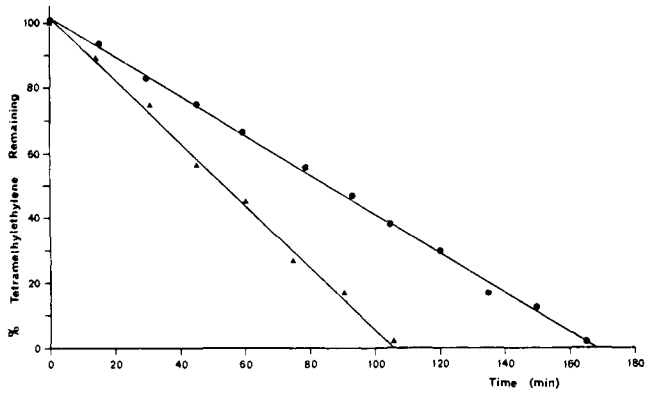

Figure 1. Quantum yield studies with (HP)-Rose Bengal. Photooxidation of tetramethylethylene using a 400-W General Electric high-pressure sodium lamp: (▲) reaction solution with 0.07 M tetramethylethylene and 4.91×10^{-5} M soluble Rose Bengal in methanol; (●) reaction solution with 0.07 M tetramethylethylene and 10 mg/mL of (HP)-Rose Bengal in methanol. Identical apparatus was used in both cases, and controls showed that the path length was sufficient to ensure absorption of all of the incident light.

detectable absorption due to free Rose Bengal indicating that (HP)-Rose Bengal functions as a true heterogeneous sensitizer and is stable to photooxidative conditions. Indeed, it proved possible to reuse the sensitizer repeatedly with no apparent loss of efficiency.

In view of the satisfactory behavior of (HP)-Rose Bengal in water, we were prompted to compare the effectiveness of (HP)-Rose Bengal with that of (P)-Rose Bengal in a variety of solvents. Photooxidation of tetramethylethylene using standard suspensions of the two sensitizers showed that both sensitizers behaved very similarly in polar solvents such as acetone, dichloromethane, or methanol.[14] The quantum yield for singlet oxygen formation was determined for (HP)-Rose Bengal in methanol by comparison of the zero-order rates of photooxygenation of tetramethylethylene using this sensitizer and soluble Rose Bengal (Figure 1). The assumption of a steady-state concentration of singlet oxygen[1b] and the known quantum yield of 0.76 for its formation from soluble Rose Bengal in methanol[15] lead to a value of 0.48 for the quantum yield of singlet oxygen formation from (HP)-Rose Bengal in methanol. This figure compares very favorably with the value of 0.43 determined for (P)-Rose Bengal in dichloromethane.[1b] In contrast to (P)-Rose Bengal, however, we have found that (HP)-Rose Bengal is not useful as a sensitizer in nonpolar media such as dioxane, toluene, or octane, in accord with its hydrophilic nature.

A comparison of the relative effectiveness of the two heterogeneous sensitizers in water has been made. We have found that adequate suspensions of (P)-Rose Bengal result if 0.05% TWEEN 80 is added to permit wetting of this polymer. In the presence of TWEEN 80, using 10-mg/mL suspensions of both polymers, 9,10-anthracenedipropionic acid sodium salt (10^{-4} M) undergoes 63% reaction in 1 min with (HP)-Rose Bengal, whereas only 4% reaction occurs with (P)-Rose Bengal. Clearly the hydrophilic polymer-bound sensitizer is superior under these conditions and should provide a valuable alternative for photooxygenations in aqueous media.

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(12) With the anthracene, substrate concentrations were 0.1–1 mM.
(13) In these cases the light path is longer, allowing small quantities of the sensitizer to be used.
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(21) Address correspondence to Celanese Research Company, Summit, N.J. 07901.

A. Paul Schaap,* Arthur L. Thayer, K. A. Zaklika
Department of Chemistry, Wayne State University
Detroit, Michigan 48202

Peter C. Valenti*[21]
Hydron Laboratories, Inc.
New Brunswick, New Jersey 08902
Received February 20, 1979

4a-Hydroperoxyflavin N-Oxidation of Tertiary Amines

Sir:

