Monoamine Oxidase B-Catalyzed Reactions of *cis*- and *trans*-5-Aminomethyl-3-(4-Methoxyphenyl)dihydrofuran-2(3*H*)-ones. Evidence for a Reversible Redox Reaction

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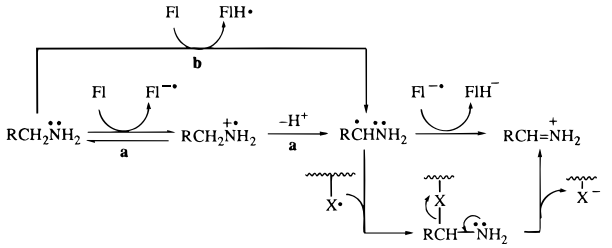
Abstract: Monoamine oxidase B (MAO B) was previously shown to catalyze the decarboxylation of *cis*- (**1**) and *trans*-5-(aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one hydrochloride (**2**) (Silverman, R. B.; Zhou, J. J. P.; Ding, C. Z.; Lu, X. *J. Am. Chem. Soc.* **1995**, *117*, 12895−12896). By [^{14}C]-labeling of the aryl methoxyl group, it is now shown that the decarboxylated product is 4-(4-methoxyphenyl)butanal (**7**), which is in the same oxidation state as the substrate. Two other products are produced, 4-carboxy-4-(4-methoxyphenyl)-butanal (**8**), and 5-formyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (**9**). Only **9** is an oxidation product; **7** and **8** are in the same oxidation state as the substrate (**1** or **2**). No products are detected under strictly anaerobic conditions. All of these products can be rationalized as arising from the formation of an α-carbon radical, generated either by single-electron amine oxidation and loss of a proton or direct hydrogen atom abstraction to **10** (Scheme 5). This intermediate then can undergo second electron oxidation and hydrolysis of the iminium ion to give **9** (the normal oxidation product). However, it also can suffer either homolytic C−O bond cleavage, decarboxylation, and electron return from the active site to give **7** or heterolytic cleavage and electron return from the active site to give **8**. 5-(4-Methoxyphenyl)tetrahydrofuran-2-ol (**14**), an oxidation product from the intermediate that leads to **7**, is not detected. These results suggest that MAO B can catalyze reversible redox reactions.

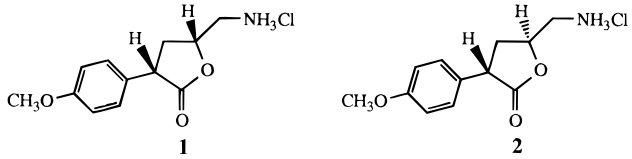
Introduction

Monoamine oxidase (EC 1.4.3.4; MAO) is a flavoenzyme that is important in the degradation of a variety of biogenic amines. Considerable evidence has been presented that is consistent with an electron-transfer mechanism for this enzyme (Scheme 1, pathway a),[1] although others have suggested a direct hydrogen atom abstraction mechanism (Scheme 1, pathway b)[2] and a nucleophilic mechanism.[3] Recently, it has been proposed that MAO contains a redox-active disulfide at the active site and that electrons from the substrate are transferred initially to the disulfide and from the disulfide to the flavin.[4] This intriguing result may explain why a flavin semiquinone intermediate has not yet been detected during enzymatic turnover.[2] The first electron transfer to the flavin depicted in pathway a is generally written as a reversible reaction to rationalize the isotope effect on C−H bond cleavage that is often observed, although there is no evidence that this step is reversible. To provide chemical evidence for the intermediacy of an α-radical during MAO-catalyzed amine oxidation (by either pathway a or b), two different approaches were taken. In the first approach,

Scheme 1



it was shown that MAO B-catalyzed oxidation of (aminomethyl)cubane led to destruction of the cubane structure, supporting the generation of a cubylcarbinyl radical.[5] Secondly, *cis*- (**1**)



and *trans*-5-(aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one hydrochloride (**2**) were synthesized with [^{14}C] labels at the carbonyl carbon atoms, and it was shown that MAO B catalyzed the decarboxylation of both analogues; the mechanism proposed for this decarboxylation reaction proceeds via α-radical **3** (drawn via an amine radical cation intermediate) as shown in Scheme 2.[6] The fate of radical **4**, however, was not clear. In

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(1) (a) Silverman, R. B. *Acc. Chem. Res.* **1995**, *28*, 335−342. (b) Silverman, R. B. In *Advances in Electron-Transfer Chemistry;* Mariano, P. S., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2, pp 177−213.

