Stereoselective Ring Opening of 1-Phenylcyclopropylamine Catalyzed by Monoamine Oxidase-B

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Abstract: The inactivation of monoamine oxidase (MAO) by 1-phenylcyclopropylamine (1-PCPA, **1**) has been proposed (Silverman, R. B.; Zieske, P. A. *Biochemistry* **1985**, *24*, 2128–2138) to proceed via a radical mechanism involving ring opening of an unstable cyclopropylaminyl radical (Scheme I). This intermediate has been shown to partition between attachment to the N-5 position of the covalently-bound flavin cofactor, which results in irreversible inactivation, and attachment to an active-site cysteine residue, which results in an unstable adduct that hydrolyzes to release acrylophenone and active enzyme over time. The stereochemistry and mechanism of the ring-opening step is investigated in this paper. The enantiomerically pure dideuterated 1-PCPA analogues **5** and **6** were prepared and used to inactivate MAO. Extractions of the acrylophenone metabolites released on decomposition of the reversible cysteine adducts that were formed during inactivation by **5** and by **6** were subjected to GCMS analysis. It was found that the inactivation by the *R*-isomer (**5**) produced 66% of [β,β-$^2H_2$]acrylophenone, 19% of [α-^{2}H]acrylophenone, and 15% of unlabeled acrylophenone; inactivation by the *S*-isomer (**6**) resulted in formation of 4% of [β,β-$^2H_2$]acrylophenone, 6% of [α-^{2}H]-acrylophenone, and 90% of unlabeled acrylophenone. These results are indicative of a stereoselective ring opening, preferentially forming β,β-dideutero adduct **8** following inactivation by **5** (Scheme II) and the α,α-dideuterio adduct **13** following inactivation by **6** (Scheme III). Stereoselective exchange of a single α deuterium on adduct **13** is proposed to account for the complete washout of deuterium in the product and the observed deuterium isotope effect of 3.1 on the reactivation of **6**-inactivated MAO. The difference in the amount of cleavage of the two cyclopropyl bonds with each enantiomer may be due, at least in part, to a secondary deuterium isotope effect on the formation of the dideuterated carbon radical. This secondary deuterium isotope effect also provides a rationalization that favors a mechanism involving electron transfer to the aminium radical cation followed by cyclopropyl ring cleavage and then combination with an active-site cysteine radical. The results do not support a direct S_H2 mechanism. The preference for cleavage of one of the cyclopropane bonds is believed to be the result of asymmetry at the active site which is set up for stereospecific removal of the pro-*R* proton from substrates; the pro-R C–H bond of substrates corresponds to the cyclopropane bond in 1-PCPA that is preferentially broken.

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavin-dependent enzyme responsible for the oxidative deamination of biogenic and xenobiotic amines. It exists as two distinct isozymes designated MAO-A, which catalyzes the oxidation of norepinephrine and serotonin very efficiently, and MAO-B, which catalyzes the oxidation of phenylethylamine and benzylamine much more effectively than does the A isozyme. Selective inhibitors of the A isozyme are known to elicit an antidepressant effect,[1,2] and inhibitors of the B isozyme have more recently been shown to be useful in the treatment of Parkinson's disease, when administered in conjunction with traditional L-dopa therapy.[3]

Over the last decade we have provided evidence for a single-electron-transfer mechanism for monoamine oxidase.[4] The earlier evidence was derived from studies on the mechanism-based inactivation[5] of MAO by a variety of cyclopropylamine analogues of substrates.[6-16] 1-Phenylcyclopropylamine (1-PCPA, **1**) was studied in more detail than usual,[14,16] and it was concluded that this benzylamine analogue inactivates MAO-B by the mechanism shown in Scheme I (pathway a). Initial electron transfer from the 1-PCPA amine nitrogen lone pair to the flavin forms the flavin semiquinone radical anion and the amine radical cation (**2**). This highly unstable cyclopropylaminyl radical then ring opens to the primary radical intermediate **3**, which partitions in a ratio of 1 to 7 between attachment to the active-site flavin radical and to an active-site cysteine radical, presumably generated by hydrogen atom transfer from an active site cysteine residue to the flavin semiquinone. Both adducts result in loss of enzyme activity. The flavin adduct, however, is stable to dialysis and denaturation, whereas the cysteine adduct spontaneously decomposes to yield active enzyme and acrylophenone (**4**). The active enzyme that is regenerated is free to repeat the above reaction cycle and partition again between the two possible adducts until, eventually, all of the inactive enzyme is in the form of the

† Department of Chemistry.

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(1) Kaiser, C.; Setler, P. E. In *Burger's Medicinal Chemistry*, 4th ed.; Wolffe, M. E., Ed.; Wiley: New York, 1981: Part III, pp 1000–1001.

(2) Berger, P. A.; Barchas, J. D. In *Psychotherapeutic Drugs*; Usdin, E., Forrest, I. S. Eds.; Marcel Dekker: New York, 1977; Part II.

(3) Tetrud, J. W.; Langston, J. W. *Science (Washington, D.C.)* **1989**, *245*, 519–522.

(4) (a) Silverman, R. B. In *Advanced in Electron Transfer Chemistry*; Mariano, P. S., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2; pp 177–213. (b) Silverman, R. B. *J. Am. Chem. Soc.* **1990**, *112*, 9364–9372 and references therein.

(5) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL; 1988; Vols. I and II.

(6) Silverman, R. B.; Hoffman, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 884–886.

(7) Silverman, R. B.; Hoffman, S. J.; Catus, W. B., III *J. Am. Chem. Soc.* **1980**, *102*, 7126–7128.

(8) Silverman, R. B.; Hoffman, S. J. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 1396–1401.

(9) Silverman, R. B. *J. Biol. Chem.* **1983**, *258*, 14766–14769.

(10) Silverman, R. B.; Yamasaki, R. B. *Biochemistry* **1984**, *23*, 1322–1332.

(11) Silverman, R. B. *Biochemistry* **1984**, *23*, 5206–5213.

(12) Silverman, R. B.; Zieske, P. A. *J. Med. Chem.* **1985**, *28*, 1953–1957.

(13) Vazquez, M. L.; Silverman, R. B. *Biochemistry* **1985**, *24*, 6538–6543.

(14) Silverman, R. B.; Zieske, P. A. *Biochemistry* **1985**, *24*, 2128–2138.

(15) Yamasaki, R. B.; Silverman, R. B. *Biochemistry* **1985**, *24*, 6543–6550.

(16) Silverman, R. B.; Zieske, P. A. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 154–159.

