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Abstract: A new strategy for the design of monoamine oxidase (MAO) inhibitors is proposed. The strategy is based on the premise that tertiary-amine containing MAO-inactivators which operate by alkylation of active site nucleophiles are activated in situ by single electron transfer (SET) to the MAO-flavin cofactor to form covalent cysteine thioether linkages to the enzyme active sites. The covalent modification of active site cysteine thiol residues by the unsaturated ketone groups in these substances was confirmed by demonstrating that they serve as active site-directed, time-dependent, nonredox based, inactivators of MAO-A and MAO-B. In the key test of the feasibility of the new MAO-inactivator design strategy, it was shown that selected tertiary-allylic and -propargylic -amino-alcohols were expected to serve as precursors of conjugated ketones in SET-promoted processes. Evidence supporting this hypothesis was gained from studies of model SET-photoreactions of members of this amino-alcohol family with 3-methyl lumiflavin (3MLF). The efficient production of 4a- and 4a,5-flavin adducts in these excited-state reactions demonstrates that amine radicals, arising by SET-oxidation of tertiary -allylic and -propargylic -amino-alcohols, fragment to generate α,β-unsaturated ketones which react rapidly with the simultaneously formed 3MLF-hydroflavin anion. The second feature of the MAO-inactivator design strategy pathway was tested by examining reactions of the MAOs with substances which contain electrophilic, conjugated enone and ynone moieties tethered to amine functions to ensure delivery to the enzyme active sites. The covalent modification of active site cysteine thiol residues by the unsaturated ketone groups in these substances was confirmed by demonstrating that they serve as active site-directed, time-dependent, nonredox based, inactivators of MAO-A and MAO-B. In the key test of the feasibility of the new MAO-inactivator design strategy, it was shown that selected tertiary-allylic and -propargylic β-amino-alcohols undergo redox reactions in the MAO-A active site which result in inactivation of the enzyme via covalent modification of a single cysteine residue. The experimental results which support the conclusions stated above are presented and discussed in this paper.

Introduction

The monoamine oxidases MAO-A and MAO-B are two structurally homologous, redox active enzymes found in the mitochondrial membranes of most mammalian tissues. The primary function of the MAOs is to regulate the concentrations of primary and secondary amines by catalyzing their oxidative deamination to produce amine and carbonyl products. Among the more important substrates of these enzymes are the neurologically active amines epinephrine, dopamine, and serotonin. As a consequence, the MAOs play key roles in regulating the level of these neurotransmitters in the human brain. MAO-inhibitors cause an elevation in the levels of the neuroactive amines and are thus of medicinal importance. MAO-A which preferentially oxidizes serotonin is selectively and irreversibly inhibited by the tertiary propargylic amine clorgyline (1), an agent used in the treatment of depression. On the other hand, MAO-B is preferentially inactivated by the inhibitor deprenyl (2) which is used in conjunction with L-DOPA to treat Parkinson’s disease.

Both MAO-A and MAO-B contain a flavin (FAD) cofactor, covalently bound through a cysteine thioether linkage to the 8-position of the isoalloxazine ring. The chemical steps of

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MAO catalysis, represented in Scheme 1, involve simultaneous oxidation of the amine substrate and reduction of the oxidized flavin cofactor to generate imine and 1,5-dihydroflavin products. Hydrolysis of the imine and flavin oxidation by molecular oxygen then complete the catalytic cycle.

The first partial reaction, oxidation of the amine substrate, has been the focus of much mechanistic speculation. While some investigators favor a single electron transfer (SET)/radical mechanism for the oxidation of primary and secondary amines, the polar addition–elimination mechanism originally proposed by Hamilton is equally feasible (Scheme 2). Tertiary amines, on the other hand, because of their reduced nucleophilicity and increased electron-donor ability, are perhaps better candidates for the SET initiated mechanistic pathway.

Much of what is known about the chemical mechanism of the amine oxidation partial reaction catalyzed by MAO has derived from the results of studies of the mechanism(s) for the amine oxidation partial reaction catalyzed by MAO has derived from the results of studies of the mechanism(s) for the oxidation of primary and secondary amines, the polar addition–elimination mechanism originally proposed by Hamilton is equally feasible (Scheme 2). Tertiary amines, on the other hand, because of their reduced nucleophilicity and increased electron-donor ability, are perhaps better candidates for the SET initiated mechanistic pathway.

There seems to be agreement that the respective catalytic and inactivation of the enzyme by mechanism based inhibitors.10,11

Based on the documented retro-aldol type fragmentation reactivity of β-hydroxy-aminium radicals, we anticipated that tertiary β-allylic and β-propargylic β-amino alcohols of general structure would serve as precursors of conjugated ketones in SET-promoted processes (Scheme 4) and, consequently, as MAO-inactivators.

