was washed with aqueous NaOH (3 × 30 mL) and water (2 × 30 mL), dried over anhydrous $MgSO_4$, and filtered, and the filtrate was evaporated in vacuo. The residue was purified by chromatographic separation with hexane as eluent to give **49**.

(*E*)-Trimethyl(1-phenyl-1,3-butadien-1-yl)silane (49a). According to the general procedure, **1a** (0.269 g, 1.0 mmol) was converted to **49a** (0.142 g, 70%): IR (neat) ν 3065, 2956, 1600, 1565, 1494, 1248, 941, 898, 836, 701 cm^{-1}; ^1H NMR (250 MHz) δ[34] 0.10 (s, 9 H), 5.08 (dd, J = 1.9, 9.9 Hz, 1 H), 5.30 (dd, J = 1.9, 16.9 Hz, 1 H), 6.25 (quasi dt, J = 16.9, 10.3 Hz, 1 H), 6.53 (d, J = 10.7 Hz, 1 H), 6.96-6.99 (m, 2 H), 7.20-7.35 (m, 3 H); ^{13}C NMR (62.5 MHz) δ -1.6, 118.5, 125.6, 127.9 (two overlapping signals), 134.5, 138.9, 142.3, 147.8; accurate mass calcd for $C_{13}H_{18}Si$ 202.1178, found 202.1178.

(*E*)-Trimethyl[1-(2-naphthyl)-1,3-butadien-1-yl]silane (49b). Following the general procedure, **1b** (0.319 g, 1 mmol) was transformed to **49b** (0.204 g, 81%): mp 52-54 °C; IR (KBr) ν 3054, 2956, 1728, 1628, 1597, 1562, 1502, 1247, 931, 864, 853, 838, 807, 742 cm^{-1}; ^1H NMR δ 0.13 (s, 9 H), 5.07 (dd, J = 1.9, 10.0 Hz, 1 H), 5.33 (dd, J = 1.9, 16.9 Hz, 1 H), 6.26 (quasi dt, J = 16.9, 10.3 Hz, 1 H), 6.60 (d, J = 10.6 Hz, 1 H), 7.13 (dd, J = 1.6, 8.4 Hz, 1 H), 7.41-7.48 (m, 3 H), 7.78-7.85 (m, 3 H); ^{13}C NMR δ -1.15, 118.8, 125.2, 125.9, 127.1, 127.6, 127.7, 131.9, 133.5, 134.6, 139.3, 140.0, 147,8; accurate mass calcd for $C_{17}H_{20}Si$ 252.1334, found 252.1331. Anal. Calcd: C, 80.89; H, 7.99. Found: C, 80.29; H, 8.10.

(*E*)-Trimethyl[1-(1-naphthyl)-1,3-butadien-1-yl]silane (49c). Via the general procedure, **1c** (0.319 g, 1 mmol) was converted to **49c** (0.199 g, 70%): IR (neat) ν 3057, 2955, 1589, 1577, 1561, 1504, 1403, 1390, 1248, 928, 898, 839, 791, 774, 755 cm^{-1}; ^1H NMR (250 MHz) δ 0.07 (s, 9 H), 4.98 (dd, J = 1.8, 9.9 Hz, 1 H), 5.30 (dd, J = 1.8, 17.0 Hz, 1 H), 5.91 (quasi dt, J = 17.0, 10.3 Hz, 1 H), 6.78 (d, J = 10.6 Hz, 1 H), 7.04 (dd, J = 1.1, 7.0 Hz, 1 H), 7.40-7.48 (m, 3 H), 7.71-7.86 (m, 3 H); ^{13}C NMR (62.5 MHz) δ -1.5, 118.8, 124.6, 125.3, 125.4, 125.6, 126.0, 126.5, 128.2, 131.4, 133.7, 134.8, 139.8, 140.5, 145.9; accurate mass calcd for $C_{17}H_{20}Si$ 252.1334, found 252.1338. Anal. Calcd: C, 80.89; H, 7.99. Found: C, 80.28; H, 8.11.

(*E*)-Trimethyl[1-(4-methylphenyl)-1,3-butadien-1-yl]silane (49d). Via the general procedure, **1d** (0.283 g, 1 mmol) was transformed to **49d** (0.160 g, 74%): IR (neat) ν 2957, 2925, 1728, 1566, 1508, 1457, 1248, 912, 840 cm^{-1}; ^1H NMR (250 MHz) δ 0.09 (s, 9 H), 2.33 (s, 3 H), 5.07 (dd, J = 2.0, 10.1 Hz, 1 H), 5.29 (dd, J = 2.0, 16.8 Hz, 1 H), 6.26 (quasi dt, J = 16.8, 10.3 Hz, 1 H), 6.52 (d, J = 10.7 Hz, 1 H), 6.87 (d, J = 8.0 Hz, 2 H), 7.12 (d, J = 8.0 Hz, 2 H); ^{13}C NMR (62.5 MHz) δ -1.6, 21.1, 118.3, 127.8, 128.7, 134.6, 135.1, 138.8, 139.1, 147.8; accurate mass calcd for $C_{14}H_{20}Si$ 216.1334, found 216.1328.

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5-(Aminomethyl)-3-aryl-2-oxazolidinones. A Novel Class of Mechanism-Based Inactivators of Monoamine Oxidase B

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Abstract: The mechanism of inactivation of monoamine oxidase (MAO) by 5-(aminomethyl)-3-aryl-2-oxazolidinones has been investigated. (*R*)- and (*S*)-3-[4-[(3-chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (**1**) exhibit all of the properties of a mechanism-based inactivator. Several other analogues of **1** also inactivate MAO. Inactivation of MAO by (*R*)- and (*S*)-[*methoxy*-^3H]-**1** and by [*methoxy*-^3H]-3-(4-methoxyphenyl)-5-[(methylamino)methyl]-2-oxazolidinone (**15**, R = ^3H) led to incorporation of 1.0, 1.2, and 2.1 equiv of tritium per enzyme molecule after denaturation, indicating that a covalent bond between the oxazolidinones and MAO is formed. The partition ratios, determined from the amount of radioactive non-amines generated per tritium incorporated into the enzyme, were 17.6 and 10.9 for the *R* and *S* isomers, respectively. Inactivation of MAO by (*R*)- and (*S*)-[*carboxy*-^{14}C]-**1** resulted in release of 4.5 and 3.0 equiv of $^{14}CO_2$, respectively. However, in addition to the loss of $^{14}CO_2$ there also was incorporation of 1.5 and 1.0 equiv of ^{14}C, respectively, into the enzyme after denaturation. The flavin spectrum indicated that the flavin was reduced after inactivation, but upon denaturation the spectrum returned to that of the oxidized form, suggesting that attachment is to an amino acid residue, not to the flavin. 5-(Aminomethyl)-3-(4-cyanophenyl)-2-oxazolidinone inactivates MAO at a rate comparable to or faster than does the corresponding 4-methoxyphenyl analogue, suggesting that there is little or no electronic effect of ring substitution on the rate of inactivation. All of these results support an inactivation mechanism that involves one-electron oxidation of the amine to the amine radical cation, followed by proton removal to give the α radical, which can partition among three pathways (Scheme V): radical combination with an active-site amino acid residue radical to give inactive enzyme, decomposition of the oxazolidinone ring with loss of CO_2, and second electron transfer to give the corresponding aldehyde product.

Monoamine oxidase (MAO; EC 1.4.3.4) is responsible for the deactivation of amine neurotransmitters as well as the metabolism of certain exogenous amines.[1] The enzyme exists in two different isoenzymic forms, designated MAO A and MAO B, which differ in substrate specificity, distribution among tissues, and structure.[2] Nonspecific or MAO A specific inhibitors have been shown to be clinically useful as antidepressants; the clinical usefulness of MAO B specific inhibitors in the treatment of depression remains to be demonstrated.[3] Recently, however, it has become clear that there are other important uses for MAO B specific inhibitors in medicine, namely, as adjuncts to L-dopa treatment of Parkinson's disease.[4,5] Furthermore, it was shown that administration of

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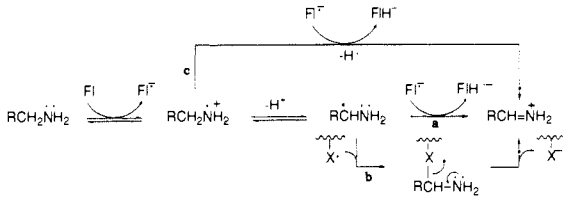
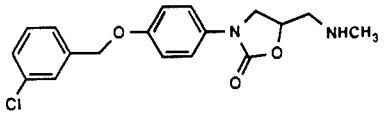
Scheme I. Proposed Mechanism for MAO-Catalyzed Amine Oxidation



MAO B inhibitors alone early in the course of this disease can alleviate the need for other drugs, presumably by slowing the progression of the disease.[4] It also has been suggested that MAO B specific inhibitors might be useful in the treatment of other neurological diseases of aging such as Alzheimer's disease.[6]

One class of inhibitors of MAO that has not received much attention until fairly recently is the oxazolidinones. Structure–activity relationship studies of a variety of oxazolidinones[7] uncovered several very potent inhibitors of MAO, some of which were MAO A selective and some of which were MAO B selective. The most potent of the MAO B inhibitors was 3-[4-[(3-chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (MD780236; **1**).[8] In addition to inhibition of MAO B, the



1

S isomer, but not the *R* isomer, of **1** also was reported to be an irreversible inactivator of MAO B;[9] however, it was later shown that both isomers were irreversible inactivators of the enzyme.[10]

Work in our laboratory over the last several years, principally with mechanism-based enzyme inactivators,[11] has demonstrated that monoamine oxidase catalyzes the oxidation of amines by a radical mechanism[12-25] (Scheme I). The fact that **1** was an irreversible inactivator of MAO suggested the possibility that it, too, was acting as a mechanism-based enzyme inactivator, and therefore, we thought that our understanding of the mechanism of MAO would be useful in determining the mechanism of inactivation of MAO by **1**. We recently proposed a novel mechanism for the inactivation of MAO by this class of compounds in which the oxazolidinone ring undergoes a radical fragmentation reaction after a one-electron oxidation of the amine by the flavin cofactor (Scheme II).[26] The fragmentation reaction results in the release of carbon dioxide and the formation of a species that is able to combine with some active-site radical (either the flavin semiquinone radical or an amino acid radical). Chemical model studies provided support for this type of reaction. Here we describe our results on the inactivation of MAO B by 5-(aminomethyl)-3-aryl-2-oxazolidinones, which indicate that this CO_2 loss mechanism is important to the turnover of the oxazolidinone analogues, but it is not relevant to the mechanism of inactivation of the enzyme by these inactivators. An alternative mechanism is proposed that is consistent with the results of these studies.