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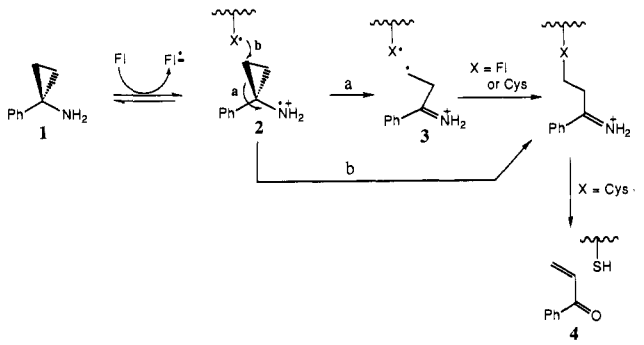
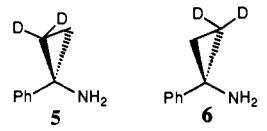
Scheme I



stable flavin adduct.[14] An alternative mechanism to these products is a radical substitution mechanism (pathway b; S_H2 mechanism) on the activated cyclopropyl ring, which leads to the same products. Cyclopropylaminyl ring cleavage is exceedingly rapid, occurring at a rate so fast that it could not be measured.[17] This rapid ring cleavage rate is support for pathway a shown in Scheme I.

Nonenzymatically, it would be expected that because of the symmetry of 1-PCPA, there would be equal probability for cleavage of the two cyclopropyl ring bonds, regardless of which mechanistic pathway in Scheme I would be followed. However, enzymes are chiral molecules, and therefore, cyclopropyl ring cleavage may be influenced by this enzyme asymmetry. In this paper we describe our investigation of the stereochemistry and mechanism of ring cleavage of 1-PCPA catalyzed by MAO with the use of the chirally dideuterated 1-PCPA analogues **5** ((*R*)-1-PCPA) and **6** ((*S*)-1-PCPA). By analysis of the acrylophenone metabolites generated by spontaneous decomposition of the enzyme cysteine adduct it is shown that, indeed, this ring cleavage is stereoselective.



Results

Syntheses of (*R*)- and (*S*)-1-PCPA (5 and 6). The syntheses of (*R*)- and (*S*)-1-phenylcyclopropylamine were carried out by the procedures of Hill et al.[18] The free amines were converted to the hydrochloride salts, **5** and **6**, by addition of HCl gas to ethereal solutions of the amines. The analytical characteristics, including the specific rotations, for both enantiomers of all intermediates prepared agreed with the literature values.[18] Although there is no reason to believe that the optical purity of **5** and **6** should be altered relative to that of the free amines, a test for their optical purity was desired. It is not practical to determine the chirality of **5** and **6** by polarimetry, but it is possible to determine the relative optical purities of deuterated compounds by Fourier transform vibrational circular dichroism, which Professor Timothy A. Keiderling (University of Illinois at Chicago) was kind enough to carry out on these compounds. Although it is not possible to determine if the two compounds are pure enantiomers by this technique, it can be concluded that the compounds have rather substantial enantiomeric excesses (from the magnitudes of the signals in terms of $\Delta A/A$) and, within experimental error (5–10%), the two compounds are of equal purities. It is highly likely, then, that they are pure (or nearly pure) enantiomers.

(17) Maeda, Y.; Ingold, K. U. *J. Am. Chem. Soc.* **1980**, *102*, 328–331.
(18) (a) Hill, R. K.; Prakash, S. R.; Wiesendanger, R.; Angst, W.; Martinoni, B.; Arigoni, D.; Liu, H.-W.; Walsh, C. T. *J. Am. Chem. Soc.* **1984**, *106*, 795–796. (b) We are grateful to Prof. Hill for providing complete experimental procedures and analyses of the compounds.

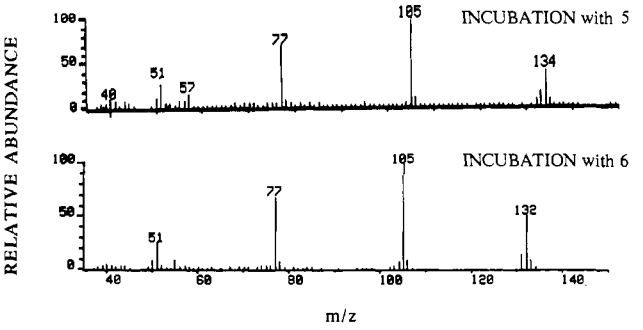
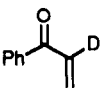
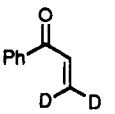


Figure 1. Determination of the relative amounts of acrylophenone, [α-^{2}H]-acrylophenone, and [β,β-$^2H_2$]acrylophenone produced on inactivation of MAO by **5** and **6**. Inactivations were carried out for 6 h in 50 mM pH 7.2 sodium phosphate buffer. The extracts of 5- and 6-inactivated MAO solutions were analyzed by GC/MS.

Table I. Relative Amounts of Metabolites Isolated from Inactivation of MAO by 1, 5, and 6 in pH 7.2 NaPi Buffer

	% abundance[a]		
			
inactivator	*m/z* 132	*m/z* 133	*m/z* 134
1	100	0	0
5	15	19	66
6	90	6	4
6[b]	94	4	2

[a] Average of two or three experiments. [b] In the absence of added catalase.

Analysis of the Acrylophenones Generated by Decomposition of the Cysteine Adducts Formed by Inactivation of MAO by 1-PCPA, (*R*)-1-PCPA, and (*S*)-1-PCPA. Following a 6-h incubation of MAO-B with 1-PCPA (**1**), (*R*)-1-PCPA (**5**), and (*S*)-1-PCPA (**6**) and extraction of the buffers with an organic solvent, the acrylophenone produced was analyzed by GC–mass spectrometry (Figure 1). The results are summarized in Table I. Inactivation by unlabeled 1-PCPA gave only unlabeled acrylophenone. Inactivation by (*R*)-1-PCPA gave a mixture of mostly [β,β-$^2H_2$]acrylophenone but also some [α-^{2}H]acrylophenone and unlabeled acrylophenone. Inactivation by the *S*-isomer, however, led to the formation of almost all unlabeled acrylophenone with minor amounts of [β,β-$^2H_2$]acrylophenone and [α-^{2}H]acrylophenone. Two control experiments, one containing MAO but no inactivator and one containing the *R*-inactivator **5** but no enzyme, both showed no acrylophenone products in the GCMS of the extracts.