(2) (a) Walker, M. C.; Edmondson, D. E. *Biochemistry* **1994**, *33*, 7088−7098. (b) Miller, J. R.; Edmondson, D. E.; Grissom, C. B. *J. Am. Chem. Soc.* **1995**, *117*, 7830−7831. (c) Jonsson, T.; Edmondson, D. E.; Klinman, J. P. *Biochemistry* **1994**, 33, 14871−14878.

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(5) Silverman, R. B.; Zhou, J. P.; Eaton, P. E. *J. Am. Chem. Soc.* **1993**, *115*, 5, 8841−8842.

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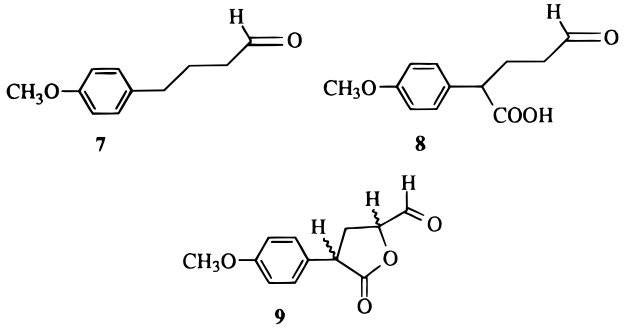
this paper, we report our investigation of the products formed by the MAO B-catalyzed reaction of **1** and **2**, which, unexpectedly, provides evidence for an apparent reversible redox reaction of MAO B and possible reversible electron transfer.

Results

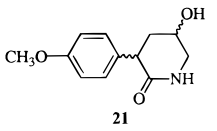
Synthesis of *cis*- (5) and *trans*-5-Aminomethyl-3-[methoxyl-^{14}C]-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one Hydrochloride (6). The *cis*-(**5**) and *trans*-5-aminomethyl-3-[methoxyl-^{14}C]-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one hydrochlorides (**6**) were synthesized by the same route previously reported for the syntheses of *cis*- and *trans*-[2-^{14}C]-5-aminomethyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one, which started from 4-methoxyphenylacetonitrile.[6,7] For the synthesis of **5** and **6**, 4-[^{14}C]methoxyphenylacetonitrile was first synthesized from 4-hydroxyphenylacetonitrile and [^{14}C]dimethyl sulfate.

Compound **7** was synthesized by pyridinium chlorochromate oxidation of 4-(4-methoxyphenyl)-1-butanol (Aldrich Chemical Co.). Compound **8** was prepared by the route shown in Scheme 3. Esterification of 4-(methoxyphenyl)acetic acid (Aldrich Chemical Co.) produced **16**, which was converted to the malonate ester (**17**) with diethyl oxalate and sodium hydride. Michael addition of the malonate ester anion to acrolein gave **18**. Following acetal protection of the aldehyde with ethylene glycol and saponification/decarboxylation, **20** was deprotected with HCl to give **8**. Aldehyde **9** was unstable under the conditions of the enzyme experiment, so the corresponding 2,4-dinitrophenylhydrazone was prepared. The cis and trans aldehydes (**9**) were synthesized from the corresponding iodides[6,7] by a DMSO oxidation.[8] Ceric ammonium nitrate oxidation of 4-(4-methoxyphenyl)butyric acid (Aldrich Chemical Co.) gave lactone **15**, which was reduced with DIBAL to the lactol **14** (Scheme 4).

Products Generated upon Incubation of 5 and 6 with MAO B. Incubation of **5** or **6** with purified beef liver MAO B[9] produced three enzyme-derived products (**7**−**9**), as detected

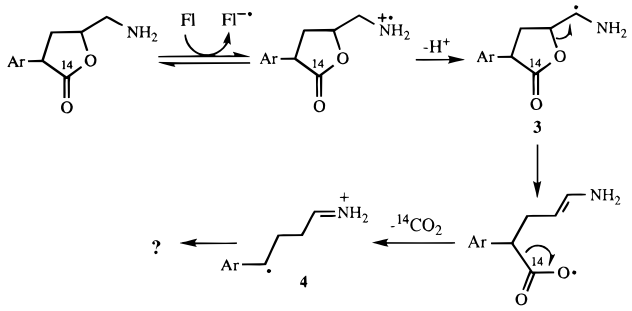


by HPLC (Figure 1). Six different radioactive products were detected. The peaks labeled A and B are diastereomers of a nonenzymatic rearrangement product of **5** and **6**, namely, **21**;[10]



D is the starting material (**5** in this experiment), C, E, and F are compounds **8**, **7**, and **9**, respectively. On the basis of the amount of enzyme used, 119 equiv of **7** (0.83 μmol of **7**/0.007 μmol of MAO B) and 9 equiv of **8** (0.065 μmol of **8**/0.007 μmol of MAO B) were produced. Because of the instability of **9**, it could not be quantified. No **14** was detected; 0.1 equiv of **14** could have been detected had it been formed. The addition of catalase to decompose any hydrogen peroxide generated during the oxidation reaction had no effect on the product formation or on their relative concentrations. When the experiment was repeated with **1** under strictly anaerobic conditions, no metabolites were detected.

