Mechanistic Analysis of the 3-Methyllumiflavin-Promoted Oxidative Deamination of Benzylamine. A Potential Model for Monoamine Oxidase Catalysis

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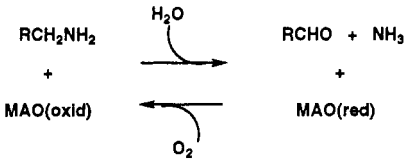
Abstract: An investigation designed to probe flavin-promoted oxidative deaminations of amines is described. 3-Methyllumiflavin (3MLF) has been found to promote clean conversion of benzylamine to *N*-benzylbenzaldimine under acid (HCl or $MgCl_2$) catalyzed, thermal (80 °C) conditions. The reaction is subject to multiple turnover. Amine structure (1° > 2° > 3°) and α-electrofugal group leaving ability ($TMS^+ > H^+$) are found to influence reactivity. In addition, the internal d-isotope effect measured for the ground-state reaction by using $PhCHDNH_2$ is found to be 4.3, a value greatly different from the 1.6 measured for the excited-state (SET) reaction run under nearly identical (20 °C rather than 80 °C) conditions. Additional mechanistic information has come from observations which show that benzylamine undergoes instantaneous reaction with N^5-ethyl-3-methyllumiflavinium perchlorate to produce a stable 4a-adduct. In addition, this adduct is transformed quantitatively to *N*-benzylbenzaldimine when treated with benzylamine at 60 °C. These results suggest that the ground-state reaction of $PhCH_2NH_2$ promoted by 3MLF follows a polar mechanism involving formation of and elimination in a covalent 4a-(*N*-benzylamino)dihydroflavin intermediate.

Flavins play an important role as cofactors in a wide variety of biological redox reactions.[1] Dehydrogenation reactions of the general type, H—X—Y—H → X=Y, represent a major family of processes mediated by the subclass of flavoenzymes known as oxidases. Included in this group are the oxidative transformations of alcohols to carbonyl products, of amines to imines, and of fatty acid esters to their α,β-unsaturated analogs.[2] Perhaps the most interesting enzymes in this class are the monoamine oxidases (MAO A and B), which catalyze oxidative deamination reactions of biogenic amines such as norepinephrine, serotonin, and dopamine.[3] The intense attention given to the MAOs results from the influential role they play in the metabolism of these neurologically active substances and the observation that MAO inhibitors are useful in the treatment of depression and related mental disorders.[4]

The overall catalytic cycle for the MAO (and related flavoenzyme) catalyzed dehydrogenation reactions consists of two stages (Scheme I). In the first, oxidative half reaction, the covalently linked flavin (FAD) moiety is converted to its reduced form ($FADH_2$) in concert with amine oxidation which provides the imine precursor of ammonia and the carbonyl product. Regeneration of oxidized form of the enzyme then occurs in the second step by the action of oxygen. Although a number of investigations have been targeted at determining the chemical mechanism for the oxidative deamination step in the cycle, little progress has been made.[3,5] As a consequence of this and of our continuing interest in amine oxidation chemistry,[6] several years ago we initiated a program to explore model chemistry occurring between amines and flavins. We felt that a full characterization of the reactions occurring in these systems might provide information about unique features of the diverse mechanistic pathways followed and, as a result, useful knowledge relevant to the MAO catalytic process.

Scheme I



In earlier efforts, we uncovered photochemical models for both catalysis of amine oxidative deamination by the MAOs[7] and MAO inactivation by cyclopropylamines.[8] In the photochemical model for catalysis, reaction of the triplet excited state of 3-methyllumiflavin (3MLF) with an amine is initiated by single-electron transfer (SET) and proceeds *via* the intermediacy of charged and neutral radical intermediates (Scheme II). The facility of the SET step here results from the strong thermodynamic/kinetic[9] driving force for SET from amine donors ($E_{1/2}(+)$ = *ca.* 0.7–1.0 V)[10] to the 3MLF triplet ($E_{1/2}^{T1}(-)$ = *ca.* + 1.3 V).[11] In this sequence, proton transfer takes place between the amine cation radical and 3MLF anion radical intermediates (from NH for 1°-

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(1) Muller, E., Ed. *Chemistry and Biochemistry of Flavins*; CRC Press: Boca Raton, FL, 1990; Vol. 1.

(2) Hamilton, G. A. In *Progress in Bioorganic Chemistry*; Kaiser, E. T., Kezdy, F. J., Eds.; Wiley: New York, 1971; Vol. 1, p 83.

(3) (a) Weyler, W.; Hsu, Y.-P. P.; Breakefield, X. O. *Pharmacol. Ther.* **1990**, *47*, 391. (b) Singer, T. P. In *Chemistry and Biochemistry of Flavins*; Muller, F., Ed.; CRC Press: Boca Raton, FL, 1990; Vol. 3, pp 437–470.

(4) *Cf.* Palfreyman, M. G.; McDonald, I. A.; Bey, P.; Schechter, P. J.; Sjoerdama, A. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1988**. Birkmayer, W.; Knoll, J.; Riederer, P.; Youdin, M. B. H. In *Monoamine Oxidase and its Selective Inhibitors*; Beckmann, H., Riederer, P., Eds.; Karger: Basel, Rinne, U.K., 1983; Vol. 95, 1.

(5) Silverman, R. B. In *Advances in Electron Transfer Chemistry*; Mariano, P. S., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2, p 177.

