with 1 M NH_4^+ HCO_2^- in DMF/H_2O (3:7), pH 6, 40 °C. 4-(*p*-Methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**4**) eluted in fractions 203-210 and 7-(*p*-methoxybenzyl)guanosine (**5**) eluted in fractions 235-246. Fractions containing the individual products were pooled and the concentration of each product was determined spectrophotometrically.

Reaction of Radiolabeled Guanosine with *p*-Methoxybenzyl Chloride at pH 4.5. To a 2-mL solution of [5'-^{3}H]guanosine (21 Ci/mmol, 1×10^{-7} M) in HoAc/Na^+AcO^- buffer (0.1 M, pH 4.5) was added 0.05 mL of a freshly prepared solution of *p*-methoxybenzyl chloride (0.68 M) in DMF. The resulting suspension was stirred at 40 °C for 24 h. At the end of this incubation, 1 mL of the resulting solution was mixed with a solution of markers for **3**-**7** and was loaded on the Aminex A-5 column which was eluted as described above for separation of the unlabeled products. The eluted fractions were mixed with PCS (Amersham/Searle) for scintillation counting. Yields for products **3** and **5**-**7**, expressed as % guanosine converted to *p*-methoxybenzylated product were 3.4, 0.34, 0.05, and 0.07%, respectively.

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The Chemistry of 1-Carba-1-deaza-N^5-ethyl-N^3-methyllumiflavins. Influence of the N^1 upon the Reactivity of Flavin 4a-Hydroperoxides

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Abstract: N^5-Ethyl-N^3-methyl-1,5-dihydro-1-deazalumiflavin (C^1-FlEtH) has been synthetized and characterized. In aqueous solution (pH 3) C^1-FlEtH reacts with 1 equiv of $^3O_2$ to provide N^5-ethyl-N^3-methyl-1-deazalumiflavinium cation (C^1-Fl_{ox}^+Et). C^1-Fl_{ox}^+Et may be reduced to C^1-FlEtH by ascorbate, dithionite, or H_2/Pd. C^1-Fl_{ox}^+Et is not photoreducible by EDTA as is Fl_{ox}^+Et. This is due to direct photolysis of C^1-Fl_{ox}^+Et with the accompanying loss of the N^5-ethyl substituent as acetaldehyde. The spectral properties of C^1-$FlEtH_2^+$, C^1-FlEtH, and C^1-$FlEt^-$ and associated pK_a's have been determined and compared to the analogous constants for $FlEtH_2^+$, FlEtH, and $FlEt^-$. A comparison of the spectral properties of Fl_{ox}^+Et and C^1-Fl_{ox}^+Et has been made. The pK_a values and the pH dependences of the rate constants for the formation and dissociation of the pseudobases (i.e., C^1-4a-FlEtOH and 4a-FlEtOH) of Fl_{ox}^+Et and C^1-Fl_{ox}^+Et have also been determined as have the rate constants (pH 3.0) for addition of β-mercaptoethanol to the 4a-positions of Fl_{ox}^+Et and C^1-Fl_{ox}^+Et (providing 4a-FlEt-SCH_2CH_2OH and C^1-4a-FlEt-SCH_2CH_2OH). Partial oxidation of C^1-FlEtH by $^3O_2$ in H_2O produces the radical C^1-FlEt· through comproportionation of C^1-Fl_{ox}^+Et and C^1-FlEtH. Evidence is presented, suggesting that the radical C^1-FlEt· possesses a higher free-energy content than does FlMe·. The oxidation of C^1-FlEtH in H_2O or *t*-BuOH with excess $^3O_2$ is autocatalytic in nature. The initial rate for reaction of C^1-FlEtH with $^3O_2$ is substantially greater than the initial rate for reaction of FlMeH with $^3O_2$. This observation is discussed in terms of the mechanism of reaction of FlRH with O_2. In DMF, C^1-FlEtH reacts with $^3O_2$ to form a 4a-hydroperoxide (i.e., C^1-4a-FlEtOOH) which is quite stable. The rate constants for solvolysis of C^1-4a-FlEtOOH and 4a-FlEtOOH in DMF have been compared. The second-order rate constants for the (a) oxidation of I^- in 95% EtOH/DMF, (b) N-oxidations of *N*,*N*-dimethylbenzylamine, *N*-methylbenzylamine, and morpholine in DMF, and (c) the S-oxidation of thioxane in DMF by C^1-4a-FlEtOOH and 4a-FlEtOOH have been determined. The flavin products for the N- and S-oxygenation reactions are the pseudobases C^1-4a-FlEtOH and 4a-FlEtOH. These reactions are quantitative. Comparison of the various rate constants indicates that C^1-4a-FlEtOOH is from 3- to 17-fold a poorer oxidizing agent than is 4a-FlEtOOH. This can be explained by the somewhat less electronegative character of the 4a-position of the 1-deazaflavin hydroperoxide. The equilibrium constants for 4a-additions and retroadditions to C^1-Fl_{ox}^+Et and Fl_{ox}^+Et are comparable, and this leads to the conclusion that the difference in free-energy contents of C^1-Fl_{ox}^+Et and Fl_{ox}^+Et (starting states) and C^1-4a-FlEtX and 4a-FlEtX (products) is the same. Due to this feature, the decrease in $\Delta G^\ddagger$ for 4a additions to Fl_{ox}^+Et, as compared to C^1-Fl_{ox}^+Et (due to the greater electronegativity of Fl_{ox}^+Et), is mirrored in a decrease in $\Delta G^\ddagger$ for dissociation of X from 4a-FlEtX as compared to C^1-4a-FlEtX. This same free-energy difference is seen in the N- and S-oxidations and oxidation of I^- supporting the contention that the greater electrophilicity at the 4a-position of 4a-FlEtOOH polarizes the $C_{4a}O$-OH bond to a greater extent in 4a-FlEtOOH than in C^1-4a-FlEtOOH, thus making 4a-FlEtOOH a better oxidant. The observation that C^1-4a-FlEtOOH differs from 4a-FlEtOOH by only 1 order of magnitude in its oxygen-transfer potential to amines and the sulfide thioxane, combined with the established propensity of 4a-FlEtOOH to enter into these reactions, suggests that the hepatic flavoprotein microsomal oxidase reconstituted with 1-carba-1-deaza FAD will retain activity, if recognized by the enzyme, in the N-oxidation of amines and the S-oxidation of sulfides.

Introduction

Molecular oxygen is reductively activated by relatively few enzymatic systems. The flavoenzyme mono- and dioxygenases represent the only cofactor requiring oxygenases which do not employ a metal ion as a requisite component of their activities. A flavoprotein monoxygenase abundant in mammalian liver and located intracellularly on the endoplasmic reticulum near the sites of protein synthesis oxidatively metabolizes tertiary amine drugs to *N*-oxides, secondary amines to hydroxylamines, and sulfide pesticides to sulfoxides.[1] It has been established (for several flavin monooxygenases) that the reductive activation of molecular oxygen produces a transient enzyme-bound flavin 4a-hydroperoxide (Enz-4a-FlHOOH, eq 1), the reactive species in the N-oxidation of amines and the S-oxidation of sulfides.[2]

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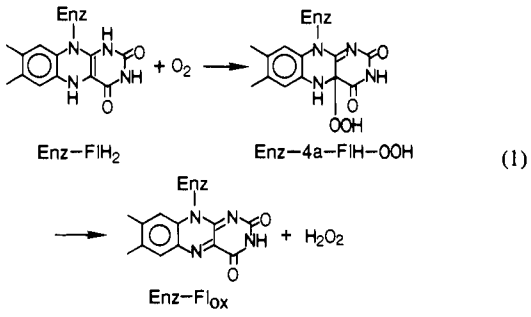
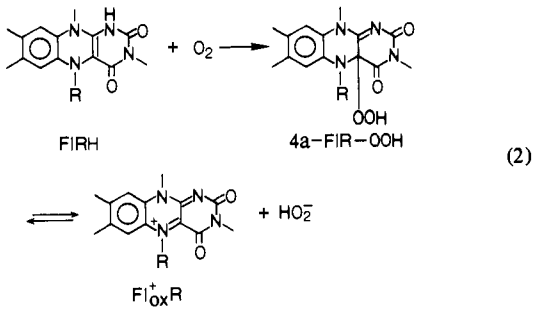
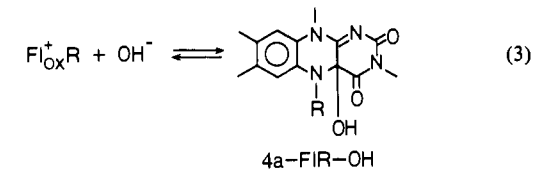


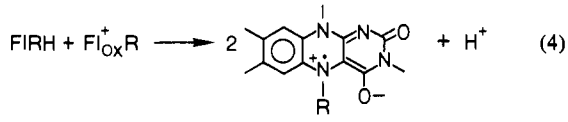
N^5-Alkyllumiflavins have been employed by us to study the reductive activation of molecular oxygen.[3] Replacement of the proton on N^5 with an alkylsubstituent does not effect the ability of the 1,5-reduced flavin to react with molecular oxygen; however, it does diminish the rate at which the resultant flavin 4a-hydroperoxide (4a-FlROOH) eliminates hydrogen peroxide (eq 2).



N^5-alkylflavin 4a-hydroperoxides (4a-FlROOH) have been isolated as stable solids and their ability to mimic biological monooxygenase reactions has been demonstrated.[4,5] The oxidized N^5-alkylflavins ($Fl_{ox}{}^+R$) are cationic, reversibly form 4a adducts with nucleophiles (eq 2 and 3), and comproportionate with reduced N^5-alkylflavins



(FlRH) in a thermodynamically favored reaction to form N^5-alkylflavin radicals (FlR·, eq 4). 1-Carba-1-deaza- (referred to



simply as 1-deaza or abbreviated by the prefix C^1) flavins have proven to be viable cofactors in flavoenzyme reactions which involve a dehydrogenation of substrate.[6b] However, mixed results have been obtained when C^1-flavins have been employed as cofactors in a few flavoenzyme monooxygenase reactions.[7] Herein we report some comparisons of $Fl_{ox}{}^+Et$, FlEtH, and FlEt· to C^1-$Fl_{ox}{}^+Et$, C^1-FlEtH, and C^1-FlEt· and compare the oxygen-transfer potential of 4a-FlEtOOH to C^1-4a-FlEtOOH in the oxidation of iodide, amines, and thioxane.