A combination of photochemical and biological studies with selected members of this family of compounds have provided preliminary results which demonstrate that (1) SET-induced photochemical reactions of these substances with the model flavin, 3-methylumiflavin, results in production of conjugated ketone intermediates, (2) conjugated ketones which are tethered to tertiary amine functions to ensure active site binding inactivate the MAOs by covalent modification of a single cysteine thiol residue, and (3) selected tertiary β-allylic and β-propargylic β-hydroxyamines inactivate MAO-A by a pathway that involves redox participation by the flavin cofactor and that results in alkylation of an active site cysteine thiol grouping.

Scheme 1

\[
\begin{align*}
& \text{H}_2\text{O}_2 \\
& \text{O}_2 \\
& \text{MAO} + \text{SCOGY} \\
& \text{RCH}_2\text{NHR}^+ \\
& \text{RCHO + H}_2\text{NR}^+ \\
& \text{MAO} + \text{SCOGY} \\
& \text{FADH}_2 \\
& \text{RCH}_2\text{NHR}^+ \\
& \text{RCHO + H}_2\text{NR}^+ \\
\end{align*}
\]

Scheme 2

\[
\begin{align*}
\text{SET - Radical Mechanism} \\
\text{MAO-FI} + \text{RCH}_2\text{NH}_2 & \xrightarrow{\text{SET}} \text{MAO-FI}^+ + \text{RCH}_2\text{NH}_2 \\
\xrightarrow{1. \text{-- H}^+} & \text{2. SET} \\
\text{MAO-FI}^+ + \text{RCH}_2\text{NH}_2 & \xrightarrow{\text{H}^+} \text{MAO-FI-H} + \text{RCH}_2\text{NH}_2 \\
\end{align*}
\]

\[
\begin{align*}
\text{Addition - Elimination Mechanism} \\
\text{MAO-FI} + \text{RCH}_2\text{NH}_2 & \xrightarrow{\text{[H]}} \text{MAO-FI}^+ + \text{RCH}_2\text{NH}_2 \\
\end{align*}
\]

Scheme 3

\[
\begin{align*}
\text{MAO-FI}^+ + \text{R}_2\text{N-CR}_2\text{E} \xrightarrow{\text{SET}} & \text{MAO-FI}^+ + \text{R}_2\text{N-CR}_2\text{E} \\
\xrightarrow{\text{E}^+} & \text{R}_2\text{N-CR}_2\text{E} \\
\end{align*}
\]

Scheme 4

\[
\begin{align*}
\text{HO} & \xrightarrow{\text{SET}} \text{HO}_2 \\
\text{R} & \xrightarrow{\text{retro-aldol fragmentation}} \text{R}_2\text{N-CR}_2\text{E} \\
\end{align*}
\]

Results

SET-Photochemical Studies. It is well-recognized that the triplet excited state of 3-methylllumiflavin (3MLF), produced by excitation and intersystem crossing, is an excellent one-electron oxidizing agent owing to its large excited-state reduction potential (ca. +1.3 V).

As such, photoreactions of this flavin serve as useful models to explore SET-induced reactions of electron donors. To determine if the amino radicals derived from tertiary β-allylic and β-propargylic β-hydroxamines do indeed undergo efficient retro-aldol type fragmentation reactions to generate electrochemical conjugated ketones, we have examined the SET-promoted photoreactions of the β-hydroxamines 4–7 with 3-methylllumiflavin.

The synthetic sequences used to prepare 4–7 are provided in Supporting Information. Photoreactions were performed by irradiating N2-purged MeOH solutions containing 3MLF and the SET-promoted photoreactions of the

indeed undergo efficient retro-aldol type fragmentation reactions -J. Am. Chem. Soc.-

which results in generation of conjugated ketone intermediates.

known silylmethylamino-enone 11 and -ynone 15 and -unsaturated carbonyl analogues bring about efficient inactivators of MAO-A over the time periods in which their α,β-unsaturated carbonyl analogues bring about complete inactivation of the enzyme, suggesting that the reactions occurring between MAO-A and 11–16 must involve the respective enone or ynone functionalities.

To demonstrate that the MAO inactivation occurs while the tethered enones/ynones are bound to the MAO-A active site, the respective enone or ynone functionalities.

Table 1. Data for Competitive Inhibition and Inactivation of MAO-A by Amino-enones 11–14, Aminoynones 15 and 16, Related Substances 17–19, and β-Hydroxamines 5–7