Results

Synthesis of 3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (1). The synthesis of **1** shown in Scheme III was modeled after the general method in a patent by Strolin Benedetti et al.[27]

Inhibition and Inactivation of MAO by (*R*)- and (*S*)-1. Both (*R*)- and (*S*)-**1** were found to be competitive inhibitors of MAO, having K_i values of 35 and 4 μM, respectively. In agreement with recently reported studies,[10] we have found that both the *R* and the *S* isomers of **1** are time-dependent inactivators of MAO; enzyme activity does not return upon exhaustive dialysis. The enzyme is protected from inactivation by the substrate cinnamylamine, but not by the external nucleophile β-mercaptoethanol. These results indicate that **1** is active-site directed and that the inactivating species does not escape the active site prior to inactivation. Efforts to determine the kinetic constants were hindered by the fact that **1** was not very soluble in buffered solutions, and incubation of MAO with **1** does not lead to complete inactivation. Estimates based on the initial rates of inactivation give values of k_{inact} = 0.011 min^{-1} and K_I = 102 μM for the *S* isomer and k_{inact} = 0.010 min^{-1} and K_I = 399 μM for the *R* isomer.

Synthesis of 3-(4-Methoxyphenyl)-5-[(methylamino)methyl]-2-oxazolidinone (15, R = H). The synthetic route to **15** is shown in Scheme III.

Inactivation of MAO by 3-(4-Methoxyphenyl)-5-[(methylamino)methyl]-2-oxazolidinone (15, R = H) and Its Corresponding [*methoxy*-^{3}H] Analogue. Compound **15** (R = H) was shown to be a time-dependent irreversible inactivator of MAO having k_{inact} = 0.006 min^{-1} and K_I = 62 mM. It is apparent, by comparison of these results with those above for **1**, that exchange of a hydrogen for a *m*-chlorophenyl group has a major effect on the activation dissociation constant. Incubation of MAO with [*methoxy*-^{3}H]-**15** (R = ^{3}H) led to complete inactivation of the enzyme and incorporation of 2.1 equiv of radioactivity into the enzyme after urea denaturation (two experiments).

Inactivation of MAO by (*R*)- and (*S*)-[*methoxy*-^{3}H]-1. Both (*R*)- and (*S*)-[*methoxy*-^{3}H]-**1** inactivated MAO with incorporation of 1.0 and 1.2 equiv of radioactivity, respectively, per inactivated enzyme. The radioactive non-amines produced during inactivation, which represent the number of molecules of inactivator that were oxidized and released from the enzyme, were isolated. The labeled *R* isomer generated 17.6 equiv of non-amines and the *S* isomer generated 13.1 equiv. Therefore, the partition ratios for inactivation of MAO by (*R*)- and (*S*)-**1** are 17.6 and 10.9, respectively.

Inactivation of MAO by (*R*)- and (*S*)-[*carbonyl*-^{14}C]-1. Inactivation of MAO by (*R*)- and (*S*)-[*carbonyl*-^{14}C]-**1** resulted in the generation of 4.5 and 3.0 equiv, respectively, of $^{14}CO_2$ per enzyme inactivated; however, the incorporation of 1.5 and 1.0 equiv of radioactivity into the enzyme after dialysis also was observed.

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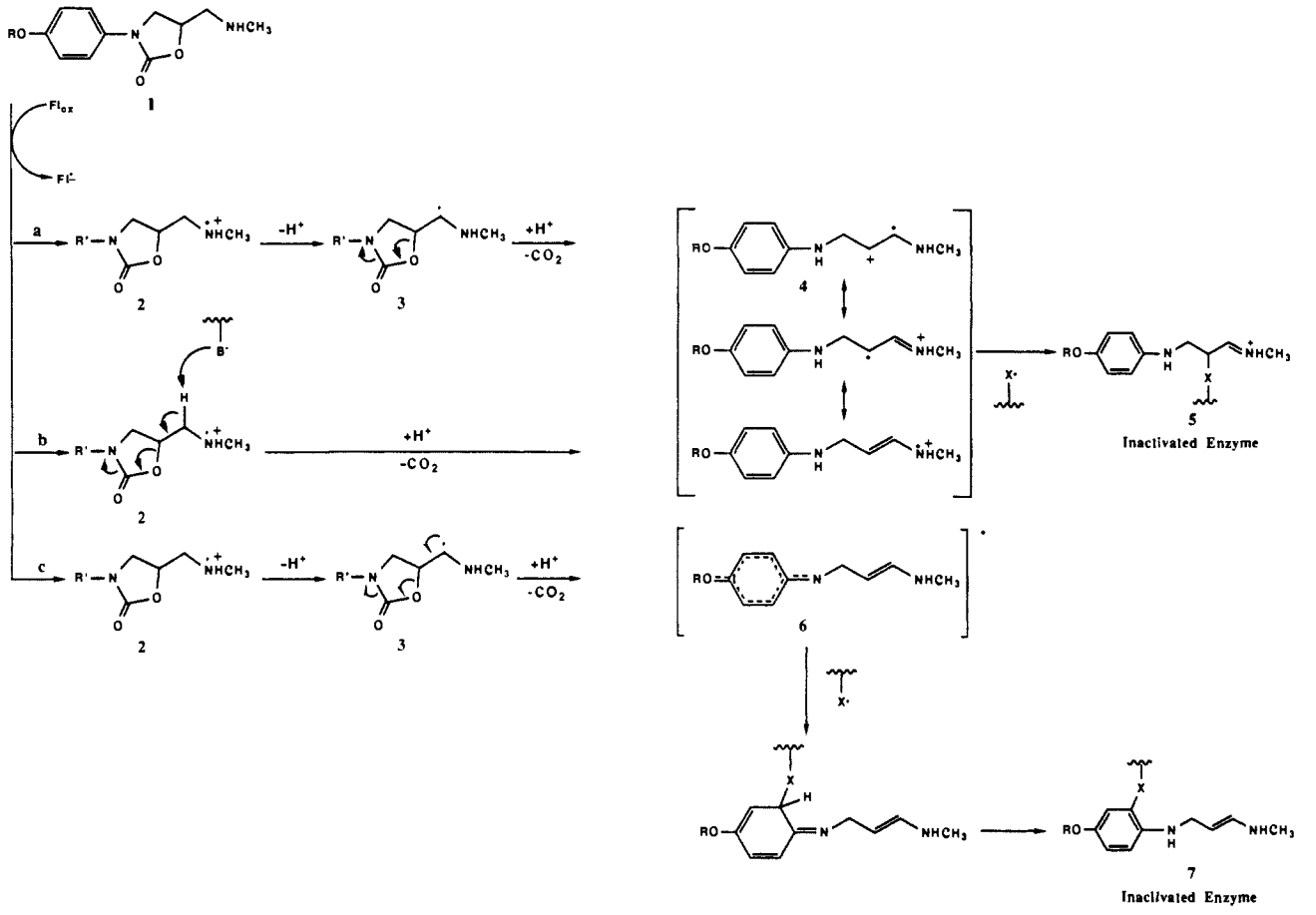
Scheme II. Proposed Mechanism of Inactivation of MAO by 5-(Aminomethyl)-3-aryl-2-oxazolidinones on the Basis of Chemical Model Studies[26]



This indicates that the oxazolidinone ring is destroyed more times than **1** produces inactivation, and that the carbonyl group is in the part of the inactivator that becomes attached to the enzyme. During inactivation the *R* isomer produced 11.2 equiv of nonamine radioactivity and the *S* isomer produced 11.0 equiv.

Synthesis of 5-(Aminomethyl)-3-(4-cyanophenyl)-2-oxazolidinone (20). Because of difficulties in carrying out the synthesis of 5-(aminomethyl)-3-(4-cyanophenyl)-2-oxazolidinone (**20**), an alternate route was used (Scheme IV). A similar procedure was developed independently by workers at Du Pont for the synthesis of a series of oxazolidinone antibiotics.[28]

Inactivation of MAO by 5-(Aminomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (17, R = H) and 5-(Aminomethyl)-3-(4-cyanophenyl)-2-oxazolidinone (20). Both **17** (R = H) and **20** were time-dependent inactivators of MAO; k_{inact} and K_I values were 0.01 min^{-1} and 49 mM for **17** (R = H) and 0.02 min^{-1} and 24 mM for **20**. These results indicate that **20** is a slightly better inactivator of MAO than is **17** (R = H).

Flavin Spectrum Change during Inactivation by 15 (R = H). After inactivation of MAO by **15** (R = H), the flavin spectrum showed that the flavin was reduced. Denaturation of the inactivated enzyme resulted in the reoxidation of the flavin under conditions in which the radioactively labeled analogue remains attached to the enzyme. This indicates that the inactivator does not become attached to the flavin, but to an amino acid residue of MAO.

