Flavin Chemical Models for Monoamine Oxidase Inactivation by Cyclopropylamines, α-Silylamines, and Hydrazines

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Abstract: Models for the inactivation of the monoamine oxidase A and B, two closely related flavoenzymes, by cyclopropylamines, α-silylamines, and hydrazines have been investigated in order to gain insight into the possible chemical mechanisms for these processes. The activated (*i.e.* high reduction potential and electrophilicity) flavin, 3-methyl-5-ethyllumiflavinium perchlorate (**5**), was employed in this effort along with *trans*-2-phenylcyclopropylamine (**1**), a host of monosubstituted hydrazines (**13-16**), and α-(trimethylsilyl)benzylamine (**9**). Admixture of **5** with **1** (25 °C, MeCN) results in instantaneous formation of the stable and completely characterized flavin−amine adducts **6** ($K_e = 2 \times 10^4$) derived by addition of the amine function in **1** to the 4a-position of **5**. Reaction of the 4a-adduct **6** with cyclopropylamine **1** (85 °C, MeCN) cleanly (80%) produces the aldimine **7** formed by condensation of the initial product, *trans*-cinnamaldehyde and amine **5**. These results demonstrate that 4a-adducts related to **6** are capable of undergoing cyclopropane ring opening reactions by polar pathways to produce electrophilic α,β-unsaturated carbonyl products. Consequently, ring opening reactions proposed for monoamine oxidase inactivation by primary and perhaps secondary cyclopropylamines can occur by polar routes and, thus, are not uniquely attributable to radical mechanistic pathways. In a similar manner, the flavinium salt **5** undergoes rapid reaction with the α-silylamine **9** to produce a stable 4a-adduct **10** ($K_e = 7 \times 10^4$). Reaction of this adduct with **9** (45 °C, MeCN) leads to initial production of *N*-[(α-trimethylsilyl)benzyl]benzaldimine (**12**) which undergoes desilylation to produce *N*-benzylbenzaldimine (**11**) under these conditions. Also, 4a-adduct **10** is rapidly converted to aldimine **11** by reaction with TBAF at 25 °C in MeCN. These results show that 4a-adducts, generated from activated flavins and α-silylamines, participate in fragmentation processes leading to silylation of nucleophiles and production of carbonyl products. This polar mechanistic pathway models the known inactivation reactions of the MAOs by α-silylamines previously attributed to SET (radical) routes. Reaction of flavinium salt **5** with phenyl- or benzylhydrazine results in formation of 4a-phenyl or -benzyl flavin adducts. For example, admixture of **5** and $PhNHNH_2$ in CH_3CN at 25 °C provides the characterizable 4a-phenyl and 4a-cyanomethyl flavins, **21** (28%) and **22** (55%), and benzene. Benzylhydrazine reacts similarly with **5** to produce only the 4a-benzyl adduct **23** (89%). Information about the mechanism for adduct formation in these reactions has come from studies with the hydrazine analogs, $NH_2NHCO_2CH_2Ph$ (**15**) and NH_2OCH_2Ph (**16**). These substances react rapidly with **5** in MeCN at 25 °C to cleanly produce stable 4a-hydrazine adducts, **17**. The results suggest that 4a-alkylation or -arylation reactions of the activated flavin **5** with hydrazines probably occur *via* the intermediacy of 4a-hydrazine flavin adducts related to **17**. Thus, a polar mechanistic model is also consistent with the known inactivation reactions of the MAOs with hydrazines which are also reported to generate 4a-flavin alkylated and arylated MAO derivatives.

Introduction

Interest in the enzymology of the monoamine oxidases,[1] flavoenzymes which catalyze oxidative deamination reactions of neurologically active amines (*e.g.* serotonin), has remained high owing to the pharmacological importance of MAO inactivators[2] and to unresolved questions about chemical mechanisms for MAO catalysis and inactivation.[3−6] In terms of the latter issue, single electron transfer (SET) radical mechanisms for the MAO catalytic and inactivation processes have gained wide acceptance.[4] This mechanism, shown for MAO catalysis in Scheme 1, involves α-CH proton transfer in ion radical intermediates formed by SET from the amine substrate to the MAO covalently bound flavin moiety. Our earlier photochemical model studies[7,8] have provided results which indicate that the chemical natures of the catalytic and inactivation reactions of this flavoenzyme are consistent with expectations based on SET radical behavior.

However, our more recent exploratory investigations of ground state models for MAO catalysis[3] have demonstrated that activated flavins (*i.e.* those with high reduction potentials and electrophilicity) promote ground state oxidative deamination reactions of primary and secondary amines by a polar (not SET radical) mechanism involving formation and elimination of intermediate 4a-covalent adducts. These observations suggest an alternative to the SET radical mechanistic proposal for MAO catalysis: specifically, that the polar catalytic mechanism (Scheme 2)[3] proposed some years ago by Hamilton[9] is possible for this enzyme.

[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1994.

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(2) Tipton, K. F.; Dostert, P.; Beneditti, M. S. *Monoamine Oxidase and Disease*; Academic Press: New York, 1984.

(3) Kim, J. M.; Bogdan, M. A.; Mariano, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 10591.

(4) Silverman, R. B.; Lu, X. *J. Am. Chem. Soc.* **1994**, *116*, 4129.

(5) Yue, K. T.; Bhattacharya, A. K.; Zhelyaskov, R. R.; Edmondson, D. E. *Arch. Biochem. Biophys.* **1993**, *300*, 178. Walker, M. C.; Edmondson, D. E. *Biochemistry* **1994**, *33*, 7088.

(6) Simpson, J. T.; Krantz, A.; Lewis, F. D.; Kokel, B. *J. Am. Chem. Soc.* **1982**, *104*, 7155.

(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.

