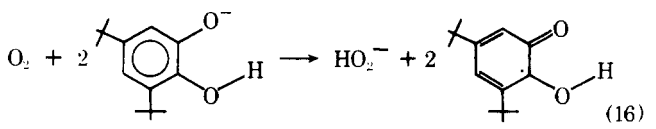


Figure 2. Plot of pseudo-first-order rate constants (k_{obsd}) vs. phenolate ion concentrations for the 1e$^-$ reduction of N^5-ethyl-3-methyllumiflavin radical ([FlEt·] = 9.80 × 10^{-5} M, *t*-BuOH solvent, 30 °C).

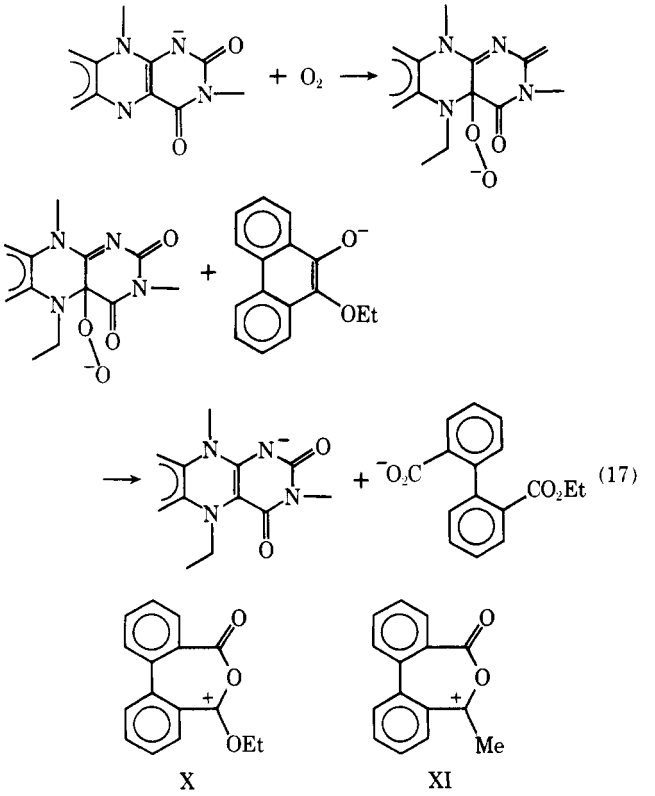


(16)

containing equimolar concentrations of VIII and *t*-BuO$^-$K$^+$ in absolute *t*-BuOH with an air-saturated ([O_2] = 3.15 × 10^{-4} M) solution of *t*-BuOH. With [VIII$^-$] in excess over [O_2] there was obtained good pseudo-first-order kinetics. The second-order rate constant for reaction of O_2 with VIII$^-$ was determined (2.32 M^{-1} s^{-1}) from a plot (Figure 3) of k_{obsd}(s^{-1}) vs. [VIII$^-$]. This value may be compared to the second-order rate constant (0.348 M^{-1} s^{-1}) for the reaction of DTBP$^-$ with O_2 under the same conditions.[7]

Discussion

A result of this study which is of biochemical relevance is the finding that the 4a-hydroperoxide obtained from the reaction of an N^5-alkylflavin with $^3O_2$ is capable of transferring both oxygen atoms to a catechol monoether anion to provide the monoester of a dicarboxylic acid (eq 17) with the regeneration of reduced flavin. We have, in effect, discovered a dihydroflavin-catalyzed dioxygenase reaction. The oxidation of Ia$^-$ to provide IVa undoubtedly occurs through the intermediacy of a peroxide formed upon dioxygen transfer from 4a-FlEtO$_2^-$ to Ia$^-$, followed by a Hock-type rearrangement (eq 10). When the OEt substituent of IX is replaced by Me, the resultant peroxide (IIb) does not provide a ring-cleavage product (IVb) but decomposes to a tertiary alcohol, IIIb (eq 4). This may be accounted for by (1) the instability of IIb in base; (2) the greater stability of X as compared to XI; (3) the inoperability of the dioxetane path of eq 9. Rearrangements of peroxide or perester intermediates similar to that of eq 10 have been suggested for the oxidative cleavage of phenols,[21]