Experimental Section

Materials. All synthetic starting materials were purchased from Aldrich. The 10% palladium on charcoal was obtained from Matheson, Coleman and Bell. Buffer salts, potassium chloride, ascorbic acid (U. S.P.) used in the kinetic experiments, *N,N*-dimethylformamide (DMF), and *tert*-butyl alcohol (*t*-BuOH) were purchased from Mallinckrodt. The acetaldehyde was purchased from Sigma. Water was purified by deionization and distillation through a Corning Model AF-3ADA still. Buffer solutions and water were outgassed with vanadous-scrubbed argon. *N,N*-Dimethylformamide (DMF) was dried over 3-Å molecular sieves, distilled onto fresh 3-Å molecular sieves under reduced pressure (ca. 10 torr, nitrogen pressure bleed), and further deoxygenated by purging with dry nitrogen (sieves and all). Oxygen-saturated solutions of DMF were prepared by purging the solvent with O_2. The *tert*-butyl alcohol (*t*-BuOH) was refluxed over calcium hydride, distilled under nitrogen, and further deoxygenated by several cycles of freeze–vacuum–thaw. The 95% ethanol was purged with nitrogen and used without further purification. Amines were obtained from commercial sources, distilled under dry nitrogen, and purged with vanadous-scrubbed argon for at least 2 h. Thioxane was distilled over the blue anion radical of benzophenone and subsequently subjected to several cycles of freeze–vacuum–thaw. Solid reagents were made anaerobic by finely powdering and alternating a vacuum and nitrogen flush. All deoxygenated materials were subsequently stored in a glovebox under nitrogen. Hydrogen peroxide was bought as both the 30% aqueous (Mallinckrodt) and the 90% (F.M.C.) solutions.

N^3-Methyl-1-deazalumiflavin (C^1-Fl_{ox}) was synthesized after the general procedures employed by Ashton et al.[8] for the synthesis of N^3-methyl-1-deazalumichrome and 1-deazariboflavin. The purity and identity of C^1-Fl_{ox} was established by TLC and mass, ultraviolet–visible, and NMR spectroscopy. The FT NMR spectrum was taken in CD_2Cl_2 and the δ values relative to internal Me_4Si were found to be 8.20 (s, 1 H, aromatic C(6)H), 7.56, (1 H, aromatic C(9)H), 5.73 (s, 1 H, C(1)H), 3.63 (s, 3 H, N^3- or N^{10}-CH_3), 3.54 (s, 3 H, N^{10}- or N^3-CH_3), 2.58 (s, 3 H, C(7) or C(8)-CH_3), and 2.49 (s, 3 H, C(7) or C(8)–CH_3). The ultraviolet–visible spectrum in methanol showed peaks at 228 (24 300) 290 (31 000), 350 (4400), and 524 nm (8700 M^{-1} cm^{-1}). **N^5-Ethyl-N^3-methyllumiflavins (FlEtH and $Fl_{ox}{}^+Et$ ClO_4^-)** were prepared from N^3-methyllumiflavin as described by Hemmerich.[9] **The 4a-Hydroperoxy-N^5-ethyl-N^3-methyllumiflavin (4a-FlEtOOH)** was synthesized as described previously.[4a] The synthesis of **N^3-methyllumiflavin** is described elsewhere.[10]

N^5-Ethyl-N^3-methyl-1,5-dihydro-1-deazalumiflavin (C^1-FlEtH). In a three-necked round-bottomed flask equipped with a rubber septum, stir bar, and gas bubbler were placed 500 mg of N^3-methyl-1-deazalumiflavin, 500 mg of 10% palladium on charcoal, 350 mL of 100% ethanol, and 1 mL of 37% hydrochloric acid. Nitrogen was bubbled through the solution for 2 h, followed by hydrogen. About 5 min after the switch to hydrogen, a color change and slight precipitation occurred corresponding to reduction of the flavin. At this time 5 mL of acetaldehyde was added with a syringe through the septum cap and the pressure of the flask was brought to about 4 psig by limiting the outflow of hydrogen and securing the fittings with rubber bands. The reaction was allowed to stir under this slight hydrogen pressure. At various times, small samples were withdrawn through the septum cap and allowed to air oxidize. Thin-layer chromatography on silica in acetonitrile showing any trace of red C^1-Fl_{ox} indicated an incomplete reaction. The product moved as the pseudobase (C^1-4a-FlEtOH) on silica or alumina and was identified as a bright green spot appearing in response to hydrochloric acid vapors. When the reaction was complete (about 8 h), the flask was sealed and placed in a glovebox under an atmosphere of nitrogen. The solution was filtered free of catalyst and solvent was removed under reduced pressure. All reagents used thereafter were deoxygenated by appropriate methods and used in the glovebox under nitrogen. Water was added to the dry material and

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(3) Kemal, C.; Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 7272.

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(5) (a) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4017. (b) Ball, S.; Bruice, T. C. *Ibid.* **1980**, *102*, 6498.

(6) (a) Spencer, R.; Fisher, J.; Walsh, C. *Biochemistry* **1977**, *16*, 3586. (b) Spencer, R.; Fisher, J.; Walsh, C. *Ibid.* **1977**, *16*, 3594.

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the pH was brought to neutrality with KOH. Four aliquots of methylene chloride were used to extract the flavin. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. Recrystallization was accomplished by dissolving the material in a minimum volume of hot methanol and allowing the crystals to form slowly in a sealed vial on a cold plate (−20 °C). The yield varied depending on the anaerobicity of the workup conditions but was typically 60%. The corrected anaerobic melting point for dihydro-N^5-ethyl-N^3-methyl-1-deazalumiflavin (C^1-FlEtH) was found to be 188–189 °C.

N^5-Ethyl-N^3-methyl-1-deazalumiflavin Radical (C^1-FlEt·). About 50 mg of C^1-FlEtH was dissolved in a minimum amount of ethanol (about 8 mL), and this was added to about 100 mL of anaerobic 0.25 M formate buffer (μ = 1.0 M, pH 4.5) in a septum-stoppered Erylenmeyer flask. A 0.5-mol equiv sample of oxygen in oxygen-saturated buffer was added with a syringe through the septum cap. The dihydroflavin was converted rapidly to the radical (yellow solution changes to a greenish-black) which precipitated. The precipitate was collected and stored under nitrogen.

Photodeethylation of C^1-$Fl_{ox}$$^+$Et. About 50 mg of C^1-FlEtH was dissolved in 10 mL of 0.10 N HCl and allowed to air oxidize to the cation C^1-$Fl_{ox}$$^+$Et. This solution was coated inside a glass bulb, and all water and HCl gases were removed by a rough vacuum (1 torr) followed by 30 min of high vacuum ($<10^{-3}$ torr). The bulb was sealed under high vacuum and irradiated with a 300 W incandescent lamp at 18 in. for 3 days. A color change of green to red was noted. A mass spectrum was then taken of the gases found in the bulb.

In a separate set of experiments, a solution of 3.33×10^{-5} M C^1-$Fl_{ox}$$^+$Et was prepared by O_2 oxidation of a solution prepared by adding 5 μL of C^1-FlEtH (2×10^{-2} M in ethanol) solution to 3.00 mL of 0.01 N HCl in a Thunberg cuvette. Vanadous-scrubbed argon saturated with 0.01 N HCl was passed through the solution for 3 h to remove all traces of remaining oxygen. The Thunberg was sealed and exposed to a 300-W incandescent light at a distance of 1 ft for 48 h while being cooled in a water-jacketed beaker. Ultraviolet and visible spectra were taken of the resulting red solution. Oxygen was added and another spectrum was recorded.

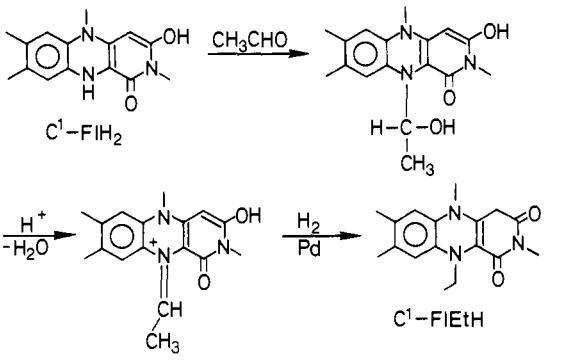
Reactions of C^1-4a-FlEtOOH. A solution of C^1-FlEtH in O_2-free DMF was added to O_2-saturated DMF. Upon mixing of the solution, the stable spectrum of C^1-4a-FlEtOOH appeared (λ_{max} at 378 nm (13 000 M^{-1} cm^{-1})). C^1-4a-FlEtOOH solutions of 1×10^{-4} M and 1×10^{-3} M in DMF were prepared in this manner. For the reaction of C^1-4a-FlEtOOH with NaI, 0.4 mL of a 1×10^{-4} M C^1-4a-FlEtOOH solution was added to 3 mL of 95% EtOH containing NaI and the change in A_{358} of the reaction mixtures recorded with time. For the reactions of C^1-4a-FlEtOOH with amines and thioxane, a measured aliquot of the reagent was added to a 1×10^{-4} M C^1-4a-FlEtOOH solution and the decrease in A_{378} observed with time. After completion of the reactions (followed at 378 nm), the visible spectrum of the final reaction mixture was recorded. In all cases, λ_{max} at 358 nm (14 000 M^{-1} cm^{-1}) was observed. This 358-nm λ_{max} is identical with that observed for the product of C^1-4a-FlEtOOH hydrolysis. Acidification of 1 mL of the final reaction mixtures with 2 mL of 1 M HCl resulted in appearance of the brilliant green C^1-$Fl_{ox}$$^+$Et ($\lambda_{max}$ = 690 nm (5800 M^{-1} cm^{-1})). The structure of the product with λ_{max} at 358 nm was thus assigned as C^1-4a-FlEtOH. Reactions of C^1-4a-FlEtOOH in *t*-BuOH were conducted by transferring 0.3 mL of a 1×10^{-3} M C^1-4a-FlEtOOH solution in DMF to 2.7 mL of *t*-BuOH. For the reaction of C^1-4a-FlEtOOH with C^1-FlEtH, the C^1-FlEtH was initially contained in 2.7 mL of *t*-BuOH.

Analysis for the N-oxidation products from the reactions of secondary amines with C^1-4a-FlEtOOH was conducted as described previously.[5] Analysis for the *N*-oxide of *N,N*-dimethylbenzylamine was conducted as described previously after thoroughly deoxygenating the final reaction mixture by purging for several hours with N_2.[5]

Metal-Free Solutions. Aqueous buffer salts were extracted for 15 min with a 0.01% solution of dithizone in CCl_4. The aqueous layer was then washed four times with fresh CCl_4, filtered, and bubbled through with nitrogen to remove traces of CCl_4. Only buffer salts giving an aqueous solution pH of less than 7 could be demetalized by this method because above this pH dithizone becomes water soluble.

