and the yellow reaction mixture was stirred for 1 h at 0 °C and 24 h at 25 °C. Aqueous workup as usual gave 430 mg of crude product as a yellow liquid. This was combined with other material obtained similarly to give 591 mg of material. Purification by medium pressure liquid chromatography (*R*-phenylglycine dinitrobenzoate bonded to aminopropyl silica (1 mmol/g); column 1 in. ×36 in., hexane/EtOAc, 99/1.5 mL/min) and column chromatography (hexane/EtOAc, 4/1) gave 170 mg (29%) of **2bd** with only a trace of **1bd** by NMR. Data on **2bd**: ^{1}H NMR (200 MHz) 7.78 (d, 2 H, J = 8.2, aromatic H ortho to SO_2), 7.30 (d, 2 H, J = 8.2, aromatic H), 5.87–5.70 (m, 1 H, =C*H*CH$_3$), 5.64–5.49 (m, 1 H, -CH$_2$C*H*=), 5.49 (s, 1 H, -SO$_2$C*H*=), 4.17 (d, 2 H, J = 6, -C*H*$_2$O-), 2.64 (q, 2 H, J = 7.3, -C*H*$_2$CH$_3$), 2.42 (s, 3 H, C$_6$H$_4$C*H*$_3$), 1.72 (d, 3 H, J = 6.4, -CHC*H*$_3$), 1.05 (t, 3 H, J = 7.3, -CH$_2$C*H*$_3$); IR (CHCl$_3$) 3025 m, 2985 m, 2952 m, 2933 m, 2890 w, 1671 w, 1599 s, 1499 w, 1465 m, 1455 m, 1404 w, 1382 m, 1348 m, 1315 s, 1305 s, 1293 s, 1150 s, 1082 s, 1016 m, 991 w, 975 w, 943 w, 908 m; MS (10 eV) m/z 280 (M$^+$, 1.2), 226 (37), 126 (10), 125 (100), 124 (35), 97 (32); high-resolution MS calcd for $C_{15}H_{20}O_3S$ 280.1133, found 280.1132.

CACR of 2bd. Treatment of **2bd** with 2.7 equiv of sodium dimsylate at 50 °C for 30 min under usual conditions for the CACR gave a 73% yield of a mixture of **3bd** and **3be** in a ratio of 35/65 by HPLC.

3.2. Axial–Equatorial Preference. Thermolysis of 1ha. Preparation of *cis*- and *trans*-4-(1,1-Dimethylethyl)-1-[2-((4-methylphenyl)sulfonyl)-1-oxoethyl]-1-(2-propen-1-yl)cyclohexane (3ha and 3ah′). A solution of 150 mg (0.4 mmol) of **1al** in 4 mL of DMSO was heated at 100 °C for 3.25 h under N_2. Usual workup and column chromatography (hexane/EtOAc, 6/1) afforded 140 mg (93%) of a mixture of **3ha** and **3ha′** in a ratio of 66/34 by analysis of the intensities of the sulfonylmethylene (-SO$_2$CH$_2$-) signal of each isomer. The isomers could not be separated, and HPLC gave inconsistent results. Recrystallization from hexane/EtOAc gave an analytical mixture of **3ha** and **3ha′**: mp 118.5–128 °C; ^{1}H NMR (200 MHz) 7.87–7.80 (m, 2 H, aromatic H ortho to SO_2), 7.35 (d, 2 H, J = 7.9, aromatic H), 5.60–5.39 (m, 1 H, C*H*=CH$_2$), 5.07–4.89 (m, 2 H, CH=C*H*$_2$), 4.28 (s, 2 H, -SO$_2$C*H*$_2$-, **3ha**), 4.25 (s, 2 H, -SO$_2$C*H*$_2$-, **3ha′**), 2.44 (s, 3 H, aryl C*H*$_3$), 2.35–2.08 (m, 4 H), 1.63–1.55 (m, 2 H), 1.25–0.74 (m, 5 H), 0.74 (s, 9 H, *t*-butyl); IR (CHCl$_3$) 3075 w, 3060 w, 3025 m, 3009 w, 2950 s, 2865 m, 1712 s, 1639 w, 1597 m, 1491 w, 1478 w, 1467 w, 1454 m, 1438 w, 1393 w, 1366 m, 1324 s, 1305 s, 1290 m, 1265 w, 1239 m, 1231 m, 1183 w, 1154 s, 1087 m, 1035 m, 1018 w, 1002 m, 980 w, 921 m, 871 w, 814 m; MS (70 eV) m/z 376 (M$^+$, 0.39), 221 (11), 220 (19), 203 (16), 179 (30), 178 (17), 155 (12), 137 (16), 123 (39), 109 (31), 107 (11), 95 (21), 91 (43), 83 (14), 81 (33), 79 (19), 69 (16), 67 (26), 65 (11), 57 (100), 55 (21), 43 (13), 41 (37). Anal. Calcd for $C_{22}H_{32}O_3S$: C, 70.21; H, 8.51; S, 8.51. Found: C, 70.25; H, 8.57; S, 8.44.

CACR of 1ha. The general procedures were followed. The diastereomeric ratios were based on NMR analysis of chromatographically homogeneous materials. See Table VII.