(17)

X XI

β-naphthoquinone,[22] and *trans*-decalin peresters.[23] Hamilton[24] has previously provided thermodynamic arguments in favor of this type of mechanism over one involving the formation and cleavage of a dioxetane. In addition, the presently favored mechanism for intradiol metallocatechol dioxygenases

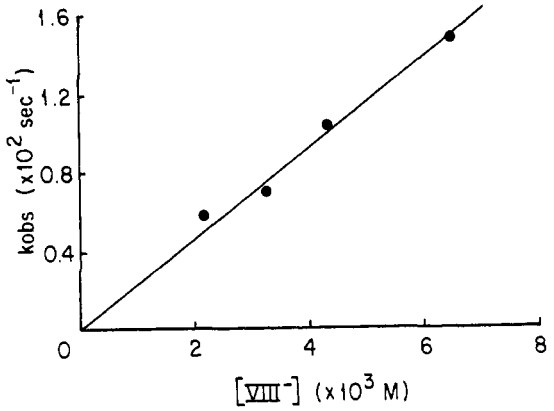


Figure 3. Plot of the pseudo-first-order rate constants (k_{obsd}) vs. the concentration of 3,5-di-*tert*-butylcatecholate anion (VIII$^-$). Oxygen concentration is 3.15×10^{-4} M in *t*-BuOH (30 °C).

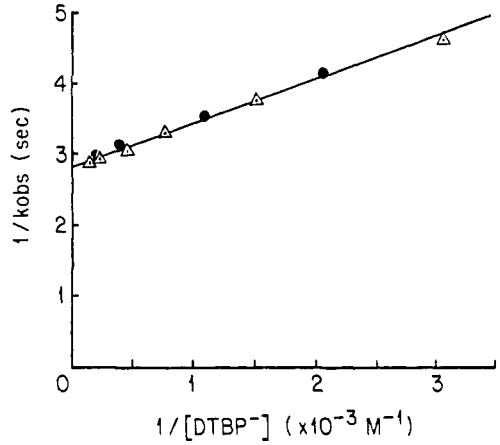


Figure 4. The reciprocal of the pseudo-first-order rate constants ($1/k_{obsd}$) vs. the reciprocal of the concentration of the anion of 2,6-di-*tert*-butyl-4-methylphenol ($1/[DTBP^-]$) for the reaction of DTBP$^-$ with the anion of N^5-ethyl-4a-hydroperoxy-3-methyllumiflavin (4a-FlEtO$_2^-$) in *t*-BuOH at 30 °C: (●) mixing of a solution of 4a-FlEtO$_2$H (5×10^{-5} M) with solutions of *t*-BuOK in the presence of DTBP (5.5×10^{-3} M) (ref 7); (△) mixing of a solution of 4a-FlEtO$_2$H (6×10^{-5} M) with solutions of DTBP containing *t*-BuOK at 6.6×10^{-3} M.

involves rearrangement through anhydride intermediate.[25,26]

Of key interest is the mechanism whereby the peroxy anion moiety of 4a-FlEtO$_2^-$ is transferred to a phenolate anion. The first example of this reaction is shown in eq 1.[7] The reaction was found to be first order in both [4a-FlEtO$_2^-$] and [DTBP$^-$] at low values of the latter and to be independent of [DTBP$^-$] at its higher molarity. In the original study[7] a *t*-BuOH solution of 4a-FlEtO$_2^-$ was stopped flow mixed with an excess of the phenol DTBP in *t*-BuOH. In a series of kinetic runs the mole fraction of DTBP present as DTBP$^-$ was increased by including increasing concentrations of *t*-BuO$^-$K$^+$. To be certain that saturation in [DTBP$^-$] was not due to a salt effect (or saturation by trace of metal) the kinetic studies of this reaction have now been repeated with a constant value of [K$^+$]. This was accomplished by stopped-flow mixing of a solution of 4a-FlEtO$_2^-$ (5.85×10^{-5} M) with solutions containing *t*-BuO$^-$K$^+$ at a constant concentration of 6.64×10^{-3} M and varying concentrations of DTBP (0.324 to 6.48×10^{-3} M). Again, saturation in DTBP$^-$ was obtained. Plots of $1/k_{obsd}$ vs. $1/$DTBP$^-$ were found to be indistinguishable when employing both sets of experimental data (Figure 4). Thus, the dioxygen transfer reaction is not unduly sensitive to the change in μ and the formation of an intermediate species from 4a-FlEtO$_2^-$ is verified. An intermediate might arise by either the covalent combination or complexation of 4a-FlEtO$_2^-$ and DTBP$^-$ (eq 18) or in a reaction involving only 4a-FlEtO$_2^-$ (eq 19):