Analysis of the Acrylophenones Generated by Decomposition of the Cysteine Adduct Formed by Inactivation of MAO by 1-PCPA in Deuterium Oxide. To determine whether the loss of deuterium from **6** was the result of exchange of deuterium during catalytic turnover, inactivation of MAO by 1-PCPA was carried out in deuterium oxide. As shown in Table II, incubation of MAO with 1-PCPA in buffered D_2O led to the formation of approximately a 2:1 mixture of undeuterated to monodeuterated acrylophenones. Under no conditions could complete exchange of the α-proton be observed. When the experiment was repeated except with prior incubation of the enzyme with [α,α-$^2H_2$]benzylamine so that deuterons would be generated *inside* of the active site of MAO, then a 1:1 mixture of undeuterated to monodeuterated acrylophenones was produced upon incubation of the enzyme with 1-PCPA (Table II). When **6** was used to inactivate the enzyme under these latter conditions, the amount of [α-^{2}H]-acrylophenone increased dramatically with a concomitant decrease

Table II. Relative Amounts of Metabolites Isolated from Inactivation of MAO by 1 in pD 7.2 NaPi Buffer in Deuterium Oxide and by 1 and 6 in pD 7.2 NaPi Buffer in Deuterium Oxide following Incubation of the MAO with [α,α-$^2H_2$]Benzylamine Hydrochloride

	% abundance[a]		
inactivator	*m/z* 132	*m/z* 133	*m/z* 134
1	63	34	2
1[b]	51	49	0

[a] Average of two or three experiments. [b] MAO preincubated with [α,α-$^2H_2$]benzylamine prior to treatment with the inactivator.

Table III. Reactivation Rates of MAO Inactivated by 1, 5, and 6 and the Corresponding Dueterium Isotope Effects

inactivator	$T_{1/2}$ (min)	isotope effect
1	42	
5	57	1.4
6	130	3.1

in the formation of unlabeled acrylophenone. There was no change in the amount of [α,α-$^2H_2$]acrylophenone produced.

Stability of [α-^{2}H]Acrylophenone under the Conditions of the Incubation Experiment. In order to determine if the unlabeled 1-PCPA produced from **5** and **6** was coming from washout of the deuterium from [α-^{2}H]acrylophenone, synthesized [α^2H]-acrylophenone was incubated with MAO at concentrations on the same order of magnitude as those theoretically present during the inactivation experiments. This experiment showed that no deuterium loss occurred after 6 h (the time of the inactivation experiments), although measurable loss did occur at longer time periods. Incubation with catalase alone over 6 h resulted in a 15% loss of deuterium from the [α^2-H]acrylophenone and incubation of [α-^{2}H]acrylophenone with MAO *and* catalase resulted in a 40% loss of deuterium. However, inactivation by **6** without added catalase present gave approximately the same result as the inactivation with catalase present, i.e., nearly exclusive formation of the unlabeled metabolite (see Table I). Therefore, it appears that the unlabeled acrylophenone is not derived from washout of the deuterium in the [α-^{2}H]acrylophenone.

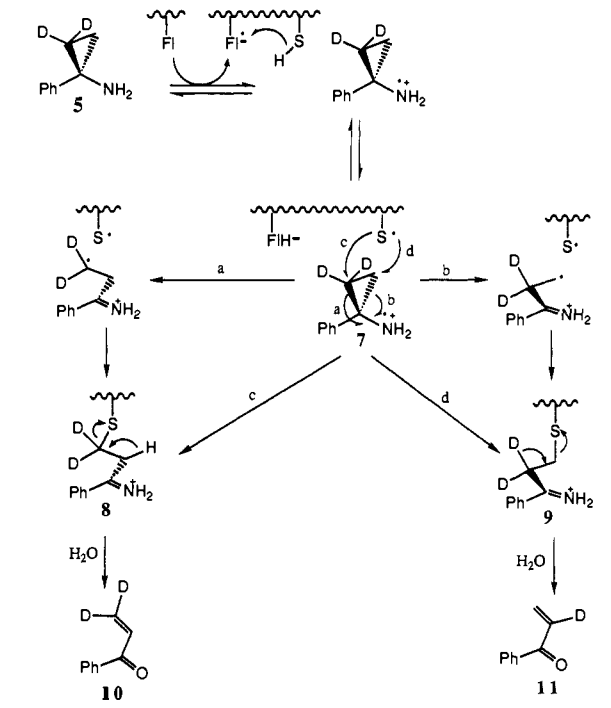
Deuterium Isotope Effect on Formation of the Acrylophenones from the Cysteine Enzyme Adducts. Following inactivation of MAO by **1**, **5**, and **6** and isolation of the inactivated enzyme by gel filtration, the rates for the return of enzyme activity (and, therefore, the formation of acrylophenone) in each case were determined. The results, summarized in Table III, indicate that the *R*-isomer exhibited a small deuterium isotope effect and the *S*-isomer showed a substantial isotope effect on the reactivation of the enzyme.

Discussion

Enzymes are well-known stereospecific catalysts for a variety of proton and group transfer reactions as well as for redox reactions. A number of years ago it was beautifully demonstrated[18,19] that the substrate for the pyridoxal 5′-phosphate-dependent enzyme 1-aminocyclopropanecarboxylic acid deaminase, namely ACPC, underwent a stereospecific cyclopropane ring cleavage in its conversion to the product, α-ketobutyric acid. The mechanism for this reaction is unclear, but it has been suggested[19] that the cyclopropane bond is cleaved open by stereospecific attack of an active site nucleophile on the pro-*S* cyclopropyl methylene of the Schiff base between ACPC and

(19) Liu, H.-W.; Auchus, R.; Walsh, C. T. *J. Am. Chem. Soc.* **1984**, *106*, 5335–5348.