(6) (a) Yoon, U. C.; Mariano, P. S. *Acc. Chem. Res.* **1992**, *25*, 233. (b) Hasegawa, E.; Xu, W.; Mariano, P. S.; Yoon, U. C.; Kim, J. U. *J. Am. Chem. Soc.* **1988**, *110*, 8099. (c) Hasegawa, E.; Brumfield, M. A.; Mariano, P. S.; Yoon, U. C. *J. Org. Chem.* **1988**, *53*, 5435. (d) Zhang, Z.-M.; Jung, Y. S.; Mariano, P. S.; Fox, M. A.; Martin, P. S.; Merkert, S. *Tetrahedron Lett.*, in press.

(7) Kim, J-M.; Cho, I.-S.; Mariano, P. S. *J. Org. Chem.* **1991**, *56*, 4943.

(8) Kim, J.-M.; Bogdan, M. A.; Mariano, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 9251.

(9) (a) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259. (b) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891.

(10) For characteristic amine oxidation potentials, see: Chow, Y. L.; Danen, W. C.; Nelsen, S. F.; Rosenblatt, D. C. *Chem. Rev.* **1978**, *78*, 243.

(11) (a) Calculated from $E_{1/2}(-)$ = –0.85 V[11b] or –0.83 V[11c] in MeCN for 3MLF and $E_{0,0}^{T1}$ = 2.1 eV[11d] for 3MLF. (b) Kim, J.-M.; Mariano, P. S. Unpublished results. (c) Mager, H.I.X.; Sazou, D.; Liu, Y. H.; Tu, S.-C.; Kadish, K. M. *J. Am. Chem. Soc.* **1988**, *110*, 3759. (d) Eweg, J. K.; Muller, F.; Visser, A. J. W. G.; Veeger, C.; Bebelaar, D.; Van Voorst, J. D. W. *Photochem. Photobiol.* **1979**, *30*, 463.

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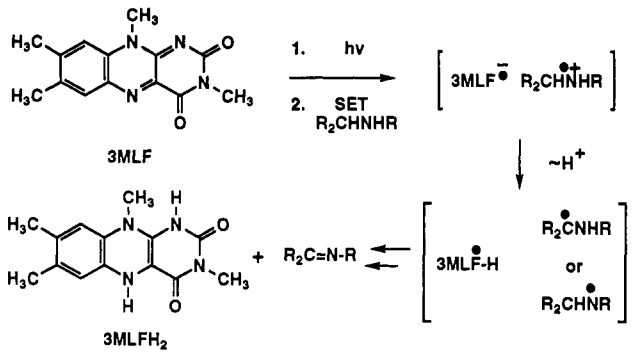
Scheme II



and 2°-amines and α-CH for 3°-amines) and is followed by either disproportionation, SET, or sequential bonding and heterolysis to produce the reduced flavin ($3MLFH_2$) and imine or iminium ion precursor of the carbonyl product.

Isolated observations of ground-state reactions between amines and flavins that mimic the MAO catalytic process have been made.[12] For example, Hamilton[12a] has shown that the flavin 10-phenylisoalloxazine participates in a thermal, base-catalyzed redox reaction with methyl α-phenylglycine to form methyl phenylglyoxylate. In a later study, Yoneda[12b] observed that 5-deazaflavin catalyzes the thermal, oxidative deamination reactions of simple primary amines. In light of the intense current interest that exists in the area of MAO biochemistry and the potential importance of mechanism-based inhibitors of these enzymes, it is surprising that only these few reports describing ground-state amine–flavin chemistry have appeared and that the mechanisms of these processes have not been explored.

Guided by our interests in this area, we recently initiated an investigation of the ground-state chemistry of flavin–amine systems. Our intent here was not only to uncover conditions that promote efficient oxidative deamination but, more importantly, to delineate fully the nature of the mechanistic pathway(s) followed in these reactions. Below, we describe the preliminary results of one phase of this study in which the chemistry of 3MLF and its N^5-ethylflavinium salt analog with selected amines has been probed. The results clearly demonstrate that (1) 3MLF promotes a clean acid-catalyzed reaction of benzylamine to produce the product of oxidative deamination and flavin reduction and (2) a polar mechanism involving the intermediacy of an amine–flavin 4a-adduct is responsible for this process.

Results

At the outset, an exploratory investigation was conducted to uncover conditions to promote 3MLF oxidative deamination of benzylamine, a typical MAO substrate.[13] We have found that this process occurs when a mixture of 3MLF (10 mM) and $PhCH_2NH_2$ (50–60 mM) in N_2-purged 2.4% H_2O–MeCN containing 10 mM HCl is heated in the dark at 80 °C (referred to below as "the standard conditions"). From the reaction (7 days, 30% conversion of BA), *N*-benzylbenzaldimine ($PhCH{=}NCH_2Ph$) is cleanly produced (85% based on 3MLF) by secondary reaction of the primary product, benzaldehyde, with $PhCH_2NH_2$. 3MLF is recovered quantitatively upon workup (in air) of the reaction mixture. The multiple-turnover nature of the process is demonstrated by the observation that 71% of $PhCH_2NH_2$ reacts to give $PhCH{=}NCH_2Ph$ (204% based on 3MLF) when the flavin and amine are treated under the standard conditions for 7 days with intermittent (3 days, 5 days) flushing of the mixture with air and then N_2. Controls have shown that $PhCH_2NH_2$ is not converted to $PhCH{=}NCH_2Ph$ when HCl or 3MLF are excluded or when the reaction is run at 25 °C. Finally, $MgCl_2$ (22 mM) can be substituted for HCl as the catalyst for the transformation of $PhCH_2NH_2$ to $PhCH{=}NCH_2Ph$ (7 days, 25% based on 3MLF). *Thus, it appears that 3MLF promotes a ground-state, acid-catalyzed oxidative deamination reaction of the primary amine $PhCH_2NH_2$.*

$$3MLF + PhCH_2NH_2 \xrightarrow[2.\ air]{1\ HCl\ or\ MgCl_2,\ N_2,\ H_2O\text{–}MeCN,\ 80\ °C} 3MLF + PhCH{=}NCH_2Ph$$

A priori, two limiting mechanisms appear reasonable for this acid-catalyzed, 3MLF-induced oxidative deamination reaction. The first involves an SET pathway which, like the photochemical route described above, proceeds via initial formation of an amine cation radical intermediate. The other is a polar route in which formation of a covalent intermediate by addition of $PhCH_2NH_2$ to N^5-protonated 3MLF is followed by elimination to generate the benzaldimine and dihydroflavin products.