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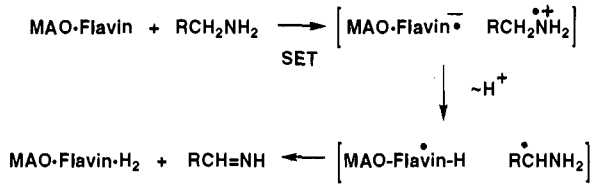
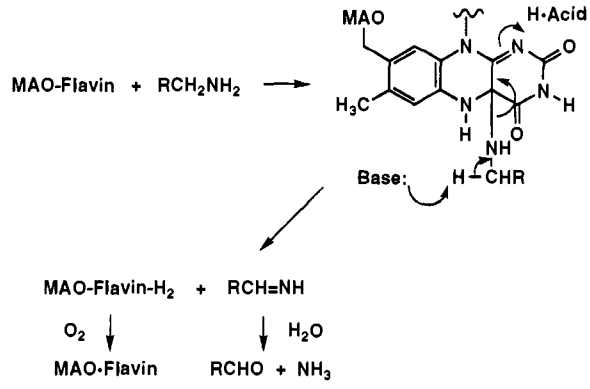
Scheme 1



Scheme 2



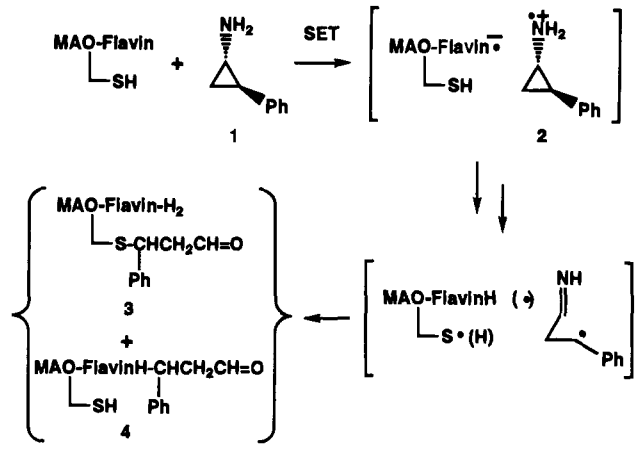
The origins of the SET radical mechanistic proposals are found principally in observations arising from studies of MAO inactivation.[4] In light of the importance of these inactivation processes to both the chemical mechanism and related pharmacological issues, it is surprising that MAO inactivation reactions have not been fully[8] scrutinized from the perspective of ground state chemical models. Consequently, we recently initiated a broad study probing the ground state chemistry of activated flavins with members of three major MAO inactivator families including cyclopropylamines, α-silylamines, and hydrazines. In this paper we report the results of this effort which demonstrate the efficient operation of polar mechanisms for chemical processes that precisely mimic MAO inactivation chemistry.

Results and Discussion

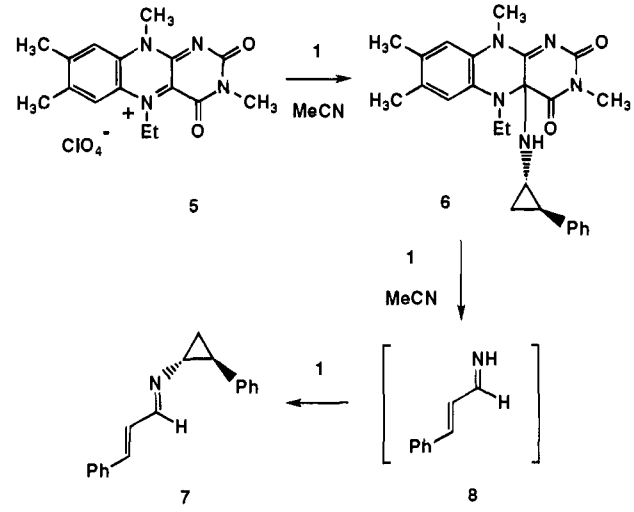
The first class of MAO inactivators modeled in this study is the cyclopropylamines,[10] represented by the antidepressant *trans*-2-phenylcyclopropylamine (tranylcypromine) (**1**). Substances in this family are thought[11] to inactivate MAO by alkylation of an active site cysteine thiol and/or flavin moiety by a pathway involving formation and ring opening of aminium radical intermediates (*e.g.*, Scheme 3). The rationale for this suggestion appears to be that cyclopropane ring opening must occur if alkylated products such as **3** and **4** are formed and that only a radical process would be responsible for this ring opening. As the observations described below show, the outcome of MAO cyclopropylamine reactions is not uniquely indigenous to SET radical chemistry.

Our studies modeling the cyclopropylamine inactivation process employed the amine **1** and 5-ethyllumiflavinium perchlorate (**5**), a substance that we[3] and others[12] have shown serves as an excellent, activated flavin model. The first relevant observation made is that addition of the amine **1** to an MeCN (25 °C) solution of flavinium salt **5** leads to instantaneous

Scheme 3



Scheme 4



formation of the stable and fully characterized (Experimental Section) 4a-adduct **6** (72% isolated, 1.6:1 mixture of diastereomers) (Scheme 4). UV monitoring of this addition reaction (Figure 1) shows the disappearance of the 415 and 558 nm bands associated with the flavinium chromophore and simultaneous development of the 345 nm band of the 4a,5-dihydroflavin adduct **6**. Analysis of the UV changes shows that the process is rapid at 25 °C and associated with a formation equilibrium constant of $K_e = 2 \times 10^4\ M^{-1}$.

Of equal importance is the finding that heating a mixture of the adduct **6** (10 mM) and **1** (110 mM) in MeCN (85 °C, N_2) leads to clean (80%) production of the known[8] *N*-cyclopropylcinnamaldimine **7**. Controls show that **7** is not produced by subjecting the reactants **1** and **6** independently to the above conditions.