Scheme II



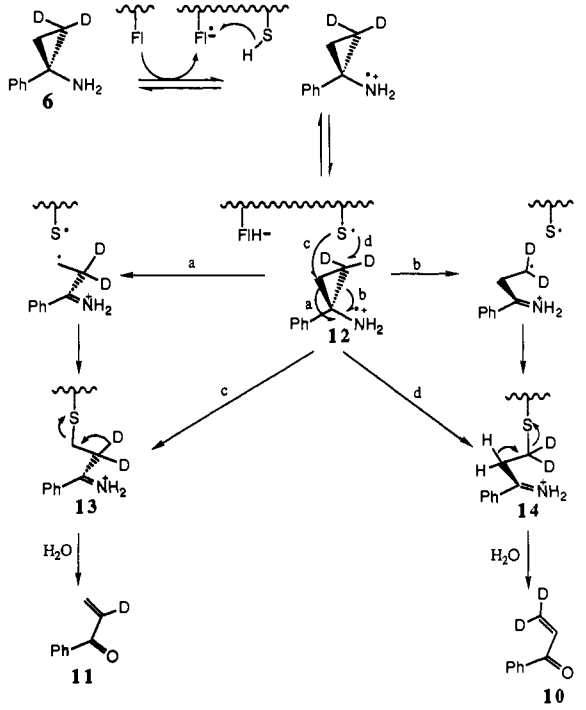
PLP. Nucleophilic reactions on activated cyclopropanes can be efficient reactions under certain circumstances, but generally they are not exceedingly rapid.[20] We wondered whether it would be possible for an enzyme to influence the stereospecificity of an exceedingly rapid cyclopropyl ring cleavage reaction and, therefore, investigated the stereochemistry of the MAO-catalyzed cleavage of 1-phenylcyclopropylamine (1-PCPA). Stereospecificity could result from the influence of the enzyme's asymmetric environment on spontaneous cyclopropylaminyl ring cleavage (Scheme I, pathway a) or by the fact that the active-site cysteine residue[16] may be closer to one methylene of the cyclopropane ring than to the other (Scheme I, pathway b). It also was of interest to obtain support for one of the two mechanistic pathways shown in Scheme I for the reaction of MAO with 1-PCPA, namely cyclopropyl ring cleavage followed by radical combination (pathway a) or a direct S_H2 mechanism (pathway b).

The approach taken to answer these questions was to use chiral dideuteration of the cyclopropyl ring as a marker for which bond breaks during the reaction. Scheme II depicts the possible stereochemical outcomes for (*R*)-1-PCPA (**5**) by both mechanisms. Following electron transfer and hydrogen atom transfer from the active-site cysteine residue, intermediate **7** would be formed which can react by several different pathways. Pathway a is the initial cleavage of the front cyclopropyl bond followed by radical combination to give adduct **8**, whereas pathway b is the same reaction except with the cleavage of the back cyclopropyl bond, which leads to **9**. Pathway c is the S_H2 reaction of the active-site cysteine radical at the front cyclopropyl carbon atom to give **8** directly and pathway d is the same reaction at the back cyclopropyl carbon atom leading to **9**. Elimination of the α-proton and the cysteine residue of **8** produces, after hydrolysis, β,β-dideuterioacrylophenone (**10**) (product of front bond cleavage). The same reaction on **9** gives α-monodeuteroacrylophenone (**11**) (product of back bond cleavage).

The same analysis for the S-isomer (**6**, **Scheme** III) indicates that when the front bond is broken, α-monodeuterioacrylophenone (**11**) is the final product, whereas when the back bond is broken, β,β-dideuterioacrylophenone (**10**) results. Therefore, when the same bond is broken in the two cyclopropyl enantiomers, opposite products (**10** or **11**) are formed. In the final analysis, then, if the

(20) Danishefsky, S. *Acc. Chem. Res.* **1979**, *12*, 66–72.

Scheme III



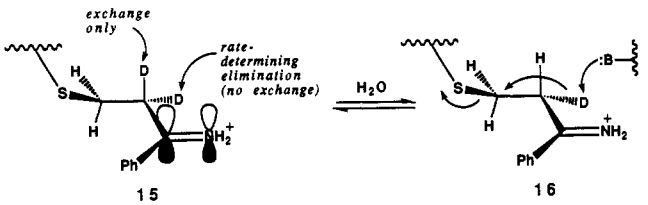
enzyme reaction is not stereoselective at all, a 1:1 mixture of these two acrylophenones would be produced and they could be quantified by GC–mass spectrometry. Stereospecificity would result in the formation of only one of the two possible acrylophenones (**10** or **11**), and stereoselectivity would give something in between these two extremes. On the assumption that cyclopropyl ring cleavage during this enzyme-catalyzed oxidation reaction is not reversible, then once the cysteine adduct is formed (**8**, **9**, **13**, or **14**), it is committed to give the corresponding acrylophenone, even though elimination of one adduct may experience a deuterium isotope effect and the other does not. The product distribution, therefore, is only a function of the commitment factor leading to **8**, **9**, **13**, or **14**.

With these possible outcomes in mind, the inactivation of MAO-B by **1**, **5**, and **6** was carried out and the acrylophenone metabolites were isolated and quantified. The results are summarized in Table I. Several of these results are inconsistent with the above simple analysis of the problem; consequently, the enantiomeric purity of the inactivators was confirmed. Fourier transform vibrational circular dichroism measurements were made on the two dideuterated enantiomeric amines (by Professor Timothy Keiderling at the University of Illinois, Chicago), and the chiral integrity of **5** and **6** was confirmed. That being the case, there are several results that need to be rationalized.

First, an explanation is needed for why there is washout of most of the deuterium when the *S*-isomer is used. A second unexpected result is that in the case of the *S*-isomer, 96% of one bond is broken, but in the case of the *R*-isomer only about 66% of that same bond is broken. Thirdly, whereas 90% of the deuterium is washed out in the product from the *S*-isomer, only 15% of the deuterium is lost when the *R*-isomer is used. Finally, if the unlabeled acrylophenone comes from the same intermediate as does the α-monodeuteroacrylophenone (vide infra), then it appears that there is a preference for cleavage of the same bond with both enantiomers (mostly β,β-dideuteroacrylophenone is produced from the *R*-isomer and mostly unlabeled acrylophenone is produced from the *S*-isomer). What is the origin of this bond cleavage preference?

The most obvious explanation for the washout of deuterium preferentially with the *S*-isomer is that following front bond cleavage the intermediate (**13**, Scheme III), having two deuteriums

Scheme IV



α to the iminium ion, would be set up for proton exchange. When the reaction of 1-PCPA with MAO was carried out in D_2O, however, the maximum amount of exchange observed was only about one-third (Table II). Possibly, as a result of the hydrophobic nature of the active site of MAO, little deuterium exchange of the protons in the active site occurs, even after the enzyme is incubated with D_2O for extended periods of time. Inaccessibility of active sites to solvent protons is not uncommon in enzymology.[21] One way to be certain that deuterons were entering the active site, however, would be with the use of [α,α-$^2H_2$]benzylamine as a deuteron delivery system for the MAO active site. Since benzylamine is an excellent substrate for MAO, prior incubation of MAO with [α,α-$^2H_2$]benzylamine would have to generate deuterons inside the active site. However, extended incubation of MAO with [α,α-$^2H_2$]benzylamine in D_2O followed by treatment with **1** gave a maximum of only 49% of α-monodeuterated acrylophenone (Table II). Although this was a better approach than incubation of the enzyme in D_2O, it still was not totally effective for complete proton exchange. Therefore, this simple exchange mechanism does not account for the observed loss of 90% of the deuterium from **6**.