Information leading to distinction between these two mechanistic alternatives has come from experiments probing the viability of 3MLF-promoted reactions of the secondary amines $PhCH_2NHCH_3$ and $PhCH_2NHCH_2SiMe_3$ and the tertiary amine $PhCH_2NMeCH_2SiMe_3$. No reaction was observed to occur when a mixture of 3MLF and $PhCH_2NHCH_3$ was subjected to the standard reaction conditions for 7 days. In contrast, the silyl analog $PhCH_2NHCH_2SiMe_3$ was converted to a mixture of $PhCH{=}NCH_2Ph$ (31% based on 3MLF), $PhCH_2NHCH_3$ (23%), and a trace of *N*-benzylformamide ($PhCH_2NHCHO$). Formation of $PhCH_2NHCH_3$ is a consequence of acid-induced desilylation, since the silyl analog reacts under the standard conditions (7 days) in the absence of 3MLF to give $PhCH_2NHCH_3$ in the same (22%) yield. Finally, the tertiary α-silylamine, $PhCH_2NMeCH_2SiMe_3$, does not undergo oxidative deamination with 3MLF under the standard conditions. The lack of reactivity of this substance is not due to its greater basicity (*vs* the 1°- and 2°-amines, which would cause a diminished concentration of the reactive intermediate, 3MLF-H^+).[14] This is demonstrated by an experiment in which an equimolar (30 mM) mixture of this amine and $PhCH_2NH_2$ is treated with 3MLF (10 mM) under the standard conditions for 6 days. In this event, $PhCH_2NMeCH_2SiMe_3$ remained unreacted while 20% of $PhCH_2NH_2$ was transformed to $PhCH{=}NCH_2Ph$ (31% based on 3MLF). The combined results indicate that amine reactivity in the 3MLF-induced oxidative deamination process is influenced by nucleophilicity (not oxidation potential) and α-electrofugal group leaving ability ($Me_3Si^+ > H^+$) of the amine substrates. *Specifically, the unreactivity of $PhCH_2NMeCH_2SiMe_3$ is inharmonious with expectations if an SET mechanism were operable, since this substance possesses the lowest oxidation potential in the series*[15] *and its cation radical is known to undergo facile desilylation.*[7,16]

$$3MLF + PhCH_2NHCH_2TMS \xrightarrow[2.\ air]{1.\ HCl,\ N_2,\ H_2O\text{–}MeCN,\ 80\ °C} \left\{ \begin{array}{c} 3MLF + PhCH{=}NCH_2Ph \\ PhCH_2NHCH_3 + PhCH_2NHCHO \end{array} \right\}$$

Additional mechanistic information has come from an analysis of the kinetic deuterium isotope effect for the 3MLF-promoted

(12) (a) Brown, L. E.; Hamilton, G. A. *J. Am. Chem. Soc.* **1970**, *92*, 7225. (b) Yoneda, F.; Sakuma, Y.; Kodokawa, Y.; Koshiro, A. *Chem. Lett.* **1979**, 1467. (c) Shinkai and his co-workers[12d] have also observed that mixtures of NAD analogs and flavins induce oxidative deamination of benzylamine by unspecified catalytic mechanism(s). (d) Shinkai, S.; Kuroda, H.; Manabe, O. *J. Chem. Soc., Chem. Commun.* **1981**, 391.

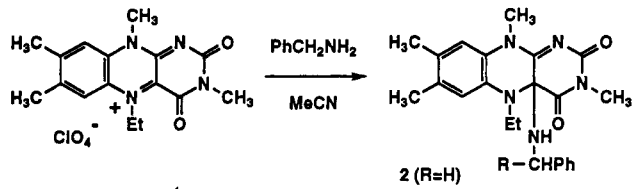
(13) Tabor, C. W.; Tabor, H.; Rosenthal, S. M. *J. Biol. Chem.* **1954**, *208*, 645.

(14) (a) The pK_a of 3MLF-H^+ is *ca.* 0.2[14b] as compared to ca. 9 for the amines. (b) Suelter, C. H.; Metzler, D. E. *Biochim. Biophys. Acta* **1960**, *44*, 23.

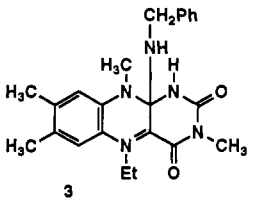
(15) Cooper, B. E.; Owen, W. J. *J. Organomet. Chem.* **1971**, *29*, 33.

(16) As we have shown previously,[6] α-silyl tertiary amine cation radicals undergo rapid and selective silyl cation transfer to silophiles such as MeCN or H_2O when they are not paired with strongly basic anion radicals.