These results demonstrate that primary cyclopropylamines add rapidly to the 4a-position of activated flavins to give 4a-adducts with intact cyclopropane rings. Moreover, as expected based upon the fact that hydroflavin anions are good leaving groups and that cyclopropylcarbinyl cation-like ring opening reactions are common, the formed adducts undergo cyclopropane ring cleavage processes to produce α,β-unsaturated carbonyl (imine) products. Several mechanisms appear possible for this fragmentation/elimination reaction. One route involves simultaneous cyclopropane ring opening and hydroflavin anion departure to generate an ion pair which is then transformed to the dihydroflavin and α,β-usaturated imine products by an amine facilitated proton transfer. The process could also be promoted

(9) Hamilton, G. A. In *Progress in Bioorganic Chemistry*; Kaiser, E. T., Kezdy, F. J., Eds.; Wiley; New York, 1971; Vol. 1, p 83.
(10) Paech, C.; Salach, J. L.; Singer, T. P. In *Monoamine Oxidase Structure, Function and Altered Function*; Singer, T. P., VonKorff, R. W., Murphy, D. C., Eds.; Academic Press: New York, 1979, p 39.
(11) Silverman, R. B. *J. Biol. Chem.* **1983**, *258*, 14766.
(12) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 6498.

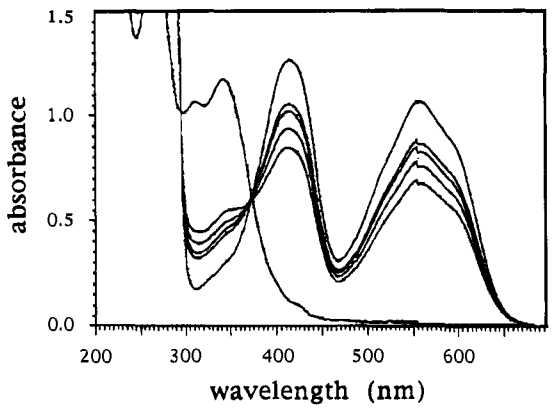
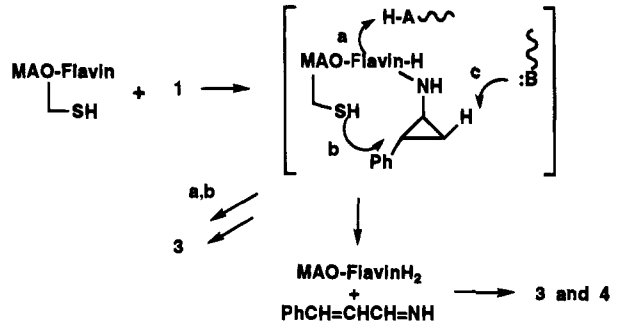


Figure 1. UV−visible spectra of solutions of the flavinium salt **5** (0.13 mM) and cyclopropylamine **1** (0, 0.03, 0.04, 0.06, 0.08, and 2.6 mM corresponding to decreasing absorbance at 558 nm and increasing absorbance at 345 nm) in MeCN at 25 °C.

Scheme 5



by amine-induced deprotonation occurring in concert with cyclopropane ring cleavage and hydroflavin anion elimination. A final alternative employs the amine as a nucleophile, promoting ring opening by attack at the cyclopropane benzylic center along with hydroflavin departure.

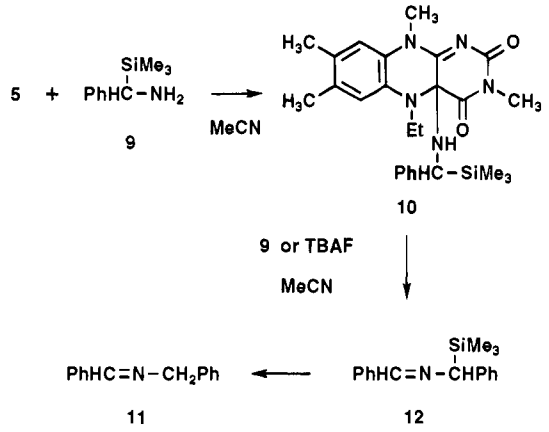
It is interesting that chemistry of this type occurring in the MAO active site and following addition of **1** to the flavin moiety would lead (1) to formation of an electrophilic α,β-unsaturated imine which can serve to alkylate either a dihydroflavin or thiol nucleophile by Michael addition or (2) to direct alkylation of a thiol by SH nucleophilic assisted cyclopropane ring cleavage (Scheme 5).

These observations suggest that the SET radical mechanism[8] is not unique in rationalizing the outcome of cyclopropylamine inactivations of the MAOs. A specific issue that needs to be addressed in this regard relates to how the enzyme might activate the fragmentation chemistry of a 4a-adduct related to **6**. Importantly, simultaneous protonation of the departing hydroflavin anion at N-1 by an acid enzyme residue would facilitate this process. This type of catalysis is well within the perspective of enzyme chemistry and is probably responsible for assisting the MAO induced dehydrogenation reaction of primary amines occurring by either a polar or SET pathway. If cyclopropane ring cleavage happens in the enzyme 4a-adduct in concert with ring deprotonation to give the unsaturated imine directly, catalysis by a basic residue would be required. Potential candidates for this function are the histidine[13] or carboxylate[14] groups which have been previously identified as essential residues in the MAOs by use of chemical modification techniques. It should be noted that both the polar and SET

(13) Hiramatsu, A.; Tsurushin, S.; Yasunobu, K. T. *Eur. J. Biochem.* **1975**, *57*, 587.

(14) See ref 1, p 396 for a description of unpublished observations.

Scheme 6



mechanisms for catalysis require participation by an active site base to deprotonate either an intermediate amine flavin 4a-adduct or an amine cation radical.[15] A final, reasonable mode for facilitating the fragmentation reaction involes direct attack on the benzylic cyclopropane ring center by the active site thiol nucleophile. This would directly furnish the cysteine covalent modification proposed to be associated with inactivation of MAO by **1**.

