Peters, R. G., Jones, W. C., & Cromartie, T. H. (1981) *Biochemistry 20*, 2564–2571.
Philips, M., Roustan, C., Fattoum, A., & Pradel, L.-A. (1978) *Biochim. Biophys. Acta 523*, 368–376.
Philips, M., Pho, D. B., & Pradel, L.-A. (1979) *Biochim. Biophys. Acta 566*, 296–304.
Pompon, D., & Lederer, F. (1976) *Eur. J. Biochem. 68*, 415–423.
Pompon, D., & Lederer, F. (1978) *Eur. J. Biochem. 90*, 563–569.
Pompon, D., & Lederer, F. (1982) *Eur. J. Biochem. 129*, 143–147.
Pompon, D., & Lederer, F. (1983) *Eur. J. Biochem. 131*, 359–365.
Pompon, D., Iwatsubo, M., & Lederer, F. (1980) *Eur. J. Biochem. 104*, 479–488.
Ray, W. J., Jr., & Koshland, D. E. (1961) *J. Biol. Chem. 236*, 1973–1979.
Riordan, J. F., McElvany, K. D., & Borders, C. L., Jr. (1977) *Science (Washington, D.C.) 195*, 884–886.
Shoun, H., Beppu, T., & Arima, K. (1980) *J. Biol. Chem. 255*, 9319–9324.
Somlo, M., & Slonimski, P. (1966) *Bull. Soc. Chim. Biol. 48*, 1221–1249.
Takahashi, K. (1968) *J. Biol. Chem. 243*, 6171–6179.
Takahashi, K. (1977a) *J. Biochem. (Tokyo) 81*, 395–402.
Takahashi, K. (1977b) *J. Biochem. (Tokyo) 81*, 403–414.

Effect of α-Methylation on Inactivation of Monoamine Oxidase by *N*-Cyclopropylbenzylamine†

Richard B. Silverman

ABSTRACT: Monoamine oxidase (MAO) was shown previously [Silverman, R. B., & Hoffman, S. J. (1980) *J. Am. Chem. Soc. 102*, 7126–7128] to catalyze the oxidation of *N*-cyclopropylbenzylamine (N-CBA) at two sites on the molecule. Oxidation at the benzyl methylene gave benzaldehyde and cyclopropylamine; oxidation of the cyclopropyl group, which involved cyclopropyl ring cleavage, led to inactivation of the enzyme. In this paper it is shown that methylation of the benzyl methylene dramatically alters this partition ratio in favor of enzyme inactivation. Contrary to a previous report [Alles, G., & Heegaard, E. V. (1943) *J. Biol. Chem. 147*, 487–503], it is shown here that α-methylbenzylamine *is* a substrate for MAO; consequently, *N*-cyclopropyl-α-methylbenzylamine (N-CαMBA) is a good candidate for mechanism-based inactivation. *N*-Cyclopropyl[7-^{14}C]benzylamine, *N*-cyclopropyl-α-methyl[*phenyl*-^{14}C]benzylamine, *N*-[1-^{3}H]-cyclopropylbenzylamine, and *N*-[1-^{3}H]cyclopropyl-α-methylbenzylamine are synthesized, and product formation following MAO inactivation is quantified. The results obtained with these compounds indicate that with N-CαMBA, α-methylbenzyl oxidation (which produces acetophenone and cyclopropylamine) is only 1% that of cyclopropyl oxidation (which gives enzyme inactivation), whereas with N-CBA the amount of oxidation at the corresponding sites is equal. It also is shown that the K_i values for (*R*)-(+)- and (*S*)-(−)-α-methylbenzylamine are similar, suggesting that dimethylation of N-CBA should not interfere with binding to MAO. *N*-Cyclopropyl-α,α-dimethylbenzylamine, which should be incapable of benzyl oxidation, is prepared and shown to be a mechanism-based inhibitor of MAO whose properties differ slightly from those of N-CBA and N-CαMBA. These results show that α-substitution of N-CBA has a profound effect on the partition ratio and present a new approach to rational drug design based on physical organic chemical principles.

