The effect of excess amine on the reaction rate was also studied. Sufficient trimethylamine was added to form 9-BBN·NMe$_3$ and provide 50% excess NMe$_3$. The results are shown graphically in Figure 1. The percentage of complex formation of (9-BBN)$_2$ with various amines was determined by the IR method described previously.[11a]

Competitive Studies of Alkene Pairs. The relative reactivities of alkenes toward (9-BBN)$_2$ in both CCl$_4$ and THF were studied by using the competitive method. The reaction procedure has been described previously.[1b] Several alkene pairs were chosen for comparison. A summary of the results is given in Table III.

Registry No. (9-BBN)$_2$, 21205-91-4; 9-BBN·THF, 76422-63-4; 9-BBN·SMe$_2$, 64045-91-6; 9-BBN·NMe$_3$, 64070-34-4; 9-BBN·*N*-methylpiperidine, 83605-84-9; 9-BBN·(2-Mepy), 70338-10-2; 9-BBN·TMEDA, 64045-93-8; 2-(9-BBN)·TMEDA, 83605-85-0; SMe$_2$, 75-18-3; CCl$_4$, 56-23-5; THF, 109-99-9; 2,5-dimethyl-THF, 1003-38-9; 1-hexene, 592-41-6; 2-methyl-1-pentene, 763-29-1; 3,3-dimethyl-1-butene, 558-37-2; cyclopentene, 142-29-0; 2-methyl-2-butene, 513-35-9; 1-methylcyclopentene, 693-89-0; *cis*-3-hexene, 7642-09-3; *cis*-4,4-dimethyl-2-pentene, 762-63-0; hexanal, 66-25-1; cyclohexanone, 108-94-1; cyclohexane, 110-82-7; benzene, 71-43-2; diethyl ether, 60-29-7.

Photochemical and Photophysical Studies of Amines with Excited Flavins. Relevance to the Mechanism of Action of the Flavin-Dependent Monoamine Oxidase‡

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Contribution from the Departments of Chemistry and Pharmacological Sciences, State University of New York, Stony Brook, New York 11794, and the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. Received November 30, 1981

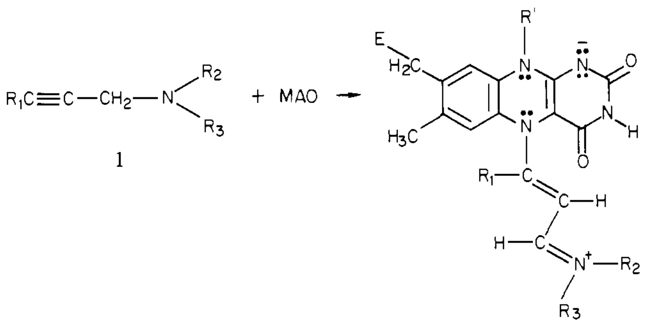
Abstract: The photochemical reactions between 3-methyllumiflavin (3-MLF) and β,γ-acetylenic, β,γ,δ-allenic, and saturated amines, in aqueous and nonhydroxylic solvents, have been investigated. The fluorescence of 3-MLF in nonhydroxylic solvents is efficiently quenched by all amines studied, with values of k_s (the rate constant for quenching of singlet 3-MLF) near the calculated rate of diffusion. Quenching rates in aqueous solution are pH and concentration dependent and indicate that the free amine is required for the observation of fluorescence quenching. Whereas amine quenching of singlet 3-MLF is nonproductive, quenching of triplet 3-MLF leads to adducts that can be isolated from the reaction of the allenic and β,γ-acetylenic amines but that are unstable in the case of saturated amines. The isolated products from β,γ,δ-allenic amines **2a** and **2b** are flavocyanines. The reactions of β,γ-acetylenic amines with triplet 3-MLF give more complicated product mixtures that include flavocyanines and C_{4a},N_5 adducts in similar amounts. These studies are consistent with a pathway involving one-electron transfer from amine to triplet 3-MLF, followed by successive proton and one-electron transfers leading to reduced flavin and iminium ion intermediates.

Introduction

Reactions between flavins and amines are of contemporary interest and can be induced photochemically[2a,d] or by the action of the flavoenzyme mitochondrial monoamine oxidase[2b,c] (E.C. 1.4.3.4, MAO) according to eq 1. A number of β,γ-unsaturated

$$\text{E-Fl} + R_1CH_2NR_2R_3 \rightarrow \text{E-FlH}^- + R_1CH{=}N^+R_2R_3 \xrightarrow{H_2O} R_1CHO + R_2NHR_3 \quad (1)$$

amines[3-5] not only serve as substrates but are also "suicide inhibitors"[6-8] of this enzyme, in that they become covalently linked to the flavin prosthetic group during the course of catalysis. In fact, treatment of MAO with β,γ-acetylenic amines (**1**) leads irreversibly to intensely absorbing adducts that have been characterized as flavocyanines[5,9,10] (eq 2). (Flavocyanines have also been prepared photochemically from the model flavin, 3-methyllumiflavin (3-MLF), and tertiary β,γ-acetylenic amines, but in this case C_{4a},N_5 adducts are formed in substantial amounts (eq 3).[4]) By comparison, β,γ,δ-allenic amines that are strict analogues of acetylenic suicide inhibitors do not yield flavocyanines when they are incubated with MAO but give a product possessing the spectrum of a "reduced (alkylated) flavin".[5,11]



E = apoprotein (2)
R' = ribityl-5'-phosphate AMP residue

To gain a deeper understanding of suicide inhibition of flavoenzymes, it is of interest to model events leading to covalent and irreversible attachment of such latent inhibitors to the target flavin prosthetic group. Although the means of activating the components in the dark, enzymatic process, and in the light-induced

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‡Dedicated to Professor William von Eggers Doering on the occasion of his 65th birthday.

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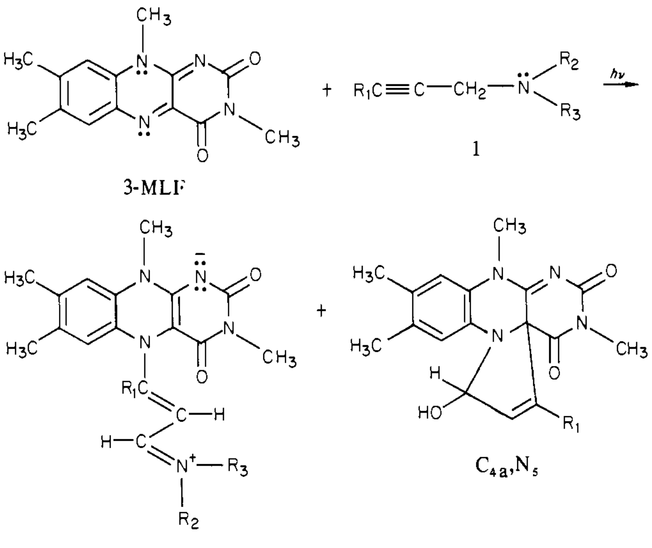