<table>
<thead>
<tr>
<th>compd</th>
<th>$K_i$ (mM)</th>
<th>$K_{\text{inact}}$ (mM)</th>
<th>$k_{\text{inact}} \times 10^2$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>4.8 ± 0.5</td>
<td>13.8 ± 0.3</td>
<td>9.5 ± 0.1</td>
</tr>
<tr>
<td>12</td>
<td>4.1 ± 0.5</td>
<td>7.6 ± 0.5</td>
<td>13.0 ± 0.3</td>
</tr>
<tr>
<td>13</td>
<td>1.5 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>14</td>
<td>4.0 ± 0.6</td>
<td>6.0 ± 0.2</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>15</td>
<td>2.8 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>16</td>
<td>0.4 ± 0.3</td>
<td>3.7 ± 0.5</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>17</td>
<td>1.0 ± 0.1</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>18</td>
<td>0.8 ± 0.2</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>19</td>
<td>1.9 ± 0.2</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>5</td>
<td>2.1 ± 0.2</td>
<td>13.9 ± 0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>(+)-(R)-5</td>
<td>1.4 ± 0.1</td>
<td>11.8 ± 0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>(−)-(S)-5</td>
<td>2.3 ± 0.4</td>
<td>2.5 ± 0.5</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>6</td>
<td>0.4 ± 0.03</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>7</td>
<td>2.0 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>0.7 ± 0.08</td>
</tr>
</tbody>
</table>

* Not determined.

The ability of the amino-enones and -ynones to serve as inactivators of MAO-A was examined next. MAO-A (50 mM) in Na$_2$HPO$_4$ buffer (pH 7.2) containing 0.2% Triton-X at 25 °C was independently reacted with 11–16. Catalytic activity was monitored periodically by using the kynuramine assay. As exemplified in Figure 1 for the case of amino-enone 11, MAO-A inactivation occurs in a time and concentration dependent manner. Kitz-Wilson replots of the reciprocals of the apparent rate constants for the inactivation processes vs the reciprocals of the inactivator concentrations provide the rate constants for inactivation, $k_{\text{inact}}$, and the inactivation dissociation constants, $K_{\text{inact}}$, listed in Table 1. Significantly, the amino-ketones 17 and 18 and the tertiary α-silylamine 19 do not serve as efficient inactivators of MAO-A over the time periods in which their α,β-unsaturated carbonyl analogues bring about complete inactivation of the enzyme, suggesting that the reactions occurring between MAO-A and 11–16 must involve the respective enone or ynone functionalities.

The synthetic sequences used to prepare 4–7 are provided in Supporting Information. Photoreactions were performed by irradiating N$_2$-purged MeOH solutions containing 3MLF and the allylic and propargylic amino alcohols with Uranium glass filtered-light ($\lambda > 320$ nm, ensuring that 3MLF is the primary light absorbing species). The progress of each photoreaction was monitored by UV-visible spectroscopy, and irradiation was terminated when the absorbance at 450 nm associated with 3MLF reached ca. 20% of its original value. Product separation was accomplished by either silica gel TLC or precipitation (in the case of flavin 4a-adducts). The results in terms of products and yields are summarized in Schemes 5. The efficient formation of flavin adducts in these photoreactions is consistent with the proposed retro-aldol type reaction of intermediate tertiary β-allylic and β-propargylic β-hydroxaminium radicals which results in generation of conjugated ketone intermediates.

Determination of Enzymatic Inhibition and Inactivation Constants for Tethered Amino-Enones and Ynones.

The known silylmethylamino-enone 11 and -ynone 15 and their homologues and non-silicon containing analogues 12, 14, and 16 were prepared (Supporting Information) in order to determine if, and how, active site-bound, conjugated ketones inactivate the MAOs. Accordingly, these substances were first tested as inhibitors of the MAOA-catalyzed oxidation reaction of kynuramine. Double reciprocal plots of the initial velocities versus kynuramine concentration at various inhibitor concentrations showed that 11–16 are all reversible, competitive inhibitors of this enzyme. The inhibition constants, $K_i$, determined for these substances as well as for the saturated amino-ketones


(20) (a) Kitz, W.; Wilson, B. J. Biol. Chem. 1962, 237, 3245. (b) Lineweaver H.; Burke, D. J. Am. Chem. Soc. 1934, 56, 658.


(22) Ellman, G. L. Arch. Biochem. Biophys. 1959, 82, 70.
Design of Monoamine Oxidase Inactivators

Table 2. Free-Thiol Determinations on MAO-A and Its Derivatives Arising by Inactivations with Aminoenones 11 and 12. Aminoynone 15, and Hydroxyamines 5 and 7

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of Thiols</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO-A</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>MAO-A + 11</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>MAO-A + 12</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>MAO-A + 15</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>MAO-A + 5</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>MAO-A + 7</td>
<td>5.6 ± 0.1</td>
</tr>
</tbody>
</table>

* Determined by use of the DTNB titration method (ref 25) and three independent experiments each.

flavine moiety. UV-visible spectroscopic methods were used to distinguish between the two mechanistic pathways for MAO-A inactivation. Accordingly, three deoxygenated, sealed cuvettes containing MAO-A alone, MAO-A and the aminoynone 11, and MAO-A and kynuramine were incubated for a 2 h time period leading to ca. 97% inactivation of the enzyme. The solution containing MAO-A and kynuramine, as expected, experiences a rapid decrease in the flavin absorbance in the 350–550 nm region as a result of reduction to the 1,5-dihydro form. In contrast, the oxidized flavin spectrum of the solution containing MAO-A and 11 remains unchanged during this time period. Thus, the flavin cofactor in MAO-A is not redox active in the reaction with 11.