$$\text{4a-FlEtO}_2^- + \text{DTBP}^- \underset{k_2}{\overset{k_1}{\rightleftarrows}} X_1 \xrightarrow{k_3} \text{products} \qquad (18)$$

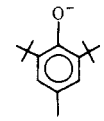
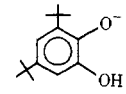
$$\text{4a-FlEtO}_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} X_2 \xrightarrow{k_3[\text{DTBP}^-]} \text{products} \qquad (19)$$

No reasonable structures are apparent for an intermediate compound which could be formed between 4a-FlEtO$_2^-$ and DTBP$^-$ and it was argued[7] that complex formation between the two negatively charged species would not be favorable in *t*-BuOH. In addition, evidence of a kinetic nature could not be found for the complexation of the neutral phenol with neutral 4a-FlEtO$_2$H. In this study we find that the reaction of VIII$^-$ with 4a-FlEtO$_2^-$ is also first order in [VIII$^-$] at its lower values, becoming independent of [VIII$^-$] at higher concentrations. Assuming the sequence of eq 19 with X_2 at steady state provides the equation

$$1/k_{obsd} = k_2/k_1k_3[I^-] + 1/k_1 \qquad (20)$$

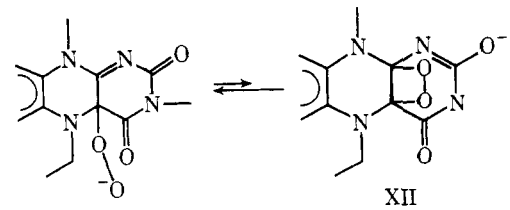
Plots of $1/k_{obsd}$ vs. $1/$[VIII$^-$] and $1/k_{obsd}$ vs. $1/$[DTBP$^-$] are provided in Figures 1 and 4. The derived rate constants are given in Table IV. Within experimental error the values of k_1 determined with DTBP$^-$ and VIII$^-$ are identical. This finding

Table IV

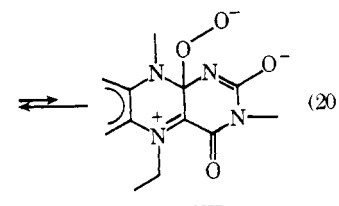
	k_1, s^{-1}	k_2/k_1k_3, s M	k_2/k_3, M
	0.36	6.2×10^{-4}	2.2×10^{-4}
	0.37	7.7×10^{-4}	2.8×10^{-4}

supports the sequence of eq 19. It is of some little surprise to find that the values of the partition coefficients (k_2/k_3) determined with both phenolate ions are also very similar. Assuming that eq 19 represents the sequence of events, k_2 is common to both reactions and VIII$^-$ undergoes reaction with X_2 about 1.3 times slower than does DTBP$^-$. With the liberal assumption that the finding with DTBP$^-$ and VIII$^-$ of a common value of k_1 will prove general, one might draw the conclusion that the sequence of eq 19 is correct and that 4a-FlEtO$_2^-$ is spontaneously converted into an intermediate or intermediates which peroxidize the phenolate anions with about the same rate. The various possibilities will be considered.