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Registry No. 1aa, 109787-35-1; **1ab**, 109787-39-5; **1ac**, 109889-21-6; **1ad**, 109787-38-4; **1af**, 109787-43-1; **1ba**, 109787-47-5; **1bd**, 87039-98-3; **1be**, 87039-99-4; **1bg**, 123209-39-2; **1ca**, 123209-23-4; **1cb**, 123209-26-7; **1da**, 123209-25-6; **1ea**, 109787-55-5; **1eb**, 123209-27-8; **1eh**, 91873-76-6; **1ei**, 109787-57-7; **1ej**, 109787-58-8; **1ek**, 91873-77-7; **1em**, 109787-59-9; **1fa**, 109787-63-5; **1fh**, 109787-64-6; **1fi**, 109787-65-7; **1ga**, 109787-67-9; **1gh**, 91873-78-8; **1gk**, 91873-79-9; **1ha**, 123209-45-0; **2aa**, 109787-36-2; **2ad**, 123209-24-5; **2bd**, 123209-44-9; **3aa**, 80945-31-9; **3ab**, 82352-35-0; **3ac**, 82352-34-9; **3ad**, 82352-33-8; **3ba**, 123209-28-9; **3bd**, 87040-00-4; **3be**, 87040-01-5; **3bg**, 123209-41-6; **3ca**, 80945-33-1; **3cb**, 123209-30-3; **3da**, 123209-29-0; **3ea**, 82352-36-1; **3eb**, 109787-56-6; **3eh**, 91873-80-2; **3i**, 123209-34-7; **3ek**, 91873-81-3; **3em**, 123209-33-6; **3fa**, 123209-31-4; **3fh**, 123209-35-8; **3fi**, 123209-36-9; **3ga**, 123209-32-5; **3gh**, 91873-82-4; **3gk**, 9173-83-5; *cis*-**3ha**, 123209-46-1; *trans*-**3ha**, 123209-47-2; **4ab**, 123209-37-0; **5**, 123209-38-1; **6**, 82352-37-2; **7**, 123209-40-5; **9bd**, 123209-42-7; **9be**, 123209-43-8; **10**, 66947-20-4; **13**, 123209-62-1; **14**, 51620-81-6; **15**, 123209-61-0; **16**, 123209-48-3; (*E*)-**18aa**, 123209-51-8; (*Z*)-**18aa**, 123209-52-9; (*E*)-**18ad**, 123209-53-0; (*Z*)-**18ad**, 123209-54-1; **19**, 123209-49-4; **20**, 123209-49-4; **21aa**, 123209-55-2; **21ad**, 123209-56-3; MeCH=CH$_2$OH, 6117-91-5; *syn*-2,3-dimethyl-4-pentenoic acid, 58367-54-7; *anti*-2,3-dimethyl-4-pentenoic acid, 58367-53-6; 2-(2-propenyloxy)propenal, 70265-44-0; 2-(2-propenyloxy)propenal diethyl acetal, 123209-57-4; 2-(2-propenyloxy)propenal oxime, 123209-58-5; 2-(2-propenyloxy)-2-propenyl methanesulfonate, 123209-59-6; 2-(2-propenyloxy)-2-propenol, 123209-60-9; thiophenol, 108-98-5; 1-phenylsulfenyl-2-propyne, 5651-88-7; 1-phenylsulfenyl-1,2-propadiene, 1595-38-6; 2-chloro-3-phenylsulfenyl-1-propene, 4834-59-7; chloramine-T, 127-65-1; allyl alcohol, 107-18-6; phenylsulfonylacetone, 5000-44-2.

Supplementary Material Available: A description of the general experimental methods and the preparations, full characterizations, and reactions of compounds containing other stabilizing groups (**13**, **14**, **15**, **17aa**, **18aa**, **18ad**, **19**, **20**, **21aa**, and **21ad**) (9 pages). Ordering information is given on any current masthead page.

Model Studies for the Mechanism of Inactivation of Monoamine Oxidase by 5-(Aminomethyl)-3-aryl-2-oxazolidinones

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Abstract: 3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (MD 780236) has been reported to be an irreversible inactivator of monoamine oxidase (MAO), but the mechanism of inactivation is not known. A mechanism is now proposed that involves one-electron transfer to give the corresponding amine radical cation, removal of an α-proton, and decomposition of the oxazolidinone ring with loss of CO_2 to another radical which attaches to an enzyme active site radical. Chemical model studies for the proposed inactivation mechanisms are reported. Treatment of 3-(4-methoxyphenyl)-5-(chloromethyl)-2-oxazolidinone with tributylstannane and AIBN at 190 °C gave *N*-allylanisidine (6%) and CO_2 in addition to the hydrogen atom rebound product 3-(4-methoxyphenyl)-5-methyl-2-oxazolidinone. A high yield of *N*-allylanisidine was obtained by treatment of the corresponding bromo analogue with zinc, magnesium, or *n*-butyllithium. These studies support a radical inactivation mechanism that may proceed through additional carbon radical or carbanion intermediates. MD 780236 labeled with ^{14}C at the carbonyl of the oxazolidinone ring inactivates MAO with loss of $^{14}CO_2$, consistent with the model study results.