Scheme 2



Discussion

MAO catalyzes the oxidation of a variety of primary, secondary, and tertiary amines. However, upon reaction of MAO B with either **5** or **6** three enzyme-derived products are formed, two of which, **7** and **8**, are in the same oxidation state as **5** and **6**. The formation of these metabolites can be accounted for by an elaboration of the mechanism shown in Scheme 1 for MAO B-catalyzed decarboxylation of **5** and **6** (Scheme 5). Following either one-electron transfer and proton transfer or direct hydrogen atom abstraction (not specifically shown in Scheme 5) intermediate **10** could decompose by a homolytic (pathway a) or a heterolytic (pathway b) cleavage to **11** or **13**, respectively. Homolytic C−C bond cleavage of **11** leads to decarboxylation and formation of intermediate **12**, as previously reported.[6] To get to **7** from **12** a one-electron *reduction* is required, which could occur by electron transfer from the flavin semiquinone to the benzylic radical (followed by proton transfer and hydrolysis). This returns the oxidation state to that of the substrate. The corresponding oxidation of **12**, i.e., electron transfer from **12** to the flavin semiquinone, apparently, is not favorable, since that would lead to **14**, which was not detected (a fraction of an equivalent could have been detected). Heterolytic cleavage of **10** (pathway b) would give **13**; to get to **8** from **13**, again, a one-electron *reduction* by electron transfer from the flavin semiquinone is required. Compound **8**, but not the major product, compound **7**, also could arise from a nucleophilic mechanism. The expected normal oxidation product **9** can arise from **10** by second electron transfer (pathway c) and hydrolysis of the resultant iminium ion.

Since **7** and **8** return the flavin to the oxidized form, if cleavage of the C−O bond of **10** (pathways a and b) is faster than electron transfer to the iminium ion (pathway c), then even under anaerobic conditions, products **7** and **8** should be detected. When the reaction was carried out anaerobically, no products were detected, suggesting that formation of **9**, which gives reduced flavin and inactivates the enzyme, is a much faster process than cleavage of the C−O bond. This same conclusion was arrived at previously[6] when **1** and **2** were [^{14}C]-labeled at the lactone carbonyl carbon; only 0.5 and 6 equiv of $^{14}CO_2$ was

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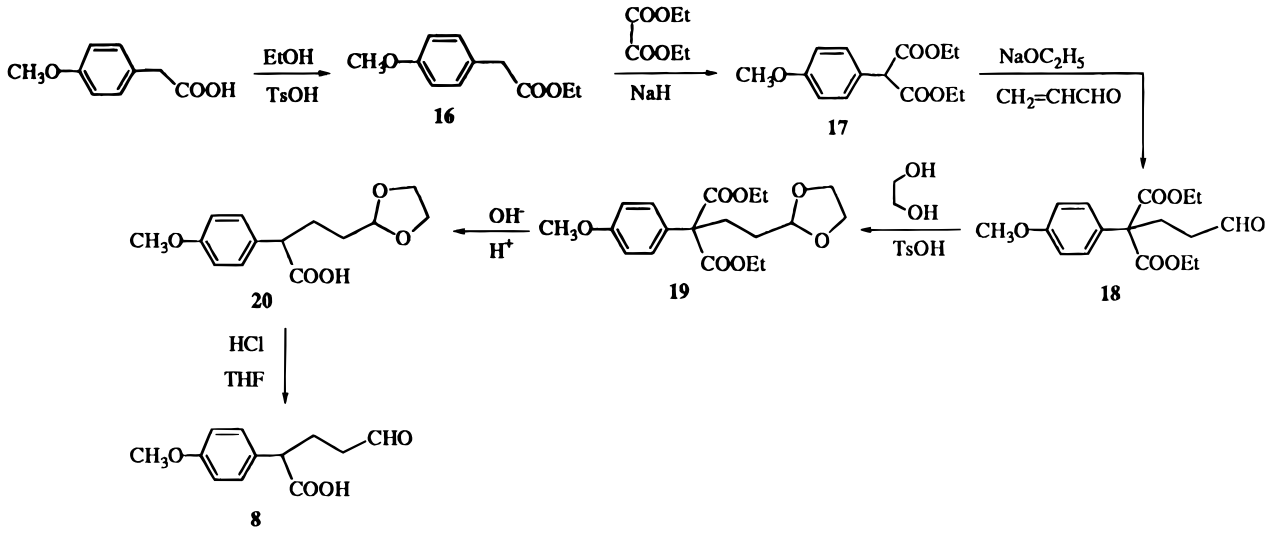
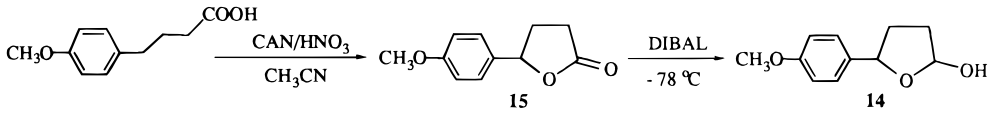
(10) Lu, X.; Silverman, R. B. *Bioorg. Med. Chem.* in press.