1a, $R_1 = R_2 = R_3 = CH_3$ (3)
b, $R_1 = R_2 = CH_3$; $R_3 = CH_2Ph$
c, $R_1 = H$; $R_2 = R_3 = CH_3$
d, $R_1 = H$; $R_2 = CH_3$; $R_3 = CH_2Ph$

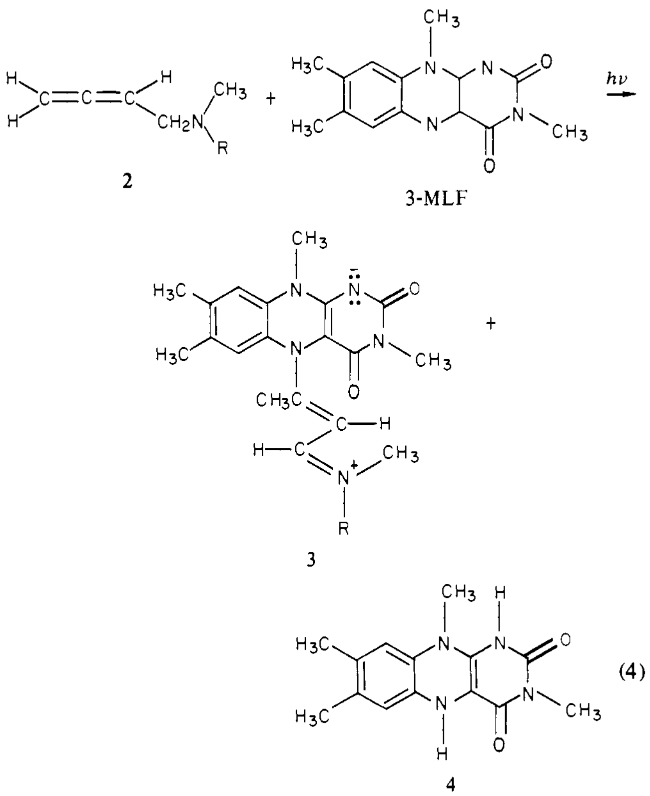
reaction are obviously distinct, the sequence of electron- and hydrogen-transfer processes may be common to both systems and give rise to analogous intermediates. A comparison of the reactions of β,γ,δ-allenic, β,γ-acetylenic, and simple saturated amines was thus undertaken to clarify the mechanism of the photochemical reaction of 3-MLF with amines.

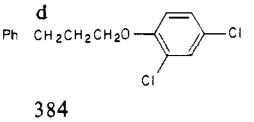
Results

Saturated Amines. Irradiation (365 or 436 nm) of degassed acetonitrile solutions of 3-MLF (6×10^{-5} M) in the presence of excess (5×10^{-4} M) triethylamine, tripropylamine, or *N,N*-dimethylbenzylamine results in bleaching of the 3-MLF 445-nm absorption band and disappearance of the 533-nm fluorescence. A slight excess of amine (ca. 1.2 equiv) is sufficient to effect complete conversion of 3-MLF. Upon admission of oxygen to the irradiated solutions, 90–95% of the initial 3-MLF absorption and fluorescence are promptly regenerated. The photoproducts were not identified.

Irradiation (365 nm) of 3-MLF (3×10^{-4} M) and tripropylamine (3×10^{-2} M) in oxygenated acetonitrile solution results in slower bleaching of the 3-MLF 445-nm absorption than is observed for degassed solutions. After complete or irreversible bleaching of 3-MLF absorption, the irradiated solution was analyzed by GC–MS for amine oxidation products. Nearly equal amounts of propanal and dipropylamine (5×10^{-3} M) are formed along with a smaller amount of propylidene-*n*-propylamine (2.5×10^{-3} M). The total yield of volatile amine oxidation products corresponds to the oxidation of ca. 25 amine molecules per initial 3-MLF molecule.

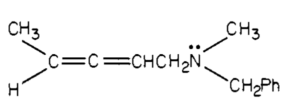
Unsaturated Amines. We attempted to obtain a stable compound that could serve as a model for the "reduced flavin-like" adduct of MAO and allenic amines[5,11] by irradiating **2a** or **2b** (eq 4, R = CH_3 or CH_2Ph) in the presence of 3-MLF with Pyrex-filtered light. Surprisingly, flavocyanines **3a** and **3b** were produced in a photoreaction that was isosbestic. The most striking example, the reaction of **2a**, displays isosbestic points at 411, 280, and 253 nm (Figure 1), and possesses a solvent-dependent electronic spectrum and a pK_a that is very typical of flavocyanines[2a] (see Experimental Section). As in the case of the pargyline (**1d**) adduct,[2a] 3-MLF can be regenerated from flavocyanine **3** by treatment with morpholine, but the latter reaction occurs more slowly.



R	a	b	c	d	e
	CH_3	CH_2Ph	$CH(CH_3)CH_2Ph$		H
λ_{max}(3), nm	376	383	385	384	382

Besides flavocyanine **3a**, ca. 20–30% of 1,5-dihydrolumiflavin **4**, a component unstable to oxygen, is formed as a coproduct (eq 4). The formation of **4** limits the yield of flavocyanine under anaerobic conditions, but by recycling the system (admitting oxygen to regenerate 3-MLF and then purging with argon, followed by rephotolysis) greater than 90% conversions of 3-MLF to **3a** can be obtained. Thus the reaction of **2a** with 3-MLF is preparatively useful and leads to essentially a single photoadduct.

The photoreaction to give high yields of flavocyanine also appears to be quite general for unencumbered tertiary and secondary allenic amines, as we have observed results similar to that of **2a**, for the *N*-buta-2,3-dienylamines **2b**–**e**, and *N*-penta-2,3-dienyl-*N*-methyl-*N*-benzylamine (**5**; eq 4). However, flavocyanine formation is dependent upon the pattern of substitution of the allene, as the geminally substituted allenes **6** and **7** do not produce the characteristically intense flavocyanine absorption at λ_{max} 380–390 nm.[2a,12]



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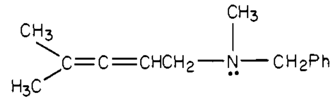

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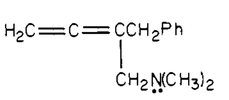

7

Although **6** and **7** react with 3-MLF at rates similar to those of **2a**–**e** and **5**, their photoproducts display reduced flavin-like spectra.[13b] Interestingly, absorption at 447 nm is substantially restored (60–70%) when the photoproduct of **6** and 3-MLF is treated with oxygen and allowed to stand at room temperature.

Primary allenic amines $H_2C{=}C{=}CHCH_2NH_2$ (**8**) or $CH_3CH{=}C{=}CHCH_2NH_2$ (**9**)) are slower to react by at least an order

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[12] Maycock, A. L. *J. Am. Chem. Soc.* **1975**, *97*, 2270.