To demonstrate that amino-enone inactivation is a consequence of alkylation of an MAO cysteine thiol function, the number of free thiols within the enzyme before and following inactivation was determined by using the 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) titration method. Accordingly, native MAO-A was found to contain seven free thiol functions in agreement with the seven known (i.e., predicted from the gene sequence) free cysteine residues of this enzyme (Table 2). In contrast, DTNB titrations of the enzymes, inactivated by the amino-enones 11 and 12, showed that both contain ca. 6-titratable thiol moieties (Table 2). However, the amino-ynone 15 serves as a much less selective alkylating agent for MAO-A, indicated by the data in Table 2; complete inactivation of MAO-A by 15 results in a modified enzyme that contains ca. 1-free thiol only. The combined results demonstrate conclusively that the major, if not exclusive, process involved in MAO-inactivation by the amino-enones (and perhaps -ynones) is active site thiol alkylation.

Determination of MAO Binding Constants for the β-Allylic- and β-Propargylic-β-hydroxyamines. The β-hydroxyamines 5–7, although lacking phenolic functionality, are structural analogues of the aryl-ethanolamine containing MAO inhibitors/substrates, amphetamine, epinephrine, and norepinephrine. Thus, modest binding affinities to the MAO active sites were expected. Indeed, the steady-state initial velocity data listed in Table 1 show that 5–7 are reversible competitive inhibitors of MAO-A, with dissociation constants which are of comparable magnitude to those of other tertiary amines (e.g., $K_i = 7.1 \text{ mM}$ for MAO-B by N,N-dimethyl-N-(2-phenylethyl)-amine$^{26}$ and sterically crowded primary amines (e.g., $K_i = 0.2 \text{ mM}$ for MAO-B by 1-phenylcyclopentylamine$^{27}$).

To determine if the individual enantiomers of these β-amino alcohols have different MAO-A binding affinities, resolution of 5 was carried out. Separation of the antipodes was performed

and (S)-stereoselectivity in binding the allylic (Supporting Information). As indicated by the similar magnitudes of the dissociation constants for MAO inactivation with the (R)- and (S)-enantiomers of \(\text{S}-\text{amphetamine} \) (Table 1), the enzyme displays little stereoselectivity in binding the allylic \(\beta\)-amino alcohol enantiopodes. This is not an unexpected result based on the observation\(^{28}\) that MAO-A displays only a small binding preference for the (R)- (\(K_i = 0.1\) mM) vs the (S)- (\(K_i = 0.11\) mM) enantiomers of N-methylamphetamine.

![Figure 1](image1.png)

**Figure 1.** A plot of the natural log of the percent activity remaining vs time for inactivation of MAO-A by the amino-enone \(\text{S}\)- MAO-A (8.5 \(\mu\)M was incubated with \(\text{S}\) (0, square; 1, diamond; 2.5, circle; 5.0, triangle; 7.5, filled square; 10.0, filled diamond mM) in 50 mM \(\text{Na}_2\text{HPO}_4\) (pH 7.2) solutions containing 0.2% Triton-X at 25 °C. Aliquots (50 \(\mu\)L) were removed periodically and assayed for MAO-activity by use of the kynuramine procedure.

by HPLC through the use of a Chiralcel OJ column or by crystallization of and regeneration from the (\(\text{S}\)-dibenzoyl-tartrate salt. Absolute stereochecmical assignments to (\(+\))-5 and (\(-\))-5 as (\(R\)) and (\(S\)), respectively, were made based on X-ray crystallographic analysis of the tartrate salt \(\text{S}\) of (\(-\))-5 (Supporting Information). As indicated by the similar magnitudes of the inhibition constants exhibited by the (\(R\))-enantiomer and (\(S\))-enantiomer of 5 (Table 1), the enzyme displays little stereoselectivity in binding the allylic \(\beta\)-amino alcohol enantiopodes. This is not an unexpected result based on the observation\(^{28}\) that MAO-A displays only a small binding preference for the (\(R\))- (\(K_i = 0.1\) mM) vs the (\(S\))- (\(K_i = 0.11\) mM) enantiomers of N-methylamphetamine.

![Figure 2](image2.png)

**Figure 2.** Plot of the natural log of the percent activity remaining vs time for inactivation of MAO-A by \(\text{S}\). MAO-A (10 mM) was incubated with \(\text{S}\) (0, square; 1, diamond; 2, circle; 4, triangle; 8, filled square; 10 mM, filled diamond) in 50 mM \(\text{Na}_2\text{HPO}_4\) buffer (pH 7.2) at 25 °C. MAO-A activity was monitored by use of the kynuramine assay.