Scheme 3



Scheme 4



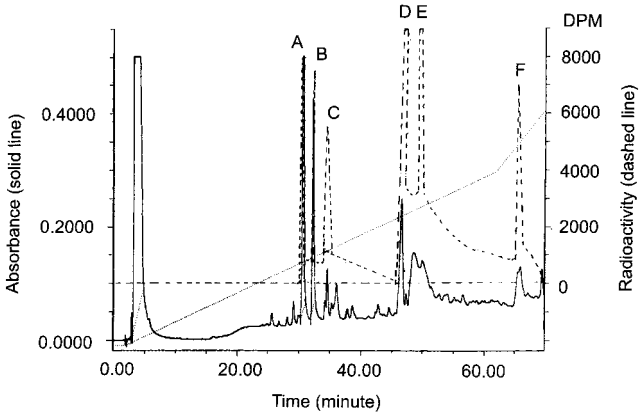


Figure 1. HPLC of metabolites from incubation of MAO B with either **5** or **6**. Peaks A and B are diastereomers of a nonenzymatic rearrangement product[10], peak C is **8**, peak D is starting material (**5**), peak E is **7**, and peak F is **9**.

trapped (presumably leading to the formation of **7**) upon inactivation of MAO B by the cis- and trans compounds, respectively, but 146 and 281 equiv of nonamines (the sum of **7**−**9**), respectively, were produced. Presumably, the reason that not even 1 equiv of **9** was detected was because of its instability.

Both the conversion of **12** to **7** and **13** to **8** support a reversible redox reaction that may involve an electron-transfer mechanism from the substrate to the flavin or from the substrate to a redox-active disulfide.[4] This provides strong evidence that a reversible initial electron transfer from the amine substrate to the flavin is plausible and reasonable and suggests that the MAO-catalyzed oxidation of amines may be completely reversible.

Experimental Section

General Methods. NMR spectra were recorded on either a Varian 300-MHz or a Varian Unity Plus 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. Thin-layer chromatography was performed on EM/UV silica gel plates with a UV indicator. Mass spectra were obtained on a VG Instruments VG70-250SE high-resolution spectrometer. UV spectra were recorded on a Perkin-Elmer Lambda 10 UV/vis spectrometer. Radioactivity was measured on a TRI-CARB 2100TR liquid scintillation analyzer. Column chromatography was peiformed with Merck silica gel (230−400 mesh). [^{14}C]-Dimethyl sulfate was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO) Other chemicals were purchased from Aldrich Chemical Co. Biochemicals and enzymes were purchased from Sigma Chemical Co.