Migration of the peroxy anion moiety from the 4a to the 10a position to yield X_2 as a 10a-peroxyflavin (XIII) would be



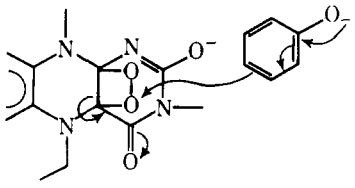
XII



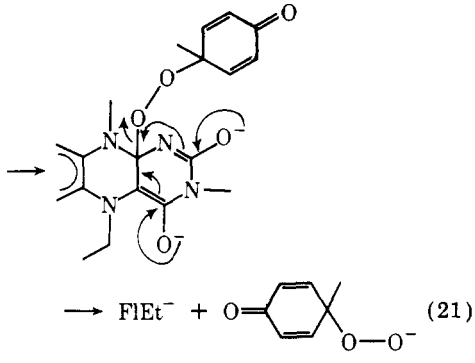
(20)

XIII

highly endothermic and should not be expected. This view is supported by the observation that the rate constants for reaction of the 4a-peroxy anion of 10-(2′,6′-dimethylphenyl)-5-ethyl-3-methylisoalloxazine (XIV) and 4a-$FlEtO_2^-$ with $DTBP^-$ do not differ greatly.[27] The 10a position of XIII is sterically hindered[28] to nucleophilic attack and rearrangement to the 10a-peroxyflavin would be even more endothermic than in the rearrangement of 4a-$FlEtO_2^-$. The hypothetical dioxetane (XII) may be considered as X_2. The structure of XII is sterically feasible even when a bulky substituent, such as the 2′,6′-dimethyl moiety, occupies the 10 position. This is due to the fact that the >C=C< bond between C_{4a} and C_4 provides a butterfly configuration much as in dihydroflavin and steric compression between the 10a and 10 substituents is greatly reduced. Nucleophilic attack of PhO^- upon the dioxetane XII



XII



$$\longrightarrow FlEt^- + O{=}\langle\rangle{-}O{-}O^- \qquad (21)$$

to produce $^-OOPh{=}O$ would require C—O rather than O—O bond scission. We know of no instances in which the opening of a dioxetane has been shown to involve C-O bond cleavage. However, the structure of XII is rather unique possessing, as it does, an electron sink in the >C=O function at the 4a position of the isoalloxazine ring.

In the mechanism of eq 19 the species X_2 might represent (O_2 + $FlEt^-$) or ($O_2^-\cdot$ + FlEt·). The formation of these intermediates could occur simply by the reversal of the reaction of $^3O_2$ with 1,5-dihydroflavin:[5]

$$FlEtOO^- \rightarrow FlEt\cdot \uparrow\downarrow O_2^-\cdot \rightarrow FlEt\cdot \uparrow\uparrow O_2^-\cdot \rightarrow FlEt^-O_2 \rightarrow FlEt_2^- + O_2 \qquad (22)$$

If X_2 represented completely dissociated $FlEt^-$ + O_2, the peroxidation would take place in a second-order reaction between phenolate anion and O_2. This possibility was eliminated in the previous study by an independent determination of the rate constant for the reaction of eq 23b. The second-order rate

$$4a\text{-}FlEtO_2^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} FlEt^- + O_2 \qquad (23a)$$