A second group of MAO inactivators consists of primary amines bearing α-trialkylsilyl functions.[16−18] The time and concentration dependent, irreversible inhibition caused by these substances has been explained previously[17,18] in terms of an SET radical route involving silyl transfer from α-silylaminium radicals to MAO-active site nucleophiles. This speculation rests solely on precedent gained from SET photochemical studies of α-silylamines where amine cation radical desilylation processes have been detected.[19]

In order to determine if silyl transfer processes of this type in silylamine flavin ground state chemistry are unique for SET pathways, we have investigated the reactions of the silylbenzylamine **9** with activated flavin **5**. As anticipated,[3] admixture of **9** and **5** in MeCN at 25 °C results in instantaneous formation of the completely characterized (Experimental Section) 4a-adduct **10** (78%, 1.4:1 mixture of diastereomers, λ_{max}(MeCN) = 345 nm, $K_e = 7 \times 10^4$ M^{-1}) (Scheme 6). Significantly, reaction of this adduct (93 mM) with **9** (119 mM) at 45 °C yields initially the aldimine **12** which is transformed under the reaction conditions (19 h) to the non-TMS aldimine **11**. When the superior silophilic reagent TBAF (93 mM) is reacted with **10**, imine **11** is efficiently (78%) generated (presumably *via* **12**) under less vigorous (*i.e.* 25 °C) conditions.

These observations show that primary α-silylamines also add rapidly to activated flavins to yield 4a-adducts which are structured to undergo familiar[20] fragmentation reactions to produce dihydroflavins, aldimines, and silylated nucleophile products. Consequently, a polar pathway of this type (Scheme 7), actrivated by attack of a nucleophilic enzyme residue (see above), is fully consistent with the observations made in studies

(15) (a) It is unlikely that a hydroflavin anion is sufficiently basic to deprotonate amine cation radicals (ref 15b). (b) Dinnocenzo, J. P.; Banach, T. E. *J. Am. Chem. Soc.* **1989**, *111*, 8646. Zhang, X. M.; Yeh, S. R.; Hong, S.; Freccero, M.; Albini, A.; Falvey, D. E.; Mariano, P. S. *J. Am. Chem. Soc.* **1994**, *116*, 4211.

(16) Danzin, C.; Collard, J. N.; Marchal, P.; Schirlin, D. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 540.

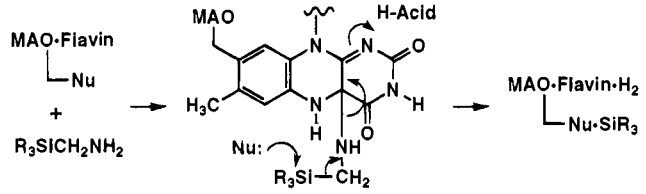
(17) Silverman, R. B.; Banik, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 2219.

(18) Banik, G. M.; Silverman, R. B. *J. Am. Chem. Soc.* **1990**, *112*, 4499.

(19) Hasegawa, E.; Xu, W.; Mariano, P. S.; Yoon, U. C.; Kim, J. U. *J. Am. Chem. Soc.* **1988**, *110*, 8099.

(20) Whitmore, F. C.; Sommer, L. H.; Gold, J.; Van Strien, R. E. *J. Am. Chem. Soc.* **1947**, *69*, 1551.

Scheme 7



of MAO inactivations by α-silylamines. Here again, SET radical mechanisms are not unique in this regard.

A third family of MAO inactivators, widely studied owing to their antidepressant properties,[21] is comprised of alkyl- and arylhydrazines. Enzymological efforts by Hellerman[22] and Singer[23] and their co-workers have shown that these processes involve initial formation of a diazene and the reduced MAO flavin and that this is followed by oxygen-dependent inactivation resulting in the production of at least two covalently modified MAOs. The major product from MAO inactivation by phenylhydrazene has been identified as a flavin 4a-phenyl adduct. No evidence is yet available to aid in the identity of the chemical mechanisms for both the initial redox reaction and ultimate alkylation process (*i.e.* diazene + MAO flavin) involved in hydrazine inactivations. We have explored the chemistry of the activated flavin **5** with a series of hydrazines and hydrazine analogs in order to uncover and understand mechanistic models for this chemistry.

The substrates used in this effort include phenylhydrazine (**13**), benzylhydrazine (**14**), benzyl carbazate (**15**), and *O*-benzylhydroxylamine (**16**). Preparative reaction of the flavinium salt **5** with hydrazine **13** in MeCN at 25 °C under an air atmosphere leads to generation of the 4a-phenyl and 4a-cyanomethyl flavin adducts, **21** (mp 216−217 °C) and **22** (mp 210−214 °C), in respective isolated yields of 28% and 55%, along with benzene (Scheme 8). The adduct ratio changed from 0.6 (**21**:**22**) to 6 (**21**:**22**-d_2) when the solvent was varied from CH_3CN to CD_3CN which gives a *d*-isotope effect on the product ratio of *ca.* 24. In contrast, benzylhydrazine reacts with flavinium salt **5** in MeCN under aerobic condition to yield the 4a-benzyl adduct **23** exclusively (89% isolated). Also, this latter process conducted under a limited-oxygen atmosphere gives rise to the air unstable 1,5-dihydro-5-ethyllumiflavin (**18**) (38%, precipitate) along with the adduct **23** (31%).