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**Determination of the Thermodynamic and Kinetic Constants for MAO Inactivation with the \(\beta\)-Allylic- and \(\beta\)-Propargylic-\(\beta\)-Hydroxaminines.** The \(\beta\)-allylic and \(\beta\)-propargylic \(\beta\)-hydroxaminines \(\text{S} \) – \(\text{S}\) were tested as irreversible inhibitors of MAO-A by use of time dependent reactions in which catalytic activity was assayed by use of the kynuramine method.\(^{19}\) The apparent rate constants for inactivation, derived from the slopes of plots of the natural log of activity remaining vs time (Figure 2 for \(\text{S}\)), were evaluated. From the Y-intercepts of double reciprocal plots\(^{20}\) of apparent \(k_{\text{inact}}\) value vs inactivator concentration, the \(k_{\text{inact}}\) value were derived, and from the X-intercepts, the dissociation constants, \(K_{\text{inact}}\), for MAO-inactivator were determined. The inactivation constants determined in this manner for \(\text{S}\) and \(\text{S}\) are recorded in Table 1. Interestingly, the primary \(\beta\)-amino alcohol \(\text{S}\) was found not to be an MAO-A inactivator.

To determine if MAO-A inactivation by the \(\beta\)-amino alcohols \(\text{S}\) and \(\text{S}\) is associated with covalent modification of the enzyme, active site protection experiments were carried out. Specifically, the inactivation reactions were carried out with 16 mM of each inactivator and in the presence or absence of saturating (\(+\))- (\(S\)-amphetamine (1.6 mM). As expected for mechanism-based inactivation, (\(+\))- (\(S\)-amphetamine completely blocks the reactions of \(\text{S}\) and \(\text{S}\) (16 mM) with this enzyme. In addition, application of the DTNB titration method shows that MAO-A inactivated by both the allylic- and propargylic-tertiary amino alcohols, \(\text{S}\) and \(\text{S}\), contains one less cysteine-thiol function than the native enzyme (see Table 2).

UV-visible spectroscopic monitoring of the reaction of MAO-A with \(\beta\)-amino alcohol \(\text{S}\) has provided information about the redox-participation of the flavin cofactor in the process. As shown by the spectra reproduced in Figure 3, anaerobic reaction of MAO-A with \(\text{S}\) is associated with a change from the typical enzyme oxidized flavin spectrum with a maximum at ca. 450 nm to one that contains a maximum at ca. 410 nm. This change, which is reversed by admission of oxygen into the reaction solution, is not consistent with complete reduction of the flavin residue in the enzyme to produce either the 1.5-dihydro form.

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or a 4a- or N5-adduct. In fact, previous efforts have shown that the 410 nm absorption band is associated with an anionic flavin-semiquinone radical. This species might be a metastable product in the \( \beta \)-amino alcohol inactivation pathway (see below) suggesting that the flavin cofactor of MAO-A is involved in the mechanism for inactivation by 5 and 7.

As shown in Scheme 4, fragmentation of aminium radicals derived from the allylic and propargylic \( \beta \)-amino alcohols 5 and 7 forms a dimethylaminomethyl radical which would be further transformed by oxidation and subsequent hydrolysis to \( N,N \)-dimethylaniline. Dabsyl-chloride treatment of the crude mixture obtained from reaction of MAO-A with amino-alcohol 5 leads to isolation of the dimethylamine derived sulfonamide characterized by TLC, HPLC, and \( ^1H \) NMR analyses.

Finally, MAO-inactivation by the allylic- and propargylic-amino alcohols 5 and 7 appears to be selective for MAO-A. For example, although 5 is a modest competitive inhibitor of MAO-B (\( K_I = 2.7 \) mM), neither it nor its acetylene analogue 7 are inactivators of the B-enzyme.

**Discussion**

The results presented above demonstrate the validity of the proposed strategy for MAO-inactivator design. It is clear that SET-photochemical processes can serve as a predictive method for the identification of tertiary amines which are capable of reacting with the MAOs to generate electrophilic products in the enzyme active site region. The observations made in the current photochemical study confirm that tertiary amines which are capable of reacting with the MAOs to generate electrophilic products in the enzyme active site region. These substances serve as affinity labeling agents owing to the presence of both the amine functions which lead to active site binding and the \( \alpha, \beta \)-unsaturated ketone groups which provide Michael addition reactivity. The active nucleophile in these inactivation processes is a cysteine thiol which earlier chemical studies have identified as being required for catalytic activity.