Additional evidence against a simple exchange mechanism was procured. If, in fact, loss of deuterium from intermediate **13** is derived from a rapid exchange of the deuteriums α to the iminium ion, followed by a rate-determining elimination of the cysteine residue to give acrylophenone, then there should be no deuterium isotope effect on the elimination step because all of the deuterium would have been washed out prior to elimination. We have previously demonstrated that the cysteine adduct can be isolated and the return of enzyme activity can be followed with time.[14] When this experiment was carried out with the *S*-isomer, it was found, however, that there is a kinetic isotope effect on reactivation of the enzyme and, consequently, on the formation of acrylophenone (Table III). Therefore, this isotope effect on reactivation excludes initial deuterium washout followed by rate-determining elimination. Another explanation for these results is that initial rate-limiting elimination to α-monodeuterioacrylophenone occurs, but under the conditions of the experiment, the deuterium exchanges out of the α-monodeuterioacrylophenone in a rapid second step. This mechanism also was tested experimentally. If this is the case, then the deuterium of α-monodeuterioacrylophenone should be exchangeable under the conditions of the experiment. Incubation of MAO with α-monodeuterioacrylophenone under the conditions of the above experiment led to little or no loss of deuterium. This experiment suggests that loss of deuterium is not the result of initial rate-determining elimination followed by deuterium exchange from the product. If loss of both deuteriums is not occurring by washout followed by elimination or by elimination followed by washout, then what is the explanation? One possibility is depicted in Scheme IV (formed as shown in Scheme III). If the cysteine adduct is relatively rigid (**15**), then only one of the two C–D bonds is parallel with the iminium p-orbitals and stereospecific washout of that one deuterium to give **16** would result. The other C–D bond is orthogonal to the iminium p-orbitals, and therefore, it would not be susceptible to exchange. However, this C–D bond is trans periplanar to the leaving group (the cysteine residue) and is set

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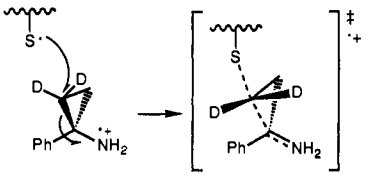
up for an E2 elimination, which would be a rate-determining step, thereby accounting for the deuterium isotope effect that is observed. Stereoselective exchange of one methylene proton is well-known in organic chemistry when a rigid cyclic system is involved. One example is the stereoselective exchange of one of the methylene protons adjacent to the carbonyl of twistan-4-one.[22] In this case exchange of the proton parallel to the carbonyl p-orbitals occurred about 300 times more rapidly than the other methylene proton which is in an orbital lying 60° out of the plane. This would be sufficient in our case to account for the nearly complete loss of the one deuteron and yet retain the second deuteron for the elimination reaction. Support for rate-limiting E2 elimination comes from the work of Ferran et al.[23] who have shown that for a *trans*-decalin iminium ion there is an isotope effect (6–7 depending upon the base) on elimination of the α-proton that is trans periplanar to the leaving group.

Regardless of which reaction mechanism is important to the formation of the cysteine adduct, it would be thought that the enantiomers should give opposite, but equivalent results. Formation of β,β-dideuterioacrylophenone from the *R*-isomer indicates that the front bond of 1-PCPA was broken (Scheme II) and formation of unlabeled acrylophenone (if it is derived from α-monodeuterioacrylophenone) from the *S*-isomer also indicates that the front bond was broken (Scheme III). Although both enantiomers have a preference for cleavage of the front bond (Table I), the *S*-isomer leads mostly to cleavage of the front bond (96:4 in favor of front bond cleavage, assuming that unlabeled and α-monodeuterioacrylophenone come from front bond cleavage), but the *R*-isomer shows only a 66:34 preference for front bond cleavage. This phenomenon of nonequivalent bond cleavage could be the result of a secondary isotope effect on the cyclopropane ring cleavage. Montgomery and Matt[24] previously reported the stereoselective cleavage of a dideuteriocyclopropylcarbinyl radical which favored cleavage of the C–C bond that did not have deuterium attached to either carbon atom. It was estimated that there would be a normal secondary kinetic isotope effect of 15% per deuterium (30% for the two deuteriums). If this secondary isotope analysis is applied to the case of 1-PCPA, then our results can be rationalized. Cleavage of the front bond of the *S*-isomer, the bond that does not have deuterium attached to either carbon atom, should be favored both from the standpoint of the enzyme asymmetry as well as on the basis of the secondary isotope effect. Therefore, these effects are additive and there is a very high preference for front bond cleavage. The front bond of the *R*-isomer, however, has both of the deuteriums attached, and therefore, this cleavage would exhibit a secondary isotope effect opposing cleavage, thereby shifting some of the cleavage to the back bond where there are no deuteriums. In this case, then, the effect of the asymmetry of the enzyme and the secondary isotope effect are competitive. This could account for the increase in the amount of unlabeled and α-monodeuterioacrylophenone from that expected for the *R*-isomer. On the basis of the results in Table I the front:back bond cleavage ratio is 24:1 for the *S*-isomer and 2:1 for the *R*-isomer. If it is assumed that there is a 30% secondary deuterium isotope effect against the cleavage of the front bond for the *R*-isomer and in favor of cleavage of the front bond for the *S*-isomer, then the real front:back ratios are 24/1.3 or 18.5 for the *S*-isomer and 2(1.3) or 2.6 for the *R*-isomer. Theoretically, these numbers, which represent the inherent asymmetry of the enzyme for preference toward front bond cleavage, should be the same for the two isomers once the secondary isotope effect is factored out. Even if the higher of the two values is used, however, it amounts to less than 2 kcal/mol of energy, which is not a large amount of energy difference considering all of the interactions involved in enzyme binding and catalysis. Possibly there is a small steric effect of the deuteriums that contributes to the difference in the preference with the two isomers.

Scheme V



The hypothesis that two different amounts of cyclopropyl bond cleavage occurs for the two enantiomers also is consistent with the observed small primary deuterium isotope effect (1.4; see Table III) observed during decomposition of the cysteine adduct from the *R*-isomer. This may correspond to the fraction of the *R*-isomer molecules that proceed by back bond cleavage.