Several additional observations provide clues about the nature of these processes. Firstly, UV monitoring of the reactions of **5** with hydrazines **13** and **14** under aerobic conditions reveals the disappearance of the 415 and 558 nm bands associated with **5**, the appearance of 4a-adduct bands at 330 nm, and the transient (*ca.* 5−15 min) development and disappearance of a 633 nm band attributable[24] to the flavinyl radical **20**. Secondly, the hydroxylamine ether **16** undergoes instantaneous reaction with **5** to cleanly produce ($K_e = 1 \times 10^4$ M^{-1}) the 4a-adduct **17** (XR = OCH_2Ph) (62% isolated, mp 53−54 °C). This adduct is stable even when placed in the presence of excess amine **16** at 70 °C for 3 days. Also, UV monitoring of the **5** + **16** reaction shows that **17** (XR = OCH_2Ph) (λ_{max} = 348 nm) is formed in the absence of a detectable quantity of the transient radical **20**. Finally, the carbazate **15** adds to **5** to give the adduct **17** (XR = $NHCO_2CH_2Ph$) (λ_{max} = 345 nm, $K_e = 5 \times 10^3$ M^{-1}) again with no transient formation of the radical **20**.

The above results suggest that a combined polar/radical (but not SET) mechanistic pathway is operable in redox reactions of activated flavins with hydrazines and that the nature of the process is dependent upon the reaction conditions (aerobic *vs.* anaerobic) and the hydrazine structure. Accordingly, it appears that hydrazines, like their less-nucleophilic primary amine analogs, undergo rapid addition to activated flavins at the 4a-position to produce adducts **17** (Scheme 8). When an electron-withdrawing group is present (*i.e.* **17** with XR = $NHCO_2Bn$) or when the α-amino group is absent (*i.e.* **17** with XR = OBn) these adducts are stable even at elevated temperatures. However, 4a-adducts derived from simple alkyl- or arylhydrazines (*i.e.* **17** with XR = NHPh or NHBn) are labile toward fragmentation and rapidly react at ambient temperature to yield 1,5-dihydroflavin and diazene products. Under aerobic conditions, the dihydroflavin **18** is transformed back to its oxidized form **5**, a process which initiates the pathway leading to 4a-alkyl or 4a-arylflavin formation. While the evidence available thus far is limited, it appears likely that diazene reactions with flavinium salt **5** involve initial formation of 4a-intermediates **19**, which extrude nitrogen homolytically to give flavinyl (**20**) and alkyl/aryl radicals. Radical coupling either directly or following H-atom transfer from solvent then gives the ultimate adducts.

The potential relationships of these observations to those made in studies[22,23] of MAO inactivation by hydrazines are intriguing. Clearly, the model chemistry mimics well the process occurring on the enzyme in terms of the nature of the ultimate products[23] formed and the requirement for oxidized flavin and diazene in order to promote inactivation.[22] Consequently, these results suggest that a mechanistic pathway like that shown in Scheme 8 is reasonable for the MAO processes involved in the hydrazine−diazene−4a-adduct cascade.

Conclusions

Observations made in the current model studies show that polar mechanisms represent reasonable alternatives to the SET radical pathways proposed earlier for primary cyclopropylamine and α-silylamine inactivation reactions of the flavin containing monoamine oxidases. Importantly, this effort has pointed out that aminocyclopropane ring cleavage and silyl-transfer, thought to be unique for radical processes, are also consistent with polar pathways for flavoenzyme reactions. Thus, thoughts about these polar routes should be incorporated into the design of experiments aimed at gaining unambiguous evidence for chemical mechanisms for MAO catalysis and inactivation.

Experimental Section

General: ^1H NMR (200, 400, 500 MHz) and ^{13}C NMR (50 MHz) were recorded on $CDCl_3$ solutions and chemical shifts are reported in ppm relative to Me_4Si or $CHCl_3$ as internal standards. IR spectra were recorded on $CHCl_3$ solutions. Melting points are reported uncorrected. Column chromatography was performed with either Merck-EM type 60 silica gel (230−400 mesh) or Alcoa type F-20 alumina (neutral, 80−200 mesh) absorbents. Preparative TLC was performed on 20 × 20 cm plates coated with Merck-EM type 60 GH-254 silica gel. All reactions were run under a dry N_2 atmosphere unless otherwise specified. All substances are isolated as oils unless otherwise noted and all compounds are judged to be >0.95% pure by ^1H and ^{13}C NMR analysis.

(21) Loomer, H. P.; Saunders, J. C.; Kline, N. S. *Am. Psychiatr. Assoc. Rep.* **1957**, *8*, 129.

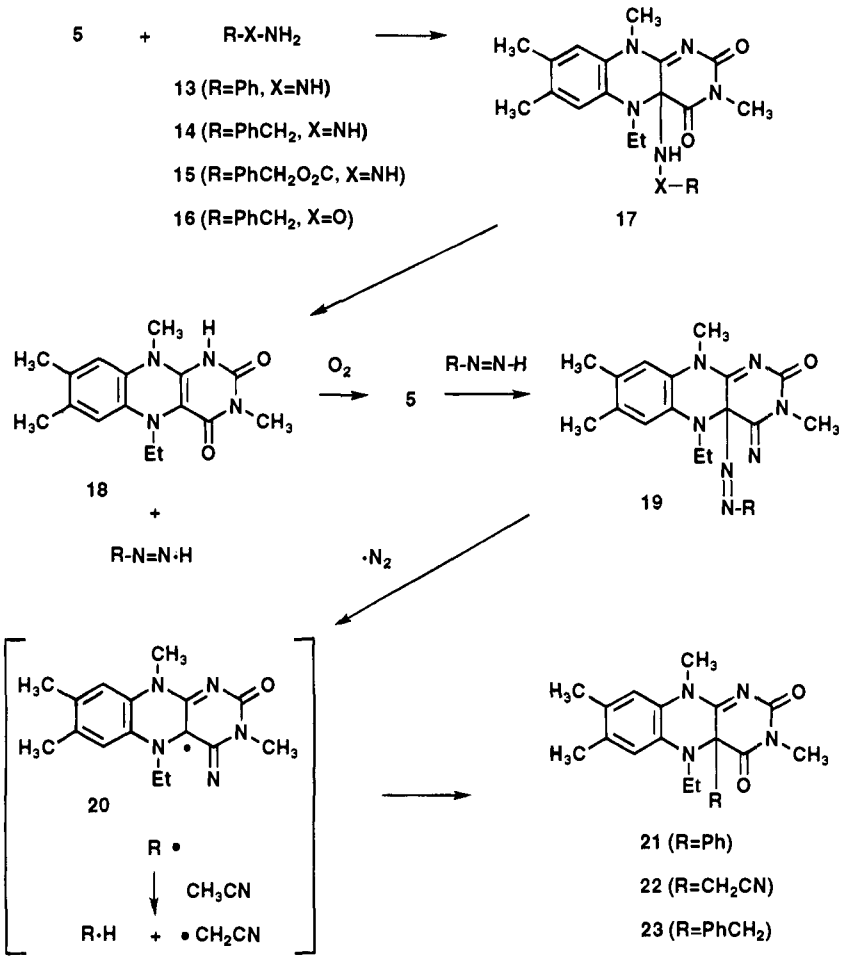
(22) Hellerman, L.; Erwin, V. G. *J. Biol. Chem.* **1968**, *243*, 5237. Patek, D. R.; Hellerman, L. *J. Biol. Chem.* **1974**, *249*, 2373. Schiele, B. C. *Ann. N.Y. Acad Sci.* **1963**, *107*, 1131.