Apparatus. Samples were weighed out on either a Mettler H51 or a Cahn RG electrobalance. Spectra were taken on a Cary Model 118C spectrophotometer or on Cary Model 15 spectrophotometer. Single wavelength kinetic measurements were made on a Durrum-Gibson Model 13001 stopped-flow spectrophotometer, on a Gilford Model 2000 recording spectrophotometer, on a Cary Model 118C spectrophotometer, or on a Cary Model 15 spectrophotometer. Spectrophotometric titrations were conducted on a Cary Model 15 spectrophotometer equipped with a titrametric cell and pH meter enclosed in a glovebox under an atmosphere of nitrogen. pH measurements were made by using a Radiometer Model 26 pH meter equipped with a standardized Model EA-125 Metrohm or GK-2302C Radiometer electrode. All calculations were performed on a Hewlett-Packard Model 9825A desk top calculator attached to Hewlett-Packard's Model 9864A digitizer and Model 9867A plotter. The time course analogue-type simulations were performed on a department-built, microprocessor-based, digital computer. Fourier transform NMR spectra were taken on a 100-MHz Varian Model XL-100 NMR spectrometer. The ESR spectra were taken on a Varian Model E4 ESR spectrometer equipped with a Varian Model V-4540 variable-temperature controller using a septum-stoppered ESR tube to exclude oxygen. Solutions of dihydro-N^5-ethyl-N^3-methyl-1-deazalumiflavin (C^1-FlEtH) in acetonitrile or ethanol and of known concentrations were kept in a septum-stoppered 5-mL Erylenmeyer flask inside a septum-stoppered, nitrogen-flushed cylindrical vessel. Aliquots of the reduced flavin were withdrawn through both septum caps with a gas-tight syringe (Hamilton) and used in the study of either the oxidized or reduced flavin forms. Oxygen concentrations in aqueous solutions were monitored by using a Yellow Springs Instrument Co. Biological Oxygen Monitor, Model 53.

Scheme I



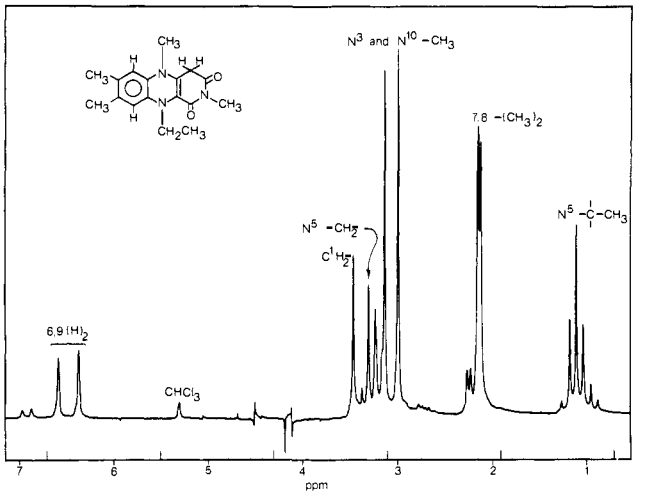
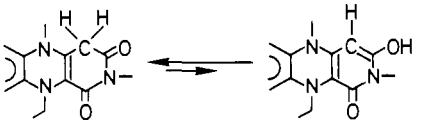


Figure 1. The 100-MHz NMR spectrum of C^1-FlEtH (CD_2Cl_2, ambient temperature). Tetramethylsilane (Me_4Si) was used as an external standard.

Results and Discussion

1,5-Dihydro-N^5-ethyl-N^3-methyl-1-deazalumiflavin (C^1-FlEtH) was synthesized by means of palladium-catalyzed hydrogenation of the N^5 imine formed on reaction of C^1-FlH_2 with acetaldehyde (Scheme I). The 100-MHz Fourier transform NMR spectrum of C^1-FlEtH in CD_2Cl_2 is shown in Figure 1. Two C^1 hydrogens are seen with a resonance at 3.46 ppm. The presence of two C^1 protons and their chemical shift indicate that the 2-position of C^1-FlEtH is predominately ketonic. Upon addition of deuterium oxide, the proton resonances at C^1 immediately disappear. Thus, keto–enol tautomerism is facile (eq 5). It is interesting to compare



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these findings in CD_2Cl_2 solvent to the results obtained by Spencer

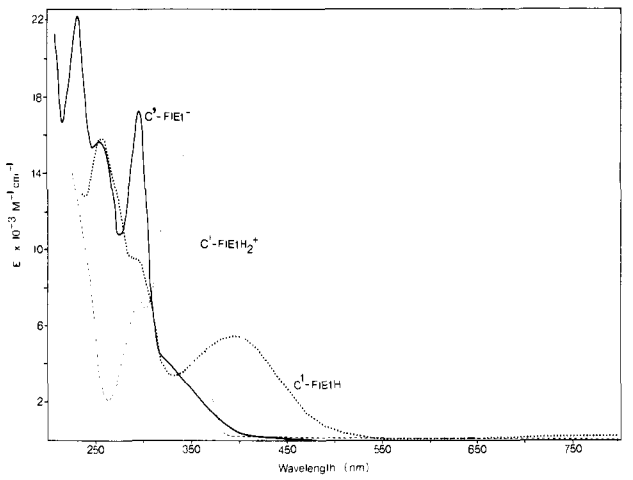


Figure 2. The UV-visible spectra of C^1-$FlEtH_2^+$, C^1-FlEtH, and C^1-$FlEt^-$ (8.03×10^{-5} M, 30 °C, μ = 1.0 M (KCl)). The spectrum of C^1-$FlEtH_2^+$ (dashed line) was recorded at pH 2.00 (0.25 M chloroacetate), that of C^1-FlEtH (dotted line) at pH 5.00 (0.25 M acetate), and the spectrum of C^1-$FlEt^-$ (solid line) at pH 8.23 (0.1 M phosphate).

et al. for dihydro-1-deazariboflavin in water.[6a] On the basis of slow and incomplete isotopic exchange of the C^1-hydrogen(s), they assigned an enol structure to the dihydro-1-deazariboflavin.

Ultraviolet-visible spectra of C^1-FlEtH at selected values of pH are shown in Figure 2. At pH values of 2.00, 5.00, and >8, the reduced N^5-ethyl-1-deazaflavin exists as the species C^1-$FlEtH_2^+$, C^1-FlEtH, and C^1-$FlEt^-$, respectively. The spectral characteristics of these acid-base species are compared to the spectral properties of the acid-base species of reduced N^5-ethyl-N^3-methyllumiflavin ($FlEtH_2^+$, FlEtH, and $FlEt^-$) in Chart I. Inspection of Chart I reveals that the spectra of C^1-$FlEtH_2^+$ and C^1-FlEtH are bathochromically shifted compared to $FlEtH_2^+$ and FlEtH. However, the spectral change which accompanies ionization of C^1-FlEtH to C^1-$FlEt^-$ is far greater than that which accompanies ionization of FlEtH to $FlEt^-$. It is believed that this is a consequence of formation of the enolate tautomer of C^1-$FlEt^-$ (see Chart I) from the predominately ketonic C^1-FlEtH. In contrast the negative charge in $FlEt^-$ is localized largely to N^1, so that $FlEt^-$ possesses an electronic configuration similar to that of FlEtH. It may be noted that the visible spectrum of the neutral 1,5-dihydro-1-deaza-riboflavin shows a λ_{max} at 480 nm (2000 M^{-1} cm^{-1})[6a] where as 1,5-dihydroriboflavin has no visible λ_{max}. Perhaps exchange of ribitol at N(10) by a methyl substituent and/or the proton at N(5) by an ethyl substituent brings about a change in conformation of the C^1-1,5-dihydroisoalloxazine ring that reflects itself in the visible absorption spectra and the proton-exchange rate at C^1.

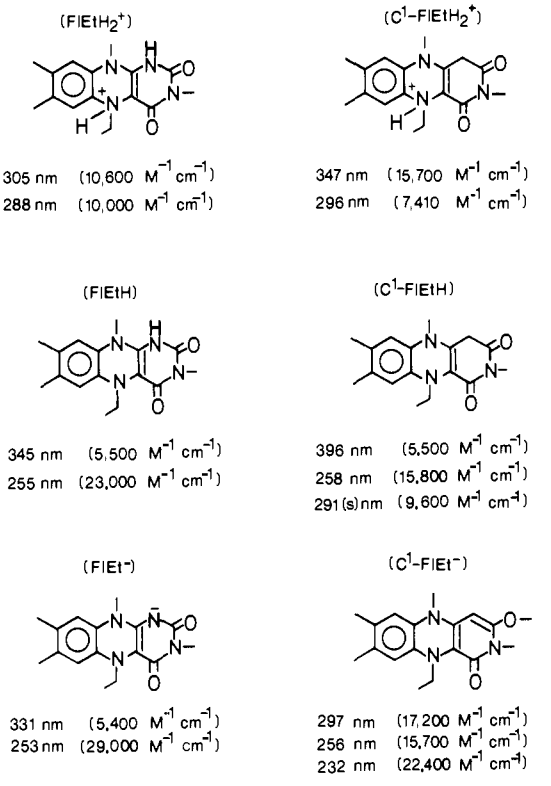
Spectrophotometric titration of C^1-FlEtH was conducted in water at 400 nm (30 °C, μ = 1.0 M) in an anaerobic cell (see Experimental Section) by using rigorously anaerobic reagents and 0.05 M ascorbic acid to assure the flavin remained reduced. As seen in Figure 2, only C^1-FlEtH absorbs appreciably at 400 nm. Neither ascorbic acid nor its univalent anion (pK_a's = 4.04 and 11.34)[11] absorb significantly in the region of 400 nm. The titration data and the simulated curve are shown in Figure 3. The observed titration curve indicates the presence of two acidic protons (eq 6). The pK_a value determined by Spencer et al. for the formation

$$C^1\text{-}FlEtH_2^+ \underset{+H^+}{\overset{pK_{a1} = 3.40, -H^+}{\rightleftharpoons}} C^1\text{-}FlEtH \underset{+H^+}{\overset{pK_{a2} = 6.62, -H^+}{\rightleftharpoons}} C^1\text{-}FlEt^- \quad (6)$$

of the reduced N^3-methyl-1-deazariboflavin anion is 6.3,[6a] and the pK_a value reported for the formation of $FlMe^-$ from FlMeH is 6.5.[3]

(11) Searjent, E. P.; Dempsey, B. "Ionization Constants of Organic Acids in Aqueous Solution" (IUPAC Chemical Data Series—No. 23); Permagon Press: New York, 1979; p 168.