Scheme III



1 2 (R=H) 2-D (R=D)



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reaction of benzylamine. We reasoned that if the ground-state reaction of the benzylamine-d_1 ($PhCHDNH_2$) with 3MLF follows an SET pathway, the internal d-isotope effect would be equivalent to that for the 3MLF-induced photochemical oxidation of this substrate. In the SET pathway, benzylic C–H bond cleavage takes place by either cation radical α-CH deprotonation or aminyl radical disproportionation (Scheme II).[7] On the other hand, C–H bond cleavage in a polar mechanism (see below) occurs in a step which mimics an E_2-elimination process. To gather isotope effect data on both a *bona fide SET* reaction[7] and the current dark 3MLF-promoted process, photolysis ($\lambda > 320$ nm, 20 °C, 1 h) and thermal (dark, 80 °C, 6 days) reactions of mixtures of 3MLF (5 mM) and $PhCHDNH_2$ (22 mM) were run in 5 mM in HCl in 2.5% H_2O–MeCN. ^1H NMR analysis of the ratios of PhCD=NCHDPh and PhCH=NCHDPh aldimine products gave directly the internal k_H/k_D values of 1.6 (20 °C) and 4.3 (80 °C) for the respective photochemical and ground-state processes. *Clearly, these results are not consistent with an SET mechanism for the ground-state acid-catalyzed, 3MLF-promoted oxidative deamination of benzylamine.*

Further clarification of the mechanistic details of the oxidative deamination reaction has come from studies conducted with N^5-ethyl-3-methyllumiflavinium perchlorate (**1**).[17] This salt is a stable, nonacidic analog of N^5-protonated 3MLF, the likely reactant in the acid-catalyzed processes described above. We anticipated that studies with **1** could potentially reveal important information about mechanism, and this has indeed turned out to be the case.

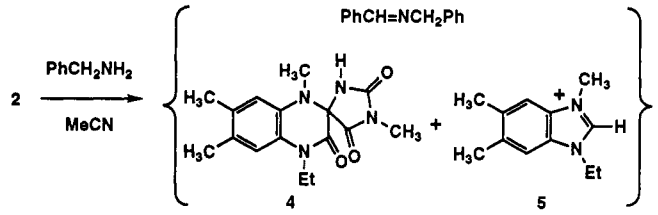
Accordingly, addition of $PhCH_2NH_2$ (final concentration 20 mM) to a MeCN solution of **1** (0.24 mM) results in an instantaneous reaction revealed by the disappearance of the absorption maxima for **1** at 558 and 415 nm and a simultaneous appearance of a new band at 341 nm (ϵ = 7000). The position of the new wavelength maximum is in the region expected for a 4a,5-dihydroflavin.[18,19] As indicated by these spectroscopic changes, a preparative dark reaction of $PhCH_2NH_2$ and **1** (2:1 molar equiv) in MeCN at 25 °C leads to rapid and efficient (80%) formation of the product responsible for the 341-nm band, which we have characterized as the 4a-benzylamino–dihydroflavin adduct **2** (mp 99–100 °C, hexane) (Scheme III). The spectroscopic properties of **2** (aided by analysis of the d_1-analog **2-D**) are in full accord with this assignment. For example, the disposition of the benzylic protons in the ^1H NMR spectrum of **2** at 3.54 and 3.63 ppm, both as doublet of doublets (J = 13.7 and 5.6 Hz), demonstrates their diastereotopic nature and shows that **2** contains a chiral center close to the benzylic center. Further,

(17) Ghilsa, S.; Hartmann, U.; Hemmerich, P.; Muller, F. *Liebigs Ann. Chem.* **1973**, 1388.

(18) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 6498.

(19) 4a,5-Dehydroflavins bearing a C-4a-alkyl substituent typically have UV-maxima in the 340–360-nm region. See ref 7 and 20b for examples of this phenomenon.

Scheme IV



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distinction between a 4a- (**2**) *vs* 10a- (**3**) adduct structure for this substance is aided by ^{13}C NMR data. In particular, the appearance of resonances for C-10a and C-4a at 155.8 and 68.8 ppm, respectively, is not expected[20] in the spectrum of a 10a-adduct. *This observation clearly shows that the preferred pathway followed in the reaction of a primary amine with an activated*[21] *flavin is one involving addition to the electrophilic 4a-position.*

Equally significant observations have been made in studies of the chemistry of the 4a-adduct **2**. Specifically, ^1H NMR analysis of the reaction of **2** (30 mM) in CD_3CN containing $PhCH_2NH_2$ (140 mM) at 60 °C in the dark reveals that **2** is converted in nearly quantitative yield to the imine $PhCH{=}NCH_2Ph$. Treatment of **2** under these conditions for 40 h (over-reaction) leads to production of $PhCH{=}NCH_2Ph$ (160% based on **2**) along with the spirohydantoin **4** (66%) and benzimidazolium salt **5** (34%) (Scheme IV). The latter substances form by a competitive, well-documented,[22] base-catalyzed decomposition of the flavinium salt. Moreover, ^1H NMR analysis of the benzaldimine products (PhCH=NCHDPh and PhCD=NCHDPh) arising from reaction of the monodeuteriated 4a-adduct **2-D** with $PhCHDNH_2$ provides an internal k_H/k_D value of 5.7 (60 °C) for this process. *These results provide clear evidence that a 4a-adduct related to 2 would be a competent intermediate in the acid-catalyzed oxidative deamination reaction occurring between 3MLF and benzylamine.*

Discussion

Several aspects of the results reported above warrant further discussion. These include issues relate to (1) the mechanistic pathway followed in the acid-catalyzed, ground-state 3MLF-promoted deamination of benzylamine, (2) the effect of amine structure and of the α-electrofugal group and isotope substitution on the rate of this process, and (3) the potential relevance of this flavin chemistry to the MAO catalytic mechanism.