Discussion

On the basis of our previous studies with inactivators of MAO[12-25] we have proposed a one-electron mechanism for amine oxidation (Scheme I). Since **1** has the general structure of MAO substrates, we predicted that it should behave as a substrate and undergo one-electron oxidation as well. Model studies were carried out[26] in order to determine the feasibility of an inactivation mechanism for **1** that followed from decomposition of the oxazolidinone ring with loss of CO_2. Several inactivation mechanisms can be envisioned for this process (Scheme II). All of the proposed pathways proceed by initial one-electron transfer from the amine to the flavin to give the amine radical cation **2** followed by proton removal. Pathways a and c involve formation of radical **3** from which either heterolytic C–O bond cleavage (pathway a) or homolytic C–O bond cleavage (pathway c) can occur, followed by attachment of the resultant radicals to an active site radical to give **5** or **7**, respectively. Pathway b is an E2-type mechanism, which could occur from the carbanionic character that is generated initially by removal of the α-proton from the amine radical cation **2**. This pathway leads to the same radical intermediate formed by pathway a. All three proposed pathways would result in the loss of CO_2 from the inactivator molecule and could lead to attachment of the remainder of the molecule to the active site (**5** or **7**).

In order to obtain support for any of these inactivation mechanisms, it was necessary to show (1) that the 5-(aminomethyl)-3-aryl-2-oxazolidinones were irreversible inactivators of MAO, (2) that the oxazolidinone carbonyl was released as CO_2, and (3) that the molecules, exclusive of the oxazolidinone carbonyl, became attached to the enzyme. Several different 5-(aminomethyl)-3-aryl-2-oxazolidinones were synthesized and all were shown to inactivate MAO. It was not necessary for the amino group to be methylated or for the aromatic ring to be electron rich, such as in the case of **1**, for these compounds to be MAO inactivators. Both (*R*)- and (*S*)-**1** inactivated MAO, as did the corresponding 4-methoxy analogue **15** (R = H), the electron-deficient analogue **20**, and the primary amines **17** (R = H) and **20**. It is apparent from the difference in the K_I values for **1** (399 and 102 μM for the *R* and *S* isomers, respectively) and for **15** (R = H) (62 mM) that the 3-chlorophenyl group is important for efficient binding, but it is likely that the inactivation mechanisms are the same.

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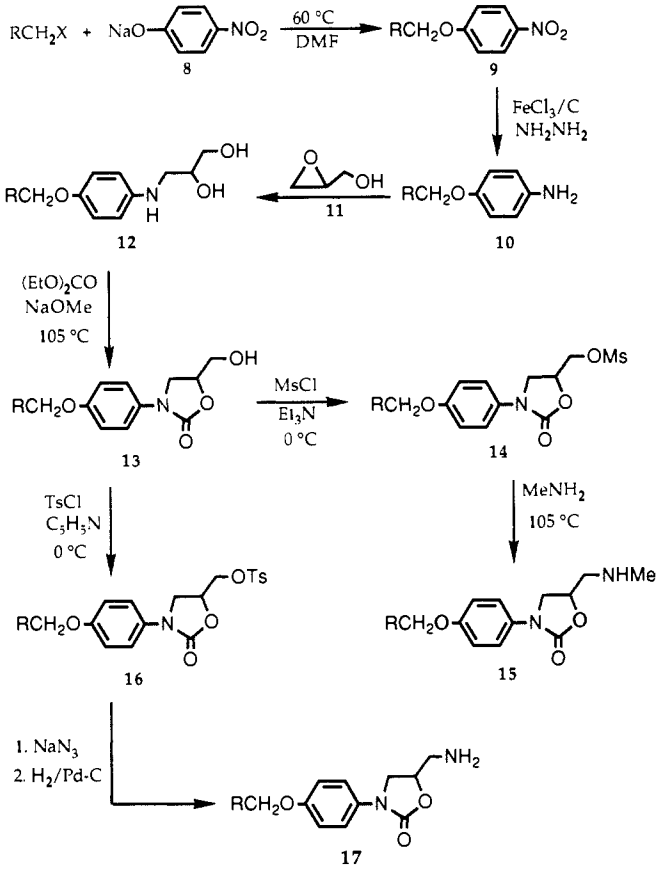
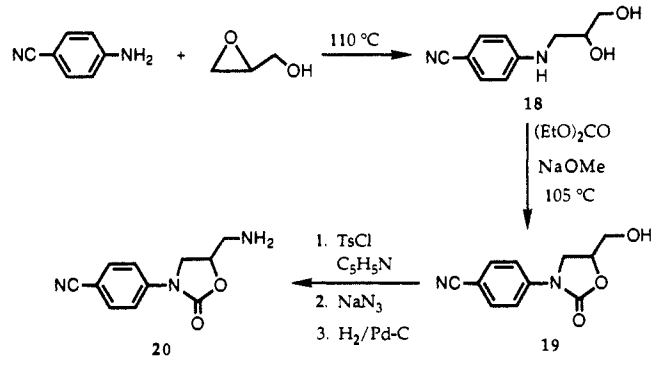
Scheme III. Synthetic Route to 5-(Aminomethyl)-3-aryl-2-oxazolidinones



Scheme IV. Synthetic Route to **20**



Three different radioactively labeled analogues were synthesized to show that the inactivator molecules become irreversibly attached to MAO after inactivation. The *R* and *S* isomers of [*methoxy*-^{3}H]-**1** both inactivated MAO with attachment after exhaustive dialysis against 6 M urea of 1 equiv of radioactivity. Likewise, [*methoxy*-^{3}H]-**15** (R = ^{3}H) led to incorporation of 2 equiv of radioactivity into the enzyme after denaturation.

An important test of the validity of the proposed inactivation mechanisms (Scheme II) is whether CO_2 is released during inactivation by **1**. (*R*)- and (*S*)-[*carboxy*-^{14}C]-**1** were synthesized; inactivation of MAO was carried out in a closed vessel containing a base trap to collect any $^{14}CO_2$ that was generated. The *R* and *S* isomers inactivated the enzyme with release of 4.5 and 3.0 equiv, respectively, of $^{14}CO_2$. Since more than 1 equiv of CO_2 was released during inactivation, it suggests that the partition ratio, the number of molecules of inactivator converted to product per inactivation event, is greater than zero. Loss of CO_2 should be a measure of the MAO-catalyzed oxidation of **1** by one of the mechanisms in Scheme II. However, intermediates **4** or **6** (Scheme II) may be released, reduced, or further oxidized, thereby accounting for the greater than 1 equiv of CO_2 produced during inactivation. An alternative oxidation pathway for **1** and its analogues is the normal oxidation reaction, namely, its conversion to the corresponding imine followed by hydrolysis to the aldehyde, a known metabolite of **1** oxidation.[29] The amount of radioactive non-amines generated during inactivation of MAO by (*R*)- and (*S*)-[*methoxy*-^{3}H]-**1** (17.6 and 13.1 equiv, respectively) is a measure of the total number of turnovers per inactivation event (the partition ratio) and represents the total amount of metabolites produced from reactions such as those shown in Scheme II as well as from oxidation of **1** to the corresponding aldehyde. Therefore, the amount of radioactive nonamines generated from (*R*)- and (*S*)-[*methoxy*-^{3}H]-**1** should equal the amount of radioactive non-amines generated from (*R*)- and (*S*)-[*carboxy*-^{14}C]-**1** plus the amount of $^{14}CO_2$ produced. (*R*)- and (*S*)-[*carboxy*-^{14}C]-**1** generated 11.2 and 11.0 equiv of radioactive non-amines, respectively. Therefore, the total amount of ^{14}C metabolites produced by the two enantiomers is 15.7 and 14.0 equiv, respectively, which is within experimental error of the 17.6 and 13.1 equiv of total tritiated non-amines produced from (*R*)- and (*S*)-[*methoxy*-^{3}H]-**1**, respectively.

The first two criteria described above have been met and these results support the mechanisms in Scheme II. However, the third criterion, namely, that the inactivated enzyme should not contain ^{14}C from the carbonyl carbon after inactivation, was not supported. Inactivation of MAO with (*R*)- and (*S*)-[*carbonyl*-^{14}C]-**1** led to the incorporation of 1.5 and 1.0 equiv, respectively, of ^{14}C per enzyme molecule! According to the mechanisms shown in Scheme II, no ^{14}C from the oxazolidinone carbonyl group should remain attached to the inactivated enzyme; consequently, *decarboxylation is not involved in the mechanism of inactivation of MAO by the 5-(aminomethyl)-3-aryl-2-oxazolidinones. Decarboxylation must be important, however, in the MAO-catalyzed metabolism of this class of inactivators.*

There are several other inactivation mechanisms that could be considered, but the most important of these is shown in Scheme V (pathway a). This mechanism is a modification of the mechanism of inactivation that was proposed by Dostert et al.[30,31] The stability of the proposed adduct **22** would be derived from the electron-withdrawing oxygen that is at the β position. It is well-known that electron-withdrawing groups stabilize α and β sp^3 carbons in preference to sp^2 carbons, presumably because of destabilization of the electron-deficient carbonyl. For example, when electron-withdrawing substituents are β to a keto group, the corresponding hydrate or hemiacetal derivative is strongly preferred over the ketone.[32] A stable α-amino sulfide was isolated that contained a β-halogen,[33] and a fluorohydrin with a β-electron-withdrawing group was stable to chromatography.[34] A β-fluoro substituent was used to stabilize a proposed acetal intermediate in the reaction catalyzed by glycosidase, resulting in inactivation of the enzyme.[35]

If the mechanism for inactivation shown in Scheme V is correct, then it suggests that any compound containing a β-electron-withdrawing group could inactivate MAO, and a "new" class of MAO inactivators would be substrates for MAO to which strong electron-withdrawing groups are appended. This proposal cur-

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(31) The mechanism proposed in ref 30 was oxidation of the amine to the imine by an unspecified pathway followed by attack of an active-site nucleophile on the imine. The modified version shown in Scheme V takes into account previous evidence about the mechanism of the enzyme[12-25] and accounts for the decarboxylation pathway and the known aldehyde metabolite formation.