The third question about the results in Table I has to do with why *front* bond cleavage of the *S*-isomer gives almost all unlabeled acrylophenone with only a little of α-monodeuterioacrylophenone, but *back* bond cleavage of the *R*-isomer (which gives the same product as front bond cleavage of the *S*-isomer) gives about half unlabeled acrylophenone and half α-monodeuterioacrylophenone. It may be thought that since these appear to be producing the same intermediate, the same product mixture should be obtained. However, although intermediate **9** (Scheme II) and intermediate **13** (Scheme III) are enantiomers, because they are inside a chiral cavity, they are part of diastereomeric complexes. As diastereomers, they have different energies and different reactivities. Therefore, whereas **13** may undergo essentially complete washout of the deuterium that is in the C–D bond parallel to the iminium p-orbitals, **9** may not exchange as fast so that β-elimination may occur while there is still some dideuterated intermediate remaining, which leads to the formation of α-monodeuterioacrylophenone. In fact, different active-site bases may be involved in the deprotonation during the elimination step of the two adducts which could easily account for the different amounts of washout.

The secondary deuterium isotope effect argument (vide supra) also can be invoked to support a stepwise mechanism for cysteine adduct formation (pathways a and b, Schemes II and III) over the S_H2 mechanism (pathways c and d). Opening of a cyclopropane ring is in the direction of giving an intermediate having less p character (a cyclopropane ring has more p character than does an alkyl chain). This results in a less crowded transition state and, therefore, exhibits a normal secondary isotope effect (as was reported by Montgomery and Matt[24] for the cleavage of the dideuteriocyclopropylcarbinyl radical). An S_H2 mechanism, however, goes through a more crowded transition state having more p-character (five coordinate carbon), which should exhibit an *inverse* secondary isotope effect (Scheme V). If that is the case, then S_H2 attack at the front bond of the *R*-isomer (Scheme V) should be a favored process and that would lead to essentially exclusive formation of dideuterioacrylophenone, whereas, S_H2 attack of the cysteine radical at the front carbon of the *S*-isomer would have an inverse secondary isotope effect that would encourage more attack at the back carbon. In other words, *the S_H2 mechanism would give the opposite results from what are observed, and therefore is not relevant.* Therefore, it is our conclusion that the stepwise mechanism, cyclopropyl cleavage followed by radical combination, is favored over the S_H2 mechanism; the stepwise mechanism *is* consistent with our results. Given the fact that cyclopropylaminyl ring cleavage is an exceedingly rapid reaction,[17] it is reasonable that this should be a favorable process.

If the stereoselective preference for cyclopropyl bond cleavage is not arising from regioselective attack by the cysteine at one of the cyclopropane methylenes, then what is an explanation for the

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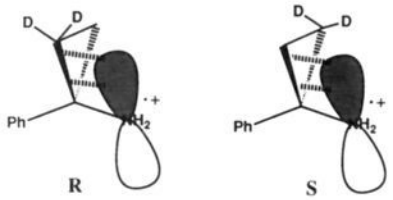

Figure 2. Preferential alignment of one cyclopropyl bond with the p-orbital of the 1-phenylcyclopropylaminium ion as a proposed driving force for the observed stereoselective ring opening.

bond cleavage preference? One possibility is that the asymmetry of the enzyme active site may force the amine radical orbital to have a preference for overlap with the front cyclopropane bond (Figure 2), which would result in a preference for bond cleavage of the front bond. It is interesting, and possibly even relevant to this observation, that MAO stereospecifically removes the pro-*R* proton of various substrates;[25] the pro-*R* proton of substrates corresponds to the front bond of 1-PCPA. A preference for stereoselective cyclopropyl bond cleavage also has been observed in nonenzymatic systems. For example, Dauben et al.[26] have found that in the case of a constrained cyclopropylcarbinyl radical, cyclopropyl ring cleavage to give the less stable radical is preferred, if the orbital containing the cyclopropylcarbinyl radical is aligned for overlap with the cyclopropane bond that leads to the formation of the less stable radical intermediate.

Conclusions

The asymmetry of the active site of MAO appears to be an important contributor to the preference for cleavage of one of the cyclopropane bonds of 1-phenylcyclopropylamine during its oxidation and subsequent attachment to an active-site cysteine residue. The evidence points to a mechanism (Scheme I, pathway a) which involves initial one-electron transfer from the amine to the flavin, hydrogen atom transfer from a cysteine residue to the flavin radical anion to give a cysteine radical, cyclopropyl ring cleavage of the 1-phenylcyclopropylaminyl radical that was generated by initial electron transfer, and combination of the resultant β-propiophenone radical with the cysteine radical to give the β-cysteinylpropiophenone adduct. When the 1-PCPA is chirally dideuterated, it is found that there is a preference for cleavage of the bond that produces **8** (Scheme II) from the *R*-isomer and **13** (Scheme III) from the *S*-isomer.

Experimental Section

Analytical Methods. Optical spectra and MAO assays were recorded on either a Perkin-Elmer Lambda 1 or Beckman DU-40 UV/vis spectrophotometer. NMR spectra were recorded on either a Varian XL-400 400-MHz spectrometer, a Varian Gemini 300 MHz spectrometer, or a Varian EM-390 90-MHz spectrometer. Chemical shift values are reported as parts per million downfield from Me_4Si as the internal standard in $CDCl_3$ and relative to DSS when D_2O was the solvent. An Orion Research Model 601 pH meter with a general combination electrode was used for pH measurements. Optical rotations were measured in 1-dm cells of 1-mL capacity using an Optical Activity AA-100 polarimeter. Analytical thin-layer chromatography was conducted on E. Merck 2.5- × 10-cm precoated plates. E. Merck silica get (230–400 mesh) ASTM was used for flash chromatography. Mass spectra were obtained on a VG Instruments VG70-250SE high-resolution mass spectrometer; the gas chromatography mass spectra (GCMS) were obtained using this instrument coupled to a Hewlett-Packard 5890 chromatograph. A Durabond DB1 15-m column was used. Typically the GC temperature was programmed from 80 to 250 °C at 15 °C/min. Elemental combustion analyses were performed by G. D. Searle & Co. (Skokie, IL).

Reagents. All reagents were purchased from Aldrich and were used without further purification except for the following: Deuterium oxide (99.9 atom % D) was purchased either from Aldrich or Cambridge Isotope Laboratories. Benzene, acetone, dichloromethane, anhydrous magnesium sulfate, hydrochloric acid, potassium hydroxide, sodium hydroxide, potassium persulfate, sodium phosphate, sodium bicarbonate, sodium chloride, and *p*-toluenesulfonic acid monohydrate were obtained from Mallinckrodt. Potassium ruthenate was purchased from Pfaltz and Bauer. Diethyl ether for reactions was distilled from sodium with benzophenone ketyl indicator under nitrogen immediately prior to use. Benzene and methylene chloride were distilled from phosphorus pentoxide under nitrogen. Triethylamine was distilled from calcium hydride. Absolute ethanol was purchased from Midwest Grain Co. Distilled water was deionized and redistilled from glass. Acrylophenone,[27] ethyl 3-hydroxy-3-phenylpropanoate,[28] 1-phenylcyclopropylamine hydrochloride[14] (**1**), and [α,α-$^2H_2$]benzylamine[29] were prepared according to literature procedures.