[13] (a) As judged by the loss of intensity of the 447-nm band of 3-MLF. (b) However, this observation may be deceptive because steric hindrance in the flavocyanines formally derived from 6 and 7 may lead to a severely distorted flavocyanine with anomalous spectral properties.

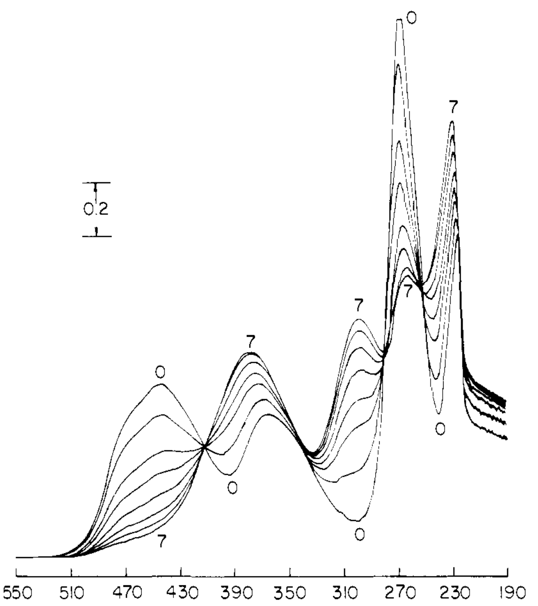

Figure 1. Time course of the Pyrex-filtered photoreaction between 3-MLF (0.5×10^{-4} M) and *N*,*N*-dimethyl-2,3-butadienylamine (**2a**) hydrochloride (0.5×10^{-3} M) in 0.1 M phosphate buffer (pH 7.2) containing 20% acetonitrile. Reaction was conducted for analytic purposes in a 3-mL cuvette, irradiating through a Pyrex-filter with a 450-W mercury lamp. Spectra were recorded at 0, 0.25, 1, 2, 4, 8, 12, and 20 min of irradiation respectively. Note isosbestic points at 412, 280, and 253 nm (*x*-axis scale is in nanometers). Similar results were obtained on a preparative scale.

Table I. Photophysical Properties of Singlet 3-MLF

solvent (ϵ)	λ_{max}, nm	ϕ_f	τ_s, ns	$10^{-7}k_f$, s^{-1}
dioxane (2.2)	531	0.59	8.56	6.9
tetrahydrofuran (7.6)	531	0.57	7.40	7.7
acetonitrile (39)	533	0.45	7.13	6.3
water (78)[a]	540	0.32	5.03	6.4

[a] 0.066 M phosphate buffer, pH 7.2 containing 20% acetonitrile.

of magnitude than their secondary or tertiary counterparts, and their photolysate possesses the spectrum of a reduced flavin.

In contrast to the specificity of the reactions of the allenic amines **2a** and **b** with 3-MLF stand the nonisosbestic photoreactions of the corresponding β,γ-acetylenic amines **1a** and **1b**, which each give a more complex reaction mixture than their allenic counterparts, containing both the C_{4a},N_5 adduct and flavocyanine as well as minor products (eq 3). The behavior of the acetylenic amines is well precedented by the work of Gärtner et al.,[2a] who have provided a large number of examples that conform to this pattern.

Fluorescence Quenching by Amines. The excited singlet state of 3-MLF is strongly fluorescent in both aqueous and nonaqueous solution.[14] The fluorescence quantum yield (ϕ_f) and energy corresponding to the fluorescence emission maximum (λ_{max}) decrease with increasing solvent polarity (Table I), as previously reported for lumiflavin.[15] Singlet lifetimes (τ_s) were measured by the time-correlated single-photon counting method. Fluorescence rate constants (k_f) are calculated from the measured quantum yields and lifetimes. The values of ϕ_f and τ_s obtained in tetrahydrofuran solution are in excellent agreement with recently reported values for 10-isopropyl-3-MLF in 2-methyltetrahydrofuran.[16] Values of k_f display little solvent sensitivity. Thus the solvent dependence of τ_s is the result of changes in the rates of intersystem crossing and nonradiative decay. The intensity of

Table II. Quenching of 3-MLF Fluorescence by Secondary and Tertiary Amines

		dioxane		acetonitrile	
amine	E_{D/D^+}[a]	$k_s\tau_s$, M^{-1} [c]	$10^{-9}k_s$, M^{-1} s^{-1}	$k_s\tau_s$, M^{-1} [c]	$10^{-9}k_s$, M^{-1} s^{-1}
Me_2NH		45	5.3		
Et_2NH	1.01[b]	31	3.6		
i-Pr_2NH	1.00[b]	22	2.6		
Me_3N	0.82[b]	32	3.7	78	11
Et_3N	0.73[b]	47	5.5	78	11
i-Pr_2NEt		51	6.0		
Me_2NCH_2Ph	0.90	61	7.1	61	8.6
$Me_2NCH_2CH{=}CH_2$	0.92	59	6.9	65	9.1
$Me_2NCH_2C{\equiv}CCH_3$ (1a)	1.01	55	6.4		
$Me_2NCH_2C{\equiv}CH$ (1c)	1.23	22	2.6	41	5.8
$PhCH_2N(CH_3)CH_2C{\equiv}CH$ (1d)				26	3.6
$Me_2NCH_2CH{=}C{=}CH_2$ (2a)		33	3.8		
$PhCH_2N(CH_3)CH_2CH{=}C{=}CH_2$ (2b)		35	4.1		

[a] Oxidation potential (V vs. Ag/AgI) in acetonitrile solution. [b] Values from ref 31. [c] From slope of linear Stern–Volmer plot for quenching of 3-MLF (6×10^{-5} M) by amines (1×10^{-3}–4×10^{-2} M).

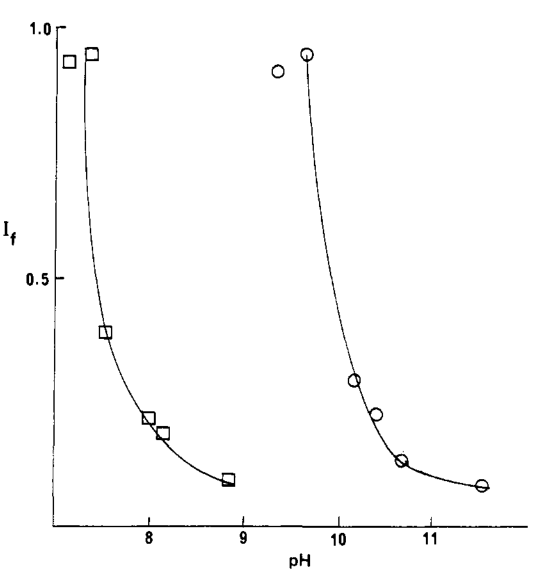

Figure 2. pH dependence of the fluorescence intensity of 3-MLF (I_f) in the presence of 0.05 M $Me_2NCH_2C{\equiv}CH$ (□) and Et_3N (○).