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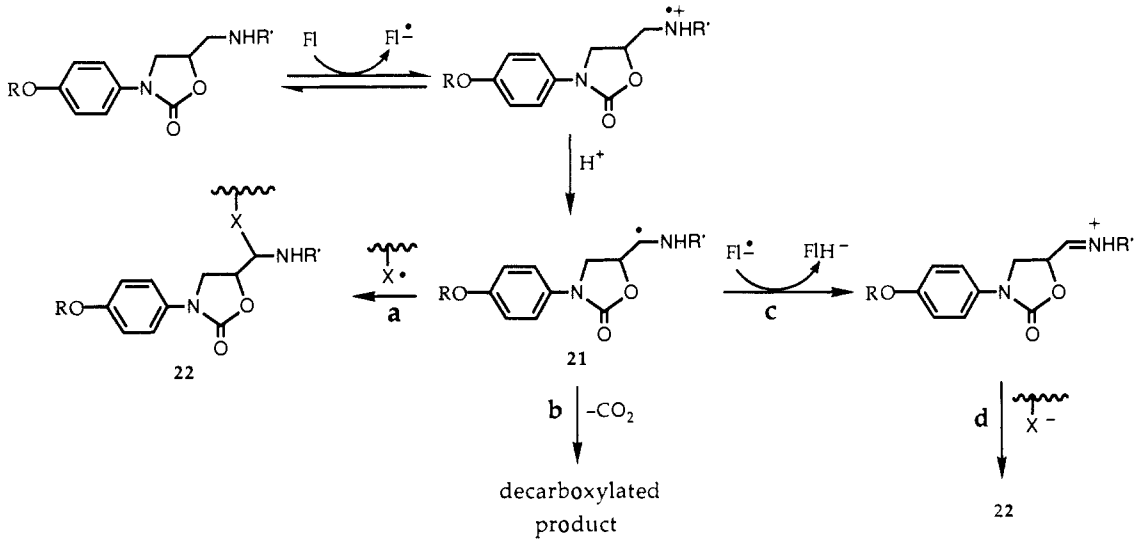
Scheme V. Potential Mechanism of Inactivation of MAO by 5-(Aminomethyl)-3-aryl-2-oxazolidinones



rently is being tested. However, we recently reported a compound that fits this description, namely, (aminomethyl)trimethylsilane, which forms a stable enzyme adduct apparently as a result of the electron-withdrawing effect of the α-trimethylsilyl group.[25] Other examples of this class of inactivators may be 2-chloro-2-phenylethylamine,[36] the inhibitors reported by the group at Hoffmann-La Roche, namely, Ro 19-6327,[37] Ro 16-6491,[38] and the antidepressant drug moclobemide,[39] as well as the anticonvulsant agent from Searle, milacemide.[40] We are currently investigating these hypotheses as well. This mechanism also may explain why more than 1 equiv of radioactivity was bound to MAO after inactivation by [*methoxy*-^{3}H]-**15** and by (*R*)-[*carbonyl*-^{14}C]-**1**. Adduct **22** (Scheme V) could be produced by addition of an active-site nucleophile to the imine of these inactivators (Scheme V, pathway d), leading to more than 1 equiv bound.

Upon inactivation of MAO by **15** (R = H), the flavin spectrum became reduced, but after denaturation it was reoxidized. This indicates that attachment of the inactivator is not to the flavin but to an amino acid residue, possibly the active-site cysteine.[22]

The results of these studies, in conjunction with our earlier work, provide evidence for the mechanism of MAO-catalyzed amine oxidation shown in Scheme I. Our studies with cyclopropylamine[12-22] and cyclobutylamine[23,24] inactivators have provided good evidence that the first step of amine oxidation by MAO involves a one-electron oxidation of the amino group. However, this earlier work has not clarified whether the mechanism proceeds from the amine radical cation by proton removal followed by α-carbon radical transfer to the flavin (pathway a), by proton removal followed by radical combination and β elimination (pathway b), or by the direct transfer of an α-hydrogen atom to the flavin (pathway c). Mariano and co-workers[41a] and Dinnocenzo and Banach[41b] have argued that hydrogen atom removal from an amine radical cation is not an energetically favorable process, but to date there is no concrete evidence for or against a hydrogen atom transfer mechanism in MAO-catalyzed amine oxidations. Our results with (aminomethyl)trimethylsilane[25] and the results described here support pathways a and b. When R in Scheme I is Me_3Si or an N-substituted 2-oxazolidinon-5-yl unit, proton removal from the amine radical cation would give a carbon radical that could partition between second electron transfer to give the aldehyde metabolites (pathway a) and radical combination with an active-site amino acid radical (presumably a cysteinyl radical[22]) to give a covalent intermediate (pathway b). In the cases of (aminomethyl)trimethylsilane and the 5-(aminomethyl)-3-aryl-2-oxazolidinones this covalent intermediate is stabilized by the α-trimethylsilyl and N-substituted 2-oxazolidinon-5-yl groups. Therefore, these compounds are acting as if they were normal substrates for MAO, but they lead to inactivation because the built-in electron-withdrawing groups stabilize the normal covalent intermediate in the enzyme reaction.

One might wonder why Mother Nature would be so careless as to design a redox enzyme with a cysteine residue near the oxidizing flavin cofactor. It may not be carelessness, however, but a clever design element that allows MAO to catalyze oxidations of a variety of substrates having different second electron oxidation potentials. After oxidation proceeds to the carbon radical intermediate (Scheme I), depending upon the oxidation potential of that species, the enzyme can continue along two different pathways. For those substrates with low oxidation potentials, second electron transfer may occur (pathway a), but for those substrates with high oxidation potentials, radical combination and β elimination could be an alternative route (pathway b). An electron-withdrawing group at the β position of a substrate increases the oxidation potential for second electron transfer and would detour the mechanism to pathway b, which results in covalent adduct formation. Since thiolate is a better leaving group than the flavin for β elimination,[18] the rate of elimination would be accelerated by covalent bond formation to a cysteine residue rather than to the flavin. This could account for the positioning of a cysteine group near the flavin cofactor.

Conclusions. The reactions that were proposed earlier on the basis of chemical model studies[26] for the inactivation of MAO by 5-(aminomethyl)-3-aryl-2-oxazolidinones do not lead to inactivation, but they are relevant to the metabolism of this class of compounds. On the basis of the experiments described here a reasonable mechanism for inactivation involves one-electron oxidation of the amine to the amine radical cation, loss of the α-proton to give the α radical, and then radical combination with an active-site amino acid radical (Scheme V, pathway a). Alternatively, the imine produced from oxidation of the amine may undergo attack by an active-site amino acid nucleophile to give the same adduct.

Experimental Section

Analytical Methods. Optical spectra and MAO assays were recorded on a Perkin-Elmer Lambda 1 UV/vis spectrophotometer or Beckman DU-40 UV/vis spectrophotometer. NMR spectra were recorded on a

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Varian XL-400 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. An Orion Research Model 601 pH meter with a general combination electrode was used for pH measurements. Mass spectra were obtained on a VG Instruments VG70-250SE high-resolution spectrometer. Thin-layer chromatography was performed using Whatman PE SIL/UV silica gel plates with UV indicator. Elemental combustion analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. Radioactivity was measured by liquid scintillation counting using a Beckman LS-3133T counter and Research Products International 3a70B scintillation cocktail. [^{14}C]Toluene (4×10^5 dpm/mL) and [^{3}H]toluene (2.22×10^6 dpm/mL, corrected for first-order decay) from New England Nuclear were used as internal standards. Radiopurity of radioactive compounds was assessed by cutting TLC plates (Merck Kieselgel 60 plates without fluorescent indicator) into strips and counting each strip with scintillation cocktail in a scintillation counter.

Reagents. All chemicals were purchased from Aldrich Chemical Co. and were used without further purification except for the following: Tritiated methyl iodide and sodium borohydride were purchased from ICN Radiochemicals; barium [^{14}C]carbonate was purchased from Research Products International Corp. Protein assays utilized the Pierce Coomassie protein assay reagent (No. 23200). In the synthetic procedures, $MgSO_4$ was used for drying organic solvents unless noted otherwise. Dialyses were carried out using Spectra Por 2 dialysis tubing (MW cutoff 12 000–14 000) purchased from Baxter Scientific Products Division.

4-[(3-Chlorophenyl)methoxy]nitrobenzene (9, R = 3-ClPh). To a stirred solution of sodium 4-nitrophenoxide (394 mg, 2.0 mmol) in dimethylformamide (5 mL) at 65 °C was added 3-chlorobenzyl chloride (270 μL, 2.1 mmol) and the resultant mixture was stirred for 6 h. The mixture was diluted with water (35 mL) and extracted with ethyl acetate (3 × 20 mL), and the organic layer was washed with brine (50 mL), dried, filtered, and rotary evaporated to yield a pale yellow solid, which was recrystallized from methanol to yield the desired product as light yellow platelets (434 mg, 82%): mp 98–100 °C; ^{1}H NMR (400 MHz) ($CDCl_3$) δ 5.12 (s, 2 H), 7.05 (m, 2 H), 7.30–7.45 (m, 4 H), 8.21 (m, 2 H); high-resolution mass spectrum calcd for $C_{13}H_{10}ClNO_3$ 263.0349, found 263.0335 (+5.3 ppm deviation).