Monoamine oxidase (MAO,[1] EC 1.4.3.4) is an important enzyme in the treatment of certain forms of depression; compounds that inhibit MAO exhibit antidepressant activity (Kaiser & Setler, 1981; Rowan et al., 1981; Baldessarini, 1977; Berger & Barchas, 1977; Tyrer, 1976). One of the earlier clinically used antidepressants, tranylcypromine, is a cyclopropylamine-containing MAO inhibitor. Recently, the mechanism of MAO inactivation by this drug was reported (Silverman, 1983), and it was shown to belong to the class of enzyme inhibitors known as mechanism-based inhibitors (Silverman & Hoffman, 1984; Walsh, 1982; Abeles & Maycock, 1976; Rando, 1974). A mechanism-based inhibitor is an unreactive compound that generally is converted to an activated form by an enzyme via its normal catalytic mechanism of action, and this altered form of the compound leads to inactivation of the enzyme. Therefore, a mechanism-based inhibitor initially acts as substrate for the enzyme, but instead of being converted into a product that is released from the enzyme, it is converted into a species that inhibits the enzyme without prior release from the active site. Numerous other cyclopropylamines, particularly the secondary amines, *N*-cyclopropyl-*N*-arylalkylamines, have been shown to be potent MAO inhibitors (Kutter & Hansch, 1969; Winn et al., 1975; Long et al., 1976; Murphy et al., 1978; Fuller et al., 1978; Silverman & Yamasaki, 1984; R. B. Silverman and M. L. Vazquez, unpublished results; Fuller et al., 1983). In our work with N-CBA (R. B. Silverman and M. L. Vazquez, unpub-

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[1] Abbreviations: MAO, monoamine oxidase; N-CBA, *N*-cyclopropylbenzylamine; N-CαMBA, *N*-cyclopropyl-α-methylbenzylamine; N-CααDMBA, *N*-cyclopropyl-α,α-dimethylbenzylamine; Cbz, carbobenzoxy group; KP_i, potassium phosphate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane.

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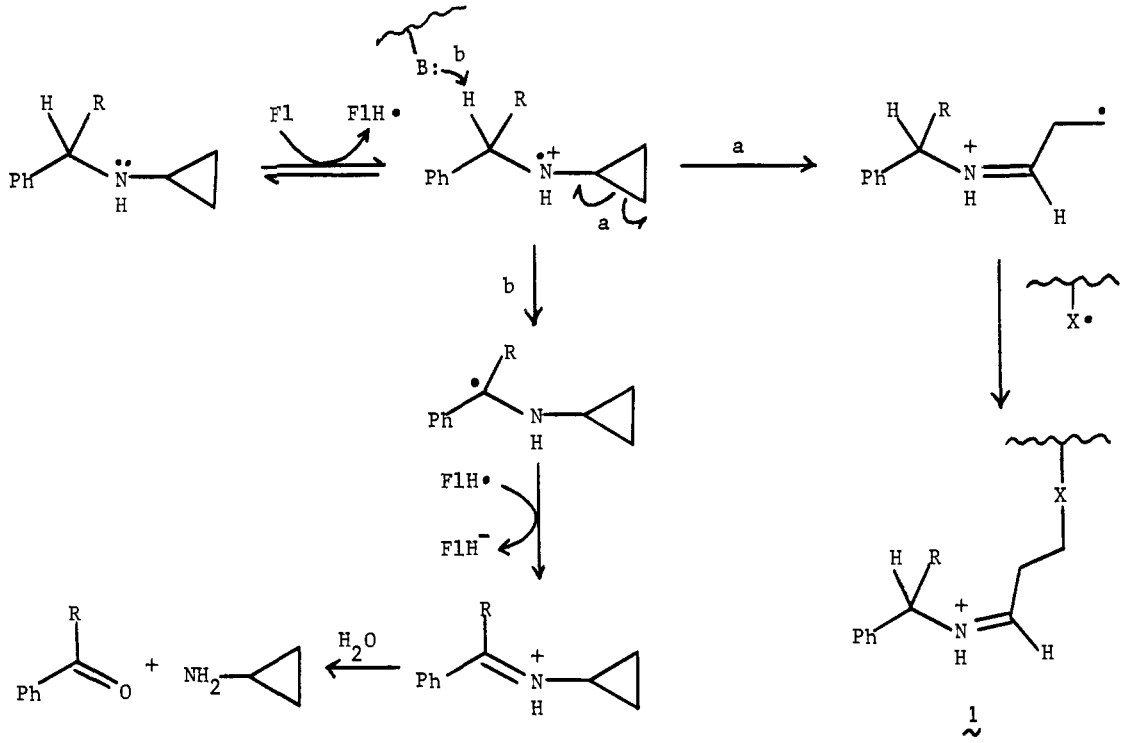
Scheme I: Proposed Mechanism for MAO Inactivation by N-CBA