Chart I



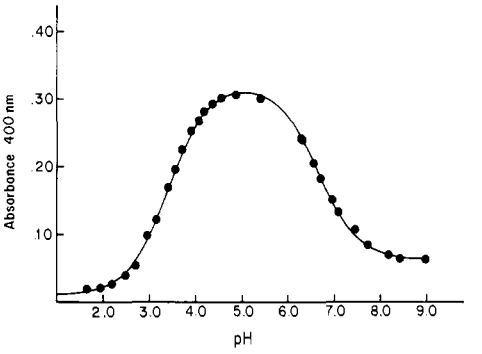
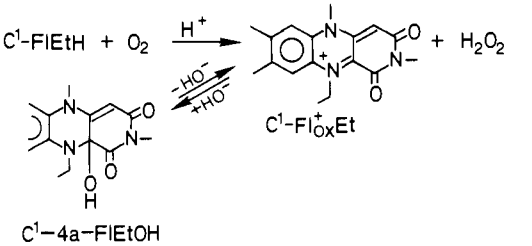


Figure 3. Spectrophotometric titration of 2×10^{-5} M C^1-FlEtH (400 nm) in the presence of 0.05 M ascorbic acid (30 °C, μ = 1.0 M (KCl)). The solid circles represent experimentally determined points, and the curve was computer generated by using ϵ_{400} values of 160 M^{-1} cm^{-1} (C^1-$FlEtH_2^+$), 4850 M^{-1} cm^{-1} (C^1-FlEtH), and 945 M^{-1} cm^{-1} (C^1-$FlEt^-$), and pK_a values of 3.40 and 6.62.

N^5-Ethyl-N^3-methyl-1-deazalumiflavinium cation (C^1-Fl_{ox}^+Et) was obtained from the oxidation of C^1-FlEtH with $^3O_2$ in acidic media. In aqueous solution, the oxidized flavin exists as the cation (C^1-Fl_{ox}^+Et) or pseudobase (C^1-4a-FlEtOH) depending on pH (see eq 7). Figure 4 shows the ultraviolet-visible spectrum of



(7)

C^1-Fl_{ox}^+Et and C^1-4a-FlEtOH. The brilliant green cation shows a maximum absorbance at 690 nm (5800 M^{-1} cm^{-1}), a bathochromic shift of about 140 nm compared to $FlEt_{ox}^+$ (λ_{max} at 548 nm (7950 M^{-1} cm^{-1})). The spectrum of C^1-4a-FlEtOH (λ_{max} at 370 nm (13 500 M^{-1} cm^{-1}), pH 6)) is also shifted bathochromically

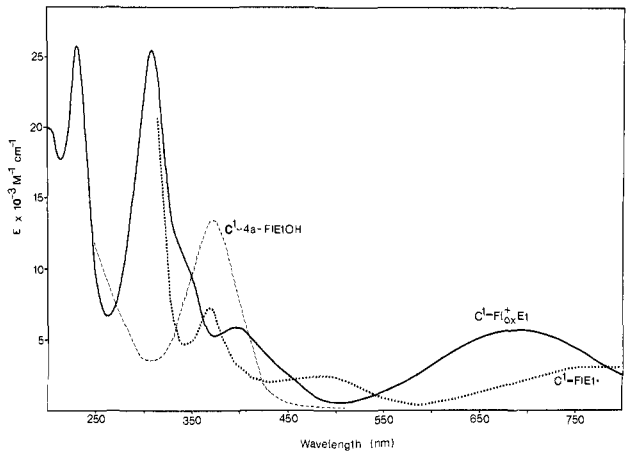
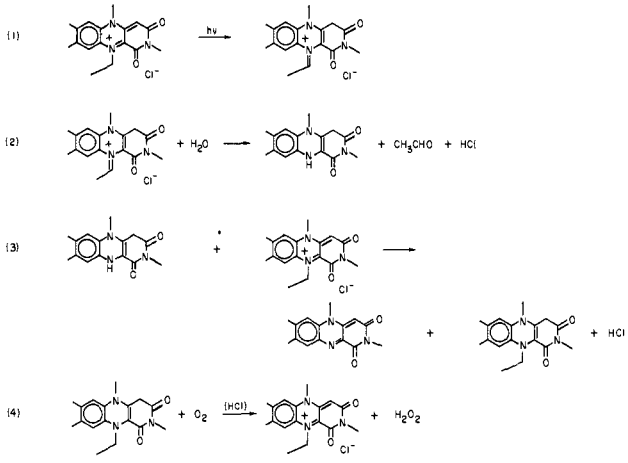


Figure 4. The UV–visible spectra of C^1-Fl_{ox}^+Et, C^1-4a-FlEtOH (8.03 × 10^{-5} M, 30 °C, μ = 1.0 M (KCl)), and C^1-FlEt· (1×10^{-4} M, 30 °C, μ = 0). The spectrum of C^1-Fl_{ox}^+Et (solid line) was recorded at pH 1 (0.1 M HCl), that of C^1-4a-FlEtOH (dashed line) at pH 6.00 (0.25 M phosphate), and that of C^1-FlEt· (dotted line) at pH 7.4 in presence of 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-*N*-oxy.

Scheme II



with respect to 4a-FlEtOH[9] ($(\lambda_{max}$ at 350 nm (9000 M^{-1} cm^{-1}), pH 5.5)). It has previously been noted that the visible spectrum of 1-deazariboflavin (λ_{max} at 535 nm (6800 M^{-1} cm^{-1})) is bathochromically shifted with respect to that of riboflavin (λ_{max} at 447 nm (12 300 M^{-1} cm^{-1})).[6a,8]

Attempts to prepare $(C^1$-$Fl_{ox}^+Et)ClO_4^-$ by oxidation of C^1-FlEtH with HNO_2 in the presence of $HClO_4$, as employed by Hemmerich[9] for the synthesis of $(Fl_{ox}^+Et)ClO_4^-$, lead to decomposition of the 1-deazaflavin. In separate experiments, it was found that C^1-Fl_{ox}^+Et is unstable in the presence of dilute HNO_2 whereas Fl_{ox}^+Et does not react with HNO_2. Since Fl_{ox}^+Et and C^1-Fl_{ox}^+Et differ only by the substitution of C^1 for N^1 it is likely that oxidation of C^1-Fl_{ox}^+Et by HNO_2 occurs at this position. Hydrogen peroxide also decomposes C^1-Fl_{ox}^+Et but not Fl_{ox}^+Et.

Reduction of C^1-Fl_{ox}^+Et by $Na_2S_2O_4$ or H_2/Pd provides C^1-FlEtH. Photolysis of solutions of C^1-Fl_{ox}^+Et in the presence of EDTA does not result in flavinium cation reduction to C^1-FlEtH (as in the photoreduction of Fl_{ox}^+Et) but results in deethylation producing N^3-methyl-1-deazalumiflavin (C^1-Fl_{ox}). Photolysis of C^1-Fl_{ox} (10^{-5} M) in the presence of excess EDTA produced only a very slow reaction (after several days, the reaction was still in initial phases). The mechanism for photodethylation of C^1-Fl_{ox}^+Et is shown in Scheme II. No accumulation of intermediates occurs in this process as isobestic points at 373, 453, and 608 nm are maintained throughout the reaction (H_2O solvent). The eventual fate of the ethyl group was found to be acetaldehyde by mass spectrum analysis (see Experimental Section). Admittance of oxygen to a solution prepared by anaerobic photolysis of C^1-Fl_{ox}^+Et is accompanied by a rapid spectral change. Prior to the admission of oxygen spectral analysis established that C^1-Fl_{ox} had been produced in 50% yield. Upon admission of oxygen, the products found represented 50% C^1-Fl_{ox}^+Et and 50% C^1-Fl_{ox}. Photodeethylation of C^1-Fl_{ox}^+Et forms C^1-FlH_2 and acetaldehyde. It may be noted that ascorbic acid reduced C^1-Fl_{ox}^+Et but not C^1-Fl_{ox}, indicating that C^1-FlH_2 has a greater reduction potential than does C^1-FlEtH. Therefore C^1-FlH_2 formed on photolysis reacts with remaining C^1-Fl_{ox}^+Et to form C^1-FlEtH and the observed C^1-Fl_{ox} in a 1:1 ratio (Scheme II). Upon admission of oxygen to the spent photolysis reaction solution, the C^1HFlEtH is oxidized to C^1-Fl_{ox}^+Et.

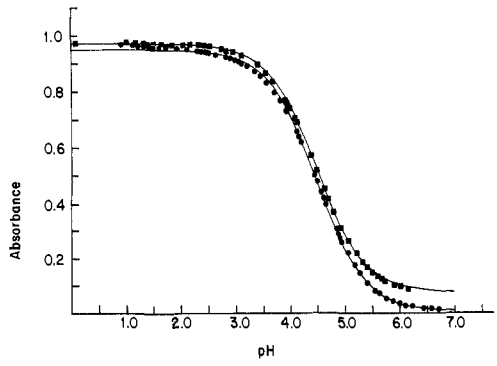


Figure 5. Spectrophotometric titration of 4.8×10^{-5} M C^1-Fl_{ox}^+Et (690 nm) and 3.58×10^{-5} M Fl_{ox}^+Et (548 nm) (30 °C, μ = 1.0 M (KCl)). The circles represent experimentally determined points for the titratio of C^1-Fl_{ox}^+Et, and the squares represent experimentally determined points for the titration of Fl_{ox}^+Et. The curves were computer generated by using ϵ_{690} of 5800 M^{-1} cm^{-1} (C^1-Fl_{ox}^+Et) and ϵ_{548} of 7950 M^{-1} cm^{-1} (Fl_{ox}^+Et) and the same pK_a value of 4.49.

A comparison of the kinetics and equilibria for pseudobase formation from the cations C^1-Fl_{ox}^+Et and Fl_{ox}^+Et has been made (eq 8 and 9). The pK_a's ($-\log(k_f^A/k_r^A)$) of eq 8 and 9 were

$$C^1\text{-}Fl_{ox}^+Et + H_2O \underset{k_r^A}{\overset{k_f^A}{\rightleftharpoons}} C^1\text{-4a-FlEtOH} + H^+ \quad (8a)$$

$$C^1\text{-}Fl_{ox}^+Et + HO^- \underset{k_r^B}{\overset{k_f^B}{\rightleftharpoons}} C^1\text{-4a-FlEtOH} \quad (8b)$$

$$Fl_{ox}^+Et + H_2O \underset{k_r^A}{\overset{k_f^A}{\rightleftharpoons}} \text{4a-FlEtOH} + H^+ \quad (9a)$$

$$Fl_{ox}^+Et + HO^- \underset{k_r^B}{\overset{k_f^B}{\rightleftharpoons}} \text{4a-FlEtOH} \quad (9b)$$

determined by spectrophotometric titration. Absorbance changes with pH were recorded at the λ_{max} of C^1-Fl_{ox}^+Et (690 nm) and the λ_{max} of Fl_{ox}^+Et (548 nm). At 30 °C and μ = 1.0 M, pK_a's associated with pseudobase formation from C^1-Fl_{ox}^+Et and Fl_{ox}^+Et are identical at 4.49 (Figure 5). At μ = 0 M (30 °C), the pK_a of C^1-Fl_{ox}^+Et was found to be 4.05. The pK_a for pseudobase formation from Fl_{ox}^+Et is slightly greater than the previously reported by Hemmerich[9] (pK_a = 4.1, μ = 0.1 M) and by Kemel and Bruice for the structurally similar $Fl_{ox}^+CD_3$ (pK_a = 4.15).[12] These results may be attributed to the favorable influence of increased ionic strength on the stabilization of charged species.