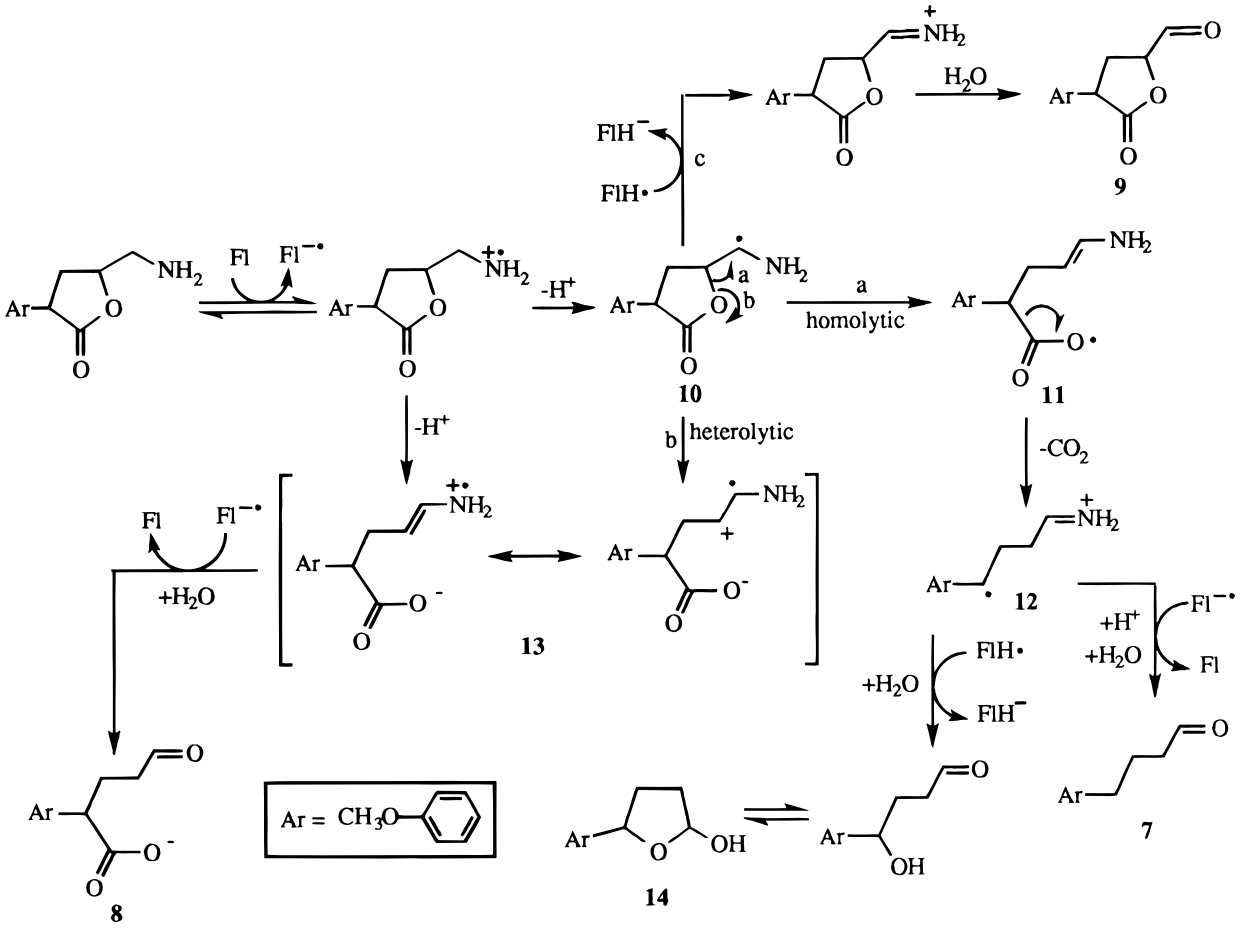
4-(4-Methoxyphenyl)butanal (7). To a stirred suspension of pyridinium chlorochromate (3.22 g, 15 mmol) and sodium acetate (0.24 g, 3 mmol) in anhydrous methylene chloride (28 mL) was added 4-(4-methoxyphenyl)-butanol (1.80 g, 10 mmol) in methylene chloride (5 mL). After 2 h of stirring, anhydrous ether (20 mL) was added, and the suspension was filtered through a pad of Celite. The filtrate was then concentrated in vacuo, and the residue was purified by flash chromatography (hexane/ethyl acetate, 4:1) to give 1.4 g (79%) of **7** as an oil. ^{1}H NMR ($CDCl_3$): δ 9.75 (s, 1 H), 7.11 (AB, 2 H), 6.85 (AB, 2 H), 3.79 (s, 3 H), 2.60 (t, 2 H), 2.47 (t, 2 H), 1.93 (m, 2 H). HRMS calcd for $C_{11}H_{14}O_2$ 178.0994, found 178.0993.

5-(4-Methoxyphenyl)dihydrofuran-2(3*H*)-ol (14). Lactone **15** was prepared by a a known procedure.[11] A mixture of 4-methoxyphenylbutanoic acid (0.97 g, 5 mmol), ceric ammonium nitrate (5.48 g, 10 mmol), 0.5 N nitric acid (10 mL), and acetonitrile (20 mL) was refluxed for 8 h. The resulting solution was cooled to room temperature and extracted with methylene chloride. The organic layer was washed successively with 10% aq sodium bicarbonate and water, dried over Na_2SO_4, and filtered. The solution was concentrated in vacuo, and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 2:1) to give 0.5 g (52%) of **15**. mp 52−53 °C; ^{1}H NMR ($CDCl_3$): δ 7.25−7.27 (AB, 2 H), 6.89−6.91 (AB, 2 H), 5.42−5.49 (m, 1 H), 3.79 (s, 3 H), 2.57−2.67 (m, 3 H), 2.18−2.22 (m, 1 H). HRMS calcd for $C_{11}H_{12}O_3$ 192.079, found 192.078.