lished results; Silverman & Hoffman, 1980), we found that, in addition to inactivation of MAO as a result of amine oxidation and cyclopropyl ring opening, another pathway involving oxidation of the benzyl methylene carbon occurred as often as cyclopropyl ring opening (Scheme I, R = H). This secondary pathway led to the formation of benzaldehyde and cyclopropylamine without inactivation of MAO. When designing potential chemotherapeutic agents, it is important to minimize the formation of metabolites that may be cytotoxic. Although partition ratios (the ratio of amount of product formed to inactivation event) have been reported in the thousands (Klinman & Krueger, 1982; Walsh, 1982), the formation of even a small amount of byproducts per inactivation may be harmful. Consequently, it would be desirable to manipulate the partition ratio for inactivation of MAO by *N*-cyclopropyl-*N*-arylalkylamines and thereby regulate the byproduct formation. In this paper, it is shown that α-substitituion of N-CBA has a profound effect on this partition ratio and leads to elimination of metabolite production.

Materials and Methods

Reagents. All reagents were bought from Aldrich Chemical Co. Diglyme was dried over KOH and distilled from lithium aluminum hydride. [U-*phenyl*-^{14}C]Acetophenone (8.79 mCi/mmol) was obtained from Pathfinder Laboratories, Inc., and [^{3}H]water (5 Ci/mL) was a product of Amersham. Research Products International 3a70B fluid or Beckman Ready-Solve was used for scintillation counting. [^{14}C]Pargyline, sp act. 3.06×10^6 dpm/μmol, was a gift of Dr. Roy McCauley (Wayne State University). Whatman 3MM paper was used for descending chromatography.

dl-N-CαMBA·HCl. The procedure described (vide infra) for *N*-CαM[*phenyl*-^{14}C] BA was followed on a larger scale to prepare N-CαMBA·HCl as shiny white crystals (65% overall yield before recrystallization): mp 199–199.5 °C dec; NMR ($CDCl_3$) δ 0.63 (m, 2 H), 1.20 (m, 2 H), 1.85 (d, 3 H), 2.09 (m, 1 H), 4.20 (q, 1 H), 7.1–7.65 (m, 5 H), 9.85 (br m, 2 H). Anal. Calcd for $C_{11}H_{16}ClN$: C, 66.83; H, 8.16; N, 7.08. Found: C, 66.66; H, 8.18; N, 7.04.

N-CααDMBA. To a solution of N-CαMBA·HCl (593 mg, 3.0 mmol) and sodium bicarbonate (1.75 g, 20.8 mmol) in water (30 mL) was added ether (30 mL). The two layers were vigorously stirred while benzyl chloroformate (0.45 mL, 3.15 mmol) was added dropwise. The mixture was stirred at room temperature for 17 h; then the ether layer was separated, and the aqueous layer was extracted with two 10-mL portions of ether. The combined ether extracts were washed with two 10-mL portions each of 1 M HCl and H_2O and then with brine (10 mL) and dried ($MgSO_4$). Evaporation of the solvent gave N-Cbz-N-CαMBA as a colorless oil (840 mg, 95%): NMR ($CDCl_3$) δ 0.58 (m, 4 H), 1.65 (d, 3 H), 2.41 (m, 1 H), 5.11 (s, 2 H), 5.23 (q, 1 H), 7.23 (s, 5 H), 7.28 (s, 5 H); TLC (silica gel; 10% ethyl acetate–*n*-hexane) R_f 0.33. To the N-Cbz-N-CαMBA under argon was added by syringe dry diglyme (5 mL) and *N*-cyclohexylisopropylamine (0.50 mL, 3.0 mmol); then the solution was stirred and cooled in a dry ice–acetone bath, and 2.6 M *n*-butyllithium in hexane (1.17 mL, 3.0 mmol) was added by syringe. The resulting dark brown solution was stirred for 15 min; then iodomethane (280 μL, 4.25 mmol) was added by syringe whereupon the reaction mixture turned milky beige. The cooling bath was removed for 15 min, and the reaction mixture was placed in an ice bath, quenched with saturated NH_4Cl (5 mL), and extracted with ether (3 × 10 mL). The aqueous layer was acidified and extracted with ether (2 × 5 mL). The combined ether extracts were washed with two portions of 10 mL each of saturated sodium bisulfite, 1 M HCl, and H_2O and then washed with brine (10 mL) and dried ($MgSO_4$). Evaporation of the solvent gave a pale yellow oil (831 mg), which was chromatographed on silica gel 60 (80 g; 2.95 × 27 cm), eluting with 5% ethyl acetate–*n*-hexane. The band with R_f 0.26 (TLC was run in 10% ethyl acetate–*n*-hexane) was collected, and the solvent was evaporated to give N-Cbz-N-CααDMBA (457 mg, 52%) as a colorless oil: NMR ($CDCl_3$) δ 0.82 (m, 4 H), 1.73 (s, 6 H), 2.62 (m, 1 H), 4.82 (s, 2 H), 7.24 (br s, 10 H). N-Cbz-N-CααDMBA (106 mg, 0.356 mmol) was dissolved in 95% ethanol (2.0 mL) and was hydrogenated over PtO_2 (7 mg) for 48 h with a H_2-filled balloon. The reaction mixture was filtered, and the solvent