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavoenzyme that catalyzes the oxidation of various biogenic amines; inactivation of this enzyme can result in an antidepressant effect.[1,2] Our mechanistic studies over the last several years[3-15] with mecha-

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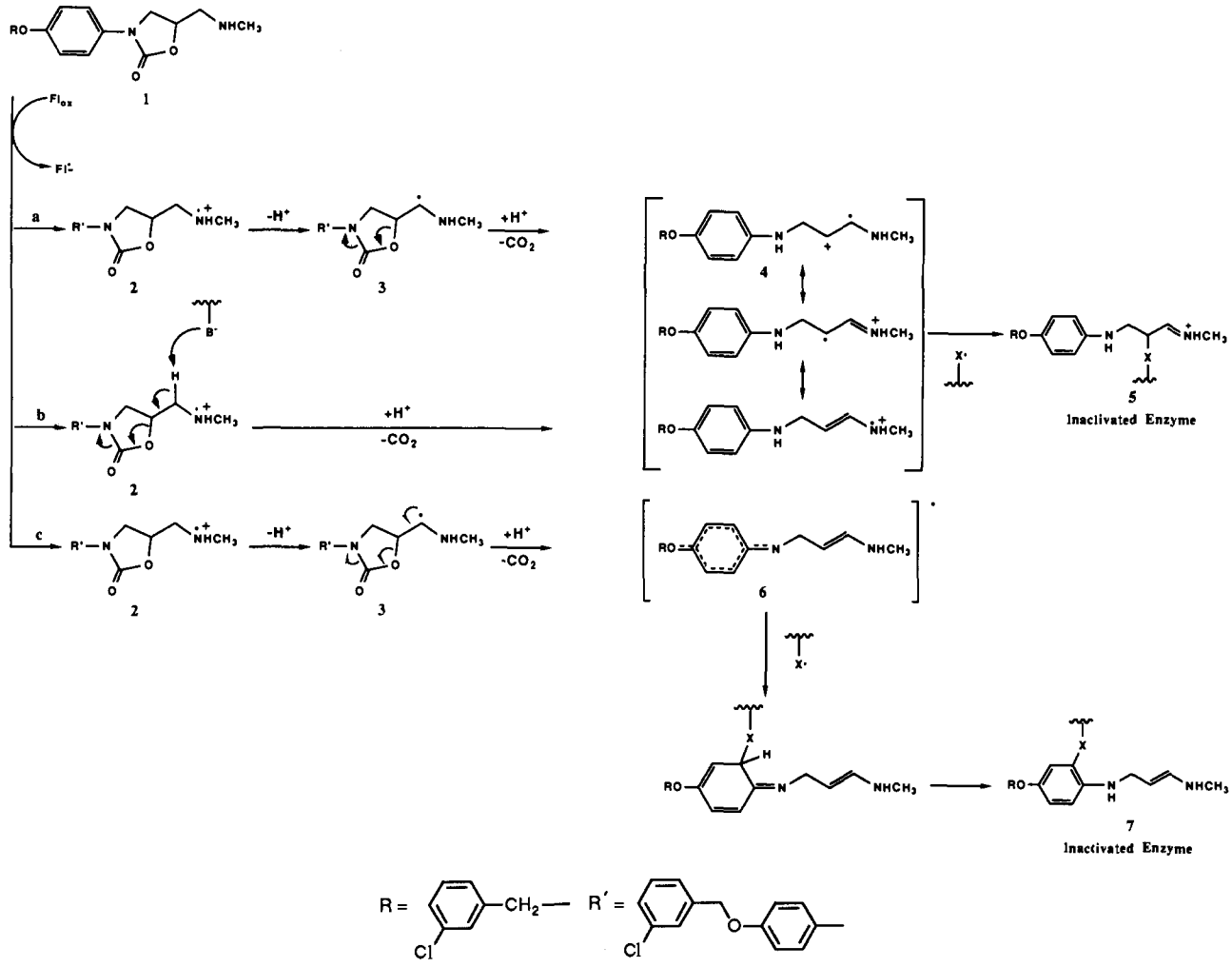
Scheme I. Proposed Mechanism of Inactivation of MAO by MD 780236



nism-based enzyme inactivators[16] of MAO support a radical mechanism for the enzyme.

Oxazolidinones that act as MAO inhibitors have been known for over 30 years.[17-19] More recently, structure–activity studies of this class of inhibitors have been reported,[20] and a promising candidate, the methanesulfonate salt of 3-[4-[(3-chlorophenyl)-methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (**1**, R = *m*-chlorobenzyl; Scheme I),[21] has emerged. Inhibition of MAO by **1** is competitive, has an irreversible component, and is selective for MAO B over MAO A.[22-24] A mechanism for inactivation of MAO by **1** was proposed[23,25] to involve oxidation of the amine bond to an imine followed by selective active site nucleophilic attack on the oxidized *S* isomer. It did not seem to us that this proposed adduct should be stable enough to result in an irreversible attachment to the enzyme. Consequently, on the basis of our previous mechanistic work with MAO,[3-15] we propose the alternative inactivation pathways shown in Scheme I. According to this scheme, **1** undergoes one-electron transfer to the radical cation **2** from which an α-proton could be removed to give radical **3**, which could decompose heterolytically (pathway a) or homolytically (pathway c). The heterolytic pathway is analogous