The observations made in our studies of the effects of amine structure, α-substituent, and isotope substitution on reactivity combine to demonstrate that the ground-state 3MLF–benzylamine reaction follows a polar mechanism.[28] As shown in Scheme V, in this sequence reversible protonation[14] (general acid catalysis) of 3MLF at N-5 is followed by nucleophilic addition of the amine to C-4a, giving the 4a-adduct **6**. The regioselectivity for the addition step (*i.e.* C-4a > C-10a) is in full accord with charge

(20) (a) For example, C-10a of 3-methyl-4a-benzyl-4a,5-dihydrolumiflavin (a substance structurally related to **2**) resonates at 155.4 ppm.[20b] (b) Walker, W. H.; Hemmerich, P.; Massey, V. *Helv. Chim. Acta* **1967**, *50*, 2269.

(21) Protonation or alkylation at N-5 of the flavin nucleus leads to an increase in both the reduction potential ($E_{1/2}(-)$ of 3MLF in MECN = –0.85 V (SCE) and $E_{1/2}(-)$ of **1** in MeCN = +0.29 V (SCE)[21b] and the electrophilicity of the system. (b) Unpublished results of Kim, J. M., and Mariano, P. S.

(22) Mager, H. I. X. *Tetrahedron Lett.* **1979**, *37*, 3549.

(23) Hemmerich, P.; Fallob, S.; Erlenmeyer, H. *Helv. Chim. Acta* **1956**, *39*, 1242. Ghisla, S.; Hartmann, U.; Hemmerich, P.; Muller, F. *Liebigs Ann. Chem.* **1973**, 1388.

(24) Padwa, A.; Dent, W. *Org. Synth.* **1988**, *67*, 133.

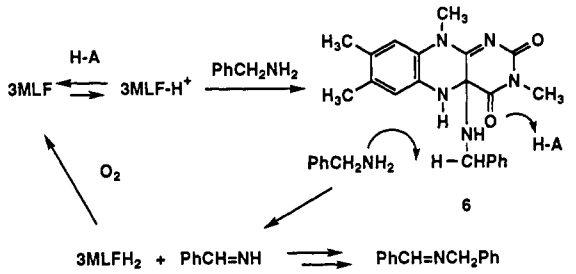
(25) Dahn, H.; Murchu, C. O. *Helv. Chim. Acta* **1970**, *53*, 1379.

(26) Nasipuri, D.; Ghosh, C. K.; Martin, R. J. L. *J. Org. Chem.* **1970**, *35*, 657.

(27) Su, D. T. T.; Thornton, E. R. *J. Am. Chem. Soc.* **1978**, *100*, 1872.

(28) It should be noted that a mechanism of this type has been invoked earlier[2,12a] to explain the base-catalyzed reaction of amino acids with flavins. However, no evidence was or has been presented to support this proposal.

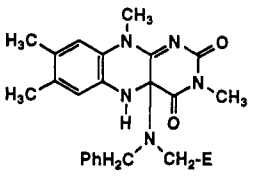
Scheme V



density considerations.[29] Moreover, the fact that the 5-ethylflavinium salt **1** undergoes rapid addition of benzylamine to efficiently produce a stable, yet even more sterically encumbered, analog of intermediate **6** strongly supports the regiochemical proposal embodied in Scheme V.

The second key step in this scheme is the elimination reaction of **6** to form the products of amine oxidation and flavin reduction. Guided by observations made in our study of the analog **2**, this elimination process is most likely both base and acid catalyzed. Accordingly, protonation at the C-4 carbonyl oxygen and amine-induced α-CH deprotonation would facilitate elimination of the dihydroflavin moiety and introduction of the C=N double bond.

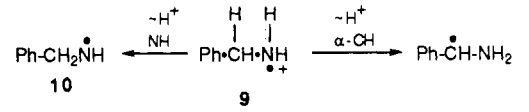
The polar mechanism is also consistent with the electrofugal group and *d*-isotope effects described above. The dramatic differences noted in the reactivity of the secondary amines $PhCH_2NHCH_3$ and $PhCH_2NHCH_2SiMe_3$ and the lack of reactivity of the tertiary amine $PhCH_2NMeCH_2SiMe_3$ are understandable in light of the predictable influence of amine structure and α-electrofugal group on the respective addition and elimination steps in the pathway. Thus, if the addition step contributes to the control of rate, reactivity should decrease in the amine series, 1° > 2° > 3°. To the extent that the elimination step governs rate, the nature of the electrofugal group being transferred will influence reactivity in the series $TMS^+ > H^+$. Numerous observations which show that TMS-transfer most often dominates deprotonation in both E_2- and E_1-eliminations[30] support the latter conclusion. Furthermore, in a recent report,[6d] we have presented evidence which suggests that α-silyl quaternary ammonium salts, serving as intermediates in Ce(IV) oxidations of tertiary α-silylamines, undergo exclusive desilylation to give iminium cations. Thus, a 4a-intermediate **7**, formed reversibly by slow addition of $PhCH_2NHCH_2SiMe_3$ to 3MLF-H^+, could undergo rapid and selective desilylation while deprotonation of the analogous, non-TMS, adduct **8** would be slow.



7 (E=$SiMe_3$)

8 (E=H)

Also consistent with the polar mechanism are the large, internal *d*-isotope effects associated with both the acid-catalyzed reaction of 3MLF with $PhCHDNH_2$ and the reaction of **2-D** with $PhCHDNH_2$. Lewis[31] and we[7] had shown earlier that photo-induced reactions of amine with flavins occur by SET mechanisms *via* the intermediacy of charged and neutral radicals. α-CH bond cleavage in the photoreaction of benzylamine with 3MLF, operating in this fashion,[7] takes place either by α-CH deprotonation in the initially formed amine cation radical **9** or by disproportionation of the radical pair **10**, depending on the relative rates of NH *vs* α-CH deprotonation of the initially formed cation radical.[32] Whichever mechanistic route is followed, the small k_H/k_D value of 1.6 at 25 °C for the photochemical reaction of $PhCHDNH_2$ with 3MLF characterizes the CH bond cleavage step in the SET-induced pathway. In contrast, the ground-state, acid-catalyzed reaction displays a large internal *d*-isotope effect of 4.6 (at 80 °C) as expected for the polar mechanism in which the CH bond is cleaved in a step which resembles a typical E_2-elimination and one that is modeled by the related fragmentation of the stable adduct **2-D**.