The results point to the operation of mechanism based pathways for MAO-A inactivation by the \( \beta \)-hydroxyamines 5 and 7. In a manner consistent with our proposal, the enzymatic sequence (Scheme 8) is most likely initiated by SET within the enzyme—inactivator complex which is then followed by retro-aldol fragmentation of the intermediate tertiary aminium radical. This step might be facilitated by the same basic residue that participates as a general base in the MAO-catalytic reaction. The \( \alpha, \beta \)-unsaturated carbonyl product formed in this manner serves as a Michael acceptor in reaction with a cysteine thiol to produce the inactivated enzyme. In the presence of oxygen, both the anionic flavin semiquinone, possibly responsible for the 410 nm absorption band under anaerobic conditions, and the dimethylaminomethyl radical are converted to the respective oxidized flavin and the iminium cation precursor of dimethyldimine.

A few additional observations made in this study are worthy of comment. The first concerns the fact that the primary amine 6, although serving as a comparable reversible competitive inhibitor of MAO-A, is not an inactivator of this enzyme. While there are a number of possible reasons for the lack of reactivity of this substance, one of these might be related to the fact that

\[ \text{Scheme 8} \]

\[ \text{Scheme 7} \]

\[ \text{Discussion} \]


primary amines often have higher oxidation potentials than their tertiary analogues. Indeed, the current photochemical studies suggest that the aminium radical derived from \( \text{pargyline} \) might arise to serve as an MAO-A inactivator may be a consequence of the fact that, like other primary and secondary amine substrates and inactivators, does not serve as an effective SET-donor to the flavin group in the enzyme.

The possibility exists that several other well-known MAO inactivation reactions, previously discussed in terms of radical coupling mechanisms, may also follow routes involving generation of and alkylation by electrophilic intermediates. For example, Silverman has shown that trans-2-phenylcyclopropylamine \( \text{22} \) reacts with MAO-B to yield a single covalent adduct via alkylation of the key cysteine residue. Bond formation in this process may well be a result of the formation of Michael addition and cinnamaldehyde (or its imine) (see Scheme 9).

In a similar way, MAO-B inactivation by the tertiary propargylic amine, pargyline \( \text{23} \) may be promoted by an initial SET step, but the known flavocyanine adduct \( \text{4} \) might arise from a local slaughter house, and was purified by a minor modification of the procedure of Salach. The alanine radical derived from \( \text{Saccharomyces cerevisiae} \) which was overexpressed with human liver MAO-A (gift from Walter Weyler). MAO-B was obtained from beef liver, purchased fresh from a local slaughter house, and was purified by thin-layer chromatography (TLC) performed with the use of 230–400 mesh silica gel, 100–200 mesh fluorisorb, or 80–200 mesh alumina. Unless otherwise noted, materials obtained from commercial sources were used without further purification. All distillations were performed under dry \( \text{N}_2 \) or Ar atmospheres unless otherwise noted.

Irradiation of 3MLF and the Amino Alcohol 4. A solution (170 mL) of 77 mg (0.26 mmol) of 3MLF and 120 mg (0.45 mmol) of the amino alcohol \( \text{4} \) in MeOH was irradiated for 1 h. The photolysate was concentrated in vacuo to give a residue which was dissolved in CHCl\(_3\) and diluted with \( n \)-hexane. The resulting precipitate was collected by filtration to give 101 mg (96%) of the adduct \( \text{8} \). Concentration of the filtrate gave 5 mg (4%) of \( \text{3-(N-benzyl-N-methyl)-aminopropanophenone} \) (9).

Irradiation of 3MLF and 4-(N,N-Dimethylamino)-3-phenylbut-1-en-3-ol (5). A solution of 71 mg (0.26 mmol) of 3MLF and 86 mg (0.45 mmol) of the amino alcohol \( \text{5} \) in MeOH (150 mL) was irradiated for 1 h. The photolysate was concentrated in vacuo to give a residue which was dissolved in 1 mL of CHCl\(_3\). To this solution was added 30 mL of \( n \)-hexane, and the resulting precipitate was collected by filtration to give 98 mg of the 4a-adduct (91%) \( \text{8} \). Irradiation of 3MLF and 4-Amino-1-buten-3-phenyl-3-ol (6). A solution of 71 mg (0.26 mmol) of 3MLF and 74 mg (0.46 mmol) of the amino alcohol \( \text{6} \) in MeOH (150 mL) was irradiated for 1 h. The photolysate was concentrated in vacuo to give a residue which was washed with dry N\(_2\) or Ar atmospheres unless otherwise noted.