A solution of **15** (0.5 g, 2.6 mmol) in toluene (4 mL) was cooled to −78 °C under a nitrogen atmosphere. Then, a 1.5 M solution of DIBAL in toluene (4 mL, 6 mmol) was added dropwise over 45 min. The resulting mixture was stirred at −78 °C for 3.5 h, and the excess DIBAL was destroyed by the addition of a solution of isopropyl alcohol (2 M) in toluene (3 mL). The solution was then allowed to warm to 0 °C, and water (0.1 mL), THF (10 mL), and a mixture of silica gel (1.5 g) and magnesium sulfate (3 g) were added successively. Between

(11) Nagarajan, A.; Porchezhiyan, V.; Srinivasaramanujam; Balasubramanian, T. R. *Ind. J. Chem.* **1985**, *24B*, 1202−1203.

Scheme 5



each addition the solution was stirred for 30 min. After filtration, the solid was washed with THF (2 × 10 mL) and methylene chloride (10 mL).The filtrate was dried over magnesium sulfate, and the solvent was evaporated under reduced pressure to give 0.4 g of **14** as an oil (80%) containing a mixture of diastereomers. ^{1}H NMR ($CDCl_3$): δ 7.38 (d, 1 H), 7.25 (AB, 2 H), 7.85 (AB, 2 H), 5.71 (t, *0.5* H), 5.59 (t, 0.5 H), 5.20 (t, 0.5 H), 4.96 (t, 0.5 H), 3.78 (s, 3 H), 2.95 (d, 0.5 H), 2.85 (d, 0.5 H), 2.41 (m, 0.5 H), 2.22 (m, 0.5 H), 2.05 (m, 1 H), 1.98 (m, 0.5 H), 1.89 (m, 0.5 H). Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.04%; H, 7.22%. Found: C, 67.78%; H, 7.41%. HRMS calcd for $C_{11}H_{14}O_3$ 194.094, found 194.094.

Ethyl 4-Methoxyphenylacetate (16). To a solution of 4-methoxyphenylacetic acid (5 g, 30 mmol) in benzene (30 mL) was added *p*-toluenesulfonic acid (60 mg) and ethanol (10 mL). The reaction was refluxed using a Dean−Stark trap to azeotropically remove the water. After 10 h, the benzene solution was cooled to room temperature, diluted with an equal volume of ether, washed with saturated NaHCO3 (2 × 20 mL), and then dried over $MgSO_4$ and filtered. The oil remaining after removal of the solvent under vacuum was **16** (5 g, 87%). ^{1}H NMR ($CDCl_3$): δ 7.21 (AB, 2 H), 6.87 (AB, 2 H), 4.15 (q, 2 H), 3.79 (s, 3 H), 3.56 (s, 2 H), 1.25 (t, 3 H). HRMS calcd for $C_{11}H_{14}O_3$ 194.094, found 194.094.

Diethyl 4-Methoxyphenylmalonate (17). This compound was prepared by a known procedure.[12] A solution of diethyl oxalate (2.92 g, 20 mmol) and **16** (3.88 g, 20 mmol) in 5 mL of anhydrous cyclohexane was added dropwise to a stirred suspension of sodium hydride (60% in oil: 800 mg, 20 mmol) in 20 mL of cyclohexane at 60 °C. Within several minutes, vigorous gas evolution and refluxing occurred. The mixture was heated for about 1 h until gas evolution ceased and was allowed to stand for 15 h at room temperature. The sodium salt of diethyl (4-methoxyphenyl)malonate precipitated as a voluminous colorless solid, which was stirred with anhydrous ether (10 mL), and the solid was collected by filtration. The solid was washed with ether (3 × 10 mL), dried in air, and stirred into a mixture of 20 mL of saturated NaCl and 5 mL of glacial acetic acid. The solution was extracted with ether (4 × 10 mL), and the combined extracts were washed with water (2 × 10 mL) and dried over Na_2SO_4. The solvent was evaporated to give 4.5 g (85%) of crude **17**, which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 12.8 (s, 0.3 H), 7.24 (AB, 1.3 H), 7.08 (d, 0.7 H), 6.84−6.91 (AB, 2 H), 6.30 (s, 0.7 H), 4.20−4.31 (m, 3.3 H), 4.13 (t, 0.7 H), 3.80 (s, 3 H), 1.23−1.32 (m, 5 H), 1.02 (t, 1 H). HRMS calcd for $C_{14}H_{18}O_5$ 266.115, found 266.115.