(23) Nagy, J.; Kenney, W. C.; Singer, T. P. *J. Biol. Chem.* **1979**, *254*, 2684.

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

(25) This substance was prepared according to the method described in: Ghilsa, S.; Hartmann, U.; Hemmerich, P.; Muller, R. *Liebigs Ann. Chem.* **1973**, 1388.

Scheme 8



General Procedure for Flavinium Perchlorate 5 Reactions. 5-Ethyllumiflavinium perchlorate (**5**)[25] and the amine or hydrazine both in MeCN were rapidly mixed at 25 °C (an instantaneous color change from purple to green occurred). 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* to give a residue which was washed with hexane and then dried *in vacuo* to provide the 4a-adduct.

Reaction of the Flavinium Perchlorate 5 with *trans*-2-Phenylcyclopropylamine (1). Preparation of 4a-Adduct 6. The above general procedure using a 2:1 molar ratio of cyclopropylamine **1** and flavinium salt **5** led to the production of the adduct **6** (58%) as a mixture of two inseparable diastereomers in the ratio of 1.6:1.

6: UV λ_{max} = 343 nm, ϵ = 7800 (MeCN); ^{1}H NMR 0.67−0.91 (m, 5H, N-5 CH_2CH_3 and C-4a cyclopropyl CH_2), 1.52 and 1.64 (m, 1H, C-4a NHC*H*−), 1.99 and 2.03 (m, 1H, C-4a benzylic), 2.22 and 2.36 (s, 3H each, C-7 and C-8 CH_3), 2.40 and 2.51 (br s, 1H, C-4a NH), 3.14 (m, 2H, N-5 CH_2CH_3), 3.29 and 3.32 (s, 3H, N-10 CH_3), 3.60 and 3.64 (s, 3H, N-3 CH_3), 6.77−7.19 (m, 7H, H-6, H-9 and C-4a Ph); ^{13}C NMR 13.0 (N-5 CH_2CH_3), 16.0 and 16.7 (C-4a cyclopropyl CH_2), 19.4 and 19.5 (C-7 and C-8 CH_3), 23.8 and 24.5 (C-4a NHCH−), 27.9 and 28.0 (N-10 CH_3), 32.6 (C-4a benzylic), 34.9 and 35.0 (N-3 CH_3), 46.2 (N-5 CH_2CH_3), 69.3 (C-4a), 116.8 (C-6), 124.2, 124.5, 125.5, 125.7, 125.8, 128.1, 140.4, 140.5 (C-9 and C-4a Ph), 130.6, 130.8, 131.3, 131.6, 132.3, 132.4, and 133.6 (C-5a, C-7, C-8, and C-9a), 155.2 and 155.5 (C-10a), 160.7 (C-2), 168.3 and 168.4 (C-4); IR 1668, 1556 cm^{-1}; EIMS *m/z* (relative intensity) 431 (M, 1), 299 (64), 271 (100), 214 (79), 186 (43), 171 (25), 119 (75), 75 (68); HRMS (EI) *m/z* 431.2310 ($C_{25}H_{29}N_5O_2$ requires 431.2321).

Reaction of the Cyclopropylamine 4a-Adduct 6. A CH_3CN (10 mL) solution containing 43 mg (0.1 mmol) of 4a-adduct **6** and 148 mg (1.1 mmol) of cyclopropylamine **1** was heated at 85 °C for 7 days in the dark under a N_2 atmosphere. The mixture was concentrated *in vacuo*, and ^{1}H NMR analysis (triphenylmethane as an internal standard) of the residue showed that **6** had been consumed and that the known[8] imine **7** had formed (80%, based on **6**) along with the C_{10a}-spirohydantoin and benzimidazolium salt, decomposition products[26] of flvinium salt **5**.

Reaction of the Flavinium Perchlorate 5 with α-(Trimethylsilyl)-benzylamine 9. Preparation of 4a-Adduct 10. By using the general procedure with a 2:1 molar ratio of benzylamine **9**[27] and flavinium salt **5** the adduct **10** (78%) was obtained as a mixture of two inseparable diastereomers in the ratio of 1.4:1.