4-[(3-Chlorophenyl)methoxy]aniline Hydrochloride (10, R = 3-ClPh). To a solution of 4-[(3-chlorophenyl)methoxy]nitrobenzene (264 mg, 1.0 mmol), $FeCl_3 \cdot 6H_2O$ (2 mg), and activated carbon (10 mg) in refluxing methanol (3 mL) was added hydrazine hydrate (80 μL, 1.64 mmol). After being stirred for 30 h, the solution was diluted with methanol and filtered, and the solvent was rotary evaporated to yield a yellow solid, which was redissolved in chloroform. The HCl salt was precipitated by bubbling HCl gas through the solution. The resulting tan solid was recrystallized from ethanol–ether to yield the desired product as off-white crystals (194 mg, 84%): mp 175–178 °C; ^{1}H NMR ($CDCl_3$) δ 4.97 (s, 2H), 6.60–6.82 (m, 4 H), 7.26–7.35 (m, 3 H), 7.42 (s, 1 H); high-resolution mass spectrum calcd for $C_{13}H_{12}ClNO$ 233.0607, found 233.0608 (0.0 ppm deviation).

(*R*)- and (*S*)-3-[[4-[(3-Chlorophenyl)methoxy]phenyl]amino]-1,2-propanediol (12, R = 3-ClPh). To a solution of 3-chloro-4-(benzyloxy)aniline (1.52 g, 6.53 mmol) in ethanol (20 mL) at 50 °C was added over 1 h a solution of (*R*)-(+)- or (*S*)-(−)-glycidol (Aldrich) (432 μL, 6.53 mmol) in ethanol (15 mL). The solution was stirred under nitrogen for 12 h and then at reflux for an additional 12 h. The solution was redissolved in ethyl acetate (35 mL) and then precipitated by the addition of hexane to give 1.36 g (68%) of a tan powder: mp 107–110 °C; ^{1}H NMR (DMSO-d_6) δ 2.82–2.90 (m, 1 H), 3.10–3.20 (m, 1 H), 3.35–3.45 (m, 2 H), 3.60–3.70 (m, 1 H), 4.58–4.65 (t, 1 H D_2O exchangeable), 4.75–4.80 (d, 1 H, exchangeable), 5.00 (s, 2 H), 5.00–5.10 (br t, 1 H, D_2O exchangeable), 6.55–6.60 (m, 2 H), 6.80–6.87 (m, 2 H), 7.38–7.53 (m, 4 H); high-resolution mass spectrum calcd for $C_{16}H_{18}ClNO_3$ 307.0975, found 307.0987 (−3.7 ppm deviation).

(*R*)- and (*S*)-3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-(hydroxymethyl)-2-oxazolidinone (13, R = 3-ClPh). To a solution of the chiral aminodiol **12** (R = 3-ClPh) (400 mg, 1.30 mmol) in diethyl carbonate (7 mL, 6.1 mmol) at 105 °C was added 25% sodium methoxide in methanol (200 μL). The reaction was stirred under nitrogen for 12 h and another addition of sodium methoxide (200 μL) was made. The solution was rotary evaporated, redissolved in methanol, and heated on a steam bath for 10 min. Removal of the solvent afforded a tan solid, which was recrystallized from ethanol to give 368 mg (85%) of a tan powder: mp 133 °C; ^{1}H NMR (DMSO-d_6) δ 3.50–3.60 (dd, 1 H), 3.62–3.70 (dd, 1 H), 3.75–3.85 (dd, 1 H), 4.00–4.10 (t, 1 H), 4.61–4.70 (m, 1 H), 5.12 (s, 2 H), 5.18–5.22 (br s, 1 H, D_2O exchangeable), 7.00–7.18 (m, 2 H), 7.37–7.55 (m, 6 H); high-resolution mass spectrum calcd for $C_{17}H_{16}ClNO_4$ 333.0768, found 333.0764, (+0.9 ppm deviation).

(*R*)- and (*S*)-3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone Hydrochloride [(*R*)- and (*S*)-15, R = 3-ClPh]. To a solution of the chiral alcohol **13** (R = 3-ClPh) (262 mg, 0.79 mmol) and triethylamine (164 μL, 1.18 mmol) in methylene chloride (5 mL) at 0–5 °C was slowly added methanesulfonyl chloride (67 μL, 0.86 mmol). This mixture was stirred under nitrogen for 30 min, then diluted with methylene chloride (35 mL), and extracted with water (2 × 30 mL), 10% HCl (2 × 30 mL), saturated sodium bicarbonate (2 × 30 mL), and brine (30 mL). The organic extract was dried, filtered, and rotary evaporated to yield a tan solid, which was recrystallized from methanol to give off-white crystals (296 mg, 91%). Methylamine gas (2 mL) was condensed (at −70 °C) into an Ace Glass threaded pressure tube (Catalog No. 8648-04) containing the mesylate and a Teflon stir bar. Cold methanol (7 mL) was added to this mixture; the tube was sealed and, after being warmed to room temperature, immersed in a 105 °C oil bath behind a safety shield. After 4 h, the reaction was cooled and the methanol was removed by rotary evaporation. The resulting brown oil was dissolved in saturated sodium bicarbonate, extracted with chloroform (3 × 25 mL), dried, and filtered. HCl gas was bubbled through the chloroform extract, and the solvent was removed by rotary evaporation to yield a white solid. This solid was recrystallized three times from ethanol–dietyl ether to give a brilliant white powder (144 mg, 48%). **(*R*)-3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone hydrochloride**: mp 225–228 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 3 H), 3.32–3.47 (m, 2 H), 3.86–3.94 (dd, 1 H), 4.18–4.26 (t, 1 H), 5.02–5.13 (m, 1 H), 5.18 (s, 2 H), 7.08–7.14 (m, 2 H), 7.40–7.57 (m, 6 H), 9.33 (br s, 2 H); high-resolution mass spectrum calcd for $C_{18}H_{19}ClN_2O_3$ 346.1084, found 346.1086, (−0.87 ppm deviation). Anal. Calcd for $C_{18}H_{20}Cl_2N_2O_3$: C, 56.41; H, 5.26; N, 7.31; Cl, 18.50. Found: C, 56.21, H, 5.33; N, 7.26; Cl, 18.75. **(*S*)-3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone hydrochloride**: mp 226–229 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 3 H), 3.32–3.47 (m, 2 H), 3.86–3.94 (dd, 1 H), 4.18–4.26 (t, 1 H), 5.02–5.13 (m, 1 H), 5.18 (s, 2 H), 7.08–7.14 (m, 2 H), 7.40–7.57 (m, 6 H), 9.33 (br s, 2 H); high-resolution mass spectrum calcd for $C_{18}H_{19}ClN_2O_3$ 346.1084, found 346.1082, (+0.29 ppm deviation). Anal. Calcd for $C_{18}H_{20}Cl_2N_2O_3$: C, 56.41; H, 5.26; N, 7.31; Cl, 18.50. Found: C, 56.09; H, 5.27; N, 7.09; Cl, 18.09.

3-[(4-Methoxyphenyl)amino]-1,2-propanediol (12, R = H). To a solution of *p*-anisidine (13.48 g, 110 mmol) in methanol (45 mL) at 50 °C was added over 1 h a solution of glycidol (3.64 mL, 54.8 mmol) in methanol (45 mL). This mixture was stirred under an argon atmosphere at 50 °C for 12 h and then refluxed for an additional 12 h. The mixture was evaporated and the excess *p*-anisidine removed by Kugelrohr distillation at 120 °C under high vacuum. The resulting light yellow solid was recrystallized from ethyl acetate–hexane to yield an off-white powder (3.3 g, 31%): mp 72–73 °C; ^{1}H NMR (DMSO-d_6) δ 2.79–2.86 (m, 2 H), 3.05–3.13 (m, 2 H), 3.35–3.40 (t, 2 H), 3.58–3.65 (m, 4 H), 3.57 (t, 1 H, D_2O exchangeable), 4.71 (d, 1 H, D_2O exchangeable), 4.95 (br t, 1 H, D_2O exchangeable), 6.50–6.75 (m, 4 H); high-resolution mass spectrum calcd for $C_{10}H_{15}NO_3$ 197.1052, found 197.1049 (+1.3 ppm deviation).

5-(Hydroxymethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (13, R = H). To a solution of **12** (R = H) (1 g, 5.1 mmol) in toluene at 105 °C was added diethyl carbonate (1.8 mL, 15 mmol) followed by 25% sodium methoxide in methanol (300 μL). The mixture was stirred under nitrogen for 3 h and then rotary evaporated under high vacuum. The resulting tan solid was recrystallized three times from ethyl acetate to yield a white powder (944 mg, 83%): mp 137–139 °C; ^{1}H NMR (DMSO-d_6) δ 3.56 (dd, 1 H), 3.67 (dd, 1 H), 3.73 (s, 3 H), 3.79 (dd, 1 H), 4.50 (t, 1 H), 4.65 (m, 1 H), 5.21 (br s, 1 H), 6.93–7.5 (m, 4 H); high-resolution mass spectrum calcd for $C_{11}H_{13}NO_4$ 223.0845, found 223.0847 (−1.3 ppm deviation).