The pH vs. log (k_{obsd}) profiles ($k_{obsd} = k_f^A + k_r^A a_H + k_f^B(K_w/a_H) + k_r^B$) for the cation–pseudobase equilibria (eq 8 and 9) are shown in Figure 6. The simulated curves fitted to the experimental points of Figure 6 were generated from the empirical eq 10 (K_w is the autoprotolysis constant for water which at 30 °C is 1.477×10^{-14} M^2). Table I lists the rate constants of eq 10 employed to generate the theoretical curve which fits the experimental points of Figure 6. The values of k'_0 are buffer dependent and, when extrapolated to zero buffer, afford k_0 (=k_1K_a

$$k_{obsd} = k'_0 + k_1 a_H + k_2 K_w / a_H \quad (10)$$

(12) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 3955.

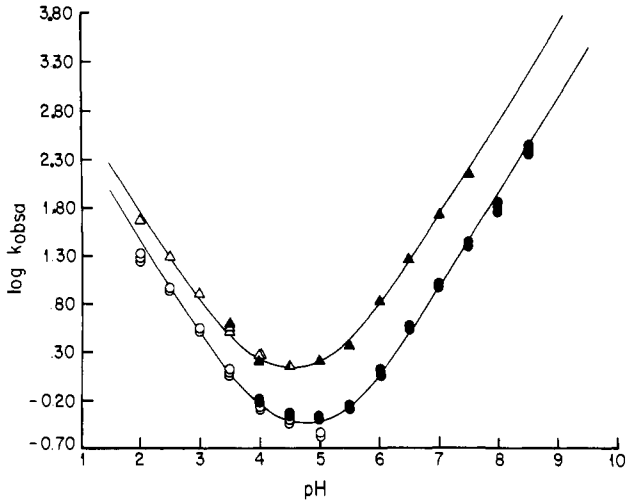


Figure 6. The pH vs. log k_{obsd} profiles for the reactions of eq 8 and 9 (30 °C, μ = 1.0 M). The triangles represent log k_{obsd} values obtained for the reaction of eq 9, and the circles represent log k_{obsd} values obtained from the reaction of eq 8. The open symbols represent log k_{obsd} values determined by mixing the pseudobase at pH 10 with acidic buffers (0.1 M) and observing the formation of cation (690 and 548 nm for the reactions of eq 8 and 9, respectively). The closed symbols represent log k_{obsd} values determined by mixing the cation at pH 3.0 with basic buffers (0.1 M) and observing the disappearance of cation. The curves were computer generated by using the values for the rate constants of eq 10 shown in Table I.

Table I. Rate Constants Found by Simulation To Fit Equation 10 to the pH vs. log k_{obsd} Profiles of Figure 6 for Pseudobase Formation

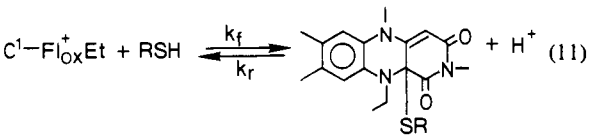
	C^1-Fl_{ox}^+Et	Fl_{ox}^+Et
k_0', s^{-1}	0.25	1.0
k_1, M^{-1} s^{-1}	3×10^3	6×10^3
k_2, M^{-1} s^{-1}	6×10^7	3.6×10^8

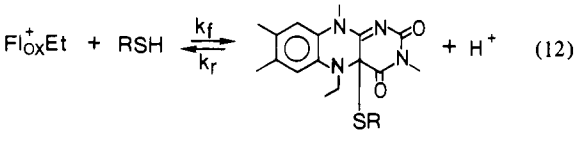
Table II. Rate Constants k_f' for Thiol Addition and k_r' for Adduct Dissociation (Eq 13) for the Reactions of C^1-Fl_{ox}^+Et (Eq 11) and Fl_{ox}^+Et (Eq 12) with Mercaptoethanol (pH 3.00, 0.25 M Chloroacetate, μ = 1.0 M, 30 °C (Metal Free))

	C^1-Fl_{ox}^+Et	Fl_{ox}^+Et
k_f', M^{-1} s^{-1}	1.6×10^3	4.5×10^3
k_r', s^{-1}	12	53

+ k_2K_w/K_a). The values of k_0 determined by buffer dilution experiments and, when calculated from the independently determined constants k_1, k_2, and K_a, are in close agreement.

Addition of mercaptoethanol to either C^1-Fl_{ox}^+Et or Fl_{ox}^+Et occurs at the 4a-position (eq 11 and 12). At pH 3.00 and under





the conditions of [mercaptoethanol] ≫ [flavinium cation], interference from pseudobase formation was minimal, and the reactions of either C^1-Fl_{ox}^+Et or Fl_{ox}^+Et with mercaptoethanol were pseudo first order to at least 3 half-lives of reaction. Plots of k_{obsd} vs. [mercaptoethanol] for the reactions of both flavinium cations

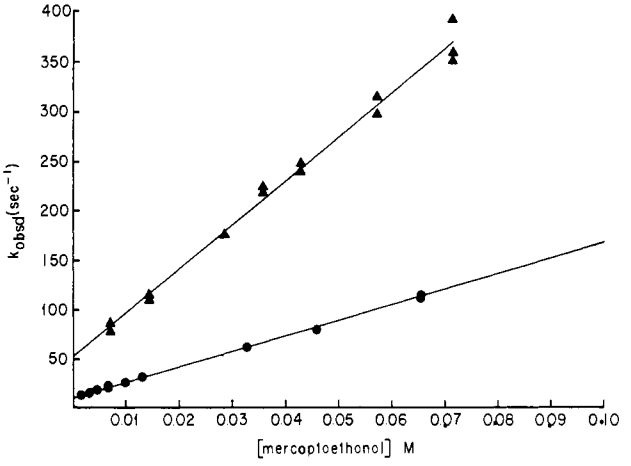


Figure 7. Plots of k_{obsd} for the reactions of C^1-Fl_{ox}^+Et (circles) and Fl_{ox}^+Et (triangles) with mercaptoethanol (pH 3.00, 30 °C, μ = 1.0 M vs. [mercaptoethanol]).

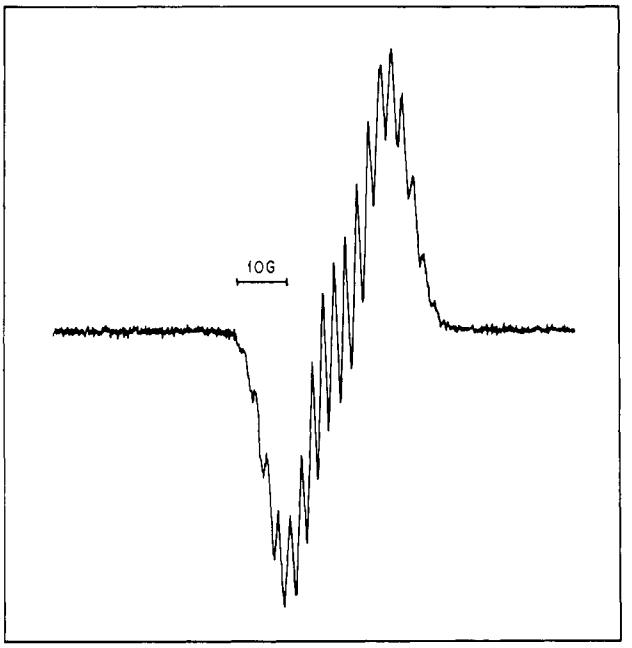


Figure 8. The ESR spectrum of C^1-FlEt· ($CHCl_3$, 25 °C).

were linear with positive intercept at [mercaptoethanol] = 0 (see Figure 7). The slopes of the lines correspond to the apparent second-order rate constants k_f' of eq 13 for thiol addition to the

$$k_{obsd} = k_f' [HOCH_2CH_2SH] + k_r' \quad (13)$$

cations and the intercepts are the observed first-order rate constants k_r' of eq 13 for adduct dissociation (see Table II). The reactions of eq 11 and 12 were found to be influenced by the presence of trace metal ion impurities. The observed rate of mercaptoethanol addition to C^1-Fl_{ox}^+Et was found to be increased by a factor of ~2 when care was not taken to remove trace metals from the buffer and the KCl employed to maintain μ. It is interesting to note that while the pseudobase pK_a's of eq 8 and 9 are identical for the two flavins, the thiol on and off rates are greater for Fl_{ox}^+Et than for C^1-Fl_{ox}^+Et.

The Radical C^1-FlEt· (λ_{max} at 775 nm (3000 M^{-1} cm^{-1}), H_2O, pH 7.4) is obtained when C^1-Fl_{ox}^+Et is partially reduced by sodium dithionite or when C^1-FlEtH is partially oxidized by $^3O_2$ or by 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-*N*-oxy (see Figure 4). Attempts to form the radical by reaction of C^1-FlEtH with ninhydrin (as found for FlEtH) gave only the oxidized flavin. An ESR spectrum of C^1-FlEt· is shown in Figure 8. The calculated *g* value is 2.002. The ESR spectrum shows a large background resonance that has no fine structure. Temperature variation

Scheme III

$$C^1\text{—FlEtH} + O_2 + H^+ \xrightarrow{k_1} C^1\text{—Fl}_{ox}{}^+\text{Et} + H_2O_2$$

$$C^1\text{—FlEtH} + C^1\text{—Fl}_{ox}{}^+\text{Et} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} 2C^1\text{—FlEt}\cdot + H^+$$

$$2C^1\text{—FlEt}\cdot + 2O_2 \xrightarrow{k_3} 2C^1\text{—Fl}_{ox}{}^+\text{Et} + 2O_2^{-}\cdot$$

$$2O_2^{-}\cdot + 2H^+ \xrightarrow{k_4} H_2O_2 + O_2$$

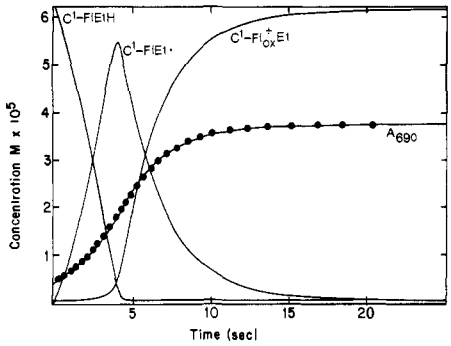


Figure 9. The time course of the reaction of C^1-FlEtH with O_2 (pH 3.00, 30 °C, μ = 1.0 M). The experimentally observed A_{690} values are shown as (●). The curves represent the concentrations of C^1-FlEtH (total, includes C^1-$FlEtH_2^+$), C^1-FlEt·, and C^1-$Fl_{ox}{}^+$Et, and the value of A_{690} calculated from the rate constants of Scheme III (described in text) using A_{690} values of 1461 M^{-1} cm^{-1} (C^1-FlEt·) and 6549 M^{-1} cm^{-1} (C^1-$Fl_{ox}{}^+$Et).

changes these spectra considerably, with the most resolved spectra at 55 °C (in chloroform) and a spectrum with no fine structure (although a strong signal is obtained) at −100 °C. Lack of fine structure is most likely due to intermolecular radical complexation at low temperature. The radical FlEt· has been prepared as a solid (see Experimental Section), and when mixed with ground KCl, it provides an ESR spectrum which is identical with that of the solution sample in chlorform at −100 °C (melting point for chloroform is −63.5 °C). The C^1-FlEt· radical probably has a structure similar to that of FlEt· which has been determined by Hemmerich et al. (see eq 4). Oxygen titration of the reduced form above pH 4.50 provides 100% radical at 0.5 mol equiv of oxygen.