10: UV λ_{max} = 345 nm, ϵ = 6500 (MeCN); ^{1}H NMR −0.44 and −0.26 (s, 9H, TMS), 0.78−0.87 (m, 3H, N-5 CH_2CH_3), 2.22, 2.26, and 2.28 (s, 6H, C-7 and C-8 CH_3), 2.52 and 2.68 (s, 3H, N-10 CH_3), 2.86 and 2.92 (s, 1H, C-4a benzylic), 2.95−3.18 (m, 2H, N-5 CH_2CH_3), 3.42 and 3.68 (s, 3H, N-3 CH_3), 6.91 (s, 1H, H-6), 6.95 (s, 1H, H-9), 6.82 and 7.10−7.27 (m, 5H, C-4a Ph); ^{13}C NMR −4.24 and −4.2 (TMS), 12.9 and 13.0 (N-5 CH_2CH_3), 19.5, 19.6, and 19.7 (C-7 and C-8 CH_3), 27.3 and 27.9 (N-10 CH_3), 31.3 and 32.5 (N-3 CH_3), 46.1 and 46.6 (N-5 CH_2CH_3), 49.8 and 50.4 (C-4a benzylic), 69.9 and 70.1 (C-4a), 116.8 and 117.3 (C-6), 125.2, 125.6, 126.0, 126.5, 127.3, 128.0, 140.0, and 141.5 (C-9 and C-4a Ph), 131.1, 131.5, 131.8, 132.1, 132.2, 132.5, 133.2, and 133.4 (C-5a, C-7, C-8, and C-9a), 155.5 (C-10a), 160.2 and 160.7 (C-2), 170.0 (C-4); IR 1680, 1570 cm^{-1}; EIMS *m/z* (relative intensity) 477 (M, 0.1), 372 (4), 343 (6), 266 (9), 179 (21), 162 (12), 106 (100); HRMS (EI) *m/z* 477.2540 ($C_{26}H_{35}N_5O_2Si$ requires 477.2560).

Reaction of the 4a-Adduct 10. A mixture of the adduct **10** (43 mM) and the amine **9** (119 mM) in CD_3CN (0.7 mL) was stirred at 45 °C for 19 h under an Ar atmosphere. ^{1}H NMR analysis showed that

(26) Mager, H. I. X. *Tetrahedron Lett.* **1979**, *37*, 3549.
(27) Tsuge, P.; Tanaka, J.; Kanemasa, S. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1191.

the imine **12** (prepared independently from benzylamine **9** and benzaldehyde) had formed (17% based on the adduct **10**). The imine **12** [-0.06 ppm (9 H, TMS), 4.40 ppm (1H, benzylic), 8.30 ppm (1H, imine)] slowly converted to the known[7] imine **11** [4.76 ppm (2H, benzylic), 8.45 ppm (1H, imine)]. The efficiency for transformation of the imine **12** to **11** was demonstrated by the observation that the independently prepared **12** slowly converted to **11** even at 25 °C in CD_3CN.

Fluoride Promoted Reaction of the Adduct 10. A CD_3CN solution (0.7 mL) containing the adduct **10** (43 mM), the amine **9** (155 mM), and tetrabutylammonium fluoride (43 mM) was stirred for 19 h at 25 °C under N_2 atmosphere. ^1H NMR analysis of the reaction mixture revealed that the adduct **10** had been consumed and the benzaldimine **11** (78% based on **10**) had formed.

Reaction of the Flavinium Salt 5 and Phenylhydrazine (13) in CH_3CN. A mixture of the flavinium salt **5** (30 mg, 0.08 mmol) and the hydrazine **13** (40 mg, 0.37 mmol) in CH_3CN (3 mL) was stirred at 25 °C for 3 days under an air atmosphere. The mixture was concentrated *in vacuo* to give a residue which was subjected to preparative TLC (silica gel, ether) to yield 8 mg (28%) of the 4a-phenyl adduct **21** (mp 216−217 °C (ether)) and 14 mg (55%) of the 4a-(cyanomethyl) adduct **22** (mp 210−214 °C (ether)), λ_{max} = 330 nm in MeCN).

21: UV λ_{max} = 330 nm, ϵ = 5500 (MeCN); ^1H NMR 0.99 (t, J = 7.1 Hz, 3H, N-5 CH_2CH_3), 2.11 and 2.25 (s, 3H each, C-7 and C-8 CH_3), 3.15 (m, 2H, N-5 CH_2CH_3), 3.24 (s, 3H, N-10 CH_3), 3.70 (s, 3H, N-3 CH_3), 6.70 (s, 1H, H-6), 6.97 (s, 1H, H-9), 7.13 and 7.43 (m, 5H, C-4a Ph); ^{13}C NMR 13.3 (N-5 CH_2CH_3), 19.4 and 19.7 (C-7 and C-8 CH_3), 28.4 (N-10 CH_3), 32.5 (N-3 CH_3), 47.3 (N-5 CH_2CH_3), 62.0 (C-4a), 117.2 (C-6), 127.3 (C-9), 126.5, 128.5, 128.9, and 135.6 (C-4a Ph), 131.8, 132.6, 133.8, and 134.0 (C-5a, C-7, C-8, and C-9a), 156.7 (C-10a), 163.9 (C-2), 167.4 (C-4); IR 1680, 1570 cm^{-1}; EIMS *m/z* (relative intensity) 376 (M, 22), 319 (68), 304 (22), 291 (29), 290 (100), 233 (10), 214 (3), 131 (4), 77 (5); HRMS (EI) *m/z* 376.1887 ($C_{22}H_{24}N_4O_2$ requires 376.1899).