Xenobiotic substances are oxidatively metabolized in the hepatic tissue by microsomal monooxygenases of the cytochrome P-450 class and by flavomonooxygenases. The N-oxidation of amines in animals is a function of the hepatic flavomonooxygenases. Hepatic monooxygenase activities toward amines have become of particular concern[1-3] owing to the fact that people are increasingly subjected to numerous pharmacologically active nitrogen compounds (nicotine, tranquilizers, antihistamines, narcotics, hallucinogens, tropic alkaloids, ephedrine and derivatives, etc.) and the N-oxidation of arylamines and arylamides is a prerequisite in the conversion of these agents into their ultimate carcinogenic derivatives.[4]

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Table I. Product Yields From the Spontaneous (k_1) and DMA-Dependent (k_2[DMA]) Reactions of 4a-FlEtOOH

[DMA] × 10^2 M	product yields, % 4a-FlEtOH[a]	product yields, % *N*-oxide	calcd yield of *n*-oxides $k_2[DMA]/(k_1 + k_2[DMA])$ × 100
0	21	0	0
6.7	46	50	44
16.7		68	67
33.3	85	77	80
66.7	100	80	88

[a] The yield of 4a-FlEtOH includes that of FlEt·. Both are converted into Fl_{ox}+Et under the conditions of analysis.[12]

Evidence has been presented in support of the involvement of enzyme bound 4a-hydroperoxyflavin (4a-FlHOOH) as a precursor to the flavoprotein monooxygenase event.[5] The synthesis of 5-alkyl-4a-hydroperoxyflavins (4a-FlROOH) has been reported.[6,7] The 4a-FlROOH compounds have been shown to possess monooxygenase activity in the oxidation of aldehydes[6,8,9] (a chemiluminescent model for bacterial luciferase) and to exhibit dioxygenase activity toward the anion of 2,6-di-*tert*-butyl-4-methylphenol.[10] Herein we report our preliminary studies of the N-oxidation of tertiary amines by 4a-FlEtOOH.

The reactions of 4a-FlEtOOH with tertiary amines (*N*,*N*-dimethylaniline (DMA) and *N*,*N*-dimethylbenzylamine (DMB)) were conducted[11] in absolute and oxygen free *t*-BuOH (30 °C) by following the disappearance of 4a-FlEtOOH at 370 nm. Under the experimental conditions of [tertiary amine] ≫ [4a-FlEtOOH], the decrease in A_{370} was first order to more than 2 half-lives. A plot of the determined pseudo-first-order rate constants (k_{obsd}) vs. the concentration of the tertiary amine employed was linear with positive intercept in k_{obsd} at [tertiary amine] = 0 (for DMA, see Figure 1). The values of the rate constants of eq 1 were determined from the intercept and slope, respectively, of Figure 1 as $k_1 = 8 \times 10^{-5}$ s^{-1} and $k_2 = 9.6 \times 10^{-4}$ M^{-1} s^{-1}:

$$\frac{-d[\text{4a-FlEtOOH}]}{dt} = (k_1 + k_2[\text{DMA}])[\text{4a-FlEtOOH}] \quad (1)$$

The more basic *N*,*N*-dimethylbenzylamine (DMB) was also found to react with 4a-FlEtOOH in a process first order in both components to yield quantitatively the flavin pseudobase (4a-FlEtOH) and the *N*-oxide of DMB.[12] The second-order rate constant for reaction of DMB with 4a-FlEtOOH exceeds that for reaction of DMA with the flavin hydroperoxide by 1.25 × 10^4, a factor which may be compared with the ratio 7.6 × 10^3 for the K_a of their conjugate acids in water ($\beta_{nuc} = 1.1$). Product analyses[12] for the *N*-oxide of DMA and for the pseudobase of *N*-ethyllumiflavinium cation (4a-FlEtOH) as a function of DMA concentration are provided in Table I. Inspection of Table I reveals that the percent yield of the *N*-oxide of DMA may be calculated on the assumption that the bimolecular reaction of DMA with 4a-FlEtOOH converts the latter into its *N*-oxide (eq 2).

Experiments in which 4a-FlEtOO$^-$ was generated by addition of [*t*-BuO$^-$] = [4a-FlEtOOH] did not result in the formation of the *N*-oxide of DMA. The dissolution of solid NaO_2 in *t*-BuOH 1 M in DMA under anaerobic conditions also did not lead to the formation of the *N*-oxide. It has been tentatively proposed that the transfer of the peroxide anion moiety of 4a-FlEtOO$^-$ to 2,6-di-*tert*-butyl-4-methyl phenolate anion (DTBP$^-$)[10] involves the formation of O_2^{-}· from 4a-FlEtOO$^-$. That neither 4a-FlEtOO$^-$ or O_2^{-}· provides the *N*-oxide of DMA rules out such a radical process for the N-oxidation reaction. Another possible mechanism for the N-