was evaporated to a colorless oil, which was taken up in ether (5 mL) and extracted with three 1-mL portions of 0.1 N HCl. The ether was evaporated to give unreacted starting material and another product (30.5 mg), which were again hydrogenated as above. The aqueous extracts were combined and were washed with ether (2 × 5 mL); then the water was evaporated in vacuo at room temperature to give a colorless oil (50 mg), which was taken up in H_2O (1 mL), added to 10 mL of 0.1 N NaOH, and extracted with ether (3 × 5 mL). The combined ether extracts were dried (K_2CO_3), and the solvent was evaporated to a colorless oil (30 mg), which was chromatographed on silica gel 60 (5 g; 1.05 × 12.5 cm), eluting with 1:1 ethyl acetate–*n*-hexane. The material having R_f 0.24 was collected in 1-mL fractions 11–33, which were combined, and the solvents were evaporated. The resulting colorless oil was dissolved in ether (5 mL) and extracted with three 1-mL portions of 0.1 N HCl. The combined aqueous extracts were combined and washed with ether, and the H_2O was evaporated in vacuo at room temperature to give a colorless oil (30 mg, 40%), which was crystallized and recrystallized from chloroform–*n*-hexane. The product was obtained as fine white needles: mp 178–181 °C dec; NMR (D_2O) δ 0.76 (m, 4 H), 1.89 (s, 6 H), 2.45 (m, 1 H), 4.75 (s, HDO), 7.60 (m, 5 H). Anal. Calcd for $C_{12}H_{18}ClN$: C, 68.07; H, 8.57; N, 6.62. Found: C, 67.73; H, 8.20; N, 6.45.

N-CαM[phenyl-^{14}*C]BA·HCl.* [U-*phenyl-*^{14}C]Acetophenone (0.25 mCi) in 125 μL of benzene was diluted with chloroform (0.5 mL) containing acetophenone (29 μL, 0.25 mmol). Cyclopropylamine (20 μL, 0.275 mmol) was added followed by 4-Å molecular sieves, and the flask was flushed with argon and stoppered. The reaction mixture was slowly stirred for 18 h, and then additional chloroform was added, the mixture was filtered, and the sieves were washed with chloroform. The combined chloroform filtrates were evaporated to a pale yellow liquid, which was dissolved in methanol (0.5 mL) and cooled in an ice bath. Sodium cyanoborohydride (11 mg, 0.175 mmol) and then 5 N HCl (100 μL) were added; the reaction mixture was flushed with argon and stirred at room temperature for 26 h. Rotary evaporation of the solvent gave a white solid, which was triturated with chloroform to which $MgSO_4$ was added. After filtration, the residue was washed with chloroform, and the combined chloroform filtrates were evaporated to an off-white solid (39 mg, 80%). Recrystallization twice from chloroform–ethyl acetate gave the product as shiny white crystals, sp act. 1.92 × 10^6 dpm/μmol, which was radiopure and chemically pure by descending paper chromatography (12:3:5 *n*-butanol–acetic acid–water), and all of the radioactivity comigrated with carrier N-CαMBA.

N-[1-^{3}*H]CαMBA·HCl.* This compound was prepared as shiny white crystals, sp act. 4.77 × 10^6 dpm/μmol, by the same procedure described (vide supra) for the synthesis of *N*-CαM[*phenyl-*^{14}C]BA·HCl with the substitution of *N*-[1-^{3}H]cyclopropylamine (Silverman & Hoffman, 1981) for cyclopropylamine in stoichiometric amounts. The product was radiopure and chemically pure by descending paper chromatography [8:2 *n*-butanol–water (top layer)] and all of the radioactivity comigrated with carrier N-CαMBA.