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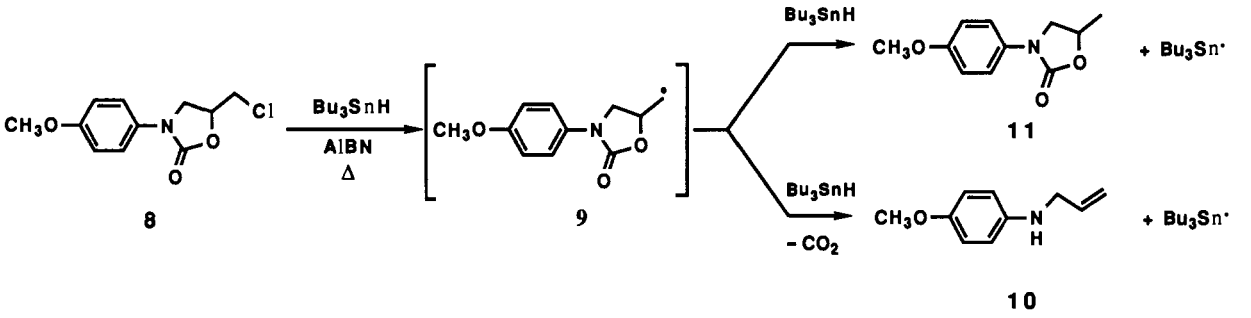
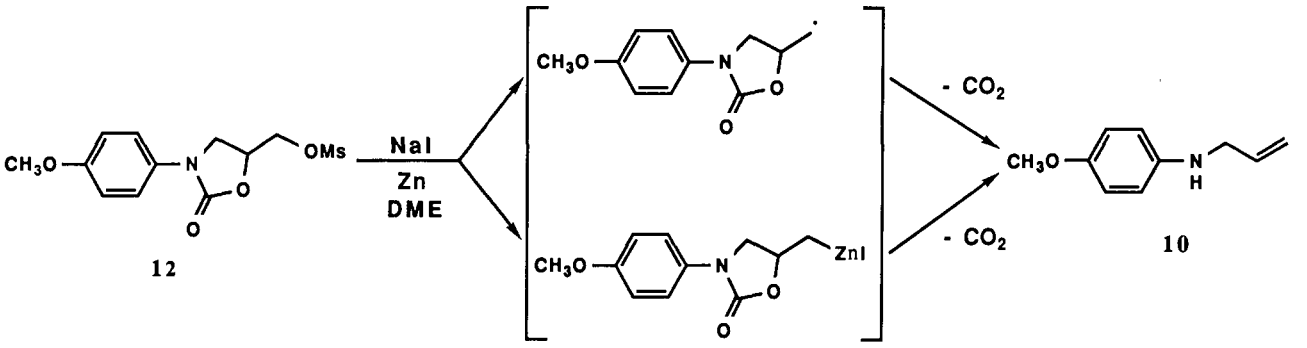
Scheme II. Model Reaction for Pathways a and c in Scheme I



8 9 11 10

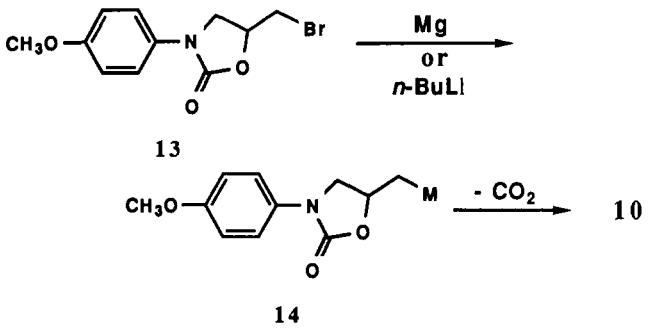
Scheme III. Reaction of 5-[(Mesyloxy)methyl]-3-(4-methoxyphenyl)-2-oxazolidinone with Sodium Iodide and Zinc Metal



12 10

to the mechanism for the conversion of ethylene glycol to acetaldehyde by Fenton's reagent,[26] a mechanism that has been implicated in the inactivation of ribonucleotide reductase by 2′-deoxy-2′-halonucleotides.[27] Pathway a would result in the loss of CO_2 and the formation of radical **4**, which could combine with an active site radical (either the flavin radical anion or an amino acid radical[12]) to inactivate the enzyme (**5**). The homolytic pathway c also results in the generation of CO_2 but gives a different radical (**6**), which also could combine with an active site radical to inactivate the enzyme (**7**). Pathway b requires carbanionic character to be generated at the α-carbon. This carbanionic character might be enhanced in certain enzyme–inhibitor binding modes where the orbital containing the nitrogen radical is unable to attain the optimal overlap with the α-carbon–hydrogen bond.

Scheme IV. Model Reactions for Pathway b in Scheme I[a]



13 14 10

[a] M represents MgBr or Li.