A final comment is in order about the potential relationship of the observations discussed above to the mechanism for MAO catalysis of primary amine oxidative deamination. Perhaps the most biochemically relevant conclusion that can be drawn from the current work is that a polar mechanism proceeding by formation of and elimination in a 4a-intermediate cannot be dismissed[5] as a viable alternative for MAO-catalyzed reactions and related processes promoted by members of the oxidase family of flavoenzymes. In this context, the current investigation has led to results which provide a firm mechanistic foundation for Hamilton's[2] original proposal of a mechanism of this type for flavoenzyme-catalyzed dehydrogenation reactions of amines, alcohols, and acid esters. The attractive features of a polar mechanistic pathway of this type for MAO catalysis are that (1) simple acid base chemistry rather than potentially endoergic SET chemistry is used to activate[21] reaction, (2) the isotope effect of 8.0 (25 °C), measured earlier by stopped-flow techniques for the first half reaction of the MAO-catalyzed reaction of benzylamine ($PhCH_2NH_2$ *vs* $PhCD_2NH_2$),[33] is more in line[34] with the internal k_H/k_D value of 4.6–5.7 (60–80 °C) for the model ground-state (polar) reaction rather than with the photochemical (SET) pathway, and (3) the chemistry utilized is analogous to that employed by other amine oxidases which have *o*-quinones as cofactors.[35] Of course, in the absence of evidence gained from direct studies on the MAOs, the above mechanistic suggestions must remain tentative.

Experimental Section

General Methods. ^1H NMR (200, 400, 500 MHz) and ^{13}C NMR (50 MHz) spectra were recorded with $CDCl_3$ solutions unless otherwise noted, and chemical shifts are reported in ppm relative to either Me_4Si or $CHCl_3$ as internal standards. All reactions were run under a dry N_2 atmosphere unless otherwise specified. 3-Methyllumiflavin (3MLF)[23] and 5-ethyl-

(29) Mueller, F.; Jarbandham, T.; Gast, R.; Grande, H. J. In *Reactivity of Flavins*; yagi, K., Ed.; University of Tokyo Press: Tokyo, 1975; p 51.
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(31) Simpson, J. T.; Krantz, A.; Lewis, F. D.; Kokel, B. *J. Am. Chem. Soc.* **1982**, *104*, 7155.

(32) (a) On the basis of the small k_H/k_D value of 1.6 (25 °C) for the photoreaction of $PhCHDNH_2$ with 3MLF as compared to the much larger values normally associated with tertiary amine cation radical α-CH deprotonation[32b,c] we conclude that this process occurs by a route involving sequential SET–NH deprotonation[32d]–radical disproportionation. (b) Xu, W.; Zhang, X.-M.; Mariano, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 8863. (c) Dinnocenzo, J. P.; Banach, T. E. *J. Am. Chem. Soc.* **1989**, *111*, 8646. (d) Lewis, F. D.; Zebrowski, B. E.; Correa, P. E. *J. Am. Chem. Soc.* **1984**, *106*, 187. Lewis, F. D.; Reddy, G. D. *J. Am. Chem. Soc.* **1989**, *111*, 6465. Sugimoto, A.; Sumida, R.; Tamai, N.; Inoue, H.; Otsugi, Y. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3500.
(33) The k_H/k_D values for the enzymatic first half reaction[34] and the ground-state reactions are very close when consideration is given to the fact that k_H/k_D for the MAO process measured with $PhCH_2NH_2$ vs $PHCD_2NH_2$ at 25 °C is elevated by an α-secondary isotope effect on k_D while k_H/k_D values for the model reactions, measured at 60 or 80 °C, are lowered by this same secondary isotope effect on k_H.
(34) Husain, M.; Edmondson, D. F.; Singer, T. P. *Biochemistry* **1982**, *21*, 595.
(35) See for example: Hartmann, C.; Klinman, J. P. *Biofactors* **1988**, *1*, 41. Farnum, M.; Palcic, M.; Klinman, J. P. *Biochemistry* **1986**, *25*, 1898. Rodriguez, E. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1989**, *111*, 7947. Janes, S. M.; Mu, D.; Wemmer, Dr.; Smith, A. J.; Kaur, S.; Maltby, D.; Burlingame, A.L.; Klinman, J. P. *Science* **1990**, *248*, 981.

3-methyllumiflavinium perchlorate (**1**)[17] were prepared by known procedures. *N*-benzyl-*N*-[(trimethylsilyl)methyl]amine was also prepared by a known procedure.[24] Benzylamine, as its hydrochloride salt, and *N*-benzylbenzaldimine were obtained from Aldrich. All products are oils unless otherwise noted.

Acid-Catalyzed Dark Reaction of 3MLF with Benzylamine. A mixture of 3MLF (10 mM) and benzylamine (50 mM) in N_2-purged 2.4% H_2O–MeCN (20 mL) containing HCl (10 mM) was heated in the dark at 80 °C for 7 days. After being cooled to 25 °C, the mixture was concentrated *in vacuo*. After addition of triphenylmethane as an internal reference, the residue was dissolved in $CDCl_3$. ^1H NMR analysis showed that *N*-benzylbenzaldimine (85%, based on 3MLF) was the only product formed. 3MLF was recovered in >95% by workup of this mixture.

