Monoamine Oxidase B-Catalyzed Oxidation of Cinnamylamine 2,3-Oxide. Further Evidence against a Nucleophilic Mechanism

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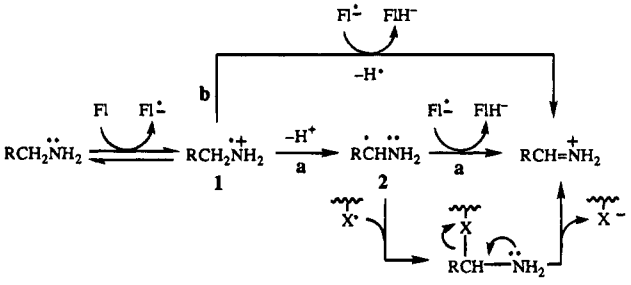
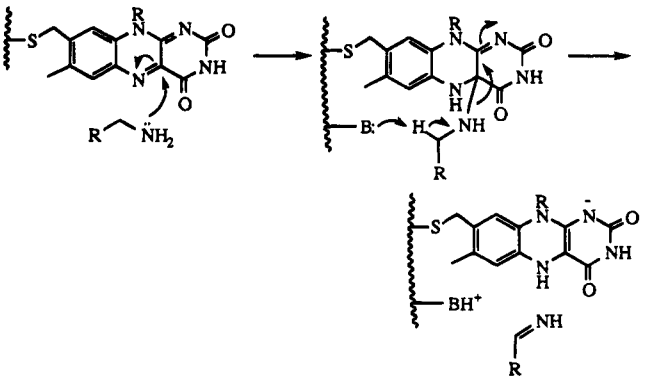
Monoamine oxidase (MAO, EC 1.4.3.4) is a flavin-containing enzyme that catalyzes the oxidative deamination of a variety of amine neurotransmitters and xenobiotics. Evidence for an initial one-electron transfer between the substrate and the enzyme to give an aminyl radical or radical cation (**1**, Scheme 1) has been provided from numerous studies with radioactively-labeled cyclopropylamine,[1] cyclobutylamine analogues,[2] and silylamines.[3] (Aminomethyl)cubane was designed as a probe to differentiate deprotonation/second electron transfer (pathway a) from hydrogen atom transfer (pathway b),[4] and on the basis of the metabolites isolated and the observation of enzyme inactivation, deprotonation to **2** was concluded to be reasonable. Recently, a nucleophilic mechanism for the oxidation of amines by MAO was proposed (Scheme 2),[5] but, on the basis of the above-cited studies and the ability of MAO to oxidize highly sterically-hindered amines, it was suggested that this mechanism is highly unlikely.[6] In the nucleophilic mechanism, following addition to the flavin, the α-proton of the substrate is removed by an active site base. This may generate carbanionic character at the α-carbon or should, at least, permit β-elimination if an appropriately-substituted group were appended. In this communication we describe studies with cinnamylamine 2,3-oxide, which was designed to test whether β-elimination occurs.

The rearrangement of 1-substituted 2,3-epoxy-3-phenylpropyl radical (Scheme 3, **3**, R = Me or Ph) was found to give only the vinyl ether product,[7] corresponding to cleavage *exclusively* of the C−C bond (pathway a); no products from cleavage of the C−O bond (pathway b) were detected. When the corresponding carbanion was generated, however, exclusive C−O bond cleavage was observed; no C−C bond cleavage was detected.[7] Therefore, cleavage of the C−O bond of the epoxide

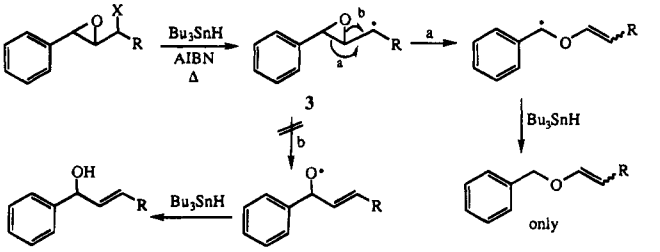
Scheme 1



Scheme 2



Scheme 3



is good evidence for a carbanion-like mechanism (either E2 or E1cB); C−C bond cleavage is consistent with a radical pathway. As a probe for the intermediacy of a carbanion-like intermediate in MAO B substrate oxidations, which would signal the possibility of a nucleophilic mechanism (Scheme 2), cinnamylamine 2,3-oxide hydrochloride (**4**, Scheme 4)[8] was tested as a substrate. Compound **4** was found to be both a very good substrate (K_m = 0.55 mM; k_{cat} = 71 min^{-1}) and an inactivator (K_I = 0.11 mM; k_{inact} = 0.055 min^{-1}) of MAO B[9] (partition ratio 1290). Inactivation was protected by substrate.[10] Three metabolites were detected and identified,[11] two directly by

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(10) The $t_{1/2}$ for inactivation of MAO B by 4.0 mM **4** was 3 min; the same experiment in the presence of 40 mM benzylamine showed that 97% of the enzyme activity remained after 12 min.

(11) In a control experiment, it was found that cinnamaldehyde 2,3-oxide reacts with hydrogen peroxide to give benzaldehyde. Hydrogen peroxide, a byproduct of the oxidation of amines by MAO (the reduced flavin is reoxidized by O_2, which is converted to H_2O_2), can be destroyed by the enzyme catalase. Consequently, it was shown that the addition of catalase (0.11 μg, 2.8 unit/μg) prevented the decomposition of cinnamaldehyde 2,3-oxide by hydrogen peroxide. To avoid the possible formation of benzaldehyde as a result of hydrogen peroxide decomposition of cinnamaldehyde 2,3-oxide, all metabolite studies were carried out in the presence of an excess of catalase. A control in which cinnamaldehyde 2,3-oxide was substituted for **4** in the presence of MAO and catalase (to which hydrogen peroxide was added to mimic the conditions of the enzyme reaction) produced *no* benzaldehyde. Therefore, all of the benzaldehyde that we observed in our enzyme studies comes from cinnamylamine 2,3-oxide, not from cinnamaldehyde 2,3-oxide.