3-MLF fluorescence in acetonitrile solution is not appreciably quenched by oxygen (1 atm) and does not decrease upon exposure to room light for several hours. The fluorescence intensity of 3-MLF in aqueous solution is independent of pH from pH 7.2 to 12.3 but decreases in acidic solution, as previously reported for lumiflavin.[17]

The fluorescence of 3-MLF (6×10^{-5} M) in nonhydroxylic solvents is efficiently quenched by all of the secondary and tertiary amines investigated. Linear Stern–Volmer[18] plots (eq 5) provide

$$I_f^0/I_f = 1 + k_s\tau_s[(\text{amine})] \qquad (5)$$

the values of $k_s\tau_s$ given in Table II, where k_s is the rate constant for quenching of singlet 3-MLF by amine. Values of k_s are calculated from these $k_s\tau_s$ values and the measured 3-MLF lifetimes (Table I). All values of k_s are near the calculated rate of diffusion (k_{diff}) in both the nonpolar solvent dioxane (ϵ 2.2, $k_{diff} = 7 \times 10^9$ M^{-1} s^{-1}) and the polar solvent acetonitrile (ϵ 39, $k_{diff} = 3 \times 10^{10}$ M^{-1} s^{-1}).[18] The spectral distribution of 3-MLF fluorescence is not altered by amine quenching.

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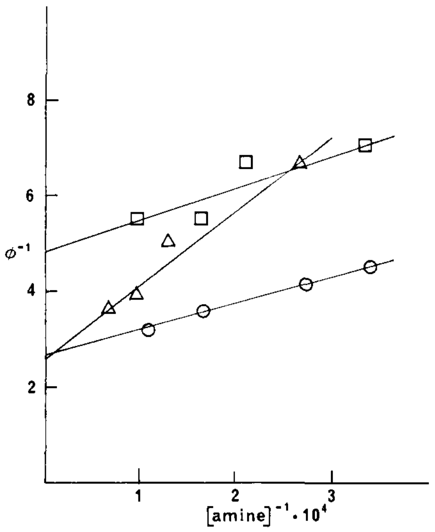

Figure 3. Dependence of the quantum yield for disappearance of 3-MLF upon amine concentration for Et_3N (○), $Me_2NCH_2CH{=}CH_2$ (△), and $PhCH_2N(CH_3)CH_2CH{=}C{=}CH_2$ (□).

Table III. Quenching of Triplet 3-MLF by Tertiary Amines in Acetonitrile Solution

amine	$\phi_{t\infty}$[a]	$10^{-4}\times k_t\tau_t$, M^{-1} [b]	$10^{-9}\times k_t^{-1}$, $M^{-1}\ s^{-1}$
Et_3N	0.38	4.5	5.8
$Me_2NCH_2CH{=}CH_2$	0.37	1.8	2.3
$PhCH_2N(CH_3)CH_2CH{=}C{=}CH_2$ (2b)	0.21	7.2	9.4

[a] Limiting quantum yield for disappearance of triplet 3-MLF from intercept of eq 7. [b] Intercept/slope (eq 7).

The fluorescence intensity of 3-MLF in aqueous solution (0.066 M phosphate buffer, pH 7.2, containing 20% acetonitrile) is unaffected by moderate concentrations (<0.01 M) of the amines listed in Table II (except pargyline (**1d**)), but is strongly quenched by amines in aqueous alkaline solution (pH 12.3). The pH dependence of 3-MLF intensity in the presence of *N*,*N*-dimethylpropargylamine (**1c**) and triethylamine (0.05 M) is shown in Figure 2. The onset of efficient fluorescence quenching occurs near the reported p*K* values of these amines, 7.05 and 10.65, respectively.[19] Thus free amine is required for the observation of fluorescence quenching, as observed by Fife and Moore[20] for quenching of singlet flavins by EDTA.

Triplet-State Quenching and Quantum Yields. Quantum yields for the disappearance of 3-MLF (6×10^{-5} M) in the presence of tertiary amines were determined by measuring the decrease in the 3-MLF absorption at 455 nm in degassed acetonitrile solution. The observed quantum yields (ϕ_{obsd}) increase with increasing amine concentration for low amine concentration ($<5\times 10^{-4}$ M) but decrease at the higher amine concentrations required for fluorescence quenching. Thus the photochemical reactions of tertiary amines with 3-MLF are triplet-state and not singlet-state reactions. Quantum yields for the triplet-state reaction (ϕ_t) can be obtained from the observed quantum yield and intersystem crossing quantum yield ($\phi_{isc} = 1 - \phi_f$)[21] according to eq 6. Least-squares fits to the data shown in Figure 3 for ϕ_t^{-1}

$$\phi_t = \phi_{obsd}/\phi_{isc} \tag{6}$$

$$\phi_t^{-1} = \phi_{t\infty}^{-1}(1 + (k_t\tau_t[\text{amine}])^{-1}) \tag{7}$$

vs. $[\text{amine}]^{-1}$ (eq 7) provide values of $k_t\tau_t$, where k_t is the rate constant for triplet quenching, τ_t is the triplet lifetime, and $\phi_{t\infty}$ is the quantum yield extrapolated to complete triplet quenching (Table III).[22] Values of k_t obtained from $k_t\tau_t$ and the reported triplet lifetime of 3-MLF ($\tau_t = 7.7 \pm 1.0$ μs[16]) given in Table III are near the rate of diffusion in acetonitrile solution.

Table IV. Quantum Yields for the Reaction of Triplet 3-MLF with Tertiary Amines

amine	ϕ_{H_2O}	ϕ_{CH_3CN}
Et_3N	0.037	0.31
Me_2NCH_2Ph	0.056	0.64
$Me_2NCH_2CH{=}CH_2$	0.009	0.45
$Me_2NCH_2C{\equiv}CCH_3$ (1a)	0.18	0.25
$Me_2NCH_2C{\equiv}CH$ (1c)	1.0	0.20
$Me_2NCH_2C{=}C{=}CH_2$ (2a)	0.074	0.25
$PhCH_2N(CH_3)CH_2CH{=}C{=}CH_2$ (2b)	0.84	0.15
$Me_2NCH_2CH{=}C{=}CHCH_3$	0.001	

Table V. Reversibility of 3-MLF–Amine Product Formation by Oxygen

amine	reversibility, %	amine	reversibility, %
Et_3N	100	$Me_2NCH_2C{\equiv}CH$	26
Me_2NCH_2Ph	100	$PhCH_2N(CH_3)\text{-}CH_2CH{=}C{=}CH_2$	63
$Me_2NCH_2CH{=}CH_2$	60	$Me_2NCH_2CH{=}C{=}CH_2$	30

Quantum yields for the disappearance of 3-MLF for a single amine concentration (5×10^{-4} M) were determined in acetonitrile and aqueous solution. Absolute values, corrected according to eq 6, are given in Table IV. The amine concentration was selected so as to attain >90% triplet quenching, on the basis of the $k_t\tau_t$ values in Table III, and <10% singlet quenching, on the basis of the $k_s\tau_s$ values in Table II for acetonitrile solution. For the amines listed in both Tables III and IV, the values of ϕ_t in acetonitrile solution agree within the limits of experimental error (±25%). The variation in ϕ_t with amine structure is more pronounced in aqueous than in acetonitrile solution. Since neither the concentration of the free amine nor the rate constants for quenching of triplet 3-MLF in aqueous solution are known, the quantum yield data in Table IV for aqueous solution is not indicative of the relative reactivities of the free amines with triplet 3-MLF. The quantum yield for reaction of triplet 3-MLF with *N*,*N*-dimethylpropargylamine decreases from 1.0 at pH 7.2 to 0.1 at pH 6.4.