$$DTBP^- + O_2 \xrightarrow{k_2} DTBP\text{-}O_2^- \qquad (23b)$$

constant so obtained was ~100-fold less than required to be compatible with the observed kinetics for peroxidation of $DTBP^-$ by 4a-$FlEtO_2^-$. From the second-order rate constant for reaction of O_2 with $VIII^-$ and a knowledge of k_1 for X_2 formation it can be calculated that the rate of reaction of O_2 with $VIII^-$ is ca. 40-fold too slow to allow the kinetic competency of X_2 = $FlEt^-$ + O_2 in the peroxidation of $VIII^-$. Though the second-order rate constants for the reaction of Ia^- and Ib^- with O_2 were not obtained, arguments against the reaction of these phenolate ions with O_2 being involved in their peroxidation by 4a-$FlEtO_2^-$ can be made. At a constant concentration of O_2 equal to ten times that of 4a-$FlEtO_2^-$ employed Ib^- provides IIIb in <9% of theory based upon $[O_2]_{initial}$ (see Results for details). No other product could be detected. Given that the peroxidation reactions with 4a-$FlEtO_2^-$ and O_2 were carried out for the same period of time and that the concentration of O_2 was at a constant concentration which exceeded that for the initial concentration of 4a-$FlEtO_2^-$ by >tenfold, the efficiency of peroxidation by 4a-$FlEtO_2^-$ exceeds that for O_2 by >100-fold. The reaction of Ia^- with O_2 under like conditions of concentration provided <12% yield of V in 10 min and a <33% yield of V in 30 min. No diphenic acid monoester (IVa) could be detected in the 10-min run and only a possible trace of this substance could be detected after 30 min. Since the steady-state oxygen concentration exceeded the initial concentration of 4a-$FlEtO_2^-$ employed by >tenfold one may conclude that the efficiency of the reactions with oxygen is <3–4% than seen with 4a-$FlEtO_2^-$. In acetonitrile Ia^- reacts with O_2 to yield both V and IVa. Thus, both 4a-$FlEtO_2^-$ and O_2 provide with Ia^- only V in *t*-BuOH but both V and IVa in acetonitrile. Like experiments with $VIII^-$ establish that the efficiency of the reaction of this phenolate anion with O_2 amounts to <4–5% of that seen with 4a-$FlEtO_2^-$. The observation that the efficiency of O_2 as a peroxidizing agent, for the various phenolate anions, is far less than with 4a-$FlEtO_2^-$ establishes that peroxidation by $FlEtO_2^-$ is not the result of the dissociation of a 4a-$FlEtO_2^-$ into $FlEt^-$ + O_2 and subsequent bimolecular reaction of phenolate ion with the free O_2 species (eq 23).

The possibility that the reaction sequence involves eq 19 with X_2 representing solvent-separated FlEt· and $O_2^-\cdot$ should be considered:

$$4a\text{-}FlEtO_2^- \overset{k_a}{\rightleftharpoons} FlEt\cdot + O_2^-\cdot \qquad (24a)$$

$$DTBP^- + FlEt\cdot \xrightarrow{k_{-a}} DTBP\cdot + FlEt^- \qquad (24b)$$

$$DTBP\cdot + O_2^-\cdot \rightarrow DTBP\text{-}O_2^- \qquad (24c)$$

To account for the saturation in the pseudo-first-order rate constant by phenolate anion it would be assumed that eq 24c would be rate limiting at low [phenolate] and eq 24a rate limiting at high [phenolate]. The reactions 24b and 24c may be studied independently.

All the phenolate ions which have been shown to be oxidized by 4a-$FlEtO_2^-$ have also been shown to reduce FlEt· to $FlEt^-$. The second-order rate constants (*t*-BuOH) follow: $DTBP^-$, minimally 10^7 M^{-1} s^{-1};[7] Ia^-, 1.02×10^3 M^{-1} s^{-1}; Ib^-, 9.97 M^{-1} s^{-1}; $VIII^-$, 1.95×10^4 M^{-1} s^{-1}. At phenolate concentrations employed in the reaction with 4a-$FlEtO_2^-$ the pseudo-first-order rate constants for phenolate ion reduction of FlEt· may be calculated as: Ia (at 6×10^{-3} M), 6.12 s^{-1}; Ib (at 7×10^{-3} M), 6.98×10^{-2} s^{-1}; VIII (at 4×10^{-3} M), 7.8 $\times 10^1$ s^{-1}. The rate constant for spontaneous decomposition of 4a-$FlEtO_2^-$ has been determined[7] to be $4.6 \pm 0.2 \times 10^{-2}$ s^{-1} (*t*-BuOH). These results establish that, at the concentrations of the various phenolate ions used in the reactions with 4a-$FlEtO_2^-$, the rate of reduction of FlEt· by Ia^-, $VIII^-$, and $DTBP^-$ is more than adequate to assure that eq 24b could be a kinetically competent component of the peroxidation reaction. In the case of Ib^-, the rate of reduction of FlEt· exceeds the rate of spontaneous decay of 4a-$FlEtO_2^-$ by a factor of 1.5. This is sufficient to account for the 70% yield of $FlEt^-$ obtained in the reaction of 4a-$FlEtO_2^-$ with Ib^-. The coupling of $O_2^-\cdot$ with phenol radical appears to be the weak link in the overall mechanism of eq 24. Thus, it has been reported[29] that in the presence of excess $O_2^-\cdot K^+$ (crown) the radical DTBP· is re-