(*R*)-(−)-[2,2-$^2H_2$]-1-Phenylcyclopropylamine Hydrochloride (5) and (*S*)-(+)-[2,2-$^2H_2$]-1-Phenylcyclopropylamine Hydrochloride (6). Upon bubbling of HCl into ether solutions of the free amines, the hydrochloride salts precipitated as white powders. Recrystallization from chloroform/hexane gave **5** and **6** as white crystals: mp 205–206 °C (for both **5** and **6**); ^1H NMR ($CDCl_3$) δ 1.15 (d, J = 6 Hz, 1 H), 1.52 (d, J = 6 Hz, 1 H), 7.26–7.48 (m, 5 H), 8.88 (s, 3 H); Fourier transform vibrational circular dichroism spectra indicated that both enantiomers were of identical enatiomeric purity within experimental error (5–10%); high-resolution mass spectrum (EI) M − 1 calcd for $C_9H_8D_2N$ 134.0939, found 134.0943 (**5**) and 134.0948 (**6**).

[2,2-$^2H_2$]-3-(Dimethylamino)-1-phenyl-1-propanone Hydrochloride. β-(Dimethylamino)propiophenone hydrochloride (5.4 g, 25 mmol) was dissolved in 14 mL of dioxane. Deuterium oxide (4 mL) and anhydrous sodium carbonate (5.7 g) were added, and the flask was fitted with a reflux condenser and drying tube. The mixture was refluxed with stirring for 20 h, the organic layer was separated and concentrated to give 4 g of an oil which was stirred with 10 mL of 20% DCl in D_2O for 1 h, and then the solvent was evaporated. Recrystallization of the residue from ethanol/acetone afforded 4 g (74%) of the product as white crystals: mp 163–164 °C; ^1H NMR ($CDCl_3$/DMSO) δ 2.84 (s, 6 H), 3.34 (s, 2 H), 7.48–7.74 (m, 3 H), 8.03 (d, 2 H).

[α-^2H]Acrylophenone. The procedure of Jaeniche and Preum[27] for the preparation of the unlabeled compound was used, starting with [2,2-$^2H_2$]-3-(dimethylamino)-1-phenyl-1-propanone hydrochloride. A yellow liquid was obtained: 90-MHz ^1H NMR ($CDCl_3$) δ 2.90 (s, 6 H), 3.50 (s, 2 H), 7.50 (m, 3 H), 8.00 (m, 2 H); high-resolution mass spectrum (EI) M^+ calcd for C_9H_7OD 133.0638, found 133.0632.

[3,3-$^2H_2$]-1-Phenyl-1,3-propanediol. Ethyl 3-hydroxy-3-phenylpropanoate (3.88 g, 20 mmol) in ether (20 mL) was cooled in an ice bath, and powdered lithium aluminum deuteride (1.5 g, 36 mmol) was added in portions with stirring under nitrogen. Stirring was continued for 2 h at room temperature, and then the reaction mixture was allowed to reflux for 0.5 h. The mixture was allowed to cool, and the excess lithium aluminum deuteride was quenched by the addition of ethyl acetate (3 mL) followed by water (2 mL). After the addition of 6 N HCl (30 mL) the organic layer was separated and the aqueous layer was extracted with ether (2 × 15 mL). The combined ether layers were washed with brine, dried over magnesium sulfate, and concentrated by rotary evaporation. The residue was purified on silica gel (ethyl acetate/hexane, 3:2) to give 1.9 g (60% yield) of product: 90-MHz ^{1} H NMR ($CDCl_3$) δ 1.72–1.98 (m, 2 H), 4.83 (t, 1 H), 7.30 (s, 5 H).

[3,3-$^2H_2$]-3-Hydroxy-1-phenyl-1-propanone. To a mixture of [3,3-$^2H_2$]-1-phenyl-1,3-propanediol (1.27 g, 8.2 mmol) and the phase-transfer reagent Adogen 464 (227.4 mg) in dichloromethane (60 mL) was added a solution of potassium ruthenate (43 mg) and potassium peroxodisulfate (8.64 g, 24 mmol) in 10% aqueous KOH (15 mL). The reaction mixture was stirred vigorously at room temperature for 4 h, the layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 25 mL). The combined organic layers were washed with water (2 × 40 mL), dried over magnesium sulfate, and concentrated by rotary evaporation. The residue was purified on silica gel (ethyl acetate/hexane, 1:1) to give 800 mg (64% yield) of the product as a pale yellow oil: bp

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95–100 °C; 90-MHz ^{1}H NMR $CDCl_3$ δ 3.19 (s, 2 H), 7.25–7.69 (m, 3 H), 7.79–8.11 (m, 2 H).

[β,β-$^2H_2$]Acrylophenone. A solution of [3,3-$^2H_2$]-3-hydroxy-1-phenyl-1-propanone (750 mg, 4.93 mmol) and *p*-toluenesulfonic acid monohydrate (150 mg) in benzene (190 mL) was heated under reflux for 4 h in a flask fitted with a Dean–Stark trap. The solution was then washed with 5% sodium bicarbonate solution (2 × 100 mL) and water (2 × 100 mL), dried over magnesium sulfate, and concentrated by rotary evaporation to give a brown oil. Kugelrohr distillation afforded 38 mg (6%) of a colorless oil: bp 95–100 °C (6 mmHg); ^{1}H NMR ($CDCl_3$) was identical to that reported in an alternative synthesis of the compound;[30] high-resolution mass spectrum (EI) M^+ calcd for $C_9H_6D_2O$ 134.0701, found 134.0700.

Enzymes and Assays. Bovine liver MAO-B was isolated according to the published method.[31] MAO activity was assayed by the method of Tabor et al.[32] Catalase (purified powder from bovine liver, type EC 1.11.1.6) was purchased from Sigma. All buffers and enzyme solutions were prepared using doubly distilled deionized water.

Inactivation of MAO-B by 5 and 6. A mixture containing 5.3 μM MAO, 2.2 mM inactivator, and 1 mg/mL of catalase in 50 mM sodium phosphate buffer pH 7.2 (4 mL total volume) was incubated in the dark for 6 h at 25 °C. Remaining enzyme activity was typically less than 10% that of a control containing no inactivator. Another control that was run contained inactivator but no enzyme.