(±)-*α-Methyl[α-*^{3}*H]benzylamine Hydrochloride.* Sodium metal (69 mg, 3.0 mmol) was added to water (1.0 mL) under argon in an ice bath. When the reaction was completed, ^3H_2O (10 μL, 50 mCi) and then α-methylbenzyl cyanide (254 μL, 2.0 mmol) were added. The reaction flask was immersed in an oil bath at 105 °C and was rapidly stirred under argon for 19 h. After being cooled in an ice bath, the reaction was quenched with concentrated HCl (450 μL), and the water was removed by low-temperature bulb-to-bulb distillation (dry ice–2-propanol). Water was added to the residue 4 times, and bulb-to-bulb distillation was carried out 4 times. Water (5 mL) was added, and then the mixture was extracted with *n*-pentane (3 × 10 mL). The combined *n*-pentane extracts were washed with water (3 × 5 mL) and then extracted with 1 N NaOH (5, 2, and 2 mL). The combined aqueous extracts were washed with *n*-pentane (3 × 10 mL) and then acidified with concentrated HCl and extracted with *n*-pentane (3 × 10 mL). The combined *n*-pentane extracts were washed with water (3 × 5 mL), dried ($MgSO_4$), and evaporated to a colorless oil (160 mg, 53%), which was dissolved in chloroform (6 mL) and immersed in an oil bath at 45 °C. To this was added concentrated H_2SO_4 (1.50 mL) followed by sodium azide (205 mg, 3.15 mmol), the reaction mixture was stirred at 40–45 °C for 3 h and cooled in an ice bath, and H_2O (5 mL) was added with stirring. The layers were separated, and the organic layer was extracted with H_2O (3 mL). The combined aqueous extracts were washed with ether (2 × 10 mL), made strongly basic with 6 N NaOH, and extracted with ether (3 × 5 mL). The combined ether extracts were washed with H_2O (3 × 5 mL) and then extracted with 1 N HCl (5, 2, and 2 mL). The acid was evaporated in vacuo at room temperature, and the residue was reevaporated from H_2O. The resulting white solid was recrystallized from chloroform–carbon tetrachloride. The first crop of crystals (23 mg) had little radioactivity and was discarded. The mother liquor was concentrated to ca. 10 mL and refrigerated, giving off-white crystals (51 mg), which were recrystallized twice from chloroform–carbon tetrachloride. The product was obtained as a white powder, sp act. 6.43 × 10^5 dpm/μmol, which was radiopure and chemically pure by descending paper chromatography [8:2 *n*-butanol–H_2O (top layer)], and all of the radioactivity comigrated with carrier α-methylbenzylamine hydrochloride.

Enzyme and Assays. MAO was purified from bovine or porcine liver mitochondria by the method of Salach (1979) and stored at high concentration (25–35 mg/mL) in 50 mM KP_i, pH 7.2 buffer in the refrigerator. The specific activity varied from preparation to preparation but was in the 2.8–4.0 units/mg range where 1 unit of activity is the conversion of 1 μmol of benzylamine to benzaldehyde per minute at 30 °C. It was shown in a previous study (Silverman & Yamasaki, 1984) that MAO from bovine and porcine liver purified by the Salach (1979) method is essentially equivalent for cyclopropylamine inactivation studies. MAO was assayed in 20 mM Tris-HCl, pH 9.0 buffer containing 1 mM benzylamine by the method of Tabor et al. (1954). Protein concentration was assayed by the method of Lowry (1951).

Release of Tritium from α-Methyl[α-^{3}*H]benzylamine.* MAO (5.1 mg) in 1.4 mL of 50 mM KP_i, pH 7.2 buffer containing 1.37 mM α-methyl[α-^{3}H]benzylamine was incubated at 30 °C. At 30, 60, 90, 120, and 180 min, 250-μL aliquots were removed, were added to 25 μL of 20% trichloroacetic acid, and then were applied to a column (0.5 × 2.5 cm) of Dowex 50 (H^+). The column was washed with 2 × 1.0 mL of water, and the combined eluents were collected in a scintillation vial, diluted with 10 mL of scintillation fluid, and counted. A nonenzyme control was run concurrently.

Oxidation of α-Methylbenzylamine. MAO (0.5 mg) was incubated in a stoppered cuvette with 1.37 mM α-methylbenzylamine in 50 mM KP_i, pH 7.2 buffer at 23 °C. The absorption spectrum from 550 to 400 nm was recorded after 5 days relative to a control solution containing no α-methylbenzylamine, and then the solutions were equilibrated with

air, and the spectrum was recorded.

Inactivation of MAO by N-CαMBA and N-CααDMBA. MAO (4 μM) was incubated with various concentrations of N-CαMBA (50, 40, 30, 25, 20, and 10 μM) or N-CααDMBA (100, 50, 30, and 20 μM) at 25 °C. At various times, 10 μL was removed and diluted with 490 μL of assay solution, and enzyme activity was measured.