The disappearance of 3-MLF upon irradiation with amines in acetonitrile solution can be reversed by the admission of air into the cell. The reversibility varies from 100% to 25% regeneration of the starting 3-MLF as measured by the absorbance at 445 nm (Table V).

Discussion

All of the secondary and tertiary amines investigated quench the singlet state of 3-MLF with rate constants at or near the diffusion-controlled limit in nonhydroxylic solvents (Table II). Similarly, the quantum yield and more limited kinetic data for the reaction of triplet 3-MLF with amines in acetonitrile solution (Tables III, IV) show little variation with amine structure. These results are indicative of rate-determining one-electron transfer from the amine to excited singlet or triplet 3-MLF to yield a radical ion pair (eq 8).[23,24] The quenching of aromatic hydro-

$$\text{3-MLF}^* + R_3N{:} \rightarrow \text{3-MLF}^{-}\cdot + R_3N^{+}\cdot \tag{8}$$

$$\Delta G_{ET} = -E^* - E_{A/A^-} + E_{D/D^+} - 0.06\ \text{eV} \tag{9}$$

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carbon and carbonyl excited states by amines is known to occur via one-electron transfer in cases where the free energy for electron transfer, calculated by using Weller's equation (eq 9),[23] is exothermic. With values of 2.6 and 2.1 eV for the singlet and triplet energies of 3-MLF (E^*),[16] –0.04 V for the reduction potential of 3-MLF (E_{A/A^-}), and 0.73 for the oxidation potential of triethylamine (E_{D/D^+}),[25] the calculated values for electron transfer from triethylamine to singlet and triplet 3-MLF are –1.9 and –1.4 eV, respectively. Since variations in secondary and tertiary amine oxidation potential with structure are relatively small (±0.3 V),[25] it is not surprising that for all of the amines investigated the rate of quenching of both singlet and triplet 3-MLF is at or near the diffusion-controlled limit in nonhydroxylic solvents. Small variations in k_s with amine structure such as the decrease with α branching for secondary amines (Table II) indicate that the rate of electron transfer may be sensitive to steric as well as electronic effects.[26]

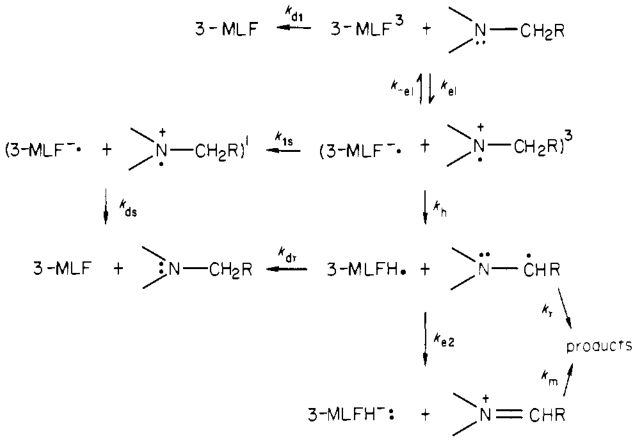
Direct spectroscopic evidence for one-electron transfer quenching of triplet flavin has recently been reported by Heelis and Phillips.[27] Subsequent to the decay of the triplet state of the flavin in the presence of various quenchers, a new transient is observed (λ_{max} 370 nm), which decays to form the neutral flavin radical. On the basis of its spectral characteristics, the new transient is proposed to be the flavin radical anion.

A one-electron transfer mechanism for quenching of both singlet and triplet 3-MLF by amines provides a plausible explanation for the efficient formation of products from the triplet-state (Table IV) in contrast to the singlet-state process. Singlet radical ion pair reactions must compete with highly exothermic back-electron transfer, which repopulates the reactant ground states.[23] Back-electron transfer in the triplet radical ion pairs is in general slow owing to its spin-forbidden nature. The rate of back-electron transfer for singlet radical ion pairs is highly dependent upon the energy gap between the radical ion pair and ground states and is expected to be large in cases such as 3-MLF/amines for which this gap is small (ca. 0.8 eV).[28] In cases where this gap is large (>2 eV, e.g., for many aromatic hydrocarbons) chemical reactions and exciplex fluorescence are observed to compete with back-electron transfer for singlet radical ion pairs and exciplexes.

The observation of efficient chemical reactions resulting from quenching of triplet but not singlet 3-MLF by amines finds analogy in the reactions of singlet and triplet fluorenone with amines.[29] Quantum yields for photoreduction of triplet fluorenone by tertiary amines are high (ca. 1.0) whereas little or no photoreduction occurs upon singlet quenching. Peters[30] has recently observed by picosecond spectroscopy that the singlet radical ion pair formed from fluorenone and the tertiary amine 1,4-diazabicyclo[2.2.2]octane decays within 60 ps of its formation. Thus the absence of photoreduction in the case of singlet fluorenone and amines is due to the short lifetime of the singlet radical ion pair.

One-electron oxidation of amines renders the α hydrogens of the resulting cation radical far more acidic than those of the neutral amine.[31] Photoreduction of aromatic hydrocarbons and carbonyls by amines is known to involve transfer of the α hydrogen to the excited species via an electron-transfer, proton-transfer mechanism.[32,33] Evidence for moderately efficient proton transfer from the amine cation radicals to 3-MLF anion radical is provided by the quantum yields for disappearance of 3-MLF in degassed acetonitrile solution with both saturated and β,γ-unsaturated amines (Table IV). Quantum yields in aqueous solution display much larger variation, reflecting the need for an unprotonated amine in order to obtain triplet quenching as well as singlet quenching (Figure 2). The quantum yields for the least basic amines (e.g., **1c** and **2b**) are higher in aqueous than in acetonitrile solution, possibly due to water-catalyzed proton transfer in the triplet radical ion pair. Bryce-Smith and co-workers[34] have observed that the photoaddition of tertiary amines to benzene is catalyzed by proton donors.