The reaction of C^1-FlEtH with excess oxygen in aqueous solution (pH 3.00) forms oxidized N^5-ethyl-N^3-methyl-1-deazalumiflavinium cation (C^1-$Fl_{ox}{}^+$Et) and H_2O_2 (see eq 7). The cation C^1-$Fl_{ox}{}^+$Et, though oxidizable by H_2O_2, is stable for extended periods of time in the presence of the low values of $[H_2O_2]$ generated in these experiments (i.e., $\sim 10^{-4}$ M). Oxygen titration established that 1 mol of oxygen is consumed on reaction with 1 mol of dihydroflavin. Iodometric analysis confirmed that the product of reduction of $^3O_2$ is H_2O_2 (~93%). The rate of reaction of C^1-FlEtH with O_2 was found to decrease with increasing acidity. It has been observed previously that the rate of $^3O_2$ oxidation of 1,5-dihydro-N^3-methyllumiflavin (FlH_2), 1,5-dihydro-N^5-methyl-N^3-methyllumiflavin (FlMeH),[3] 1,5-dihydroriboflavin, and 1,5-dihydro-1-deazariboflavin[6a] also decrease with decreasing pH. The order of reactivity with O_2 is thus C^1-$FlEt^-$ ≫ C^1-FlEtH > C^1-$FlEtH_2^+$ as noted with other flavins. C^1-FlEtH oxidation is not subject to buffer catalysis. The same observation has been made previously with the oxidation of FlMeH.[3] The time course for oxidation of C^1-FlEtH was found to fit the reaction sequence of Scheme III, identical with the reaction sequence proposed by Kemal and Bruice for the autoxidation of FlMeH.[3] The rate constants found to give a good fit to the sequence of reactions in Scheme III over a range of oxygen concentrations (0.25 M chloroacetate, pH 3.00; μ = 1.0 M (KCl); 30 °C; 690 nm) were determined by digital simulation (Figure 9) to be k_1 = 65 M^{-1} s^{-1}, $K_{pH} = k_2/k_{-2}$ = 1 × 10^6 M^{-1} s^{-1}/2 × 10^5 M^{-1} s^{-1} = 5, and k_3 = 630 M^{-1} s^{-1}. The value of k_1 = 65 M^{-1} s^{-1} for reaction of C^1-FlEtH with O_2 (pH 3.00, 30 °C) can be compared to the value of 29 M^{-1} s^{-1} reported for reaction of FlMeH with O_2 (pH 4.85, 30 °C).[3] Thus C^1-FlEtH is more reactive with O_2 than is FlMeH. Spencerr et al. have reported that 1,5-dihydro-1-deazariboflavin

Scheme IV

$$FlRH + O_2 \rightleftharpoons FlRH^{+}\cdot + O_2^{-}\cdot$$

$$FlRH^{+}\cdot \rightleftharpoons FlR\cdot + H^+$$

$$FlR\cdot + O_2^{-}\cdot \rightleftharpoons 4a\text{—}FlROO^-$$

$$4a\text{—}FlROO^- + H^+ \rightleftharpoons 4a\text{—}FlROOH$$

$$4a\text{—}FlROOH + H^+ \rightleftharpoons Fl_{ox}{}^+R + H_2O_2$$

is oxidized by O_2 more rapidly than is 1,5-dihydroriboflavin.[6a] The k_2 value of 10^6 M^{-1} s^{-1} for the comproportionation of C^1-FlEtH with C^1-$Fl_{ox}{}^+$Et is much less than the value of ≫10^7 M^{-1} s^{-1} for the comproportionation of FlEtH with $Fl_{ox}{}^+$Et (or FlMeH with $Fl_{ox}{}^+$Me). The k_2 value of 2 × 10^5 M^{-1} s^{-1} for C^1-FlEt· disproportionation (pH 3) also contrasts with that for disproportionation of FlEt· (or FlMe·) which is too small to detect at any pH. That is comproportionation of FlEtH with $Fl_{ox}{}^+$Et is irreversible. The k_3 value of 630 M^{-1} s^{-1} for the reaction of C^1-FlEt· with O_2 (pH 3) is much greater than the value of 8 M^{-1} s^{-1} reported for the reaction of FlMe· with O_2 (independent of pH between 4.85 and 7). These observations suggest that the C^1-FlEt· is of higher free energy (ΔG) relative to C^1-FlEtH and C^1-$Fl_{ox}{}^+$Et than is FlEt· (relative to FlEtH and $Fl_{ox}{}^+$Et). This suggestion is further supported by the observation that ninhydrin oxidizes FlEtH to FlEt· but oxidizes C^1-FlEtH to C^1-$Fl_{ox}{}^+$Et. It has been suggested elsewhere[3] that the reaction of FlRH with O_2 (k_1 of Scheme III) occurs via the sequence of reactions shown in Scheme IV. Support for this mechanism of Scheme IV has come from recent experiments demonstrating that $O_2^{-}\cdot$ can either couple with FlEt· to form 4a-FlEtOO$^-$ or reduce FlEt· to yield $FlEt^-$ and O_2.[13] The other reactions of Scheme IV have been described elsewhere.[3,4,14] Perhaps the greater reactivity of O_2 with C^1-FlEtH than with FlMeH (discussed above) is due to a faster rate of coupling of C^1-FlEt· with $O_2^{-}\cdot$ than is the case for the coupling of FlMe· with $O_2^{-}\cdot$.

Oxidation of C^1-FlEtH with oxygen (DMF) is rapid and yields C^1-4a-FlEtOOH quantitatively (eq 14). The hydroperoxide is

$$C^1\text{—FlEtH} + O_2 \xrightarrow{DMF} \text{4a-FlEtOOH} \qquad (14)$$

quite stable in DMF. Oxidation of FlEtH with O_2 in DMF yields a maximum of 50% 4a-FlEtOOH (determined iodometrically). That 4a-FlEtOOH is obtained in a lower yield than is C^1-4a-FlEtOOH may be attributed to the fact that decomposition of 4a-FlEtOOH in DMF is competitive with its formation from FlEtH and $^3O_2$. Thus, the reaction of oxygen in DMF with C^1-FlEtH is faster than that with FlEtH, and the resultant C^1-4a-FlEtOOH adduct is far more stable than is the 4a-FlEtOOH. In *t*-BuOH the product of C^1-4a-FlEtH oxidation (λ_{max} at 366 nm) seems to be the pseudobase C^1-4a-FlEtOH. Iodometric analysis indicated that neither C^1-4a-FlEtOOH, H_2O_2, nor *t*-BuOOH were produced in the reaction. Acidification generated C^1-$Fl_{ox}{}^+$Et in quantitative yield (eq 7). Oxidation in *t*-BuOH gives a UV–visible spectrum (λ_{max} at 366 nm) identical with oxidation in a small amount of water followed by addition of *t*-BuOH. Plots of A_{366} vs. time were found to be sigmoidal, a feature characteristic of an autocatalytic process. Although C^1-4a-FlEtOOH is not obtained on O_2 oxidation of C^1-FlEtH in *t*-BuOH, it may be prepared in DMF and diluted into *t*-BuOH without decomposition. The inability to prepare C^1-4a-FlEtOOH in *t*-BuOH was found to be due to the fact that C^1-FlEtH reacts with C^1-4a-FlEtOOH to yield (exclusively) C^1-4a-FlEtOH at a rate sufficiently rapid

(13) Nanni, E. J., Jr.; Sawyer, D. T.; Ball, S. S.; Bruice, T. C. *J. Am. Chem. Soc.* **1981**, *103*, 2797.
(14) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 1635.

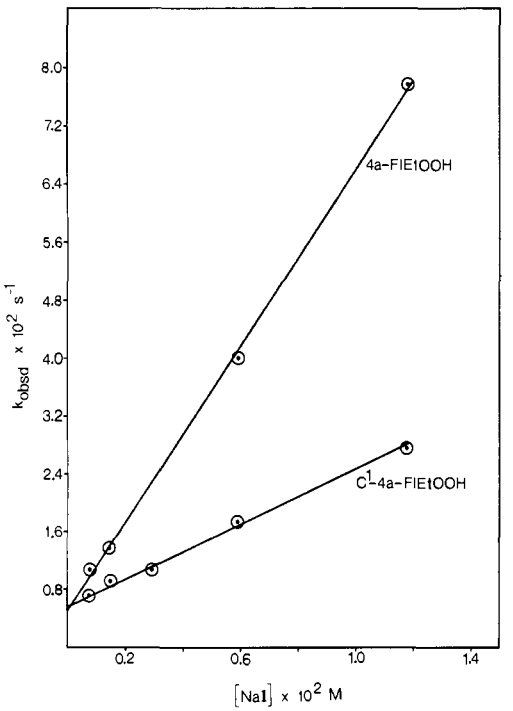


Figure 10. Plots of k_{obsd} (eq 19) for the reactions of C¹-4a-FlEtOOH and 4a-FlEtOOH with NaI in 95% EtOH/DMF (v/v, 3.0/0.4) at 30 °C vs. [NaI].