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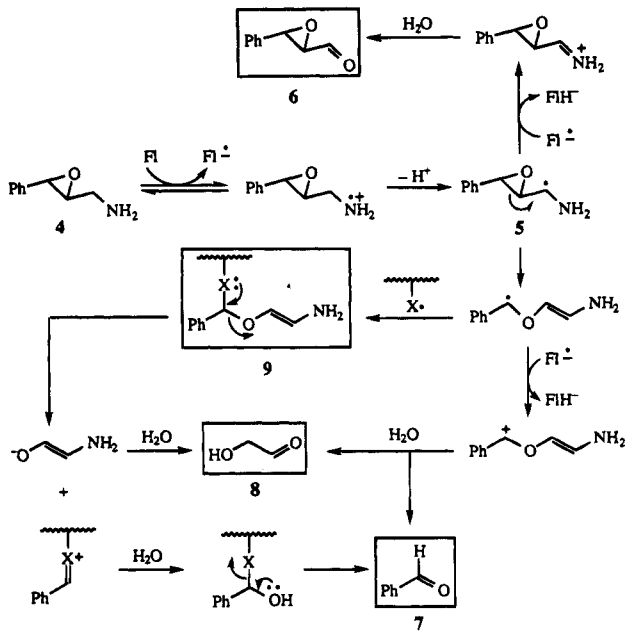
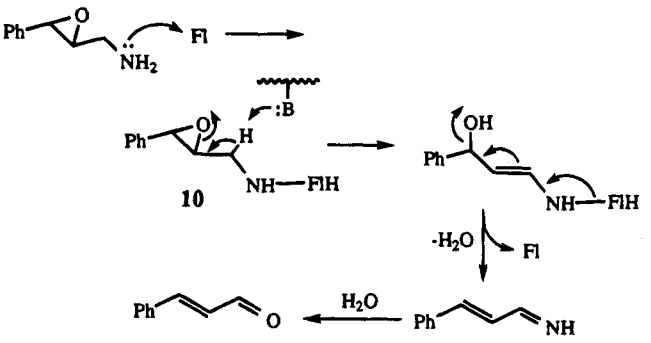
Scheme 4



HPLC and one after derivatization. The two metabolites identified directly were cinnamaldehyde 2,3-oxide (**6**)[12] and benzaldehyde (**7**) (the ratio of **6**:**7** = 110); no cinnamaldehyde was detected. Following Centricon filtration of the inactivated enzyme, the filtrate was treated with 2,4-dinitrophenylhydrazine solution and was heated to 70 °C for 1 h.[13] HPLC showed that the osazone, the 2,4-dinitrophenylhydrazone of glyoxal (OHC-CHO), which forms by oxidation of glycolaldehyde (**8**) to glyoxal followed by derivatization,[14] and the 2,4-dinitrophenylhydrazone of benzaldehyde were produced; no 2,4-dinitrophenylhydrazone of cinnamaldehyde or of any other compound was detected. When the enzyme reaction was repeated with cinnamaldehyde 2,3-oxide instead of **4**, no benzaldehyde was detected;[11] therefore, benzaldehyde is not the product of hydrolysis of cinnamaldehyde 2,3-oxide.

Cinnamaldehyde, which was shown to be stable under the conditions of the enzyme experiment, could be formed by a C−O bond cleavage from a nucleophilic addition mechanism as shown in Scheme 5. From intermediate **10** it is reasonable

Scheme 5



to assume that, at least, some cleavage of the epoxide would occur. The inability to detect the formation of any cinnamaldehyde is evidence against this nucleophilic pathway. It is interesting that *trans*-2-phenylcyclopropylamine, the carbon isostere of cinnamylamine 2,3-oxide, is a substrate for MAO B that does not undergo cyclopropyl ring cleavage.[15] This may be the result of different orbital alignment for the two molecules in the active site of MAO, possibly resulting from hydrogen bonding with the epoxide nonbonded electrons, such that the epoxide cleavage is favored over that for the corresponding cyclopropane analogue.

Inactivation is thought to be the result of the formation of **9** (Scheme 4). This adduct should be relatively stable at high pH and less stable at low pH. Dialysis of the inactivated enzyme in different pH buffers for 20 h resulted in the recovery of 14% of enzyme activity at pH 9, 19% at pH 7, and 45% at pH 5, consistent with structure **9**. Furthermore, treatment of the inactivated enzyme with acidic 2,4-dinitrophenylhydrazine gave the expected decomposition products of **9**, namely, the hydrazones of benzaldehyde and glyoxal.[16]

In summary, these studies provide further evidence against a nucleophilic mechanism for MAO-catalyzed amine oxidation.

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(16) MAO B (200 μL; 178 μM) was incubated with 24 mM **4** in 100 mM potassium phosphate buffer, pH 7.2, until complete inactivation occurred. The inactivated enzyme was dialyzed against 50 mM potassium phosphate buffer, pH 7.2, with several changes of buffer over 24 h and then 2,4-dinitrophenylhydrazine reagent was added and the mixture was heated to 70 °C for 3 h. Following centrifugation, an aliquot was analyzed by reversed-phase HPLC (C_{18} silica gel; 40% water in acetonitrile; 254 nm) and compared with standard 2,4-dinitrophenylhydrazones of benzaldehyde and glycolaldehyde.