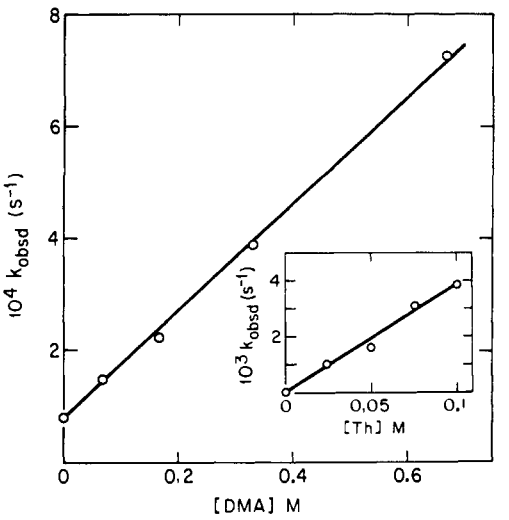
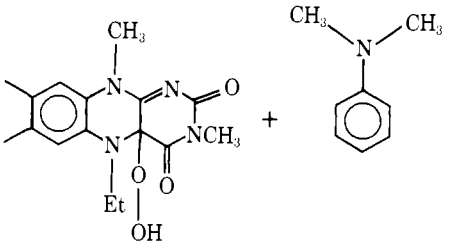
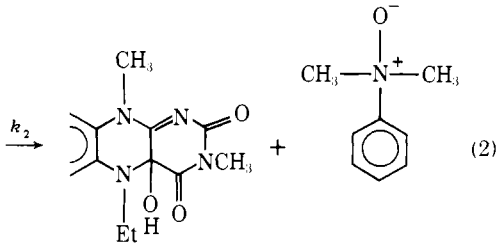


Figure 1. Plot of the pseudo-first-order rate constants (k_{obsd}; 30 °C, absolute *t*-BuOH) for the reaction of 4a-FlEtOOH with *N*,*N*-dimethylaniline vs. the concentration of *N*,*N*-dimethylaniline ([DMA]). Inset to figure is a plot of k_{obsd} (30 °C, absolute dioxane) for the reaction of 4a-FlEtOOH with thioxane vs. thioxane concentration ([Th]).



4a-FlEtOOH

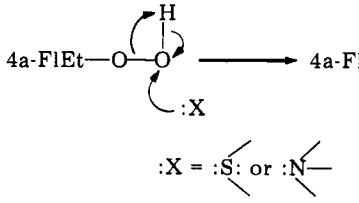


4a-FlEtOH (2)

oxidation reaction involves hydrogen peroxide (eq 3) as the oxidizing agent. Since the literature[13] procedure for the synthesis of DMA *N*-oxide involves the reaction of H_2O_2 with DMA, this reaction was looked into in some detail. At 30 °C under anaerobic conditions with [H_2O_2] = (0.2–0.7 M) ≫ [DMA] = (3 × 10^{-4} M), no *N*-oxide was formed after more than 1 week. With [DMA] = (0.07–0.7 M) ≫ [H_2O_2] = (8 × 10^{-4} M), DMA was not found to increase the rate of anaerobic decomposition of H_2O_2 relative to a control run containing no DMA.[14] Also, no *N*-oxide was present at the end of a 3-week period. When H_2O_2 was combined with excess DMA and an equivalent amount of *t*-BuOK in *t*-BuOH, no *N*-oxide was formed in a period of time >7 half-lives for the reaction of equivalent concentrations of 4a-FlEtOOH and DMA. In independent experiments, *N*-oxide was found to be sufficiently stable under the various reaction conditions such that its formation would have been easily detected. It was also found that *t*-BuOOH does not react with DMA in absolute

$$\text{4a-FlEtOOH} \rightleftharpoons Fl_{ox}{}^{+}\text{Et} + HO_2^{-}$$
$$HO_2^{-} + t\text{-BuOH} \rightleftharpoons H_2O_2 + t\text{-BuO}^{-} \quad (3)$$