Scheme I. Mechanism of Reaction of Triplet 3-MLF with Tertiary Amines



Further evidence for the formation of an α-aminoalkyl radical in the reactions of triplet 3-MLF with tertiary amines is provided by the formation of the same products from tripropylamine in oxygenated solutions as reported by Davidson and co-workers[35] for photooxidation by triplet dyes and carbonyl compounds. Like these sensitizers, 3-MLF is recycled in these reactions, allowing the conversion of ca. 25 mol of amine to oxidation products per mol of 3-MLF consumed. Davidson[35] proposes that the reaction of the α-aminoalkyl radical with molecular oxygen leads to the formation of amine oxidation products (propanal, dipropylamine, propylidene-*n*-propylamine).

Unsaturated Amines. The reaction of triplet 3-MLF with β,γ-unsaturated (tertiary) amines results in the formation of covalent adducts (vide supra). Formation of adducts via the triplet and not the singlet state of 3-MLF rules out possible reaction mechanisms involving concerted cycloaddition or ene reactions[36] between the excited flavin and ground-state amine. The addition of triplet flavin to the γ carbon of unoxidized allenic and acetylenic amines is also improbable since (a) this path is unavailable to saturated amines, yet the similar quantum yields for reaction of triplet 3-MLF with saturated and unsaturated amines support a common photochemical mechanism, and (b) the allenic (primary) amines do not form flavocyanines and are much poorer substrates than the corresponding tertiary amines. If a radical addition mechanism were operative, triplet 3-MLF would be expected to add to the less hindered primary allenic amines, at least as fast as it adds to tertiary allenic amines.

Alternatively, a hydrogen atom α to nitrogen could be transferred directly to triplet 3-MLF. Although the bond strength and accessibility of the α-CH in allenic (primary) amines make it at

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least as favorable for abstraction by triplet 3-MLF, as compared with the corresponding hydrogen atom in allenic (tertiary) amines, the allenic (tertiary) amines are much more efficient quenchers of triplet 3-MLF than are their primary allenic counterparts. Consequently, it is unlikely that direct hydrogen transfer is responsible for quenching of the triplet 3-MLF.

Mechanistic Considerations. (A) The Photochemical Reaction. A plausible mechanism for the reaction of the triplet 3-MLF with both saturated and unsaturated amines is outlined in Scheme I. Irreversible electron transfer ($k_{\rm el} \gg k_{\rm -el}$) accounts for the observation of diffusion-controlled triplet quenching in acetonitrile solution (Table III). The resultant triplet radical ion pair can undergo either a proton transfer to yield a radical pair ($k_{\rm h}$) or intersystem crossing to the singlet radical ion pair ($k_{\rm is}$), which rapidly decays to the ground state ($k_{\rm ds}$). The radical pair can react further to yield products ($k_{\rm r}$ or $k_{\rm e2}$, vide infra) or can disproportionate to yield starting materials ($k_{\rm dr}$). Thus the observed quantum yields are dependent upon the 3-MLF intersystem crossing quantum yield (eq 6), the efficiency of triplet quenching by amine to yield the radical ion pair, the efficiency of radical pair formation from the triplet radical ion pair, and the efficiency of product formation from the radical pair (eq 10). The observed

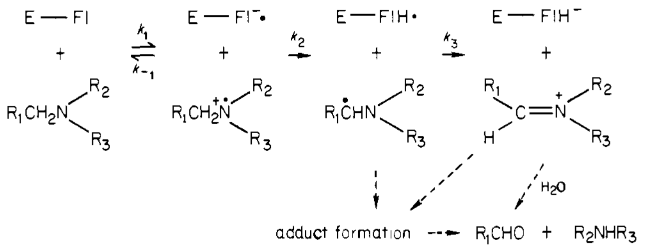
$$\Phi_{\rm obsd} = \Phi_{\rm isc}\left(\frac{k_{\rm dt}}{k_{\rm dt} + k_{\rm el}}\right)\left(\frac{k_{\rm h}}{k_{\rm h} + k_{\rm is}}\right)\left(\frac{k_{\rm r} + k_{\rm e2}}{k_{\rm r} + k_{\rm e2} + k_{\rm dr}}\right) \quad (10)$$

quantum yield thus provides only a lower bound for the efficiency of any one step in the overall reaction mechanism. It is interesting to note that the highest quantum yields are observed in aqueous solution for $Me_2NCH_2C{\equiv}CH$ (**1c**) and $PhCH_2N(CH_3)CH_2HC{=}C{=}CH_2$ (**2b**) (Table IV). Both of these amines are sufficiently basic to provide a significant concentration of free amine at pH 7.2. Both amine cation radicals have highly acidic α hydrogens, promoting the efficient formation of the radical pair. Finally, both amines efficiently form flavin–amine photoadducts (eq 3, 4). All three factors are necessary for the observation of a high quantum yield.

The reaction of triplet 3-MLF with unsaturated amines yields a radical pair that can react further to form covalent adducts either directly via radical combination ($k_{\rm r}$) or via a second one-electron transfer ($k_{\rm e2}$) to generate a conjugated iminium ion to which reduced flavin can add in Michael fashion ($k_{\rm m}$; Scheme I). Both the propargyl (**1**) and allenic (**2**) amines can form unusually stable α-aminoalkyl radicals owing to conjugation with both the electron-donating nitrogen nonbonding orbital and the electron-withdrawing π orbital. Such "merostabilized" radicals are known to be efficient free radical scavengers.[37] However, free radical coupling of α-aminopropargyl radicals is known to occur preferentially at the α vs. γ carbon.[37,33a] Thus a radical coupling mechanism would require some special interaction or conformation for the α-aminoalkyl–flavin radical pair in order to yield the γ-coupling product required for flavocyanine formation. The extraordinarily low oxidation potential of α-aminoalkyl radicals and the low second reduction potential of flavins[38] lead us to favor the electron-transfer Michael addition pathway for covalent bond formation (Scheme I, $k_{\rm e2}$, $k_{\rm m}$). This pathway also accounts for covalent bonding to the γ vs. α carbon of the unsaturated amines.

The products of the reaction of triplet 3-MLF with saturated tertiary amines have not been characterized owing to their rapid and quantitative reaction with oxygen to regenerate 3-MLF (Table V). The most likely possibilities are fully reduced flavin or an unstable covalent adduct. By analogy to the reactions of unsaturated amines with triplet 3-MLF, covalent adduct formation with saturated amines might occur via either radical coupling or a second one-electron transfer followed by Michael addition (Scheme I). In either case, the adduct formed would be a geminal diamine, which should be hydrolytically unstable and may react with oxygen by a mechanism similar to that of fully reduced flavin.