When the reaction was run under the conditions described above except that intermittent (3 days, 5 days) flushing of the mixture with air and then N_2 was included, a 204% yield (based on 3MLF) of *N*-benzylbenzaldimine was measured by ^1H NMR analysis. Similar dark reactions conducted at 25 °C or in the absence of HCl yielded only trace quantities of *N*-benzylbenzaldimine. When $MgCl_2$ (22 mM) was substituted for HCl for a reaction conducted under otherwise the same conditions, 25% (based on 3MLF) *N*-benzylbenzaldimine was produced.

Reaction of 3MLF with *N*-Benzyl-*N*-[(trimethylsilyl)methyl]amine. A mixture of 3MLF (11 mM) and $PhCH_2NHCH_2SiMe_3$ (40 mM) in N_2-purged 2.4% H_2O–MeCN (20 mL) containing HCl (14 mM) was stirred in the dark at 80 °C for 7 days. The mixture was concentrated *in vacuo*, made basic with $NaHCO_3$, and extracted with $CHCl_3$. The $CHCl_3$ extracts were concentrated *in vacuo*. ^1H NMR analysis of the residue showed the presence of *N*-benzylbenzaldimine (31%, based on 3MLF), *N*-benzylmethylamine (23%, based on the starting amine), and a trace of *N*-benzylformamide.

A similar reaction under an air atmosphere yielded *N*-benzylformamide (55%, based on 3MLF), *N*-benzylbenzaldimine (41%, based on 3MLF), and *N*-benzylmethylamine (12%, based on the starting amine). The reaction in the absence of 3MLF under air provided *N*-benzylmethylamine (15%, based on the starting amine) and a trace of *N*-benzylbenzaldimine. A control reaction in the absence of 3MLF provided *N*-benzylmethylamine (22%, based on the starting amine) and a trace of *N*-benzylbenzaldimine.

Preparation of *N*-Benzyl-α-d_1-amine.[25] To a suspension of 2.0 g (48 mmol) of $LiAlD_4$ in 70 mL of anhydrous ether at 0 °C was added a solution of 10.2 g (96 mmol) of benzaldehyde in 40 mL of ether. After addition was complete (1 h), the solution was stirred at reflux for 3 h. After quenching with H_2O, the mixture was filtered, and the filtrate was dried over Na_2SO_4 and concentrated *in vacuo* to give 10.4 g (99%) of benzyl-α-d_1 alcohol:[26] ^1H NMR 2.15 (s, 1H, OH), 4.55 (t, J = 1.9 Hz, 1H, benzylic), 7.17–7.37 (m, 5H, aromatic Ph).

To benzyl-α-d_1 alcohol (5.0 g, 45.9 mmol) was added phophorus tribromide (2.2 mL, 23.2 mmol) at 0 °C. The mixture was stirred at 110 °C for 1.5 h and at 25 °C for 12 h, cooled to 0 °C, diluted with H_2O, and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were concentrated *in vacuo*, and the residue was subjected to column chromatography on silica gel (ether) to yield 7.1 g (90%) of benzyl-α-d_1 bromide:[27] ^1H NMR 4.42 (t, J = 1.5 Hz, 1H, benzylic), 7.20–7.40 (m, 5H, aromatic Ph).

A mixture of benzyl-α-d_1-bromide (5.2 g, 30 mmol) and potassium phthalimide (5.9 g, 32 mmol) in dry DMF (30 mL) was stirred at 120 °C for 5.0 h, cooled to 0 °C, poured into water, and extracted with ether. The ethereal extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The white solid obtained was recrystallized from *n*-hexane to give 4.1 g (58%) of (benzyl-α-d_1)phthalimide (mp 116 °C): ^1H NMR 4.70 (br s, 1H, benzylic), 7.05–7.30 and 7.52–7.75 (m, 9H, aromatic); ^{13}C NMR 41.0 (t, J = 20.1 Hz, benzylic), 123.0, 127.5, 128.3, 128.3, 131.7, 133.6 and 135.9 (aromatic), 167.6 (C=O); IR 1773, 1721, 1392 cm^{-1}; EIMS m/z (relative intensity) 238 (M, 100), 220 (32), 209 (28), 130 (17), 105 (56), 92 (22), 76 (49),51 (17); HRMS (EI) m/z 238.0865 ($C_{15}H_{10}D_1NO_2$ requires 238.0853).

A mixture of (benzyl-α-d_1)phthalimide (0.67 g, 2.8 mmol) and hydrazine hydrate (100%, 255 μl, 5.3 mmol) in EtOH (20 mL) was stirred at reflux for 1.5 h. After the mixture was cooled to 25 °C, H_2O was added, and the mixture was concentrated *in vacuo*. The residue was diluted with H_2O and 2 mL of concentrated HCl, stirred at reflux for 2h, cooled to 0 °C, and filtered. The filtrate was concentrated *in vacuo* to give a white solid which was dissolved in H_2O, basified with NaOH (pH *ca.* 10), and extracted with $CHCl_3$. The $CHCl_3$ extracts were concentrated *in vacuo* to give 0.25 g (82%) of *N*-benzyl-α-d_1-amine:[25] ^1H NMR 1.48 (s, 2H, NH_2), 3.83 (t, J = 2.0 Hz, 1H, benzylic), 7.30 (m, 5H, aromatic).

Internal Kinetic Isotope Effect for the Photochemical and Thermal Reactions of 3MLF and $PhCHDNH_2$. Photoreaction. A solution (10 ml) containing 3MLF (4.6 mM), *N*-benzyl-α-d_1-amine (22 mM), and concentrated HCl (4.8 mM) in 2.4% H_2O–MeCN was irradiated with uranium glass (>320 nm) filtered-light for 1 h under a N_2 atmosphere. The photolysate was concentrated *in vacuo*, and the residue was analyzed by ^1H NMR spectroscopy. A ratio of 1.62:1 (average of two experiments) for the benzaldimines PhCD=NCHDPh and PhCH=NCHDPh was determined by comparing the integrals of the resonances at 8.38 ppm (imine C—H) and 4.80 ppm (benzylic C—H).