3-(4-Methoxyphenyl)-5-[(mesyloxy)methyl]-2-oxazolidinone (14, R = H). To a solution of the alcohol (200 mg, 0.9 mmol) and triethylamine (190 μL, 1.35 mmol) in methylene chloride (5 mL) at 0–5 °C was slowly added methane sulfonyl chloride (77 μL, 1.00 mmol). The mixture was stirred at 0 °C under argon for 30 min, diluted with methylene chloride (25 mL), and extracted with water (2 × 25 mL), 10% HCl (2 × 25 mL), saturated sodium bicarbonate (2 × 25 mL), and brine (25 mL). The organic layer was dried, filtered, and rotary evaporated to yield a tan solid, which was recrystallized from methanol to yield off-white crystals (251 mg, 93%): mp 127–130 °C; ^{1}H NMR ($CDCl_3$) δ 3.13 (s, 3 H), 3.71 (s, 3 H), 3.93 (m, 1 H), 4.14 (t, 1 H), 4.47 (m, 2 H), 4.90 (m, 1 H), 6.9–7.46 (m, 4 H); high-resolution mass spectrum calcd for $C_{12}H_{15}NO_6S$ 301.0620, found 301.0624, (+1.5 ppm deviation).

3-(4-Methoxyphenyl)-5-[(methylamino)methyl]-2-oxazolidinone Hydrochloride (15, R = H). Methylamine gas (2 mL) was condensed into an Ace Glass threaded pressure tube (Catalog No. 8648-04) containing

14 (R = H) (200 mg, 0.66 mmol) and a Teflon stir bar. To this solution was added cold methanol (7 mL) and the tube was sealed. After being warmed to room temperature, the pressure tube was immersed for 4 h in a 105 °C oil bath, which was placed behind a safety shield. The mixture was then cooled and evaporated to yield a brown oil. This oil was dissolved in saturated bicarbonate (30 mL) and extracted with chloroform (3 × 20 mL). The chloroform extract was dried, filtered, and rotary evaporated to yield a clear oil. This oil was redissolved in chloroform, and HCl gas was bubbled through the solution. The solvent was removed to yield a white solid, which was recrystallized three times from ethanol–diethyl ether to give brilliant white crystals (81 mg, 45%): mp 217–220 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.29 (m, 2 H), 3.75 (s, 3 H), 3.89 (dd, 1 H), 4.18 (t, 1 H), 5.08 (m, 1 H), 6.99 (m, 2 H), 7.46 (m, 2 H), 9.44 (br s, 2 H). Anal. Calcd for $C_{12}H_{17}ClN_2O_3$: C, 52.85; H, 6.28; N, 10.27; Cl, 13.00. Found: C, 52.85; H, 6.37; N, 10.23; Cl, 12.84.

3-[4-([^{3}H]Methoxy)phenyl]-5-[(methylamino)methyl]-2-oxazolidinone Hydrochloride (15, R = ^{3}H). To *p*-nitrophenol (139 mg, 1.0 mmol) and potassium carbonate (154 mg, 1.2 mmol) in a 2-mL Wheaton vial was added an acetone solution (1 mL) of C^3H_3I (25 mCi, 94 mCi/mmol). The vial that contained the radioactive methyl iodide was rinsed with acetone (500 μL) and this wash was added to the reaction vial, followed by an aliquot of nonradioactive methyl iodide (34 μL, 0.55 mmol). The vial was sealed and immersed in a 70 °C oil bath for 6 h. The reaction was cooled, an additional aliquot of nonradioactive methyl iodide (34 μL, 0.55 mmol) was added, and the mixture was stirred for an additional 17 h at 70 °C. The reaction mixture was filtered to remove sodium iodide and excess potassium carbonate, and the solvent was removed in vacuo. The resulting yellow oil was redissolved in chloroform (20 mL) and was filtered a second time. After the chloroform was removed in vacuo, the resulting yellow oil was dissolved in ethyl acetate (7 mL) and 10% Pd on carbon (80 mg) was added. The solution was evacuated and purged with hydrogen gas three times and then stirred protected from the light under hydrogen gas (1 atm) for 19 h. The mixture was filtered and evaporated in vacuo. The radioactive *p*-anisidine was carried on to the final product by the method previously described. All of the radioactivity in the final product was found to comigrate with unlabeled carrier compound. The specific activity of the final amine hydrochloride was 2.04 × 10^7 dpm/μmol (9.27 mCi/mmol).

5-(Aminomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone Hydrochloride (17, R = H). To a solution of **13** (R = H) (402 mg, 1.80 mmol) in pyridine (5 mL) at 0–5 °C was added tosyl chloride (344 mg, 1.80 mmol). The mixture was stirred under an atmosphere of nitrogen for 1 h and then stored overnight at 4 °C. The reaction was diluted with ethyl acetate (25 mL) and washed with 10% HCl (2 × 20 mL), saturated sodium bicarbonate (2 × 20 mL), and brine (20 mL). The organic layer was dried, filtered, and rotary evaporated to yield a white solid, which was dissolved in dimethylformamide (3 mL). This solution was warmed to 40 °C followed by the addition of sodium azide (585 mg, 9.0 mmol). The reaction was stirred for 12 h, diluted with 1:1 ethyl acetate–methylene chloride (30 mL), and filtered to remove excess sodium azide. The solvents were removed by rotary evaporation under high vacuum, and the resulting light yellow oil was dissolved in 3:1 methanol–ethyl acetate (4 mL). 10% Pd on carbon was added (30 mg); the solution was evacuated, purged with hydrogen gas three times, and then stirred under hydrogen gas (1 atm) for 5 h. The solution was then filtered, rotary evaporated, and redissolved in chloroform. HCl gas was bubbled through the solution, the solvent was removed, and the resulting white solid was recrystallized three times from ethanol–diethyl ether to yield a brilliant white powder (246 mg, 53%): mp 240–245 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.22 (m, 2 H), 3.74 (s, 3 H), 3.87 (m, 1 H), 4.15 (t, 1 H), 4.95 (m, 1 H), 6.98–7.45 (m, 4 H), 8.48 (br s, 3 H); high-resolution mass spectrum calcd for $C_{11}H_{14}N_2O_3$ 222.1004, found 222.1004 (0.0 ppm deviation).

3-[(4-Cyanophenyl)amino]-1,2-propanediol (18). A mixture of *p*-aminobenzonitrile (3 g, 25.4 mmol) and glycidol (0.94 mL, 14.1 mmol) was heated neat at 110 °C for 2.5 h. The thick orange oil was dissolved in ethyl acetate (50 mL) with warming on a steam bath, and a product was precipitated with hexane. The resulting light yellow solid was recrystallized from ethyl acetate–hexane to yield an off-white powder (0.5 g, 10%): mp 108–111 °C; ^{1}H NMR (DMSO-d_6) δ 2.98 (m, 1 H), 3.22 (m, 1 H), 3.33–3.67 (m, 3 H, 1 H, D_2O exchangeable), 3.61 (m, 1 H), 4.65 (br t, 1 H, D_2O exchangeable), 4.85 (d, 1 H, D_2O exchangeable), 6.65–7.43 (m, 4 H); high-resolution mass spectrum calcd for $C_{10}H_{12}N_2O_2$ 192.0899, found 192.0893 (+2.3 ppm deviation).

3-(4-Cyanophenyl)-5-(hydroxymethyl)-2-oxazolidinone (19). To a mixture of **18** (245 mg, 1.27 mmol) in diethyl carbonate (5 mL, 34.7 mmol) at 105 °C was added 25% sodium methoxide in methanol (100 μL). After the mixture was stirred for 13 h, another addition of 25% sodium methoxide was made (100 μL). After 5 h, thin-layer chromatography (100% ethyl acetate) showed that all of the starting amino diol (which slowly turns brown upon irradiation at 254 nm) was consumed, and that two new products had been formed (R_f 0.80 and 0.47). The reaction was cooled, and the diethyl carbonate was removed by rotary evaporation. The light orange oil was redissolved in methanol and heated for 15 min on a steam bath. Thin-layer chromatography of this mixture revealed only one product (R_f 0.47). The methanol was removed by rotary evaporation and the tan solid was recrsytallized from ethyl acetate–hexane to give a white powder (222 mg, 80%): mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ 3.57 (m, 1 H), 3.69 (m, 1 H), 3.88 (dd, 1 H), 4.13 (t, 1 H), 4.75 (m, 1 H), 5.25 (br s, 1 H, D_2O exchangeable), 7.76–7.89 (m, 4 H); high-resolution mass spectrum calcd for $C_{11}H_{10}N_2O_3$ 218.0691, found 218.0692 (0.0 ppm deviation).

5-(Aminomethyl)-3-(4-cyanophenyl)-2-oxazolidinone Hydrochloride (20). To a solution of **19** (400 mg, 1.83 mmol) in dry pyridine (5 mL) at 0–5 °C was added tosyl chloride (350 mg, 1.83 mmol). This solution was stirred for 1 h at 0–5 °C and then was stored overnight at 4 °C. The mixture was diluted with ethyl acetate (30 mL) and was extracted with 10% HCl (2 × 30 mL), saturated sodium bicarbonate (2 × 30 mL), and brine (30 mL). The organic layer was dried, filtered, and evaporated to yield a clear oil. This material was dissolved in dry dimethylformamide (3 mL) at 40 °C, and sodium azide (400 mg, 6.15 mmol) was added with stirring. This mixture was stirred for 12 h, diluted with 1:1 ethyl acetate–methylene chloride (10 mL), and filtered to remove the excess sodium azide. The solvents were removed by rotary evaporation under high vacuum and the resulting yellow oil was dissolved in 3:1 methanol–ethyl acetate (4 mL). 10% Pd on carbon was added (30 mg); the solution was evacuated and purged with hydrogen gas three times and then was stirred under hydrogen gas (1 atm) for 5 h. The mixture was filtered, evaporated, and redissolved in chloroform (50 mL). HCl gas was bubbled through the solution, followed by removal of the solvent to yield a white solid, which was recrystallized from ethanol–diethyl ether three times to give a white powder (231 mg, 50%): mp 228–232 °C; ^{1}H NMR (DMSO-d_6) δ 3.25–3.35 (m, 2 H), 3.93 (m, 1 H), 4.25 (t, 1 H), 5.00 (m, 1 H), 7.72–7.91 (m, 4 H), 8.38 (br s, 3 H); high-resolution mass spectrum calcd for $C_{11}H_{11}N_3O$ 217.0851, found 217.0851.