4,4-Dicarbethoxy-4-(4-methoxyphenyl)butanal (18). This compound was prepared by the procedure of Warner and Moe.[13] Compound **17** (2.66 g, 10 mmol) was added to a solution of sodium (6 mg) in absolute ethanol (20 mL). The solution was cooled to 0 °C, and acrolein (1.12 g, 20 mmol) was added dropwise. The reaction temperature was maintained at approximately 5 °C during the reaction. After the reaction was complete (2 h), the reaction mixture was neutralized with glacial acetic acid. The solvent was removed under vacuum, and the residue was dissolved in benzene (15 mL) and then washed with water (3 × 15 mL). The organic solution was dried over anhydrous sodium sulfate. Removal of the solvent gave **18** as an oil (2.1 g, 65%). ^{1}H NMR ($CDCl_3$) δ 9.65 (s, 1 H), 7.39 (AB, 2 H), 6.86 (AB 2 H), 4.08−4.30 (m, 4 H), 3.77 (s, 3 H), 2.20−2.70 (m, 4 H), 1.20−1.28 (m, 6 H). HRMS calcd for $C_{17}H_{22}0_6$ 322.142, found 322.142.

3,3-Dicarbethoxy-2-[4-(4-methoxyphenyl)butyl]-1,3-dioxolane (19). A mixture of compound **18** (2 g, 6.2 mmol), *p*-toluenesulfonic acid (60 mg), and ethylene glycol (5 g, 80 mmol) was suspended in benzene (40 mL) and refluxed using a Dean−Stark trap. After 5 h of reflux,

(12) Tietze, L. F.; Eicher, Th. *Reaction and Syntheses in the Organic Chemistry Laboratory;* University Science Books: Mill Valley, CA, 1989; p 182.

(13) Warner, D. T.; Moe, O. A. *J. Am. Chem. Soc.* **1948**, *70*, 3470−3472.

the suspension was washed with saturated sodium carbonate and then dried over $MgSO_4$ and filtered. Removal of the solvent gave **19** as an oil (2 g, 90%). ^{1}H NMR ($CDCl_3$): δ 7.43 (AB, 2 H), 6.86 (AB, 2 H), 4.85 (t, 1 H), 4.18−4.28 (m, 4 H), 3.92 (t, 2 H), 3.82 (t, 2 H), 3.77 (s, 3 H), 2.30−2.52 (m, 2 H), 1.55−1.70 (m, 1 H), 1.35−1.50 (m, 1 H), 1.20−1.37 (m, 6 H). HRMS calcd for $C_{19}H_{26}O_7$ 366.168, found 366.130.

3-Carboxy-2-[4-(4-methoxyphenyl)butyl]-1,3-dioxolane (20). A mixture of compound **19** (400 mg, 1.1 mmol), potassium hydroxide (560 mg, 10 mmol), water (3 mL), and ethanol (4 mL) was refluxed for 2 h. After a clear solution was formed, 8 mL of water was added and the solution was washed with ether (3 × 7 mL). Then, the solution was acidified with 10% aqueous HCl to pH 2−3. The acidic solution was extracted with ether (3 × 10 mL), and the combined extracts were dried over $MgSO_4$, filtered, and concentrated under vacuum, giving **20** as an oil (200 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 7.21 (AB, 2 H), 6.81 (AB, 2 H), 4.85 (t, 1 H), 3.95 (t, 2 H), 3.82 (t, 2 H), 3.77 (s, 2 H), 3.55 (t, 1 H), 2.10−2.23 (m, 1 H), 1.82−1.97 (m, 1 H), 1.54−1.72 (m, 2 H). HRMS calcd for $C_{14}H_{18}O_5$ 266.115, found 266.115.

4-Carboxy-4-(4-methoxyphenyl)butanal (8). Compound **20** (100 mg, 0.38 mmol) was deprotected to the aldehyde with 1 N aqueous HCl in THF at room temperature for 20 h. After evaporation in vacuo, the remaining oil contained a small amount of starting material and an impurity. Treatment of the crude **8** with 2,4-dinitrophenylhydrazine reagent gave the 2,4-dinitrophenylhydrazone (**8a**) (**8**) ^{1}H NMR ($CDCl_3$): δ 9.71 (s 1 H), 7.21 (AB, 2 H), 6.88 (AB, 2 H), 3.77 (s, 3 H), 3.56 (t, 1 H), 2.41 (m, 1 H), 2.33 (m, 0.5 H), 2.10 (m, 1 H), 1.89 (m, 0.5 H), 1.61 (m, 1 H). HRMS calcd for $C_{12}H_{14}O_4$ 222.089, found 222.089. (**8a**) ^{1}H NMR ($CDCl_3$): δ 11.0 (s, 1 H), 9.10 (s, 1 H), 8.25 (d, 1 H), 7.85 (d, 1 H), 7.42 (t, 1 H), 7.21 (AB, 2 H), 6.85 (AB, 2 H), 4.12 (m, 0.5 H), 3.80 (s, 3 H), 3.61 (m, 0.5 H), 2.41 (m, 2 H), 2.10 (m, 1 H), 1.21 (m, 1 H). Anal. Calcd for $C_{18}H_{18}O_7N_4$: C, 53.73%; H, 4.48%; N, 13.93%. Found: C, 53.44%; H, 4.45%; N, 13.84%.