Scheme II. Proposed Mechanism for Enzymatic Amine Oxidation



The formation of potentially oxidatively unstable α-coupling products could also account for the partially reversible nature of the reaction of triplet 3-MLF with unsaturated amines (Table V). Photoxidation of β,γ-unsaturated amines by singlet *trans*-stilbene is known to yield both the more stable "merostabilized" and the less stable α-aminomethyl radicals, which can only yield α-coupling products.[33a]

(B) The Enzymatic Reaction. The mechanism in the case of the enzyme monoamine oxidase is more problematical, as it is not obvious how the components are activated for reaction. With assumption of values in the range of 0.0 to +0.25 V for the unknown reduction potential of MAO and 1.5 V for the oxidation potential of naturally occurring primary amine substrates,[25] electron transfer from amine to MAO would be endothermic by 1.25–1.50 V. Thus the barrier to electron transfer would appear to be formidable; however, enzymes are known to utilize binding energy to lower activation energies to values significantly less than those calculated or measured for nonenzymatic prototype reactions. It is possible to envision a rate-accelerating catalytic role for MAO that is difficult to mimic in model systems. The combination of (a) lowering the oxidation potential of the amine, perhaps by flattening the bonds to nitrogen,[31] and (b) raising the reduction potential of the flavin (flavins are known to be environmentally sensitive) can be accomplished by utilizing the binding energy of the enzyme–substrate complex to distort the potentially reactive components. Enzyme–substrate binding may also orient the amine lone pair and the α-C–H so as to optimize orbital overlap for both the electron-transfer and proton-transfer processes. Finally, enzyme–substrate binding provides a longer interaction time than is possible for 3-MLF, thus allowing substantially slower reactions to occur for the enzymatic vs. nonenzymatic oxidation.

The idea of simple rate-determining electron transfer for MAO oxidation of amine has been suggested[39] but cannot be general since many MAO-catalyzed reactions exhibit positive isotope effects ($k_{\rm H}/k_{\rm D} > 1$) when the α carbon is substituted with deuterium.[40,41] A mechanism for enzymatic amine oxidation that is in keeping with the observed isotope effects and our observation for photochemical amine oxidation is shown in Scheme II. Slow electron transfer to give a radical ion pair may be reversible ($k_{-1} > k_2$) and thus not rate determining. Highly reversible electron transfer is not unreasonable in view of the highly exothermic nature of this process. Depending on the relative magnitude of k_{-1} and k_2, isotope effects between 1 and 7 might be observed.

Regio- and Chemoselectivity of the Flavin Reactions. β,γ-Acetylenic amines **1a** and **1b** give flavocyanines upon incubation with MAO (eq 1) and with triplet 3-MLF lead to a complex mixture of products that includes flavocyanines and C_{4a},N_5 adducts (eq 2). The photochemical reactions of 3-MLF with allenic amines **2a** and **2b** lead to essentially a single adduct, the flavocyanine **3**, which differs from the allenic amine–MAO adduct (which has the spectrum of a reduced (alkylated) flavin).[5] Despite the differences in the reaction course, a reasonable working hypothesis can be formulated that rationalizes the formation of the known products. The primary assumption is that the enzymatic inhibition

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and the photoreaction resemble the turnover of substrate by enzyme in that they are all redox reactions leading to imines and reduced flavin. Hence both the C_{4a},N_5 and flavocyanine adducts can be accounted for by assuming a competition between the nucleophilic C_{4a} and N_5 atoms of the reduced flavin, for the strongly electrophilic acetylenic or (allenic) iminium ion.[2d] If this view is tenable, then the matter of which products are formed is reduced to considerations of factors determining regio- and chemoselectivity in the system.

The greater regioselectivity of the photochemical addition of simple allenic vs. acetylenic amines with 3-MLF may reflect the steric and symmetry properties of the cumulene and acetylene functionalities. The cylindrically symmetrical triple bond may be accessible to attack by both C_{4a} and N_5 atoms, whereas the allene may have very specific orientational demands for orbital overlap, preferring the more sterically accessible N_5 position of the flavin to the relatively encumbered C_{4a} locus. The formation of distinct enzymatic and photochemical adducts from amines **2a** and **b**, with the flavin prosthetic group and 3-MLF, respectively, can probably be traced to the forces that determine binding at the active site of the enzyme (and thus site-specific attack on the flavin prosthetic group). These forces are certain to be different from those that determine the structures of the excited-state complexes, radical pairs, or radical ion pairs formed from triplet flavins with amines, and are likely to be highly constraining. The fact that little is known regarding the nature of such photochemical and enzymatic complexes limits our ability to predict regio- and chemoselectivity.

Experimental Section

Materials. *N*,*N*-Diisopropylethylamine (Aldrich), triethylamine (Matheson), *N*,*N*-dimethylallylamine (Eastman), *N*,*N*-dimethylpropargylamine (Aldrich), *N*,*N*-dimethylbenzylamine (Aldrich), trimethylamine (Eastman), tri-*n*-propylamine (Aldrich), dimethylamine (Eastman), diethylamine (Aldrich), and diisopropylamine (Aldrich) were fractionally distilled prior to use. Those amines that boil below 100 °C were distilled from sodium hydroxide. 3-Methylumiflavin was prepared by the method of Hemmerich.[42] Allenic amines were synthesized according to procedures developed by Claesson.[43]

Solvents. *p*-Dioxane (Aldrich 99+%) was used as received. Tetrahydrofuran (Aldrich 99.9%) was refluxed over sodium and distilled immediately before use. Acetonitrile (Aldrich 99+%; Burdick and Jackson, distilled in glass) was refluxed over calcium hydride and distilled immediately before use. Buffered aqueous acetonitrile was prepared from water that had been passed over a series of ion-exchange columns. Monobasic sodium phosphate (Mallinckrodt) was dissolved in water, and the resulting solution was basified with sodium hydroxide to pH 7.2. The solution was then made to be 20% by volume of acetonitrile.

General Procedures. Melting points were determined in open glass capillaries by using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Model 727 spectrophotometer. NMR spectra were recorded with Varian Associates EM-360 and HFT-80 instruments and a Bruker 360-MHz spectrometer with tetramethylsilane as the internal standard. Fluorescence spectra were recorded on a Hitachi Perkin-Elmer MPF 44A spectrophotometer equipped with a corrected spectra microprocessor unit. Fluorescence quantum yields were measured relative to quinine sulfate.[44] The fluorescence lifetime of 3-MLF was measured by using time-correlated photon counting. The 445-nm line of a mode-locked argon ion laser was used as the excitation source.

Quantum yield measurements were conducted on an optical bench with a Bausch and Lomb SP 200-W super-pressure short arc mercury lamp interfaced with a Bausch and Lomb 0.25-m high-intensity grating monochromator. Solutions were degassed by nitrogen bubbling and maintained at 22 ± 1 °C during irradiation. Light intensities at 435 nm were measured by potassium ferrioxalate actinometry.[45] The change in 3-MLF concentration was determined by measuring the decrease in absorption at 440 nm at 20% conversion and is corrected for the small absorbance of photoproducts at this wavelength.