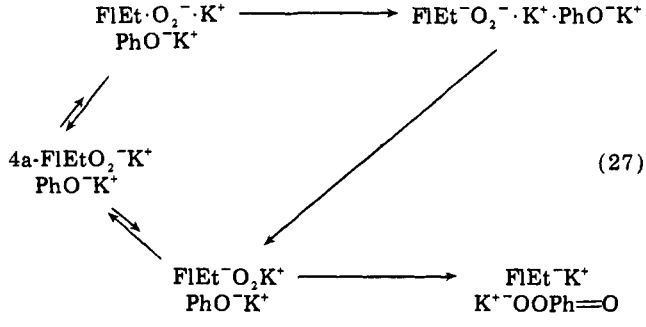
duced to $DTBP^-$. There appears to be in the literature no documented example of $O_2^-\cdot$ coupling with a radical species to yield a peroxide.[30] Formation of the phenol-derived peroxides would not, so it appears, result from the reaction of molecular oxygen derived from 4a-$FlEtO_2^-$ with phenolate anion nor from the coupling of the radical, derived by $1e^-$ abstraction from phenolate anion, with superoxide. The intermediate X_2 must, therefore, represent a compound or complex of oxygen and dihydroflavin. The dioxetane mechanism of eq 21 must be considered as a possibility. Another possibility is that phenolate ion reacts with an O_2 species which has not dissociated from the flavin moiety (eq 25).

$$\text{4a-FlEtO}_2^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \{\text{FlEt}^-\text{O}_2\} \xrightarrow{k_3[\text{PhO}^-]} \text{product} \qquad (25)$$

The oxygen would not, in this instance, be as closely associated with the flavin as in the dioxetane XII and might possess some singlet character. Charge-transfer complexes of oxygen have been proposed to be involved in bimolecular and intromolecular oxygen transfer reactions.[31] Because the values of k_3 determined for $DTBP^-$ and $VIII^-$ are quite similar the capture of X_2 by phenolate ion may be diffusion controlled.

$$\text{4a-FlEtO}_2^- \underset{2.2 \text{ to } 2.8 \times 10^5}{\overset{0.36}{\rightleftharpoons}} X_2 \xrightarrow{10^9[\text{PhO}^-]} \text{product} \qquad (26)$$

An alternative would involve the preassociation of 4a-$FlEtO_2^-K^+$ with $PhO_2^-K^+$ in a loose complex within a solvent cage. Dissociation of 4a-$FlEtO_2^-$ within this solvent cage could then result in the peroxidation reaction prior to solvent rearrangement (eq 27). If this were the case, then the equilibrium



(27)

constant for complex formation would be only slightly dependent upon the nature of the phenolate anion ($DTBP^-$ vs. $VIII^-$) while the equilibrium constants for complex formation would be 5.85×10^2 for $DTBPK^+$ and 3.63×10^2 for $VIII^-K^+$.