Results and Discussion

In order to test the mechanistic hypotheses shown in Scheme I, chemical model studies for the radical and carbanion intermediates were carried out. All of the models for the inactivation of MAO depicted in Scheme I are based on the assumption from previous work with inactivators of MAO[3-15] that the first step in the MAO-catalyzed oxidation of amines is the one-electron transfer from the amine to the flavin, resulting in the formation of the amine radical cation (in the case of **1** then **2** would be formed). The first model was designed to test whether generation of radical character adjacent to the *N*-(4-alkoxyphenyl)oxazolidinone ring (intermediate **3**, pathways a and c, Scheme I) would induce decomposition of the heterocycle with loss of CO_2. A radical related to **3**, namely, **9** (Scheme II), was chemically generated by heating chloride **8**, tributylstannane, and a catalytic amount of azobisisobutyronitrile (AIBN).[28] The desired radical began to form at about 180 °C. When the reaction mixture was heated neat at 190 °C for 7 h, all of the starting chloride was consumed, and two products, isolated by silica gel chromatography, accounted for 86% of the total mass. The predicted decarboxylation product, *N*-allylanisidine (**10**), and the product of hydrogen atom rebound (**11**) were obtained in a 6:94 ratio. The predominance of **11** is not surprising, since it is well-known that high concentrations of tributylstannane favor intermolecular hydrogen atom abstraction rather than intramolecular processes.[29,30] Substitution of hexabutylditin[31] for tributylstannane did not improve the reaction. The bromine analogue of **8** in refluxing benzene gave only **11**, suggesting that oxazolidinone decomposition is not a facile process. Control reactions showed that neither heating **8** without tributylstannane and AIBN nor heating **11** with tributylstannane and AIBN resulted in formation of **10**. Loss of CO_2 was detected by carrying out the reaction under a slow stream of nitrogen, which was passed through an aqueous solution of lead acetate. Precipitation of lead carbonate was observed, but this could not be quantified. However, when an amount of sodium bicarbonate equivalent to the amount of CO_2 that would have been generated in the above reaction was added to the lead acetate solution, a similar precipitate resulted. A control experiment, carried out in the absence of **8**, gave no precipitate.

It has been reported that the reduction of organic iodides (prepared in situ from the mesylates and sodium iodide) with zinc[32] proceeds, at least partially, by a one-electron process involving

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a radical intermediate.[33] When the mesylate analogue **12** (Scheme III) was heated with sodium iodide and zinc dust in refluxing dimethoxyethane, the sole product obtained was the decarboxylation product **10** in a nearly quantitative yield. The mechanism for the reaction of alkyl halides with zinc is unknown, however, and may involve a carbanionic intermediate[33a] (Scheme III). Therefore, it is not clear whether the zinc reaction is a model for pathways a and c (as is the reaction of the alkyl chloride with tributyltin hydride and AIBN) or for pathway b. Neither of the above models, however, differentiates pathway a from c.

Additional chemical models for the carbanionic pathway b, namely, the reactions of bromide **13** (Scheme IV) with magnesium turnings or with *n*-butyllithium, also were carried out. These reagents are expected to produce the corresponding organomagnesium[33b] or organolithium intermediates (**14**). In both cases a high yield of *N*-allylanisidine (**10**) was obtained, suggesting that the generation of carbanion character adjacent to the oxazolidinone ring also would lead to heterocycle decomposition with loss of CO_2 as depicted in pathway b (Scheme I).

Our preliminary results with MAO are consistent with these chemical model studies. Compound **1** (R = CH_3) was found to be a time-dependent inactivator of MAO (K_I = 62 mM; k_{inact} = 0.006 min^{-1}), indicating that the 4-methoxy model compounds are relevant to the enzyme inactivation mechanism by MD 780236. Compound **1** (R = *m*-chlorobenzyl), labeled in the oxazolidinone ring with ^{14}C at the carbonyl carbon,[34] irreversibly inactivates MAO with no incorporation of radioactivity into the enzyme but with concomitant release of $^{14}CO_2$.

Although it appears that the anionic decarboxylation pathway b is the more favorable chemical process, the efficiency of this decomposition reaction in the active site of MAO depends upon the amount of carbanionic character on the α-carbon following formation of the amine radical cation.[3-15] The radical decomposition pathways a and c appear to be more energetically demanding reactions; the geometric requirements for these fragmentations may be quite strict, but there may be enzyme binding modes that align the appropriate orbitals in an optimal fashion that favors these decomposition reactions.

Conclusions. Model studies for potential mechanisms of inactivation of monoamine oxidase by MD 780236 (Scheme I) indicate that the oxazolidinone ring can undergo fragmentation when either radical or carbanionic character is adjacent to it. Both pathways produce an intermediate radical that could attach to an active site radical, thereby inactivating the enzyme.

Experimental Section

Reagents. All reagents are from Aldrich Chemical Co. except for azobisisobutyronitrile, which was purchased from Fluka. All chemicals were used without further purification unless noted otherwise.