Reactivation of N-CαMBA- and N-CααDMBA-Inactivated MAO by Benzylamine. MAO (4 μM) was divided into two equal portions; half was incubated at 25 °C with 100 μM N-CαMBA until no activity remained (1.25 h); the other half contained no N-CαMBA. After dialysis of each enzyme solution against two changes of 1 L of 20 mM KP_i, pH 7.0 buffer at 4 °C, both were taken to 1 mM in benzylamine. At time intervals, 10-μL aliquots were removed from each half and assayed as above. The same experiment was carried out by substituting N-CααDMBA for N-CαMBA.

Change in the Optical Spectrum during Inactivation of MAO by N-CαMBA. The optical spectrum of MAO (250 μg) in 500 μL of 40 mM Tris-HCl, pH 9.0 buffer was recorded vs. buffer; then 5.3 μL of a 506 μM solution of N-CαMBA (5.3 μM final concentration) was added. Spectra were recorded at 2, 10, 25, and 82 min. Urea was added to give a concentration of 8 M, and the spectrum was recorded.

Change in the Optical Spectrum during Inactivation of MAO by N-CααDMBA, during Reactivation by Benzylamine, and after Urea Treatment. MAO (1.1 mg) in 745 μL of 50 mM HEPES, pH 7.2 buffer was treated with 5 μL of a 15 mM solution of N-CααDMBA (final concentration 100 μM). Spectra were recorded at 2, 8, 15, and 105 min. Benzylamine (1 μL) was added to give a final concentration of 1.0 mM, and the spectrum was recorded at 1 and 4 h. The solution was dialyzed against 50 mM HEPES, pH 7.2 buffer containing 1 mM benzylamine for 15 h, and the spectrum was recorded. Urea was added to 8 M; then after 40 min, the spectrum was recorded. A similar experiment was carried out in 20 mM KP_i, pH 7.0 buffer.

Inactivation of MAO by N-[1-^{3}H]CαMBA and N-[1-^{3}H]-CBA. All solutions were prepared in 30 mM Tris-HCl, pH 9.0 buffer except where noted. MAO (ca. 35 mg/mL) in 50 mM KP_i, pH 7.2 buffer was diluted 7-fold with Tris buffer and then divided into two 550-μL portions and two 183-μL portions. To one 550-μL portion was added 50 μL of a 24 mM solution of N-[1-^{3}H]CαMBA, to the other 550-μL portion was added 50 μL of a 24 mM solution of N-[1-^{3}H]CBA, to one 183-μL portion was added 16.7 μL of a 1.7 mM solution of [^{14}C]pargyline, and to the other 183-μL portion was added buffer. Nonenzyme control reactions also were run concurrently. All solutions were allowed to incubate at 25 °C for 3 h; then each was microdialyzed against two 3-mL portions of 20 mM KP_i, pH 7.0 buffer at room temperature (4 h total). The two 3-mL outer portions of the microdialyses (95% of the counts from the control reactions were removed) were combined and were applied to columns of Dowex 50 (H^+) (0.5 × 5.0 cm), eluting first with water, collecting 2.0-mL fractions until no more radioactivity ([^{3}H]acrolein) was eluted (ca. 12 mL), and then with 1.5 N HCl, collecting the 2.0-mL fractions corresponding to cyclopropylamine hydrochloride (the first 12–14 mL). Aliquots of each fraction (0.50 mL) were removed for scintillation counting (10 mL of Ready-Solve). The water was evaporated from the remainder of the combined fractions in vacuo below room temperature. Descending chromatography was performed on the residues eluting with 12:3:5 *n*-butanol–acetic acid–water. The fraction of counts corresponding to cyclopropylamine (R_f 0.56), minus the counts in the corresponding nonenzyme control cyclopropylamine fractions, was used to determine the actual amount of cyclopropylamine produced during inactivation by N-[1-^{3}H]-CαMBA and N-[1-^{3}H]CBA. The inactivated enzyme solutions were dialyzed against four 300-mL portions of 20 mM KP_i, pH 7.0 buffer over 18 h, and then aliquots were removed for scintillation counting (10 mL of Ready-Solve), enzyme activity determinations, and protein concentration assays.