Experimental Section

General. All reactions were run under N\(_2\) or Ar atmospheres unless otherwise noted and magnesium sulfate was used as a drying agent. All new compounds were obtained as oils in \( >90\% \) purity (by \( ^1\text{H} \) and \( ^13\text{C} \) NMR) unless otherwise noted. \( ^1\text{H} \) NMR (200, 400, 500 MHz) and \( ^13\text{C} \) NMR (50 MHz) spectra were recorded on CDC\(_3\) solutions unless otherwise noted and chemical shifts are reported in parts per million relative to CHCl\(_3\) (7.24 for \( ^1\text{H} \) NMR and 77.00 for \( ^13\text{C} \) NMR) as an internal standard. Coupling constants are given in Hertz (Hz). \( ^13\text{C} \) NMR resonance assignments were aided by use of the DEPT technique to determine number of attached hydrogens. Mass spectrometric data was obtained by use of either electron impact (EI) or chemical ionization (CI) techniques and fragments are recorded as \( m/z \) (relative intensity). Infrared (IR) spectroscopic data are recorded in units of \( \text{cm}^{-1} \). Optical rotations were recorded by using the sodium D-line (589 nm). Thin-layer chromatography (TLC) was performed on 0.25 mm silica coated glass or plastic plates. Preparative TLC was performed on 20 \times 20 cm plates coated with silica gel. Column chromatography was performed with the use of 230–400 mesh silica gel, 100–200 mesh fluorisorb, or 80–200 mesh alumina. Unless otherwise noted, materials obtained from commercial sources were used without further purification. All distillations were performed under dry N\(_2\) or Ar atmospheres unless otherwise noted. All reaction solvents were dried and distilled prior to use. The solvent used for photo-reactions (MeOH) was of spectrograde quality.

Preparative photochemical reactions were conducted by using an apparatus consisting of a 450W Hanovia medium pressure, mercury lamp surrounded by a Uranium glass filter (\( \lambda > 330 \text{ nm} \)) within a quartz, water cooled well immersed in the reaction solution (ca. 15 \( ^\circ \text{C} \) reaction temperature). In each case, the photolysis solution was purged with deoxygenated N\(_2\) before and during irradiation.

MAO-A (\( K_M = 0.12 \text{ mM} \), \( k_{cat} = 35 \text{ min}^{-1} \)) was purified from the yeast \( \text{Saccharomyces cerevisiae} \) which was overexpressed with human liver MAO-A (gift from Walter Weyler). MAO-B was obtained from beef liver, purchased fresh from a local slaughter house, and was purified by a minor modification of the procedure of Salach.36

Irradiation of 3MLF and the Amino Alcohol 4. A solution (170 mL) of 77 mg (0.26 mmol) of 3MLF and 120 mg (0.45 mmol) of the amino alcohol 4 in MeOH was irradiated for 1 h. The photolysate was concentrated in vacuo to give a residue which was dissolved in CHCl\(_3\) and diluted with \( n \)-hexane. The resulting precipitate was collected by filtration to give 101 mg (96%) of the adduct 8. Concentration of the filtrate gave 5 mg (4%) of 3-(N-benzyl-N-methyl)-aminopropanophenone (9).
dissolved in ether. Filtration gave 17 mg (24%) of 3-MLF as the precipitate. Concentration of the filtrate in vacuo gave a residue which was subjected to preparative TLC on silica gel (ether) to provide 55 mg (68%) of the 4a-adduct.\(^{8}\)

**Irradiation of 3MLF and 4-(N,N-Dimethylamino)-3-phenylbut-1-yn-3-ol (7).** A solution of 50 mg (0.19 mmol) of 3MLF and 105 mg (0.56 mmol) of the amino alcohols 7 in 150 mL of MeOH was irradiated for 2 h. The photolyzed was concentrated in vacuo giving a residue which was mixed with ether, followed by filtration to give 26 mg of 3-MLF as the precipitate. The filtrate was concentrated in vacuo giving a residue which was subjected to preparative TLC on silica gel (ether) to yield 27 mg of recovered amino alcohol and 18 mg (50%) of the adduct.\(^{9}\)

**Time Dependent Inactivation of MAO-A.** Aliquots of an MAO-A stock solution were added to solutions containing varying concentrations of the inactivators in 50 mM sodium phosphate buffer (pH 7.2), containing 0.2% Triton X-100. After mixing, the samples were incubated at 25 °C and periodically assayed for enzyme activity by measuring the increase in absorbance at 250 nm. Reactions were initiated by adding MAO-A (final concentrations in individual cuvettes that were then sealed with rubber septa. These reactions were incubated at 25 °C and periodically assayed for enzyme activity by measuring the increase in absorbance at 250 nm. One unit of enzyme activity equals that amount needed to form 1 nmol of product per minute.

**Inhibition of MAO-A Catalysis of Kynuramine Oxidation.** Initial velocities for MAO-A catalyzed oxidation of kynuramine were determined by measuring the change in absorbance at 314 nm. The enzyme activity of MAO-B was measured by use of a modified procedure of Tabor.\(^{60}\) MAO-B in 50 mM sodium phosphate buffer (pH 7.2) containing 0.2% Triton X-100 at 25 °C was mixed with benzylamine as the substrate. The rate of reaction to form benzaldehyde was measured by monitoring the increase in absorbance at 250 nm. One unit of enzyme activity equals that amount needed to form 1 nmol of product per minute.