22: UV λ_{max} = 330 nm (MeCN); ^1H NMR 0.86 (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), 2.58 (d, J = 16.6 Hz, 1H, C-4a CH_2), 2.76 (d, J = 16.6 Hz, 1H, C-4a CH_2), 3.05 (m, 2H, N-5 CH_2CH_3), 3.42 (s, 3H, N-10 CH_3), 3.68 (s, 3H, N-3 CH_3), 6.98 (s, 1H, H-6), 7.03 (s, 1H, H-9); ^{13}C NMR 12.6 (N-5 CH_2CH_3), 19.5 and 19.8 (C-7 and C-8 CH_3), 25.5 (C-4a CH_2), 28.5 (N-10 CH_3), 32.9 (N-3 CH_3), 47.6 (N-5 CH_2CH_3), 58.5 (C-4a), 113.7 (C-4a CN), 117.5 (C-6), 127.0 (C-9), 129.9, 131.0, 133.0, 135.1 (C-5a, C-7, C-8, and C-9a), 155.1 (C-10a), 160.3 (C-2), 166.1 (C-4); IR 1690, 1575 cm^{-1}; EIMS *m/z* (relative intensity) 339 (M, 15), 299 (100), 271 (84), 214 (26), 185 (10), 103 (5); HRMS (EI) *m/z* 339.1704 ($C_{18}H_{21}N_5O_2$ requires 339.1695).

Reaction of the Flavinium Salt 5 and Benzylhydrazine (14) in CD_3CN. A mixture of the flavinium salt **5** (21 mg, 0.05 mmol) and the hydrazine **14** (48 mg, 0.39 mmol) in CD_3CN (3 mL) was stirred at 25 °C for 18 h under an air atmosphere. The mixture was concentrated *in vacuo* to give a residue which was subjected to preparative TLC (silica gel, ether) to yield 18 mg (89%) of the C-4a benzyl adduct **23**.

23: UV λ_{max} = 330 nm (MeCN); ^1H NMR 0.88 (t, J = 7.1 Hz, 3H, N-5 CH_2CH_3), 2.26 and 2.30 (s, 3H each, C-7 and C-8 CH_3), 2.86 (d, J = 12.8 Hz, 1H, C-4a benzylic), 2.94−3.09 (m, 3H, C-4a benzylic and N-5 CH_2CH_3), 3.01 (s, 3H, N-10 CH_3), 3.68 (s, 3H, N-3 CH_3), 6.81 (m, 2H, C-4a Ph), 7.02 (s, 1H, H-6), 7.11 (s, 1H, H-9), 7.17 (m, 3H, C-4a Ph); ^{13}C NMR 12.7 (N-5 CH_2CH_3), 19.4 and 19.8 (C-7 and C-8 CH_3), 27.6 (N-10 CH_3), 32.3 (N-3 CH_3), 43.5 (C-4a benzylic), 47.9 (N-5 CH_2CH_3), 64.0 (C-4a), 117.0 (C-6), 127.6 (C-9), 128.0, 128.4, and 129.3 (C-4a Ph), 131.8, 132.4, 133.9, and 134.3 (C-5a, C-7, C-8, C-9a, and C-4a Ph), 155.4 (C-10a), 163.1 (C-2), 168.1 (C-4); IR 1680, 1565 cm^{-1}; EIMS *m/z* (relative intensity) 390 (M, 10), 299 (100), 214 (42), 186 (16), 105 (23); HRMS (EI) *m/z* 390.2047 ($C_{23}H_{26}N_4O_2$ requires 390.2056).

When a similar reaction (3 days) was run under limited air (NMR tube) conditions, the dihydroflavin **18** crystallized from the reaction mixture (38%, mp 155−160 °C). ^1H NMR spectroscopic data for **18** matched those reported.[25] The remaining solution was concentrated *in vacuo* and the residue subjected to preparative TLC (silica gel, ether) to give 4a-benzyl adduct **23** (31%).

Reaction of the Flavinium Perchlorate 5 with *O*-Benzylhydroxylamine (16). Preparation of 4a-Adduct 17 (XR = OCH_2Ph). The 4a-benzyloxyamine adduct **17** (XR = OCH_2Ph) (62%, mp 53−55 °C (hexane)) was obtained. by the general procedure using a 2:1 molar ratio of the hydroxylamine ether **16** and flavinium salt **5**.

17 (XR = OCH_2Ph): UV λ_{max} = 348 nm, ϵ = 7000 (MeCN); ^1H NMR 0.83 (t, J = 7.2 Hz, 3H, N-5 CH_2CH_3), 2.23 and 2.27 (s, 3H each, C-7 and C-8 CH_3), 3.21 (q, J = 7.2 Hz, 2H, N-5 CH_2CH_3), 3.38 (s, 3H, N-10 CH_3), 3.50 (s, 3H, N-3 CH_3), 4.30 (ABq, J = 11.4 Hz, 2H, C-4a benzylic), 6.83 (s, 1H, H-6), 6.90 (s, 1H, H-9), 7.03 and 7.24 (m, 5H, C-4a Ph); ^{13}C NMR 13.1 (N-5 CH_2CH_3), 19.5 and 19.7 (C-7 and C-8 CH_3), 28.3 (N-10 CH_3), 32.6 (N-3 CH_3), 46.4 (N-5 CH_2CH_3), 69.9 (C-4a), 77.2 (C-4a benzylic), 117.1 (C-6), 123.3 (C-9), 128.0, 128.2, and 129.1 (C-4a Ph), 130.7, 131.4, 132.1, 133.6, and 136.1 (C-5a, C-7, C-8, C-9a, and C-4a Ph), 155.7 (C-10a), 159.6 (C-2), 168.1 (C-4); IR 1677, 1570 cm^{-1}; CIMS *m/z* (relative intensity) 422 (M + 1, 0.5), 299 (100), 271 (93), 214 (40); HRMS (CI) *m/z* 422.2162 ($C_{23}H_{28}N_5O_3$ requires 422.2192).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds **6**, **10**, **21**, **22**, **23**, and **17** along with UV−visible spectra of mixtures of flavinium salt **5** with varying concentrations of amines **9**, **15**, and **16** (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS. See any current masthead page for ordering information.

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