Isolation of Acrylophenone Metabolites Formed during MAO-B Inactivation by 5,6, and Controls. Each of the inactivation mixtures and controls described above was saturated with sodium chloride and extracted with dichloromethane (3 × 10 mL). The combined extracts were washed with brine (15 mL), 0.5 M HCl (2 × 15 mL), saturated sodium bicarbonate solution (15 mL), and then brine again (2 × 15 mL). After being dried over magnesium sulfate, the extracts were concentrated by rotary evaporation at 25 °C to ca. 1 mL. The sample was concentrated further by passing a stream of argon over it to give a final volume of 2–10 μL immediately prior to GCMS analysis. A Durabond DB1 15 m column was used in the GC. Typically the GC temperature was programmed from 80 to 250 °C at 15 °C/min. Acrylophenone, [α-^{2}H]acrylophenone, and [β,β-$^2H_2$]acrylophenone eluted from the column as one peak in the GC trace, with the slightly faster-running deuterated analogues toward the front of the peak and the unlabeled acrylophenone toward the back. To determine the relative amounts of each metabolite, the entire peak area in the GC trace was scanned at the single mass ions corresponding to the molecular ion peaks, and the resultant peak areas were integrated. Finally, the contribution from the M + 1 and M – 1 peaks of the neighboring ions were subtracted out, based on the M + 1 and M – 1 peaks observed in the GCMS of standard acrylophenone.[33,34]

Isolation of Acrylophenone Metabolites Formed during Inactivation of MAO by 1 in D_2O. Deuterated sodium phosphate buffer was prepared by first dissolving sodium phosphate monohydrate in a small amount of deuterium oxide and then removing the solvent by rotary evaporation. This was repeated twice, and then the exchanged salt was taken to volume with deuterium oxide and brought to pD 6.8 with sodium deuteroxide. Catalase (8 mg) was dissolved in 1.5 mL of D_2O and lyophilized. 1-PCPA (**1**) was dissolved in 1.5 mL of D_2O, and the solvent was removed by rotary evaporation (three times). MAO (5.0 mg in 1 mL) was dialyzed in 40 mL of the deuterated buffer (three changes, at least 4 h per change) at 25 °C. The MAO solution was divided into two equal portions; the final inactivator solutions contained 4 mg of catalase, 2.5 mg of MAO, and 1.5 mg of inactivator in a total volume of 4 mL. Inactivations and isolation of metabolites were carried out exactly as described in Isolation of Acrylophenone Metabolites Formed during MAO-B Inactivation by **5**, **6**, and Controls.

Isolation of Acrylophenone Metabolites Formed during Inactivation by 1 and 6 of MAO-B Previously Incubated with [α,α-$^2H_2$]Benzylamine in D_2O. MAO, catalase, and inactivators were subjected to deuterium exchange in a similar fashion to that described in Isolation of Acrylophenone Metabolites Formed during Inactivation of MAO-B by **1** in D_2O. [α,α-$^2H_2$]Benzylamine hydrochloride was dissolved in a small volume of D_2O and concentrated by rotary evaporation. MAO (7.5 mg) was then incubated with 12 mg of the catalase and 25.3 mg of [α,α-$^2H_2$]benzylamine hydrochloride in 12 mL of deuterated buffer for 24 h at room temperature. The mixture was transferred to dialysis tubing and was dialyzed against 100 mL of the deuterated buffer (four changes, at least 4 h per change). Each of the inactivations was then set up containing 5.3 μM MAO, 1.5 mM inactivator, and 1 mg/mL catalase in a total volume of 4 mL buffer. After 6 h in the dark at room temperature, the solutions were worked up in an identical fashion to that described in Isolation of Acrylophenone Metabolites Formed During MAO-B Inactivation by 5, 6, and Controls.

Incubation of [α,α-^{2}H]Acrylophenone with MAO and/or Catalase. In a typical experiment, 40 μg of [α-^{2}H]acrylophenone was incubated in the dark with 5.3 μM MAO and/or 4 mg of catalase in 50 mM sodium phosphate buffer pH 7.2 (4-mL total volume) for a specified period of time. The mixture then was extracted with dichloromethane (3 × 10 mL), and the combined extracts were washed twice with brine (2 × 15 mL). After being dried over magnesium sulfate, the extracts were concentrated and submitted for GCMS analysis as described above.

Rate of Reactivation of MAO-B Reversibly Inactivated by 1, 5, and 6. A mixture containing 2 μM MAO and 3 mM **1** in 50 mM sodium phosphate, pH 7.2 buffer (134 μL total volume) was incubated at 25 °C for 90 min. The solution was then mixed with 67 μL of 2 mg/mL Blue Dextran solution in the same buffer and applied to Sephadex G-25 (0.9 × 16.5 cm) previously equilibrated with 20 mM sodium phosphate buffer, pH 7.2, and eluted with the same buffer at a flow rate of 0.5 mL/min at 25 °C. A 0.5-mL fraction containing the majority of the Blue Dextran (and MAO) was collected and was incubated at 25 °C. Aliquots were removed periodically and assayed for enzyme activity. Pseudo-first-order reactivation of the inactivated enzyme was observed. This procedure was repeated using the inactivators **5** and **6**.

Acknowledgments. We are very grateful to Professor Richard K. Hill (University of Georgia) for providing us with his experimental procedures for the syntheses and analyses of the enantiomers of [2,2-$^2H_2$]-1-phenylcyclopropylamine and to Professor Timothy A. Keiderling and Mr. Baoliang Wang (University of Illinois at Chicago) for carrying out the Fourier transform vibrational circular dichroism studies on **5** and **6**. Acknowledgement also is made to Mr. Nicholas Andrew for preliminary synthetic studies. The GCMS analyses were carried out by Dr. Hoying (Doris) Hung and Saman Shafaie in the Analytical Services Laboratory of the Department of Chemistry at Northwestern University. We are also very grateful to the National Institutes of Health (Grant GM32634) for financial support of this research.

Supplementary Material Available: Vibrational circular dichroism (top) and IR absorption (bottom) spectra of (*R*)- (solid line) and (*S*)- (dashed line) [2,2-$^2H_2$]-1-phenylcyclopropylamine hydrochlorides in $CHCl_3$. Spectra have been normalized to correct for concentration differences in the R- and S-samples. The spectra are not corrected for baseline to illustrate the mirror image quality of the spectra. The band marked with an * is due to a baseline artifact, and the gap marked with a # is obscured by the solvent absorption (1 page). Ordering information is given on any current masthead page.

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