**Sulfhydryl Titration of MAOs.** Solutions of MAO A with and without added inactivator in 100 mM sodium phosphate buffer (pH 7.2) containing 10% glycerol and 0.2% Triton X-100 were incubated at 25 °C. When the enzyme activity was <5% of the control activity, the solutions were dialyzed for 4 h against three changes (500 mL) of 100 mM sodium phosphate buffer (pH 7.2), containing 10% glycerol and 0.2% Triton X-100. The dialyzed solutions were assayed for enzyme activity and protein content.

Thiol titrations with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) were performed according to a modification of the literature procedure.\(^{25}\) A 200 μL aliquot of either the control or inactivated MAO A solution was added to a solution of 380 μL of deionized water, 200 μL of 100 mM sodium phosphate buffer (pH 8.0), and 100 μL of 20% sodium dodecyl sulfate (NaDodSO₄) with 1 mg/mL EDTA. The absorbance of each solution at 412 nm was recorded as an enzyme absorbance background reading. The blank absorbance was zeroed by using a solution of 380 μL deionized water, 200 μL of 100 mM sodium phosphate buffer (pH 8.0), 100 μL of 20% NaDodSO₄ with 1 mg/mL EDTA, and 200 μL of 10% glyceraldehyde in 100 mM sodium phosphate buffer (pH 7.2), containing 0.2% Triton X-100. A 20 μL aliquot of DTNB solution was added to the solution used to prezero the spectrometer, and the absorbance was measured and used as a DTNB absorbance background. The total amount of free 5-mercapto-2-nitrobenzoate produced was calculated from the absorbance at 412 nm of the DTNB treated MAO-A solution minus the two background readings. The assay was performed in triplicate.

**Changes in the Flavin UV–Visible Spectrum upon Inactivation of MAO-A.** Solutions of MAO-A (25 μM) in 50 mM potassium phosphate buffer, at pH 7.2 containing 0.2% Triton X-100, were placed in individual cuvettes that were then sealed with rubber septa. These cuvettes were repeatedly purged with argon and evacuated. A solution of each inactivator was then added by using an airtight syringe, and the UV–visible spectra were recorded periodically over 27 min time period. These experiments were performed in duplicate.

**Dimethylamino Production in the Reaction of MAO-A with β-Hydroxynime 5.** MAO-A (100 μM) was inactivated with β-hydroxynime at 25 °C in 50 mM sodium phosphate buffer, at pH 7.2, containing 5% glycerol and 0.2% Triton X-100. The mixture was then made basic by addition of saturated NaHCO₃ and mixed with 1 mL of a 2 mg/mL solution of dabsyl chloride in acetonitrile.\(^{25}\) The solution was kept at 25 °C for 1 h and extracted with chloroform. The extracts were dried, and a 10 μL portion was removed for HPLC analysis (Beckman Ultrasphere ODS C-18 column (0.46 cm × 25 cm), eluted with hexane:2-propanol (80:20) at a flow rate of 1 mL/min). TLC (ether) was performed with dabsyl chloride \(R_f = 0.76, \text{β}-\text{amino alcohol 5} \left(R_f = 0.52 \right)\), and \(N,N\text{-dimethyl}dabsylamide \left(R_f = 0.64 \right)\). The remaining solution was subjected to preparative TLC (ether). The band that comigrated with \(N,N\text{-dimethyl}dabsylamide \left(R_f = 0.64 \right)\) was collected and subjected to \(^1H\) NMR analysis. A control containing 25 mM \(S\) in 50 mM sodium phosphate buffer, at pH 7.2, containing 5% glycerol and 0.2% Triton X-100 was also treated with dabsyl chloride in the same manner.

**Inhibition of MAO-B Catalyzed Oxidation of Benzyline.** Initial velocities for MAO-B catalyzed oxidation of benzyline were determined by monitoring the changes in absorbance at 250 nm. Reactions were initiated by adding aliquots of MAO-B to solutions containing varying concentrations of benzyline and the inhibitors in 50 mM sodium phosphate buffer at pH 7.2 containing 0.2% Triton X-100 at 25 °C. The \(K_i\) value for each inactivator was determined by use of the Lineweaver–Burk\(^{60}\) plot and Cleland kinetic computer analysis\(^{35}\) methodologies. All \(K_i\) determinations were made in duplicate.

**Effect of Amphetamine on the Inactivation of MAO-A.** Solutions of MAO-A in 50 mM sodium phosphate buffer at pH 7.2 with 0.2% Triton X-100 at 25 °C containing either amphetamine, amphetamine and the inactivator, or only inactivator were incubated at 25 °C. Aliquots were removed at various time intervals, and enzyme activity was assayed by use of the kynuramine assay procedure.
Time Dependent Inactivation of MAO-B. Aliquots of an MAO-B stock solution were added to solutions containing varying concentrations of inactivators in 50 mM sodium phosphate buffer (pH 7.2), containing 0.2% Triton X-100. The samples were incubated at 25 °C and periodically assayed for enzyme activity by use of the benzylamine assay procedure. All assays were performed in duplicate.

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