Monoamine Oxidase-Catalyzed Oxidative Decarboxylation of *cis*- and *trans*-5-Aminomethyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one. Evidence for the Intermediacy of an α-Radical

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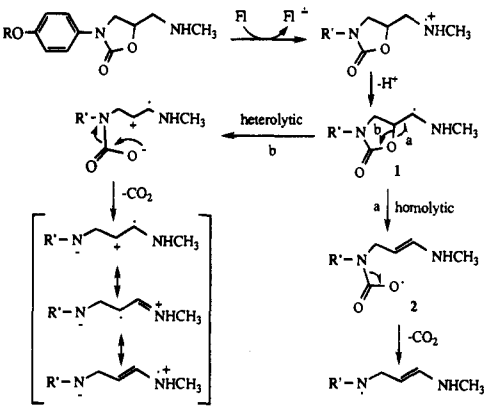
Monoamine oxidase (EC 1.4.3.1, MAO) catalyzes the oxidation of a variety of primary, secondary, and tertiary amines to give the corresponding imines.[1] Most of the mechanistic studies support a radical mechanism.[2,3] Previously, we found that a class of oxazolidinone inactivators of MAO underwent MAO-catalyzed excision of CO_2 from the oxazolidinone ring by a route that did not lead to enzyme inactivation.[4] Two mechanisms were proposed to account for this loss of CO_2, both proceeding from an α-radical (**1**, Scheme 1). Homolytic C−O bond cleavage (pathway a) leads to a carboxyl radical (**2**), which is known to undergo rapid decarboxylation.[5] However, because of anion stabilization by the oxazolidinone nitrogen, decarboxylation also could have arisen from a heterolytic elimination route (pathway b).

The corresponding lactone also was shown to be a time-dependent inactivator of MAO,[6] whose inactivation mechanism was hypothesized to be the result of formation of a covalent adduct at the α-position to the enzyme; the electron-withdrawing effect of the lactone ring was proposed to be responsible for adduct stabilization.[7]

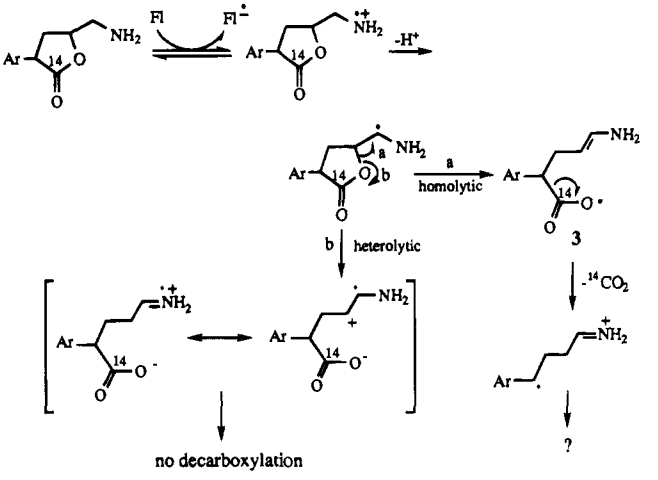
It occurred to us that excision of CO_2 from the lactone also should be possible, but only if an α-radical is generated and only if homolytic C−O bond cleavage occurs (Scheme 2, pathway a). As in the case of the oxazolidinone, this would generate a carboxyl radical (**3**), which is known to undergo rapid decarboxylation to the carbon radical.[5] Heterolytic C−O bond cleavage from the α-radical (Scheme 2, pathway b), however, would produce a reactive species without loss of CO_2 because of the stability of alkyl carboxylates. α-Carbanion formation, by any mechanism, would lead to heterolytic C−O bond cleavage to give the carboxylate, which, also, would not undergo decarboxylation (Scheme 3). Loss of CO_2, therefore, is a signal for both α-radical formation and homolytic C−O bond cleavage.

To determine if CO_2 excision occurs during MAO-catalyzed oxidation of the lactone, [2-^{14}C]-*cis*- (**4**) and [2-^{14}C]-*trans*-5-aminomethyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (**5**) were synthesized by the route shown in Scheme 4.[8] Incubation of homogeneous beef liver MAO with **4** and **5** resulted in the production of 0.5 and 6 equiv of $^{14}CO_2$, respectively,[9] per inactivation event. Since homolytic C−O bond cleavage depends upon appropriate overlap between the orbital of the

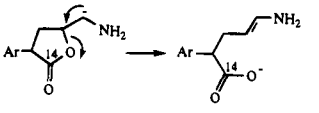
Scheme 1. Homolytic versus Heterolytic Mechanisms for Excision of CO_2 from Oxazolidinones



Scheme 2. Homolytic versus Heterolytic Mechanisms for MAO-Catalyzed Oxidation of 5-Aminomethyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-ones



Scheme 3. Fate of a Carbanion Intermediate



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(8) Starting from 1 mCi of K^{14}CN on a 1.0 mmol scale, 40 mg of **4** and 21 mg of **5** were obtained radiopure with specific activities of 1.0 mCi/mmol.