The oxidation potentials of the amines and the reduction potential of 3-MLF were measured with the assistance of John Begemann (Northwestern) by cyclic voltammetry in acetonitrile vs. Ag/AgI in a three-electrode cell with Pt working and auxilliary electrodes. The measurements were computer controlled and run at a scan rate of 1 V/s. The solutions were 0.1 M in tetra-*n*-butylammonium perchlorate and 10^{-4} M in 3-MLF or 10^{-3} M in amine. They were deoxygenated by bubbling with nitrogen prior to measurement. The first and second irreversible oxidation peaks of 3-MLF were observed at −0.038 and −0.338 V, respectively.

The products of 3-MLF-sensitized oxidation of tripropylamine in the presence of oxygen were identified by comparison of GC–MS retention times and fragmentation patterns with authentic samples of propanal, dipropylamine, and propylidene-*n*-propylamine. A 10 ft × $^1/_8$ in. column containing 8% Carbowax 1000 and 2% KOH on Chromosorb G provided product separation. Mass spectra were recorded with a Hewlett-Packard 5958A GC–MS.

Photolyses of 3-Methyllumiflavin with Amines. Typically, 3-methyllumiflavin and a 10-fold excess of amine (0.5×10^{-3} M) in 20% acetonitrile–80% (0.1 M) phosphate buffer, pH 7.2, was irradiated with Pyrex-filtered light from a 450-W mercury lamp, and the reaction was worked up according to Gärtner.[2a] The general pattern in the photoreaction of tertiary amines containing buta-2,3-dienyl and penta-2,3-dienyl residues is the formation of flavocyanines (as evidenced by intense absorption ϵ 20 000, λ_{max} 375–385 nm) and minor amounts of 1,5-dihydro-3-MLF (**4**).

For buta-2,3-dienyldimethylamine (**2a**) 20–30% of **4** is formed during the reaction. But by recycling the reaction mixture (admitting oxygen, purging with argon followed by rephotolysis), we obtained greater than 90% yields of a yellow-brown powder that was characterized as 5-(3′-(dimethyliminio)-1′-methylpropenyl)-3-methyl-1,5-dihydrolumiflavin betaine (3): mp 207–210 °C dec; NMR ($CDCl_3$) δ 2.22 (3 H, s), 2.28 (3 H, s, 7-, 8-CH_3), 2.36 (3 H, s, 1′-CH_3), 2.94 (3 H, s, 10-NCH_3), 3.28 (3 H, s, 3-NCH_3), 3.34 (3 H, s), 3.39 (3 H, s, $N(CH_3)_2$), 5.27–5.42 and 5.75–5.89 (br, 2′-H, two olefinic resonances split in ratio of 2:1), 6.82 (1 H, br s, 9-H), 6.97 (1 H, br, 6-H), 7.53 (1 H, d, $J_{2,3}$ = 12 Hz, 3′-H); UV λ_{max} (ϵ, mM^{-1} cm^{-1}; $CHCl_3$) 413 nm (13.0), 313 (12.6); UV (CH_3OH) 387 (16.6), 301 (15.3); UV (pH 7.2, phosphate buffer) 376 (17.3), 299 (15.8); UV (pH 2.0, HCl) 368 (19.0), 299 (12.7); UV (CF_3CO_2H) 368 (19.0), 299 (12.7); $\nu_{max}^{CHCl_3}$ 1640 cm^{-1} (2-, 4-C=O); pK_a = 4.9. The solvent dependence of the electronic spectrum of 3 is very typical of flavocyanines[2a] as is the value of the pK_a. Whereas **2a** gives almost exclusively the flavocyanine 3**a** (80% aqueous acetonitrile, pH 7.2, 0.1 M phosphate buffer, Figure 1), the irradiation of 3-MLF with the isomeric acetylenic amine, 2-butynyldimethylamine (**1a**), under essentially identical conditions gives two major isolable photochemical products, the flavocyanine 3**a** in 20% yield and 1-hydroxy-3,5,8,10,11-pentamethyl-1*H*,8*H*-benzo[*g*]pyrrolo[2,1-*e*]pteridine-4,6-dione (**12**), 32%, as yellow crystals (ether): mp 194 °C; NMR ($CDCl_3$) δ 1.79 (3 H, s, 3-CH_3), 2.19 (6 H, s, 10-, 11-CH_3), 3.27 (3 H, s, NCH_3), 3.57 (3 H, s, NCH_3), 4.47 (1 H (disappears in D_2O), d, $J_{1\text{-}OH}$ = 11.6 Hz, 1-OH), 5.60 (1 H, d (collapses with D_2O), 1-H), 5.84 (1 H, s 2-H), 6.72, 6.99 (2 s, 2 H, 9-12-H); IR ν_{max} ($CHCl_3$) 1665 (6-C=O), 1705 (4-C=O), 3450 (1-OH) cm^{-1}; UV λ_{max} (ϵ, mM^{-1} cm^{-1}) (CH_3OH) 368 nm (4.0) 200 (sh, 5.7), 279 (13.0), 234 (17.3) 214 (20.0).

Reactions were conducted for analytical purposes in a 3-mL cuvette by irradiating through a Pyrex-filter with a 450-W mercury lamp. With the above concentrations, reduction of λ_{max} 447 nm to half its initial value was accomplished in ca. 30 s for **5**, **2b–d**, 6, and 7 and in 10–20 min for primary amines **8** and **9**.

Acknowledgment. This work was supported by Grant No. 1-R01-NS13220 from the National Institutes of Health to A.K., by Biomedical Research Support Grant No. 2S07-RRO573607, and by a stipend from the donors of the Petroleum Research Fund, administered by the American Chemical Society, to B.K., for which we express our deep gratitude. We thank especially Dr. A. Claesson and C. Sahlberg of Uppsala University, Biomedical Center, Uppsala, Sweden, for the synthesis of select allenic amines. F.D.L. gratefully acknowledges support from the National Science Foundation Grant No. CHE 80-26020.

Registry No. 1a, 2588-78-5; **1b**, 2520-99-2; **1c**, 7223-38-3; **1d**, 555-57-7; **2a**·HCl, 83365-37-1; **2b**, 16719-33-8; **2c**, 73586-41-1; **2d**, 73586-42-2; **2e**, 83365-38-2; 3**a**, 74592-22-6; 3**b**, 83365-39-3; **4**, 23542-57-6; **5**, 67280-45-9; 6, 67280-44-8; **7**, 73586-40-0; **8**, 73586-36-4; **9**, 73586-37-5; **12**, 74592-23-7; 3-MLF, 18636-32-3; MAO, 9001-66-5; Et_3N, 121-44-8; Me_2NCH_2Ph, 103-83-3; $Me_2NCH_2CH{=}CH_2$, 2155-94-4; $Me_2NCH_2CH{=}C{=}CHCH_3$, 36555-45-0; tripropylamine, 102-69-2; propanal, 123-38-6; dipropylamine, 142-84-7; propylidene-*n*-propylamine, 7707-70-2.

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