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References and Notes

(1) Kemal, C.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1976,** *73,* 995.
(2) Kemal, C.; Chan, T. W.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1977,** *74,* 405.
(3) Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977,** *99,* 2387.
(4) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1977,** *99,* 7064.
(5) Kemal, C.; Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977,** *99,* 7272.
(6) Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977,** *99,* 7287.
(7) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1979,** *101,* 1635.
(8) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1979,** *101,* 4017.
(9) Hayaishi, O.; Katagiri, M.; Rothberg, S. *J. Am. Chem. Soc.* **1955,** *77,* 5450.
(10) Nishinaga, A. *Chem. Lett.* **1975,** 273. Nishinaga, A.; Tojo, T.; Matsuura, T. *J. Chem. Soc., Chem. Commun.* **1974,** 896. Tsuji, J., Takayanagi, H. *J. Am. Chem. Soc.* **1974,** *96,* 7449. Tsuji, J.; Takayanagi, H.; Sukai, J. *Tetrahedron Lett.* **1975,** 1245. Rogic, M. M.; and Demmin, T. R. *J. Am. Chem. Soc.* **1978,** *100,* 5472. Brown, D. G.; Beckmann, L.; Ashby, C. H.; Vogel, G. C.; Reinprecht, J. R. *Tetrahedron Lett.* **1977,** 1363. Matsuura, T.; Matsushima, H.; Kato, S.; Saito, I. *Tetrahedron* **1972,** *28,* 5119. Moro-Oka, Y.; Foote, C. S. *J. Am. Chem. Soc.* **1976,** *98,* 1510. Lee-Ruff, E.; Lever, A. B. P.; Rigaudy, J. *Can. J. Chem.* **1976,** *54,* 1837. Ferrand, J. C.; Johnson, D. C. *J. Org. Chem.* **1971,** *36,* 3606. Pandell, B. L. *Ibid.* **1976,** *41,* 3922.
(11) Spencer, T.; Massey, V. *J. Biol. Chem.* **1972,** *247,* 5632. Entsch, B.; Ballou, D. P.; Massey, V. *Ibid.* **1976,** *251,* 2550. Stickland, S.; Massey, V. *Ibid.* **1973,** *248,* 2953. Hastings, J. W.; Balny, C.; Le Peuch, C.; Dousou, P. *Proc. Natl. Acad. Sci. U.S.A.* **1973,** *70,* 3468.
(12) Sparrow, L. G.; Ho, P. P. K.; Sundaram, T. K.; Zach, D.; Nyns, F. J.; Snell, E. E. *J. Biol. Chem.* **1969,** *244,* 2590.
(13) Müller, E.; Schurr, K.; Scheffler, J. *J. Justus Liebigs Ann. Chem.* **1959,** *627,* 132.
(14) Eistert, B.; El-Chahawi, M. A. *Monatsh. Chem.* **1967,** *98,* 941.
(15) Roberts, R. C.; Johnson, T. B. *J. Am. Chem. Soc.* **1925,** *47,* 1399.
(16) Reid, W.; Conte, R. *Chem. Ber.* **1971,** *104,* 1573.
(17) Eistert, B.; Klein, L. *Chem. Ber.* **1968,** *101,* 900.
(18) Kharasch, M. S.; Joshi, B. S. *J. Org. Chem.* **1957,** *22,* 1439.
(19) O Neal, H. E.; Richardson, W. H. *J. Am. Chem. Soc.* **1970,** *92,* 6553.
(20) Moro-oka, Y.; Foote, C. S. *J. Am. Chem. Soc.* **1976,** *98,* 1510.
(21) Norman, R. O. C.; Smith, J. R. L. In "Oxidases and Related Redox Systems", King, T. E., Mason, H. S., Morrison, M., Eds.; Wiley: New York, 1965; Vol. 1, p 131.
(22) Karrer, P.; Schneider, L. *Helv. Chim. Acta* **1947,** *30,* 859.
(23) Criegee, R.; Kaspar, R.; Dietrich, W. *Justus Liebigs Ann. Chem.* **1948,** *560,* 127.
(24) Hamilton, G. A. "Molecular Mechansims of Oxygen Activation", Hayaishi, O., Ed.; Academic Press: New York, 1974; p 405.
(25) Que, L. Jr.; Lipscomb, J. D.; Münck, E.; Wood, J. M. *Biochim. Biophys. Acta* **1977,** *485,* 60.
(26) Loehr, T. M.; Keyes, W. E.; Loehr, J. S., "Proceedings of the 3rd International Symposium on Oxidase and Related Oxidation Systems", King, T. E., Mason, H. S., Morrison, M., Eds.; University Park Press: Baltimore, in press.
(27) Miller, A.; Bruice, T. C. *J. Chem. Soc., Chem. Commun.,* **1979,** 896; Bruice, T. C., Miller, A., *ibid,* in press.
(28) Smith, S. B.; Bruice, T. C. *J. Am. Chem. Soc.* **1975,** *97,* 2875.
(29) Nishinaga, A.; Itahara, T.; Shimizu, T.; Tomita, H.; Nishizawa, K.; Matsuara, T. *Photochem. Photobiol.* **1978,** *28,* 687. Sawyer, D., private communication.
(30) Wilshire, J.; Sawyer, D. *Acc. Chem. Res.* **1979,** *12,* 105.
(31) As an example see: Nishinaga, A.; Shimizu, T.; Matsuura, T. *J. Org. Chem.* **1979,** *44,* 2983.