(*R*)- and (*S*)-3-[[4-(Benzyloxy)phenyl]amino]-1,2-propanediol (12, R = Ph). To a stirred solution of (benzyloxy)aniline (1.61 g, 8.1 mmol) in ethanol (30 mL) at 55 °C was added a solution of either (*S*)-(−)- or (*R*)-(+)-glycidol (529 μL, 8.0 mmol) in ethanol (10 mL) over about 15 min. The reaction was heated at 55 °C for 20 h and then was refluxed for 3 h. The mixture was rotary evaporated to yield a pale yellow solid, which was redissolved in ethyl acetate (10 mL) and precipitated by the addition of hexane (40–50 mL). The product, a light tan powder, was collected by vacuum filtration to give 918 mg (42%): mp 115–118 °C; ^{1}H NMR (DMSO-d_6) δ 2.82 (m, 1 H), 3.09 (m, 1 H), 3.36 (m, 2 H), 3.60 (m, 1 H), 4.57 (t, 1 H, D_2O exchangeable), 4.72 (d, 1 H, D_2O exchangeable), 4.98 (s, 2 H), 5.02 (br t, 1 H, D_2O exchangeable), 6.53–6.80 (m, 4 H), 7.28–7.45 (m, 5 H).

(*R*)- and (*S*)-3-[4-(Benzyloxy)phenyl]-5-(hydroxymethyl)-2-oxazolidinone (13, R = Ph). To a stirred solution of (*R*)- or (*S*)-**12** (R = Ph) (800 mg, 2.67 mmol), in toluene (15 mL) at 100 °C was added diethyl carbonate (1.29 mL, 10.7 mmol), followed by the addition of 25% sodium methoxide in methanol (200 μL). The reaction was cooled to 10–15 °C, and hexane (20 mL) was added to precipitate the product. The resulting tan product (530 mg, 66%) was collected by vacuum filtration: mp 155–157 °C; ^{1}H NMR (DMSO-d_6) δ 3.50–3.60 (dd, 1 H), 3.62–3.70 (dd, 1 H), 3.75–3.85 (dd, 1 H), 4.00–4.10 (t, 1 H), 4.63–4.70 (m, 1 H), 5.12 (s, 2 H), 5.25 (br s, 1 H, D_2O exchangeable), 7.00–7.08 (m, 2 H), 7.30–7.51 (m, 7 H); high-resolution mass spectrum calcd for $C_{17}H_{17}NO_4$ 299.1158, found 299.1159 (−0.3 ppm deviation).

3-Chloro([^{3}H]benzyl) Bromide. To a stirred solution of 3-chlorobenzaldehyde (249 μL, 2.2 mmol), in THF (3 mL) at room temperature, was added NaB^3H_4 (100 mCi, 37 mCi/mg). The vial that contained the radioactive sodium borohydride was rinsed with 0.1 M NaOH (3 × 200 μL), and the washings were added to the reaction mixture. The solution was stirred under an atmosphere of nitrogen for 4.5 h, at which time nonradioactive sodium borohydride (83 mg, 2.2 mmol) was added, and the mixture stirred for an additional 2 h. Methanol (5 mL) was slowly added to the reaction, and the mixture was stirred overnight. The solution was diluted with diethyl ether (30 mL) and extracted with water (2 × 30 mL) and then brine (30 mL). The organic layer was dried, filtered, and evaporated under a stream of nitrogen. The resulting clear oil was redissolved in THF (3 mL) and cooled to 0–5 °C and phosphorus tribromide (207 μL, 2.2 mmol) was added over about 10 min. The reaction was stirred protected from the light for 1.5 h at 0–5 °C and then for an additional 11 h at room temperature. The mixture was diluted slowly with saturated sodium bicarbonate (5 mL) and stirred for 1 h. The solution was diluted with diethyl ether (30 mL) and extracted with water (30 mL) and brine (30 mL). The organic layer was dried, filtered, and evaporated under a stream of nitrogen to yield a clear oil, which was split into two batches and used in the coupling reactions with the enantiomers

of 5-(hydroxymethyl)-3-(4-hydroxyphenyl)-2-oxazolidinone (prepared as described below).

(*R*)- and (*S*)-3-[4-[(3-Chlorophenyl)[^{3}H]methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone Hydrochloride [*methoxy*-^{3}H]-15 (R = 3-ClPh)]. To a stirred solution of the (*R*)- or (*S*)-**13** (R = Ph) (299 mg, 1.0 mmol) in 3:1 methanol–ethyl acetate (7 mL) was added 10% Pd on carbon (50 mg). The solution was evacuated and purged with hydrogen gas three times and then was stirred under an atmosphere of hydrogen gas (1atm) for 5 h. The mixture was filtered and rotary evaporated to yield a brown oil [5-(hydroxymethyl)-3-(4-hydroxyphenyl)-2-oxazolidinone], which was used in the coupling reaction with tritiated 3-chlorobenzyl bromide.

To a stirred solution of the 5-(hydroxymethyl)-3-(4-hydroxyphenyl)-2-oxazolidinone prepared above and potassium carbonate (415 mg, 3.03 mmol) in 2-butanone (3 mL) was added a solution of 3-chloro[^{3}H]benzyl bromide in 2-butanone (2 mL). The mixture was heated to 65 °C, a catalytic amount of potassium iodide (2–3 mg) was added, and the reaction was stirred for 15 h. The mixture was filtered to remove excess potassium carbonate, diluted with 2-butanone (35 mL), and extracted with water (35 mL) and brine (35 mL). The organic layer was dried, filtered, and evaporated under a stream of nitrogen gas to yield a tan solid. This material was carried on to the final product in the same manner as the nonradioactive compound. All of the radioactivity in the final product was found to comigrate with unlabeled carrier compound on thin-layer chromatography (butanol–water–acetic acid 12:5:3). The specific activity of the final amine hydrochlorides were as follows: R = 2.48×10^7 dpm/μmol (11.3 mCi/mmol) and S = 2.37×10^7 dpm/μmol (10.8 mCi/mmol).

$Ag_2{}^{14}CO_3$. To a stirred solution of silver nitrate (2 g, 11.8 mmol) in deionized water (3 mL) protected from light at room temperature was added $Ba^{14}CO_3$ (19 mg, 5.405 mCi, 0.29 mCi/mg). The vial that contained the radioactive barium carbonate was rinsed with deionized water (2 mL), and the washings were added to the reaction mixture. Stirring was maintained in the dark for 4 h, at which time an addition of nonradioactive barium carbonate (178 mg, 0.90 mmol) was made. The reaction was stirred protected from light for an additional 12 h, and the resulting light yellow precipitate was collected by vacuum filtration and rinsed with deionized water (2 mL), acetone (2 mL), and diethyl ether (2 mL) under dim lighting. The product was obtained in a 98% yield (270 mg).

Diethyl [^{14}C]Carbonate. To a stirred solution of dry dimethylformamide (2.0 mL) were added ethyl iodide (500 μL, 6.25 mmol) and triethylamine (200 μL, 1.43 mmol), and the mixture was stirred at room temperature protected from the light for 15 min (until a precipitate was observed). To this solution was added the $Ag_2{}^{14}CO_3$ (198 mg, 0.98 mmol), and the resulting mixture was stirred for 6 h. An additional aliquot of triethylamine (680 μL, 4.88 mmol) was added and stirring was continued for 12 h. The mixture was then filtered, and the precipitate was washed with DMF (2 mL) and cyclohexane (2 mL). The filtrate was mixed with a 1:1 solution of potassium phosphate buffer (100 mM, pH 7.2) and brine, filtered again, and then separated in a separatory funnel. The organic layer was dried (Na_2SO_4) overnight and the cyclohexane solution of diethyl carbonate was used directly in the next step of the reaction.

(*R*)- and (*S*)-[2-^{14}C]-3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone Hydrochloride [[2-^{14}C]-15 (R = 3-ClPh)]. To a solution of the appropriate chiral **12** (R = 3-ClPh) (308 mg, 1.0 mmol) in toluene (5.5 mL) was added the cyclohexane solution of diethyl [^{14}C]carbonate followed by 25% sodium methoxide in methanol (200 μL). The solution was brought to reflux and after 20 min approximately 3 mL of solvent was removed by short-path distillation (from 78 to 90 °C). The mixture was heated for 12 h at 100 °C, at which time nonradioactive diethyl carbonate (400 μL, 3.30 mmol) was added, followed by another addition of 25% sodium methoxide in methanol (100 μL). This mixture was stirred at 110 °C for 12 h. The reaction mixture was cooled, and the product was precipitated by the addition of hexane (2–3 mL). The resulting tan powder (262 mg, 80%) was carried directly through the remaining steps as described above for the nonradioactive compounds. The specific activity of the final amine hydrochlorides were as follows: R = 3.58×10^6 dpm/μmol (1.63 mCi/mmol); S = 4.31×10^6 dpm/μmol (1.96 mCi/mmol). All of the radioactivity in the final product was shown to comigrate with unlabeled carrier product on TLC (butanol–water–acetic acid 12:5:3).