General Methods. Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 90-MHz spectrometer unless noted otherwise; chemical shifts are expressed as parts per million (δ) downfield from tetramethylsilane. Thin-layer chromatography was performed on silica gel 60F-254 coated plastic plates (Merck). Flash column chromatography utilized silica gel 60 (230–400-mesh ASTM) (Merck). Elemental combustion analyses were performed by Galbraith Laboratories Inc., Knoxville, TN.

5-(Chloromethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (8). Chloride **8** was prepared by the method of Hooz and Gilani.[35] The corresponding alcohol[36] (300 mg, 1.35 mmol) was stirred in acetonitrile (5 mL) with triphenylphosphine (708 mg, 2.70 mmol) and carbon tetrachloride (1 mL, 10.3 mmol) for 24 h at room temperature. The solvent was removed by rotary evaporation, and the residue was purified on silica gel (20 × 1.5 cm; EtOAc). Recrystallization from chloroform/*n*-hexane gave white needles: mp 105–106 °C; ^1H NMR ($CDCl_3$) δ 3.70–4.23 (m, 4 H), 3.80 (s, 3 H), 4.70–4.98 (m, 1 H), 6.85–7.55 (m, 4 H). Anal. Calcd for $C_{11}H_{12}ClNO_3$: C, 54.67; H, 5.01; Cl, 14.67; N, 5.80. found: C, 54.73; H, 5.09; Cl, 14.67; N, 5.74.

***N*-Allylanisidine (10).** The method of Takamatsu et al.[37] was followed. To *p*-anisidine (400 mg, 3.24 mmol) in anhydrous liquid ammonia (20 mL) was added allyl bromide (140 μL, 1.60 mmol). This mixture was stirred at reflux for 5.5 h, and then the solvent was allowed to evaporate. The residue was placed on a silica gel column (40 × 1.5 cm; 1:1 EtOAc/*n*-hexane), and the desired monoallylated product (11 mg, 20%) was isolated as a light yellow oil: ^1H NMR (400 MHz) ($CDCl_3$) δ 3.40 (br s, 1 H), 3.78 (m, 5 H), 5.16–5.38 (m, 2 H), 5.93–6.08 (m, 1 H), 6.60–6.85 (m, 4 H); mass spectrum (EI) 163 amu (M^+), 148 (–CH_3), 136 (—CH=CH_2), 122 (—CH_2CH=CH_2); thin-layer chromatography R_f 0.46 (1:1 EtOAc/*n*-hexane); IR (Mattson Alpha Centauri FT-IR) 2900 (br), 2700 (s), 1500 (s), 1450, 1300 cm^{-1}; high-resolution mass spectrum calcd for $C_{10}H_{13}NO$ 163.0997, found 163.0997.

3-(4-Methoxyphenyl)-5-methyl-2-oxazolidinone (11). This compound was prepared by a variation of the method of Grady and Kuivila.[38] A solution of bromide **13** (70 mg, 0.25 mmol), tributylstannane (86 μL, 0.32 mmol), and azobisisobutyronitrile (AIBN) (2 mg) in dry benzene was degassed by bubbling N_2 through the solution for 30 min. The mixture was refluxed for 8 h, and the solvent was removed by rotary evaporation. The resulting white residue was dissolved in chloroform, precipitated with *n*-hexane, and recrystallized from chloroform/*n*-hexane to yield white needles (50 mg, 96%): mp 92–94 °C; ^1H NMR ($CDCl_3$) δ 1.50 (d, 3 H), 3.44–3.66 (m, 1 H), 3.82 (s, 3 H), 4.10 (t, 1 H), 4.66–4.93 (m, 1 H), 6.89–7.53 (m, 4 H). Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 64.16; H, 6.54; N, 6.75.

5-(Bromomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (13). The bromide was prepared by the method of Hooz and Gilani.[35] The corresponding alcohol[36] (100 mg, 0.45 mmol) was stirred at room temperature in acetonitrile (2 mL) with triphenylphosphine (236 mg, 0.9 mmol) and carbon tetrabromide (446 mg, 1.35 mmol) for 24 h. The reaction mixture was then filtered to remove the triphenylphosphine oxide precipitate, and the solvent was evaporated under vacuum. The orange oily residue was purified by silica gel chromatography (40 × 1.5 cm; 1:1 EtOAc/*n*-hexane) to yield 112 mg (87%) of **13** as a white solid. This material was recrystallized from chloroform/*n*-hexane to give white needles: mp 100–101 °C; ^1H NMR ($CDCl_3$) δ 3.50–4.20 (m, 4 H), 3.80 (s, 3 H), 4.60–5.00 (m, 1 H), 6.80–7.50 (m, 4 H). Anal. Calcd for $C_{11}H_{12}BrNO_3$: C, 46.17; H, 4.23; N, 4.89; Br, 27.93. Found: C, 46.25; H, 4.20; N, 4.85; Br, 27.93.