Thermal Reaction. A solution containing the same concentration of starting materials and reagents as above was stirred at 80 °C for 6 days. ^1H NMR analysis of the crude reaction mixture gave a 4.2:1 ratio (average of two experiments) of PhCD=NCHDPh and PhCH=NCHDPh.

Reaction of the Flavinium Perchlorate 1 with Benzylamine. Preparation of 4a-Adduct 2. The 5-ethylflavinium salt **1** (51 mg, 0.13 mmol) and benzylamine (27 mg, 0.25 mmol) in MeCN (1 mL) were rapidly mixed at 25 °C (with an instantaneous color change from purple to green). Without delay, the mixture was concentrated *in vacuo* to give a residue which was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with water and concentrated *in vacuo*. The oily residue was washed with hexane to remove excess benzylamine and then dried *in vacuo* to provide 41 mg (80%) of the 4a-adduct **2** as an oil. Crystallization from *n*-hexane yielded **2** as a pale-yellow powder (mp 99–100 °C): ^1H NMR 0.88 (t, J = 7.1 Hz, 3H, N-5 CH_2CH_3), 2.02 (br t, 1H, C-4a NH), 2.25 and 2.29 (s, 3H each, C-7 and C-8 CH_3), 3.13 (m, 2H, N-5 CH_2CH_3), 3.31 (s, 3H, N-10 CH_3), 3.47 (s, 3H, N-3 CH_3), 3.54 (dd, J = 13.7, 5.6 Hz, 1H, C-4a benzylic), 3.63 (dd, J = 13.7, 5.6 Hz, 1H, C-4a benzylic), 6.89 (s, 1H, H-6), 7.01 (s, 1H, H-9), 6.84–6.86 and 7.12–7.17 (m, 5H, C-4a Ph): ^{13}C NMR 13.2 (N-5 CH_2CH_3), 19.5 and 19.7 (C-7 and C-8 CH_3), 27.9 (N-10 CH_3), 32.6 (N-3 CH_3), 46.3 (N-5 CH_2CH_3 and C-4a benzylic), 68.8 (C-4a), 117.0 (C-6), 125.2 (C-9), 127.1, 127.8, 128.2 and 138.5 (C-4a Ph), 131.3, 132.7 and 133.8 (C-5a, C-7, C-8, and C-9a), 155.8 (C-10a), 160.7 (C-2), 167.7 (C-4); IR 1670, 1560 cm^{-1}; EIMS m/z (relative intensity) 405 (M, 14), 299 (100), 271 (56), 257 (49), 214 (92), 107 (30); HRMS (EI) m/z 405.2152 ($C_{23}H_{27}N_5O_2$ requires 405.2165).

Using a similar procedure a 1:1 mixture of the two diastereomers of **2-D** was prepared: ^1H NMR 0.87 (t, J = 7.1 Hz, 3H, N-5 CH_2CH_3), 2.24 and 2.28 (s, 3H each, C-7 and C-8 CH_3), 3.13 (m, 2H, N-5 CH_2CH_3), 3.29 and 3.30 (s, 3H, N-10 CH_3), 3.46 (s, 3H, N-3 CH_3), 3.53 and 3.61 (br s, 1H, C-4a benzylic), 6.89 (s, 1H, H-6), 7.00 (s, 1H, H-9), 6.82–6.85 and 7.11–7.15 (m, 5H, C-4a Ph); ^{13}C NMR 13.1 (N-5 CH_2CH_3), 19.4 and 19.6 (C-7 and C-8 CH_3), 27.8 (N-10 CH_3), 32.4 (N-3 CH_3), 45.9 (t, J = 20.5 Hz, C-4a benzylic), 46.2 (N-5 CH_2CH_3), 68.6 (C-4a), 116.9 (C-6), 125.1 (C-9), 127.0, 127.7, 128.0 and 138.4 (C-4a Ph), 131.2, 132.6 and 133.7 (C-7, C-8, C-5a, and C-9a), 155.8 (C-10a), 160.7 (C-2), 167.7 (C-4); IR 1667, 1557 cm^{-1}; EIMS m/z (relative intensity) 406 (M, 4), 299 (35), 257 (60), 230 (32), 214 (35), 186 (17), 107 (30), 92 (100), 77 (20); HRMS (EI) m/z 406.2239 ($C_{23}H_{26}D_1N_5O_2$ requires 406.2228).

Reaction of the 4a-Adduct 2 with $PhCH_2NH_2$. A mixture of the 4a-adduct **2** (27 mM) and benzylamine (140 mM) in CD_3CN (700 μL) was let stand at 60 °C for 40 h in the dark under N_2. ^1H NMR analysis (triphenylmethane as an internal standard) showed that the adduct **2** was completely consumed and that *N*-benzylbenzaldimine had formed (160%, based on starting **2**) along with C^{10a}-spirohydantoin **4** (66%)[22] and benzimidazolium salt **5** (34%).[22]

Internal Kinetic Isotope Effect for Reaction of the 4a-Adduct 2-D with $PhCHDNH_2$. The reaction of 4a-NHCHDPh adduct **2-D** with *N*-benzyl-α-d_1-amine was performed in the manner described above. A ratio of PHCD=NCHDPh and PhCH=NCHDPh of 5.7:1 (average of two experiments) was determined by ^1H NMR spectroscopy (see above).

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