Enzyme. Bovine liver mitochondria was prepared by the method of Kearney.[42] MAO B was isolated from the mitochondria as described by Salach.[43] The enzyme was stored as a concentrated solution (20–30 mg/mL) in KP_i (50 mM, pH 7.2) at 4 °C. The specific activity varied among preparations, but ranged from 3 to 5 units/mg, where a unit of activity is defined as the conversion of 1 μmol of benzylamine to benzaldehyde per minute at pH 9.0 and 30 °C.

Enzyme Assay. MAO B was assayed in KP_i (100 mM, pH 7.2) at 25 °C with benzylamine or cinnamylamine as substrates. The turnover to the aldehyde product was measured by monitoring the increase in absorbance at 250 or 290 nm, respectively.

Preparation of Inactivator Solutions. All inactivator solutions were made by dissolving the hydrochloride salts in DMSO followed by dilution to the desired concentration with KP_i (100 mM, pH 7.2). The final concentration of DMSO in the solutions was 3%. The highest concentration of **15** (R = 3-ClPh) and its isomers that would remain homogeneous during inactivations (even if much higher concentrations of DMSO were used) was approximately 150 μM. For experiments comparing the inactivation by **17** (R = H) and **20**, a final concentration of 15% DMSO was used. Compound **15** (R = H) is freely soluble in buffered solutions; therefore, no cosolvent was used in studies with this compound. In experiments where cosolvent was used, enzyme controls were incubated in DMSO-containing buffer in order to factor out any effects of the cosolvent on the enzyme activity.

Inactivation Experiments: General Method. To an inactivator solution (765 μL) of the appropriate concentration incubated at 25 °C was added a diluted solution of MAO (35 μL of 0.30 mg/mL). After being mixed, the samples were incubated at 25 °C, periodically agitated, and then assayed for activity by removing 40 μL of the mixture and adding it to 460 μL of a benzylamine or cinnamylamine solution (1.1 mM) for assay as described above. The amount of enzyme inactivation was determined relative to control reactions containing no inactivator. Kinetic constants (k_{inact} and K_I) were determined as described by Kitz and Wilson.[44]

Inhibition Kinetics: General Procedure. The amount of inhibition of the oxidation of various concentrations of benzylamine (0.17–0.5 mM in KP_i, 100 mM, pH 7.2) by various concentrations of the isomers of **15** (R = 3-ClPh) (25, 50, and 75 μM of R; 12, 25, and 50 μM of S) was determined by adding 10 μL of a MAO solution (0.25 mg/mL) to 490 μL of an inhibitor/substrate solution at 25 °C, followed by monitoring the enzyme activity as described above. The K_i values were determined by Lineweaver–Burke[45] plots of 1/[benzylamine] vs 1/(initial velocity).

Stoichiometry of Inactivator Binding and Effect of Urea Treatment. Incubation mixtures containing MAO inactivated by the radiolabeled **15** (R = 3-ClPh) (1148 μL of 105 μM inactivator, 53 μL of a 2 mg/mL enzyme solution) were placed in dialysis bags and dialyzed against three changes (200 mL each) of potassium phosphate buffer (100 mM, pH 7.2), or until the amount of radioactivity in the dialysate reached background levels. Aliquots were removed for scintillation counting, for protein assay, and for further dialysis against 8 M urea (200 mL) for 15 h at 25 °C. Inactivation was monitored by assay of a similar incubation mixture containing unlabeled inactivator. Aliquots were removed from the urea-dialyzed sample for scintillation counting and protein assay. The original amount of active MAO was determined by titration with [^{3}H]-pargyline.[46] Calculated stoichiometries were corrected for any remaining MAO activity and are expressed as mole of radiolabel bound per mole of inactivated MAO.

Identification of CO_2 Released During Inactivation of MAO by Isomers of [2-^{14}C]-15 (R = 3-ClPh). Inactivations were carried out in 10-mL vials sealed with serum caps through which a Kontes center well (Catalog No. 882320-0000) had been inserted. The inactivation mixture consisted of 1148 μL of 105 μM carbonyl-labeled inactivator (dissolved in 3% DMSO, 100 mM KP_i, pH 7.2) and 53 μL of a dilute solution of MAO (0.3 mg/mL). The final inactivator concentration was 100 μM, and the final enzyme concentration was 13.3 μg/mL. The center well was filled with 100 μL of freshly prepared 8% KOH solution. After incubation at 25 °C for 29 h, the inactivation was quenched by addition of 100 μL of 2 M H_2SO_4, and the mixture was allowed to stand for 20 min. The center well was then removed and placed in scintillation cocktail for counting.[47] The amount of radioactive CO_2 released during the inactivation was ascertained by comparison to control reactions containing inactivator but no MAO and inactivator incubated with bovine serum albumin. The amount of enzyme inactivation was monitored by use of identical incu-

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bation mixtures containing nonradioactive inactivators. The exact amounts of CO_2 released during inactivation varied among enzyme preparations,[49] but the values reported in the text represent the average of two experiments for each inactivator. The original amount of active MAO was determined by titration with [^{3}H]pargyline.[46] Calculated stoichiometries were corrected for any remaining MAO activity and are expressed as mole of radiolabel bound per mole of inactivated MAO.

Identification of the Amount of Non-Amine Metabolites. Following incubation of MAO (44 μL of a 0.3 mg/mL enzyme solution) for 29 h with either the ^{14}C- or ^{3}H-labeled **15** (R = 3-ClPh) (965 μL of a 105 μM solution), the incubation mixture was placed directly onto a column (0.5 × 8.5 cm) of Dowex 50X-8 (200–400 mesh, H^+ form) equilibrated with deionized water. The column was eluted with deionized water (40 mL), and the eluent was collected in eight 10-mL scintillation vials. The eluent was analyzed for radioactivity by liquid scintillation counting. The amount of non-amine metabolites was determined by comparison to control values from a column run on a similar solution of inactivator incubated without MAO. The original amount of active MAO was determined by titration with [^{3}H]pargyline.[46] Calculated stoichiometries were corrected for any remaining MAO activity and are expressed as mole of radiolabel bound per mole of inactivated MAO.

Flavin Spectra of MAO Inactivated by 15 (R = H). MAO (45 μL) was incubated for 15 h in a 50 mM solution of **15** (R = H) (405 μL) (final concentration of MAO 11 μM). An assay showed that the enzyme was over 90% inactivated after that time period. The incubation mixture and a control that was incubated with buffer excluding inactivator were placed in dialysis bags and dialyzed (3 × 200 mL) against 100 mM NaP_i pH 7.2 buffer at room temperature. The dialyzed MAO solutions were placed in cuvettes and the spectra (350–550 nm) were recorded. After denaturation by addition of 13 μL of 30% sodium dodecyl sulfate, the spectra of the inactivated and control enzyme were again recorded.

(49) Although the exact amounts of radioactivity varied with different enzyme preparations, in all parallel inactivation experiments, equivalent amounts of ^{3}H and ^{14}C were incorporated into the enzyme by the correspondingly labeled inactivator analogues.

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Kinetic Energy Release Distributions as a Probe of Ligation Effects on Potential Energy Surfaces in Organometallic Reactions. Reversible Dehydrogenation of Cycloalkenes by Fe^+

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Abstract: The kinetic energy release distributions associated with dehydrogenation and double-dehydrogenation processes for Fe^+ reacting with cyclopentene, cyclohexene, cyclopentane, and cyclohexane have been obtained. Previously, dehydrogenation of alkanes and alkenes by Co^+ and Ni^+ in the gas phase has been characterized by release of more energy into product translation than can be accounted for by statistical theory. This observation is not general, however, since here we show dehydrogenations of cyclopentene and cyclohexene by Fe^+ are well described by statistical phase-space theory with the best fit of theory to experiment yielding $D_0°(Fe^+–C_5H_6) = 50 \pm 5$ kcal/mol and $D_0°(Fe^+–C_6H_8) = 66 \pm 5$ kcal/mol. For statistical theory to be applicable, it is required that there be no barrier for the reverse association reaction. The absence of a barrier in these systems is consistent with studies that indicate H/D exchange for $Fe(C_5H_6)^+$ is reversible and occurs in the presence of excess D_2 at about 5% of the Langevin collision rate. The product kinetic energy release distributions measured for the final hydrogen loss in the double dehydrogenations of cyclopentane and cyclohexane by Fe^+ are remarkably similar to those obtained for single dehydrogenations of cyclopentene and cyclohexene. This similarity is explained by participation of electronically excited Fe^+ in the first step of the double-dehydrogenation processes, which supplies the additional energy required to observe the second H_2 loss as a metastable process.

Introduction

The dehydrogenation of hydrocarbons by transition-metal ions has been extensively investigated. From studies of isotopically labeled butanes, it has been established that at least two distinct dehydrogenation mechanisms are operative. The reactions of Co^+ and Ni^+ with 2-methylpropane-*2-d_1* result in exclusive loss of HD, indicating that 1,2-dehydrogenation is the dominant route.[1] Ion-beam studies[1,2] of the dehydrogention of butane-*1,1,1,4,4,4-d_6* by Ni^+, as well as ligand exchange[2] and low-energy collision-induced dissociation experiments,[3] indicate that 1,4-dehydrogenation is the main process in that system. The reaction of Co^+ with butane-*1,1,1,4,4,4-d_6* gives a mixture of dehydrogenation products, with losses of H_2, HD, and D_2 occurring with ratios 20:35:45.[1] When the kinetic energy release distributions (KERDs) from selectively deuterated butanes were compared it was concluded[4] that 1,4-elimination was occurring, and H/D scrambling before hydrogen elimination yielded the observed isotopic ratios. The KERDs from Co^+ reacting with various isomeric butanes were distinct for 1,2- and 1,4-dehydrogenations and were all much broader than predicted by statistical theory, a result that appeared at that time to be general for dehydrogenation reactions.

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