Model Reaction for the Radical Decomposition of the Oxazolidinones (Scheme II). Chloride **8** (2.35 mg, 0.97 mmol), tributylstannane (340 μL, 1.26 mmol), and a catalytic amount of AIBN were heated at 190 °C for 7 h. The mixture was cooled, dissolved in methylene chloride, and extracted with 10% HCl (3 × 20 mL). The acid extracts were made basic and were extracted with methylene chloride (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was rotary evaporated to give a residue containing one major product (R_f 0.45; 1:1 EtOAc/*n*-hexane; silica gel), along with several polar impurities. The major product was purified by silica gel chromatography (50 × 2.0 cm; 1:1 EtOAc/*n*-hexane) to yield 8 mg of *N*-allylanisidine (**10**). The identity of this compound was confirmed by comparison of the thin-layer chromatography R_f values and 400-MHz NMR, IR, and mass spectra to those of a sample of *N*-allylanisidine prepared by the method described above.

The organic layer containing the products that did not extract into acid also contained only one major product (R_f 0.16; 1:1 EtOAc/*n*-hexane; silica gel), which was purified by silica gel chromatography (50 × 2.0 cm; 1:1 EtOAc/*n*-hexane) to yield 163 mg of **11**. The identity of this compound was confirmed by comparison of the thin-layer chromatography R_f values and NMR spectra to those of a sample of **11** prepared as described above.

Detection of CO_2 Released during the Model Reaction (Scheme II). A mixture of chloride **8** (400 mg, 1.66 mmol), tributylstannane (580 μL, 2.15 mmol), and AIBN (2 mg) was heated at 190 °C for 7 h. The head space was swept with a slow stream of nitrogen, which was passed through an aqueous lead acetate solution (150 mL, 1.2 M). During the reaction the lead acetate solution became cloudy, and a white lead carbonate precipitate settled out.

As a control reaction an amount of CO_2 equivalent to the amount that would have been generated in the above reaction was produced by acidification (1 M HCl, 109 μL) of potassium carbonate (15 mg, 0.10 mmol) and was shown to yield a similar precipitate in a lead acetate solution when swept with nitrogen gas as described above.

(33) (a) Pradhan, S. K.; Kohle, J. N.; Mistry, J. S. *Tetrahedron Lett.* **1982**, *23*, 4481–4484. (b) Recently it was shown[33c] that the reaction of alkyl bromides with magnesium proceeds via a short-lived radical intermediate. Consequently, it is not clear if this reaction is a model for pathway a or pathway b. (c) Root, K. S.; Hill, C. L.; Lawrence, L. M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 5405–5412.

(34) Synthesis to be published elsewhere.

(35) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86–87.

(36) Fauran, C.; Douzon, C. *Chim. Ther.* **1973**, *3*, 324–327.

(37) Takamatsu, N.; Inoue, S.; Kishi, Y. *Tetrahedron Lett.* **1971**, 4661–4664.

(38) Grady, G. L.; Kuivila, H. G. *J. Org. Chem.* **1969**, *34*, 2014–2016.

As an additional control, a stream of nitrogen was swept over a flask containing 600 μL of tributylstannane and AIBN (2 mg) heated at 190 °C. After 7 h no precipitate was observed in the lead acetate solution.

Control Reactions for the Above Model Reaction (Scheme II). (A) Treatment of 3-(4-Methoxyphenyl)-5-methyl-2-oxazolidinone (11) with Tributylstannane and AIBN. The methyl derivative **11** (87 mg, 0.42 mmol), tributylstannane (159 μL, 0.59 mmol), and AIBN (2 mg) were heated at 190 °C for 7 h. Thin-layer chromatography of the reaction mixture showed only unreacted starting material and some polar decomposition products at the base line. The major product was isolated by silica gel chromatography (20 × 1.5 cm; 1:1 EtOAc/*n*-hexane) and was shown to be starting material by comparison of the thin-layer chromatography R_f values and NMR spectra to those of a sample of **11** prepared as described above.

(B) Heating of 8 at 190 °C. Chloride **8** (200 mg, 0.83 mmol) was heated at 190 °C for 7 h. Thin-layer chromatography (1:1 EtOAc/*n*-hexane) showed that the major product was unchanged starting material contaminated with a small amount of polar decomposition products. The starting material was isolated by silica gel chromatography (20 × 1.5 cm; 1:1 EtOAc/*n*-hexane) to yield 194 mg (97%) of **8** as a white crystalline solid. The identity of this product was confirmed by comparison of the thin-layer chromatography R_f values and NMR spectra to those of a sample of **8** prepared as described above.

Reaction of 5-[(Mesyloxy)methyl]-3-(4-methoxyphenyl)-2-oxazolidinone with Sodium Iodide and Zinc Metal (Scheme III). Mesylate **12** was prepared from the corresponding alcohol[36] by the method of Crossland and Servis.[39] A mixture of **12** (40 mg, 0.13 mmol), NaI (99 mg, 0.66 mmol), and powdered zinc metal (85 mg, 1.3 mmol) was stirred in refluxing dimethoxyethane for 1 h. The reaction mixture was filtered to remove the excess zinc powder, diluted with water (30 mL), and extracted with chloroform (3 × 20 mL). The combined organic layer was dried ($MgSO_4$), filtered, and rotary evaporated to yield a light yellow oil, which was purified by silica gel chromatography (20 × 1.5 cm; 1:1 EtOAc/*n*-hexane), giving 20 mg (96%) of a light yellow oil (**10**). The identity of this product was confirmed by comparison of the thin-layer chromatography R_f values and NMR spectra to those of a sample of **10** prepared as described above.