to allow the trapping of C¹-4a-FlEtOOH as it is formed from C¹-FlEtH and quantitatively convert both to C¹-FlEtOH. Thus, the reaction sequence of eq 15 and 16 accounts for the observations

$$\text{C}^1\text{-FlEtH} + \text{O}_2 \rightarrow \text{C}^1\text{-4a-FlEtOOH} \quad (15)$$

$$\text{C}^1\text{-FlEtH} + \text{C}^1\text{-4a-FlEtOOH} \rightarrow 2\text{C}^1\text{-4a-FlEtOH} \quad (16)$$

described concerning the autoxidation of C¹-FlEtH in *t*-BuOH. It might be noted that the reaction of C¹-4a-FlEtOOH with C¹-FlEtH in *t*-BuOH (eq 16) differs from that for reaction of 4a-FlEtOOH with FlEtH (in methanol).[3] In the latter instance the 4a-hydroperoxide is converted to 4a-FlEtOH but the FlEtH species is $1e^-$ oxidized to provide FlEt· (eq 17).

$$\text{4a-FlEtOOH} + \text{FlEtH} \rightarrow \text{4a-FlEtOH} + \text{FlEt}\cdot + \text{HO}\cdot \quad (17)$$

The hydroperoxide C¹-4a-FlEtOOH was characterized by its absorption maximum in DMF at 378 nm (ϵ_{378} = 13 000 M^{-1} cm^{-1}) and its rapid reaction with I^- to yield I_3^- (see eq 18).

$$\text{C}^1\text{-4a-FlEtOOH} + 3\text{I}^- + 2\text{H}^+ \rightarrow \text{C}^1\text{-4a-FlEtOH} + \text{I}_3^- + \text{H}_2\text{O} \quad (18)$$

The reaction of C¹-4a-FlEtOOH with NaI (30 °C) in 95% EtOH/DMF (v/v, 3.0/0.4) was conducted by observing the production of I_3^- at 358 nm (λ_{max} of I_3^- = 358 nm (25 000 M^{-1} cm^{-1})). Under conditions of $[I^-] \gg [\text{C}^1\text{-4a-FlEtOOH}]$, the increase in absorbance at 358 nm was pseudo first order to at least 3 half-lives of the reaction. A plot of k_{obsd} vs. $[I^-]$ was linear with positive intercept at $[I^-] = 0$ (Figure 10). The value of k_2 of eq 19 (where ROOH = C¹-4a-FlEtOOH) calculated from the slope

$$\frac{\text{d}[\text{I}_3^-]}{\text{d}t} = \frac{-\text{d}[\text{ROOH}]}{\text{d}t} = (k_1 + k_2[\text{I}^-])[\text{ROOH}] \quad (19)$$

$$k_{obsd} = k_1 + k_2[\text{I}^-]$$

of the plot of k_{obsd} vs. $[I^-]$ was found to be 1.8 M^{-1} s^{-1}. This k_2 value may be compared to k_2 values of eq 19 obtained similarly for the reactions of I^- with 4a-FlEtOOH (k_2 = 6.04 M^{-1} s^{-1}) and with H_2O_2 ($k_2 = 6 \times 10^{-3}$ M^{-1} s^{-1}) and *t*-BuOOH ($k_2 = 5 \times 10^{-4}$ M^{-1} s^{-1}) under identical conditions (95% EtOH/DMF, v/v, 3.0/0.4; 30 °C). The k_1 value of eq 19 obtained from the intercepts of plots of k_{obsd} vs. $[I^-]$ was found to be 5×10^{-3} s^{-1} for both C¹-4a-FlEtOOH and 4a-FlEtOOH (Figure 10). The yield of I_3^- in the reaction of either C¹-4a-FlEtOOH or 4a-FlEtOOH with I^-, calculated from the final A_{358} values (corrected for absorbance due to C¹-4a-FlEtOR or 4a-FlEtOR, R = H and/or Et, indistinguishable by visible spectra), was found to decrease as k_{obsd} approached k_1. Additionally, when either C¹-4a-FlEtOOH or 4a-FlEtOOH was allowed to react with excess NaI and to that final reaction mixture was added H_2O_2, the k_{obsd} value for I_3^- appearance determined from the spectral change occurring after addition of H_2O_2 was found to be identical with that k_{obsd} value expected for the reaction of H_2O_2 with I^-. Thus, the reactions of C¹-4a-FlEtOOH and 4a-FlEtOOH with I^- described above are not likely to represent flavin-catalyzed oxidation of I^- by H_2O_2.

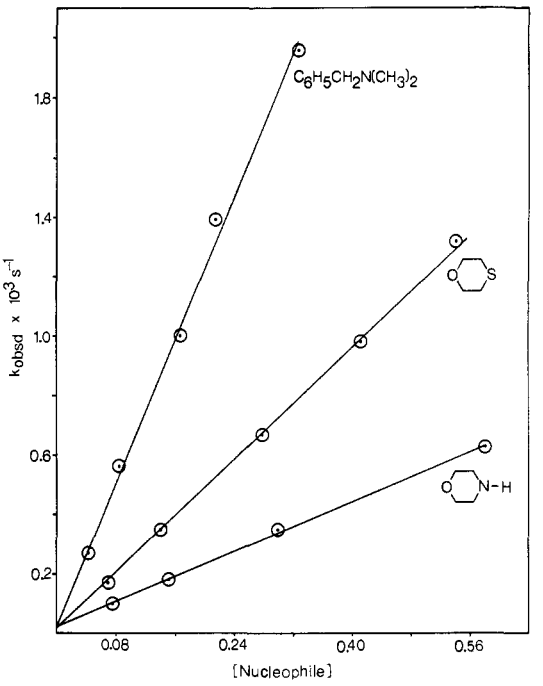
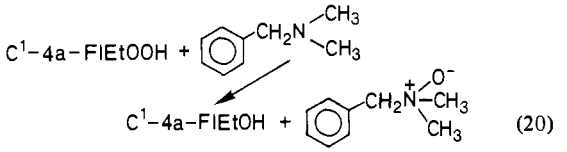


Figure 11. Plots of k_{obsd} for the reactions of C¹-4a-FlEtOOH with amines and thioxane in DMF (30 °C).

The reactions of C¹-4a-FlEtOOH (DMF) with *N*,*N*-dimethylbenzylamine (eq 20), *N*-methylbenzylamine, and morpholine



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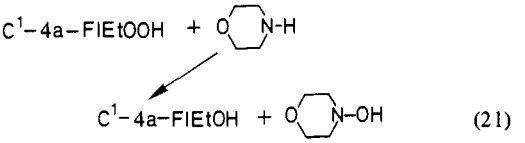


(21)

(eq 21) were investigated. In the case of the reaction of C¹-4a-FlEtOOH with the tertiary amine *N*,*N*-dimethylbenzylamine (eq 20), the products were determined as the C¹-4a-FlEtOH and the *N*-oxide of *N*,*N*-dimethylbenzylamine. The N-oxidation reaction of eq 20 is quantitative. In the case of the reactions of C¹-4a-FlEtOOH with the secondary amines *N*-methylbenzylamine and morpholine (eq 21), the products were determined as the C¹-4a-FlEtOH and the corresponding hydroxylamines. Again, the N-oxidations of the secondary amines were quantitative. The reactions of C¹-4a-FlEtOOH with the various amines were followed by observing the disappearance of C¹-4a-FlEtOOH (378 nm) with time. Under the conditions of [amine] ≫ [C¹-4a-FlEtOOH], the decrease in absorbance of the reaction mixtures at 378 nm was pseudo first order to at least 3 half-lives of reaction.

Table III. Second-Order Rate Constants k_2 (M^{-1} s^{-1}) of Eq 22 and Eq 23 for the Reactions of C^1-4a-FlEtOOH and 4a-FlEtOOH with Amines and Thioxane in DMF (30 °C)

nucleophile	10^3k_2-(C^1-4a-FlEtOOH)	10^2k_2-(4a-FlEtOOH)
$C_6H_5CH_2N(CH_3)_2$	5.9	6.9
morpholine	1.1	1.9
$C_6H_5CH_2NHCH_3$	1.5	
thioxane	2.4	4.2

Plots of the observed pseudo-first-order rate constants, k_{obsd}, vs. [amine] were linear with the same positive intercept in [amine] = 0 (Figure 11). Second-order rate constants, k_2 of eq 22 where

$$\frac{-d[ROOH]}{dt} = (k_1 + k_2[amine])[ROOH]$$

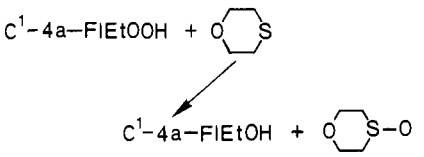
$$k_{obsd} = k_1 + k_2[amine] \quad (22)$$

ROOH = C^1-4a-FlEtOOH, determined from the slopes of plots of k_{obsd} vs. [amine] are shown in Table III. Included in Table III are k_2 values (eq 22) for the reactions of 4a-FlEtOOH in DMF with these same amines. The k_1 values of eq 22 for C^1-4a-FlEtOOH and for 4a-FlEtOOH, determined from the intercepts of the plots k_{obsd} vs. [amine] were found to be 2×10^{-5} s^{-1} and 3×10^{-4} s^{-1}, respectively.

The C^1-4a-FlEtOOH was also found to react with thioxane in DMF to yield C^1-4a-FlEtOH as the sole flavin product. Under the conditions of [thioxane] ≫ [C^1-4a-FlEtOOH], the decrease in A_{378} (C^1-4a-FlEtOOH) was pseudo first order to at least 3 half-lives of reaction and a plot of k_{obsd} vs. [thioxane] was linear with the same positive intercept (Figure 11) as that obtained in plots of k_{obsd} vs. [amine]. The value of k_2 (eq 23) obtained from

$$\frac{-d[ROOH]}{dt} = (k_1 + k_2[thioxane])[ROOH] \quad (23)$$

the slope of that plot is included in Table III along with the value of k_2 obtained similarly (DMF, 30 °C) for reaction of 4a-FlEtOOH with thioxane. Thioxane is most likely converted to its sulfoxide on reaction with C^1-4a-FlEtOOH (eq 24), as is the case for the reaction of 4a-FlEtOOH with thioxane in methanol.



(24)

At 0.7–2.8 M H_2O, hydrolysis of C^1-4a-FlEtOOH in DMF (to yield C^1-4a-FlEtOH) is pseudo first order with k_{obsd} for the reaction (2×10^{-5} s^{-1}) insensitive to the water content of the medium. As the water content is decreased (>0.4 M), k_{obsd} for the hydrolysis reaction decreases through the course of a run. These observations suggest that water acts as a trap for C^1-Fl_{ox}^+Et via eq 25 and 26

$$C^1\text{-4a-FlEtOOH} \overset{k = 2 \times 10^{-5}\ s^{-1}}{\rightleftarrows} C^1\text{-}Fl_{ox}^+Et + O_2H^- \quad (25)$$

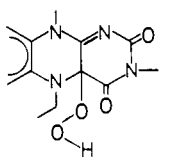
$$C^1\text{-}Fl_{ox}^+Et + H_2O \rightarrow C^1\text{-4a-FlEtOH} + H^+ \quad (26)$$

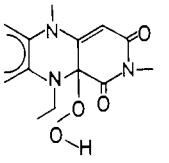
and that the k_{obsd} value of 2×10^{-5} s^{-1} represents ionization of C^1-4a-FlEtOOH (eq 25). Thus the value of k_1 (eq 22 and 23) = 2×10^{-5} s^{-1} also represents ionization of C^1-4a-FlEtOOH.