(9) In the case of **4**, MAO B (50 μL, 199 μM) in 100 mM sodium phosphate buffer, pH 7.4, was added to a 20 mL scintillation vial, which was stoppered with a serum cap with a Kontes center well (Cat. No. 882320-0000) in it. After 150 μL of KOH (8.75%) was added to the center well, a solution of **4** in 100 mM sodium phosphate buffer, pH 7.4 (1.0 mL; **4** was dissolved in 100 μL of DMSO and diluted in the buffer to give a solution containing 10% DMSO), was added to the bottom of the vial by syringe to give a final concentration of 4 mM. After the vial was shaken for 49.5 h at ambient temperature, 70% of the MAO was inactivated (relative to a control without **4**). Sulfuric acid (100 μL, 3 M) was injected into the enzyme mixture. After being shaken for 30 min, the center well was removed and counted in 10 mL of scintillation fluid (toluene/dioxane/methanol/PPO-POPOP, 162.5/25.2/81.3/11.7). The total radioactivity was 5672 cpm (6752 dpm). Radioactivity trapped in a nonenzymatic control reaction, run in parallel (70 cpm; 87 dpm), was subtracted from the experiment. The number of equivalents of CO_2 produced was determined by inactivating MAO B with [^{3}H]pargyline and setting that amount of radioactivity to 1 equiv. There was no difference in the result when the addition of sulfuric acid to quench the reaction and release the carbon dioxide was omitted from the procedure. A similar experiment was carried out with **5**, except that 109 μM MAO B was used, and the inactivator was incubated with the enzyme for 60 h; total radioactivity trapped was 41 145 cpm (46 200 dpm), and the control was 18 cpm (20 dpm). The extended incubation times were needed because of the poor substrate/inactivator activities of **4** and **5**.

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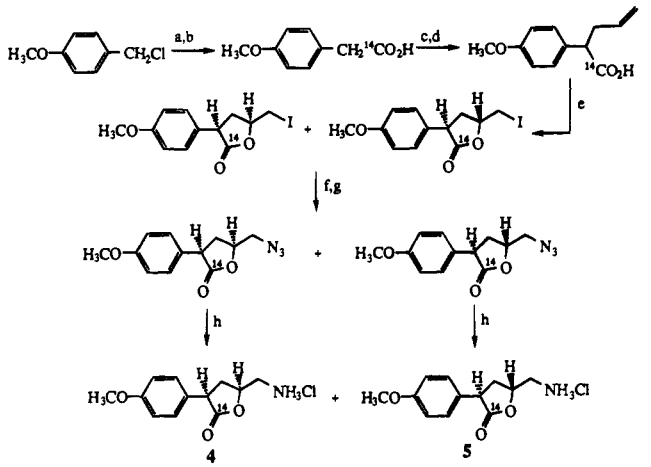
Scheme 4. Synthetic Routes to [2-^{14}C]-*cis*- (**4**) and [2-^{14}C]-*trans*-5-Aminomethyl-3-(4-methoxyphenyl)-dihydrofuran-2(3*H*)-one (**5**)[a]

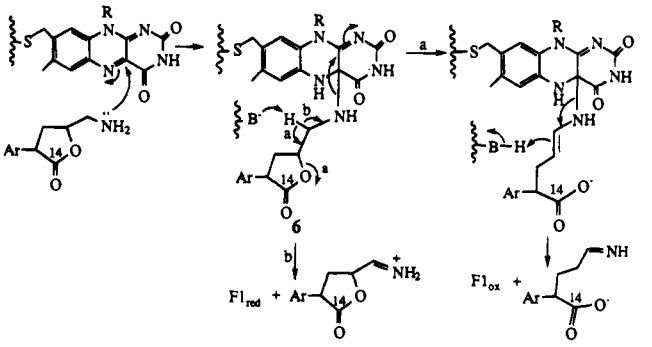


[a] (a) K^{14}CN/MeCN/18-C-6. (b) NaOH/EtOH. (c) *n*-BuLi. (d) Allyl bromide. (e) I_2/MeCN. (f) NaN_3/DMF. (g) Chromatography. (h) H_2/10% Pd–C/HCl.

C–O bond and the orbital containing the α-radical, and since **4** and **5** are diastereomers, a different amount of $^{14}CO_2$ generated from each is to be expected. Normal oxidation of these compounds to the corresponding aldehydes was determined by measuring the formation of radioactively-labeled nonamines produced, to get an indication of the relative incidence of decarboxylation versus amine oxidation (normal turnover). Compound **4** produced 146 equiv (total of 5.85×10^5 dpm) of nonamines, and **5** produced 281 equiv (total of 5.96×10^5 dpm).[10] These results indicate that decarboxylation does not occur with a high percentage of the turnovers, but this is a function of the lifetime of the radical, the orientation of the C–O bond relative to the radical-containing orbital, and the rate of C–O bond cleavage relative to second-electron oxidation. Every turnover may proceed via the α-radical, but because of the above-mentioned factors, only a small percentage of the molecules may lead to decarboxylation.

(10) Different amounts of enzyme were used for these experiments: **4**, 1.73 nmol; **5**, 0.92 nmol.

Scheme 5. Fate of a Nucleophilic Mechanism for MAO-Catalyzed Oxidation of 5-Aminomethyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-ones



These results also are further evidence against a nucleophilic mechanism for MAO.[11] Nucleophilic addition of **4** or **5** to the flavin would generate a covalent adduct (**6**, Scheme 5) that would not lead to loss of $^{14}CO_2$; deprotonation would lead either to elimination of the carboxylate and possible formation of a ^{14}C metabolite (pathway a) or to elimination of the reduced flavin and a ^{14}C metabolite (pathway b). No reasonable mechanism can account for generation of $^{14}CO_2$ from this covalent adduct.

In summary, MAO-catalyzed production of $^{14}CO_2$ from both [2-^{14}C]-**4** and [2-^{14}C]-**5** is strong evidence for the intermediacy of an α-radical and for subsequent (or concomitant) homolytic C–O bond cleavage. A nucleophilic mechanism is not consistent with these results.

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