2,4-Dinitrophenyl Hydrazone of 5-Formyl-3-(4-methoxylphenyl)-dihydrofuran-2(3*H*)-one (9). A literature procedure[8] was used to make the aldehyde from the corresponding iodide.[6,7] To a solution of 500 mg of sodium bicarbonate in DMSO (4 mL) was added the iodide compound (1 mmol) at 150 °C for 4 min. The mixture was then poured into water (12 mL), the solution was extracted with ether (4 × 10 mL), and the combined extracts were washed with water and dried over $MgSO_4$. After concentration, 2,4-dinitrophenylhydrazine reagent was added to the residue of **9** at room temperature and was stirred for 48 h. The mixture was extracted with chloroform followed by column chromatography, giving the 2,4-dinitrophenylhydrazine derivative of **9**. ^{1}H NMR ($CDCl_3$) δ 11.1 (s, 1 H), 9.16 (s, 1 H), 8.78 (s, 1 H), 8.38 (dd, 1 H), 7.90 (dd, I H), 7.21 (AB, 2 H), 6.88 (AB, 2 H), 4.70 (m, 1 H), 4.20 (m, 1 H), 4.01 (t, 1 H), 3.80 (s, 3 H), 3.48 (dd, 1 H), 3.35 (dd, 1 H).

Reaction of MAO B with 5 and 6. MAO B (140 μM, 100 μL) was incubated with 17.5 mM *cis*- (**5**) or *trans*-5-aminomethyl-3-(4-[^{14}C] methoxyphenyl)-dihydrofuran-2(3*H*)-one (**6**) in 100 mM sodium phosphate buffer, pH 7.2 (2200 μL, containing 10% of DMSO) at 25 °C until the remaining activity of MAO B was less than 10% compared to the control without inactivator, which was run simultaneously at one-fifth the scale. The incubation solution was extracted with methylene chloride (4 × 5 mL). The combined extracts were washed with water (5 mL) and evaporated. Compounds **7**−**9** were added as standards, and the residue was taken up in acetonitrile/water (6:4) and analyzed by analytical reversed-phase HPLC using a C18 column, eluting with a gradient elution between water (containing 5% acetonitrile and 0.06% trifluoroacetic acid) and acetonitrile (containing 5% water and 0.06% trifluoroacetic acid) and monitoring at 215 nm; aliquots from 0.5 min fractions were removed for scintillation counting. The experiment was repeated with nonradiolabeled **5** and **6**, and the metabolites were analyzed directly by LC/electrospray mass spectrometry. The masses corresponded to those obtained with the synthetic compounds for **7**−**9**, respectively. MS/MS fragmentation patterns of **7**−**9** also corresponded to those with the corresponding synthetic compounds.

Oxygen Dependence of the Inactivation of MAO-B by *cis*-5-Aminomethyl-3-(4- methoxyphenyl)dihydrofuran-2(3*H*)-one (5). A preincubation solution containing *cis*-5-aminomethyl-3-(4-methoxyphenyl)-dihydrofuran-2(3*H*)-one (17.5 mM, 1100 μL) in sodium phosphate buffer (100 mM, pH 7.2, containing 10% DMSO) and protocatechuate 3,4-dioxygenase (1 unit) in a 5 mL Eppendorf tube sealed with a rubber septum was frozen in liquid nitrogen, and then the system was pumped under vacuum through a needle. After 5 min, the tube was thawed at 4 °C and the system was filled with argon. The freeze−pump−thaw−argon cycle was repeated three times. Another preincubation solution in an Eppendorf tube sealed with a rubber septum, which contained MAO B (50 μL, 117 mM) and protocatechuic acid (20 μL, 12.5 mM), was deaerated by the same freeze−pump−thaw−argon process. Then the deaerated MAO B mixture was transferred to the deaerated inactivator solution with a gastight syringe. The reaction solution was incubated under argon at room temperature. After 2 days, trichloroacetic acid (1000 μL, 5 mM) was added to the incubation solution to denature the MAO B and quench the reaction. This solution was extracted with chloroform, and the extracts were analyzed by HPLC to identify the metabolites. A control experiment without protocatechuic acid and protocatechuate 3,4-dioxygenase was carried out also. In this case, the metabolites could be detected, indicating that without the enzymatic scrubbers, oxygen is present in sufficient concentration to drive the reaction. Another control reaction was carried out using benzylamine as substrate. When the anaerobic experiment was repeated, including the enzymatic oxygen scrubbers, no benzaldehyde was found by GC with flame ionization detector detection.

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