Inactivation of MAO by N-CαM[phenyl-^{14}C]BA and N-C[7-^{14}C]BA. The same procedure as described for inactivation of MAO by N-[1-^{3}H]CαMBA and N-[1-^{3}H]CBA was followed except the outer portions of the microdialyses applied to Dowex 50 were eluted with water and then with 1.5 N HCl and the fractions corresponding to α-methylbenzylamine were collected. Aliquots of each fraction were removed for scintillation counting. The HCl fractions were pooled, and the water was removed by evaporation below room temperature in vacuo. Descending chromatography (80:20 *n*-butanol–H_2O, top layer) was performed on half of each residue from the inactivated enzyme and nonenzyme control HCl fractions [R_f(N-CαMBA) 0.87, R_f(αMBA) 0.56]. The other half was chromatographed on silica gel 60 thin-layer chromatography plates, eluting with 2% aqueous ammonia in ethanol [R_f(N-CαMBA) 0.67, R_f(αMBA) 0.45].

Reduction of N-CαMBA-Inactivated MAO by Sodium Cyanoborohydride. MAO (60 μL, 35 mg/mL) was diluted 8-fold with 30 mM Tris-HCl, pH 9.0 buffer; then 50 μL of a 24 mM solution of NCαM[phenyl-^{14}C]BA was added. After incubation at 25 °C for 2.5 h, two different sets of conditions were used for the reduction on two different batches of inactivated enzyme (the second set of conditions is in parentheses). The inactivated enzyme solution was divided into two equal portions. To one portion was added a solution of 5 mg of sodium cyanoborohydride in 50 μL of 30 mM Tris-HCl, pH 9.0 buffer (or 0.2 N sodium borate, pH 9.5 buffer); to the other was added 50 μL of the corresponding buffer. Both were allowed to incubate in the refrigerator (or at room temperature) for 16 h (or 18.5 h); then each was dialyzed against 3 portions of 150 mL of 20 mM KP_i, pH 7.0 buffer over 23 h (or 7 h) and then against 100 mL of 6 M urea in the appropriate buffer. A [^{14}C]pargyline-inactivated enzyme solution served as a control. Aliquots were removed from each solution for scintillation counting (10 mL of 3a70B fluid) and protein determination.

Release of Tritium from N-[1-^{3}H]CαMBA-Inactivated MAO. MAO was inactivated with N-[1-^{3}H]CαMBA as described under Inactivation of MAO by N-[1-^{3}H]CαMBA and N-[1-^{3}H]CBA and then dialyzed against three 250-mL portions of 20 mM KP_i, pH 7.0 buffer for 6 h. No inactivator and [^{14}C]pargyline controls also were carried out. The 390 μL (11 423 dpm) of inactivated enzyme was diluted with 390 μL of 20 mM KP_i, pH 7.0 buffer containing 2.0 mM benzylamine and 10 mM β-mercaptoethanol and then was dialyzed against two 200-mL portions of this same buffer containing benzylamine and β-mercaptoethanol at room temperature for 18 h. Aliquots were removed for radioactivity determination and Lowry assays.

Results

Inhibition of MAO by (R)-(+)-, (S)-(−)-, and (±)-α-Methylbenzylamine. The inhibition of the oxidation of benzylamine at 20, 40, 60, 80, 100, 200, and 300 μM concentrations by (*R*)-(+)-, (*S*)-(−)-, and (±)-α-methylbenzylamine (100 and 200 μM) was determined. From a plot of 1/[benzylamine] vs. 1/(enzyme activity) (data not shown), the K_i values for (*R*)-(+)-, (*S*)-(−)-, and (±)-methylbenzylamine are

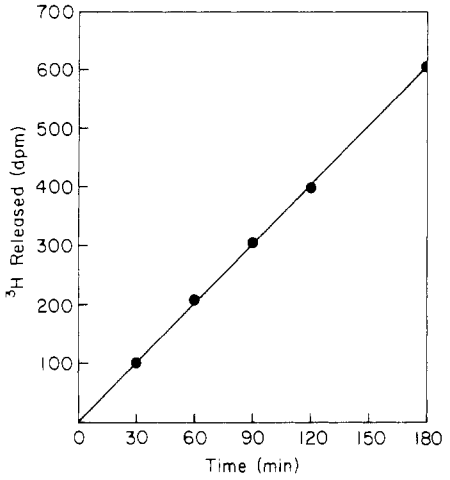


FIGURE 1: MAO-catalyzed release of tritium from α-methyl[α-^{3}H]benzylamine. See Materials and Methods for the procedure.

103 μM, 154 μM, and 122 μM, respectively.

Release of Tritium from α-Methyl[α-^{3}H]benzylamine. Figure 1 shows the release of tritium from α-methyl[α-^{3}H]-benzylamine as a function of time (minus the tritium generated from the nonenzyme control, which remained constant throughout the experiment). From this plot, it was calculated that $k_{cat} = 6.73 \times 10^{-4}$ min^{-1}.