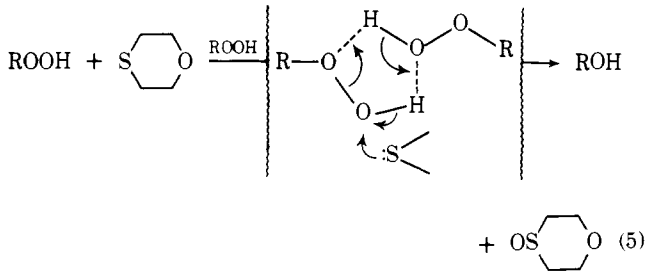
t-BuOH. Since reaction of DMA with H_2O_2 and *t*-BuOOH cannot be observed under conditions where 4a-FlEtOOH and DMA readily undergo reaction, it is not possible to compare the second-order rate constant for reaction of DMA with 4a-FlEtOOH with the rate constants for reaction of DMA with H_2O_2 and *t*-BuOOH. The mechanisms of N-oxidation of tertiary amines and S-oxidation of dialkyl sulfides represent, overall, nucleophilic displacements upon the terminal oxygen of 4a-FlEtOOH (eq 4). Employing thioxane in place of DMA



(4)

(CH_3OH solvent[8]), the ratio of the second-order rate constants for S-oxygenation is 4a-FlEtOOH:H_2O_2:*t*-BuOOH = 2×10^5:20:1.

Edwards[15] has shown that, in aprotic solvents (absolute dioxane), S-oxidations of thioxane by alkyl hydroperoxides and by hydrogen peroxide are second order in these hydroperoxide species. The second molecule of hydroperoxide was proposed to serve as an essential proton source (eq 5). The reactions of both thioxane and DMB with 4a-FlEtOOH in absolute dioxane[16] are pseudo first order in the hydroperoxide and plots of k_{obsd} vs. [thioxane] or DMB are linear. In the case of thioxane



(5)

the second-order rate constant (slope of inset to Figure 1) is 3.6×10^{-2} M^{-1} s^{-1}, while the second-order rate constant for DMB is 4.7×10^{-2} M^{-1} s^{-1}. The lack of a requirement for general acid catalysis in these S-oxidation and N-oxidation reactions by 4a-FlEtOOH is likely due to the latter's much greater oxygen transfer potential when compared with an alkyl hydroperoxide.

In summary, we have shown that 4a-FlEtOOH is a very efficient agent for the biomimetic mono-N-oxidation of tertiary amines. The efficiency of the 4a-FlEtOOH in this regard is much greater than the N-oxidizing ability of H_2O_2 or *t*-BuOOH (so much so that comparisons of the rate constants could not be made). The S-oxidation of thioxane by 4a-FlEtOOH exceeds the rate of S-oxidation by H_2O_2 by a factor of 10^5 in methanol.[8] This reaction in dry dioxane is first order in [4a-FlEtOOH] and in [thioxane]. Therefore, unlike alkyl hydroperoxides, the 4a-FlEtOOH does not require intermolecular general acid assistance for the S-oxidation (nor N-oxidation of DMB) reaction.

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(11) The 4a-FlEtOOH was prepared and analyzed as described previously.[6] DMA was distilled under a stream of N_2 and stored under N_2. *tert*-Butyl alcohol was distilled over CaH_2 and the distillate deoxygenated by several cycles of freeze–thaw.
(12) The concentration of the *N*-oxide of DMA was determined essentially as described by D. M. Ziegler and F. H. Pettit, *Biochem. Biophys. Res. Commun.*, **15**, 188 (1964). The yield of the *N*-oxide of DMB was determined by transfering 0.2 mL of the reaction mixture to 3 mL of AcOH 0.1 M in NaI. A control run employing an authentic sample of the *N*-oxide of DMB under identical conditions was conducted simultaneously. The *N*-oxide of DMB was found to react with NaI under these conditions (30 °C) with a second-order rate constant of 4×10^{-4} M^{-1} s^{-1}. The yield of *N*-oxide was determined from the amount of I_3^- formed and the identity of the oxide affirmed from the rate constant for I_3^- apperarance. The 4a-FlEtOH was determined by transferring 0.5 mL of the final reaction mixture into 2 mL of 1 M HCl. The $Fl_{ox}{}^+Et$ produced was determined by measuring the absorbance of the acidic solution at 550 nm. The 0–20% yield of FlEt· observed in the final *t*-BuOH reaction mixtures of 4a-FlEtOOH decomposition (with or without DMA) was not present in the acidified solutions. From control runs in which an authentic sample of 4a-FlEtOH was subjected to the reaction conditions and subsequent acidification as described above, an extinction coefficient for $Fl_{ox}{}^+Et$ of 6800 M^{-1} cm^{-1} in the *t*-BuOH–1 M HCl mixtures was obtained.
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Sheldon Ball, Thomas C. Bruice*
Department of Chemistry, University of California
Santa Barbara, California 93106
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