(39) Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195–3196.

Model Reaction for Carbanionic Pathway b (Scheme IV). (A) Grignard Reaction. Magnesium turnings (7 mg) were added to a solution of bromide **13** (50 mg, 0.17 mmol) in dry THF (2 mL). The solution was brought to reflux, and a small chip of iodine was added. After 1.5 h the mixture was cooled and then diluted with 5% HCl (5 mL), neutralized, and extracted with ethyl acetate. The combined organic layers were dried ($MgSO_4$) and filtered, and the solvent was evaporated to yield 26 mg (95%) of *N*-allylanisidine (**10**). The identity of the product was confirmed by comparison of the thin-layer chromatography R_f values and NMR spectra to those of a sample of **10** prepared as described above.

(B) Lithium Anion. A solution of bromide **13** (40 mg, 0.14 mmol) in dry THF (5 mL) was cooled to −78 °C, and then 113 μL of 1.6 M *n*-butyllithium was slowly added. The solution was allowed to warm slowly to 5 °C, and then 5 mL of saturated ammonium chloride was added. The solution was neutralized, diluted with 25 mL of water, and extracted with ethyl acetate. Two products were isolated by silica gel chromatography (20 × 1.5 cm; 1:1 EtOAc/*n*-hexane): **13** (6 mg, 15%) and *N*-allylanisidine (**10**) (19 mg, 85%). Products were identified by comparison of the thin-layer chromatography R_f values and NMR spectra to those of samples of **13** and **10** prepared as described above.

Release of $^{14}CO_2$ from Inactivation of Monoamine Oxidase by [*carbonyl*-^{14}C]-1. MAO (0.73 μM) was incubated for 29 h with [*carbonyl*-^{14}C]-**1** (200 μM) in a serum-capped vial with a center well (Kontes Catalog No. 882320-0000) containing 100 μL of 8 N KOH. The progress of the inactivation was followed by assaying a control reaction containing unlabeled inactivator. The enzyme reaction was quenched with 100 μL of 2 N H_2SO_4; after 30 min, the center well was placed in scintillation fluid, and the radioactivity in the center well was determined by scintillation counting. A nonenzymatic control reaction was run in parallel, and the contents of the center well were used for background radioactivity.

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Registry No. 8, 121082-79-9; **10**, 71954-46-6; **11**, 121485-50-5; **12**, 121082-76-6; **13**, 121082-86-8; MAO, 9001-66-5; MD 780236, 84269-97-6; tributylstannane, 688-73-3.

(*E*)-4-(α-Halo-*p*-tolyl)-2-oxo-3-butenoic Acids Inhibit Yeast Pyruvate Decarboxylase by a Diversity of Mechanisms: Multiple Fate for the Thiamin-Bound Enamine Intermediate

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Abstract: (*E*)-4-(*p*-Tolyl)-2-oxo-3-butenoic acid and its α-(bromomethyl) and α-(chloromethyl) derivatives have been synthesized, and their interaction with brewers' yeast pyruvate decarboxylase was evaluated. The *p*-tolyl compound was found to be a slow substrate. The bromomethyl analogue led to partial time-dependent inactivation of the enzyme, but full activity was regained eventually. This analogue was shown to lose bromide ion in an enzyme-catalyzed, and time-dependent, fashion, and in its enzyme-catalyzed reaction it was converted quantitatively to *p*-methylcinnamic acid. The chloromethyl compound led to time-dependent inactivation of the enzyme; activity was not regained even after overnight incubation. This analogue released chloride ion in a time-dependent and enzyme-catalyzed reaction and produced *p*-(chloromethyl)cinnamaldehdye and *p*-methylcinnamic acid in a ratio of 4:6. All results are consistent with decarboxylation of the compounds followed by diverse fates for the central enamine intermediate: (1) the methyl derivative undergoes normal turnover; (2) the enamine derived from the decarboxylation of the *p*-(bromomethyl) derivative undergoes halide elimination, leading to a quinone methide that tautomerizes to a 2-acylthiamin diphosphate, which upon hydrolysis regenerates active enzyme [this behavior is analogous to that in a recent report on the decarboxylation of [*p*-(bromomethyl)benzoyl]formic acid by benzoylformate decarboxylase (Reynolds, L. J.; Garcia, G. A.; Kozarich, J. W.; Kenyon, G. L. *Biochemistry* **1988**, *27*, 5530)]; (3) the enamine derived from the chloromethyl compound is partitioned between chloride elimination and turnover and, most importantly, also leads to irreversible inactivation as reported earlier for some aromatic ring substituted (*E*)-4-phenyl-2-oxo-3-butenoic acids (Kuo, D. J.; Jordan, F. *Biochemistry* **1983**, *22*, 3735).

It was demonstrated during the past few years in this laboratory that some pyruvic acid analogues that have ring-substituted styrenes in place of a methyl group are processed by the enzyme pyruvate decarboxylase (PDC, EC 4.1.1.1) and have two very useful attributes. On the one hand these compounds are slow substrates, and some among them act as mechanism-based inactivators that are useful probes of the active center environment and functionalities.[1] In addition, the enamine (or 2α-carbanion)

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