Summary

The biochemical significance of the present study revolves around a comparison of the chemistry of 4a-FlEtOOH with C^1-4a-FlEtOOH. It has previously been observed that 4a-FlEtOOH reacts with secondary and tertiary amines in *t*-BuOH to produce the same N-oxidation products found in the enzymatic oxidation of these amines by the hepatic flavoprotein microsomal oxidase[5] (see Introduction). In the case of tertiary amines, the N-oxidation products are the tertiary amine *N*-oxides. With secondary amines, the corresponding hydroxylamines are found.

Table IV. Relative Reactivities of 4a-FlEtOOH, C^1-4a-FlEtOOH, H_2O_2, and *t*-BuOOH toward Nucleophiles





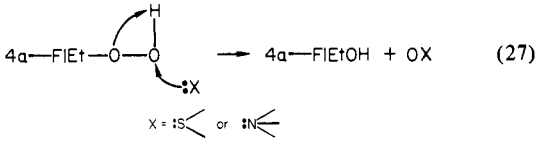
H_2O_2 +OOH

(A) (B) (C) (D)

Substrate	$1/k_r$ (relative) (A)	(B)	(C)	(D)
a I^-	1	3.3	10^3	10^4
b 3°-Amine	1	12	3.6×10^4	$>10^6$
b 2°-Amine	1	17	No Reaction	
c Alkyl Sulfide	1	17	10^4	2×10^5

[a] Relative rate constants (k_r) for the reactions of all four hydroperoxides with I^- were determined in the same medium, 95% EtOH/DMF (v/v) 3.0/0.4 (30 °C). [b] Rate constants (k_r) for the reactions of 4a-FlEtOOH with $C_6H_5CH_2N(CH_3)_2$ and morpholine were determined in both *t*-BuOH and DMF (30 °C). The rate constants (k_r) for the reactions of C^1-4a-FlEtOOH with $C_6H_5CH_2N(CH_3)_2$ and morpholine were determined in DMF (30 °C). The rate constants for the reactions of H_2O_2 and *t*-BuOOH with $C_6H_5CH_2N(CH_3)_2$ were determined in *t*-BuOH. Thus the $1/k_r$ value of 12 represents the ratio of second-order rate constants for the reactions of 4a-FlEtOOH to C^1-4a-FlEtOOH in DMF (30 °C) whereas the $1/k_r$ value of 3.6×10^4 represents the ratio of second-order rate constants for the reactions of 4a-FlEtOOH to H_2O_2 in *t*-BuOH (30 °C). [c] Rate constants (k_r) for the reactions of 4a-FlEtOOH with thioxane were determined in both methanol and DMF (30 °C). The rate constant (k_r) for the reaction of C^1-4a-FlEtOOH with thioxane was determined in DMF (30 °C). The rate constants for the reactions of H_2O_2 and *t*-BuOOH with thioxane were determined in methanol.

These are the same N-oxidation products produced by the reactions of C^1-4a-FlEtOOH in DMF with the tertiary amine *N,N*-dimethylbenzylamine (eq 20) and with the secondary amines *N*-methylbenzylamine and morpholine (eq 21). These results are consistent with the mechanism[5] proposed for the N-oxidation and S-oxidation reactions of 4a-FlEtOOH (see eq 27). Apparently,

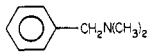


(27)

the reactivity of the flavin 4a-hydroperoxide toward nucleophiles is determined (at least in part) by the inductive polarization of the O–O bond. Thus substitution of the N^1-nitrogen of 4a-FlEtOOH for carbon (C^1-4a-FlEtOOH) results in a diminished polarization of the O–O bond which is reflected in the decreased rate constants for N-oxidation and S-oxidation reactions of C^1-4a-FlEtOOH compared to 4a-FlEtOOH (Table III). This point is further magnified by including H_2O_2 and *t*-BuOOH in the comparison. Table IV shows the relative reactivities of C^1-4a-FlEtOOH, 4a-FlEtOOH, H_2O_2, and *t*-BuOOH toward nucleophilic amines, thioxane, and iodide. In all cases, the order of reactivity is 4a-FlEtOOH > C^1-FlEtOOH ≫ H_2O_2 > *t*-BuOOH.

Free-Energy Relationships. The equilibrium constants for the addition reactions to form 4a-thiol and 4a-pseudobase adducts are essentially identical when the N^1 and the C^1 analogues are compared. Thus, the difference in free-energy contents of C^1 and N^1 flavinium cations and C^1- and N^1-4a adducts is similar. Inspection of Table V reveals that the $\Delta G^\ddagger$ values for the addition of nucleophiles to the 4a-position are more negative ($\Delta\Delta G^\ddagger$) for the N^1 flavinium cation as compared to the C^1-flavinium cation. This is also true for the oxidation reactions when 4a-FlEtOOH and C^1-4a-FlEtOOH are employed.

Table V. Free-Energy Differences ($\Delta\Delta G^{\ddagger}$ at 30 °C) for Additions and Retroadditions to the 4a-Positions of C^1-$Fl_{ox}{}^+Et$ and $Fl_{ox}{}^+Et$ and for Oxidations by C^1-4a-FlEtOOH and 4a-FlEtOOH

4a-pseudobase formation ($\Delta G^{\ddagger}_{C^1} - \Delta G^{\ddagger}_{N^1} = \Delta\Delta G^{\ddagger}$, kJ M^{-1})		
k_0,[a] s^{-1}	2.8	H_2O solvent
k_1, M^{-1} s^{-1}	0.5	H_2O solvent
k_2, M^{-1} s^{-1}	4.5	H_2O solvent
4a-thiol addition		
k_f, s^{-1}	2.6	H_2O solvent
k_r, s^{-1}	3.8	H_2O solvent
oxidations (M^{-1} s^{-1}) of		
I^-	3.0	95% EtOH
	6.1	DMF
	7.1	DMF
	7.1	DMF
4a-peroxide dissociation		
k_1, s^{-1}	6.7	DMF

[a] The k_0 values have been extrapolated to zero buffer concentration.

Inspection of Table V reveals that with a particular solvent, the values of $\Delta\Delta G^{\ddagger}$ ($=\Delta G^{\ddagger}_{C^1} - \Delta G^{\ddagger}_{N^1}$) are similar for the reactions investigated (save the acid-catalyzed process associated with k_1 of pseudobase formation). This observation supports a greater electrophilicity of the 4a-position of the N^1 flavins when compared to the C^1 flavins and a greater polarization of the peroxide moiety of the N^1-4a-FlEtOOH as compared to C^1-4a-FlEtOOH. Inspection of Table V also shows that the dissociation rate constants (for RS^-, HO^-, and HOO^-) from the 4a-position of the N^1 flavin are larger than those seen with the C^1 flavin. The ground states of C^1-$Fl_{ox}{}^+Et$ and $Fl_{ox}{}^+Et$ differ in free energy content by the same amount as the ground states of the products C^1-4a-FlEtX and 4a-FlEtX. Lowering of the free-energy content of the transition state for addition of X must also lower the ground state for dissociation of X and by the same value of $\Delta\Delta G^{\ddagger}$. Particularly noteworthy from Table V is the similarity of the $\Delta\Delta G^{\ddagger}$ for the N- and S-oxidation reactions in DMF to the $\Delta\Delta G^{\ddagger}$ for dissociation of peroxide from the C^1 and N^1 flavin hydroperoxides in that same solvent. Evidently the difference in polarization about the C_{4a}–OOH bond in 4a-FlEtOOH relative to C^1-4a-FlEtOOH is reflected in the difference in polarization about the $C_{4a}O$–OH bond and in facilitation of the N- and S-oxidation reactions.

From the present study, it is concluded that 1-carba-1-deaza FAD should, if recognized by the enzyme, serve as a cofactor for the hepatic flavoprotein microsomal oxidase in the N-oxidation of amines and the S-oxidation of sulfides.

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Conformational Barriers in Triplet 1- and 2-Naphthylcarbene. 2. Absolute Rate of Decay of Arylcarbenes by Electron Spin Resonance Spectroscopy

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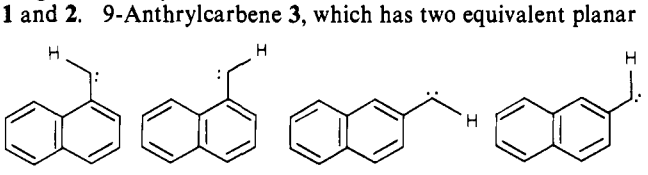
Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received November 17, 1980*

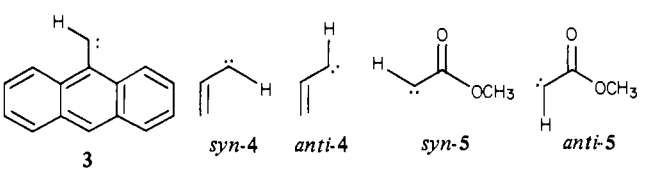
Abstract: The absolute decay of the syn and anti forms of 1- and 2-naphthylcarbene and 9-anthryl- and 2-pyrylcarbene have been measured at low temperature by ESR. The decay rates are pseudo first order and arise from reaction of the carbene with the crystalline host. The signal decay is nonexponential due to site problems in the matrix. The decay can be fitted to a log I vs. $t^{1/3}$ dependence. Matrix isotope effects reveal that the mechanism of carbene decay is by abstraction of hydrogen atom from the matrix by tunneling through a small barrier. The kinetics reveal that equilibration of the syn and anti forms of 1- and 2-naphthylcarbene is much slower than their reaction with the matrix. The activation barrier to syn–anti interconversion must be greater than 4.5–6.3 kcal/mol.

In 1965 Trozzolo et al. observed two distinct sets of triplet electron spin resonance (ESR) spectra in the low-temperature photolysis of 1- and 2-naphthyldiazomethane.[2] The spectra were assigned to the syn and anti forms of the matrix-isolated carbenes **1** and **2**. 9-Anthrylcarbene **3**, which has two equivalent planar



syn-1 *anti*-1 *syn*-**2** *anti*-**2**

forms, gives only a single spectrum. These observations confirmed previous theoretical and experimental observations that arylcarbenes are nonlinear.[3] Geometric isomerism in triplets has been observed subsequently in vinylcarbene **4** and carbomethoxycarbene[4] **5**. Interconversion of the two triplet forms may occur



3 *syn*-**4** *anti*-**4** *syn*-**5** *anti*-**5**

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