Oxidation of α-Methylbenzylamine. After 5 days, the difference spectrum showed that reduction of the flavin had occurred in the presence of α-methylbenzylamine. When air was allowed into the cuvettes, a base-line difference spectrum resulted.

Inactivation of MAO by N-CαMBA and N-CααDMBA. Incubation of MAO at each concentration of N-CαMBA showed pseudo-first-order loss of enzyme activity (data not shown). From a plot of the reciprocal inhibitor concentration vs. the half-life at each inhibitor concentration (Kitz & Wilson, 1962), $K_i = 44$ μM and k_{cat}(saturation) = 0.25 min^{-1}. Incubation of MAO at each concentration of N-CααDMBA produced inactivation that was pseudo first order only for the first half-life; afterward, an upward deviation from linearity was observed (data not shown). If the half-lives from the initial slopes are plotted against the reciprocal inhibitor concentrations (Kitz & Wilson, 1962), $K_i = 323$ μM and k_{cat}(saturation) = 4.0 min^{-1}.

Reactivation by Benzylamine of MAO Inactivated by N-CαMBA and N-CααDMBA. The kinetics of reactivation of N-CαMBA-inactivated MAO by 1.0 mM benzylamine were pseudo first order and had a half-life of 85 min; all of the enzyme activity was recovered. When MAO inactivated by N-Cα*M*[*phenyl*-^{14}C]BA was dialyzed against 20 mM KP_i, pH 7.0 buffer containing 1 mM benzylamine, 95% of the radioactivity was removed and full enzyme activity was regained. Dialysis of N-Cα*M*[*phenyl*-^{14}C]BA-inactivated enzyme against 8 M urea also removed 95% of the radioactivity. When MAO inactivated by N-[1-^{3}H]CαMBA was dialyzed against 20 mM KP_i, pH 7.0 buffer containing 2 mM benzylamine and 10 mM β-mercaptoethanol, 88% of the radioactivity was removed and full enzyme activity was regained.

Dialysis of N-CααDMBA-inactivated MAO against 20 mM KP_i, pH 7.0 buffer containing 1 mM benzylamine for 4 h resulted in regain of 64% of enzyme activity; after 16 h, no additional enzyme activity returned.

Change in Optical Spectrum during Inactivation of MAO by N-CαMBA and after Urea Treatment. A time-dependent change in the optical spectrum from that of oxidized to that of reduced flavin was observed. Upon urea denaturation, the spectrum of totally oxidized flavin was observed.

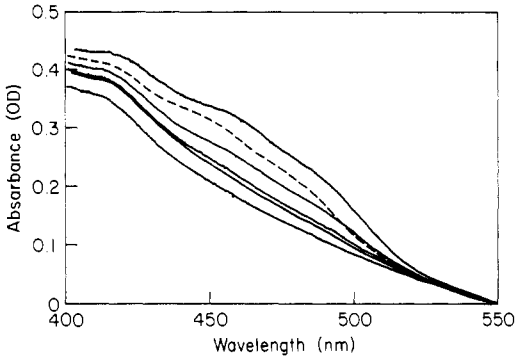
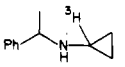
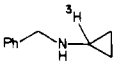
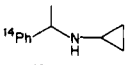
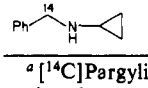


FIGURE 2: Change in optical spectrum during inactivation of MAO by N-CααDMBA and after treatment with benzylamine and urea. The solid line traces (in descending order) were obtained at 0, 2, 8, 15, and 105 min. The dashed line was obtained after the inactivated enzyme was treated with 1.0 mM benzylamine for 19 h or treated with 8 M urea for 40 min. The bottom trace was unchanged when sodium dithionite was added. See Materials and Methods for the procedure.

Table I: Inactivation of MAO by N-[1-^{3}H]CαMBA, N-[1-^{3}H]CBA, N-Cα*M*[*phenyl*-^{14}C]BA, and N-C[7-^{14}C]BA

inactivator	enzyme-bound radioactivity/enzyme	benzyl oxidation (μmol)	cyclopropyl oxidation (μmol)	benzyl/cyclopropyl oxidation
	1.20[a]	3.70×10^{-4}[c]	3.12×10^{-2}[e]	0.012
	4.03[a]	1.11×10^{-1}[c]	1.11×10^{-1}[e]	1.00
	0.14[b]	3.84×10^{-4}[d]	2.21×10^{-2}[f]	0.017
	0.31[b]	2.73×10^{-1}[d]		

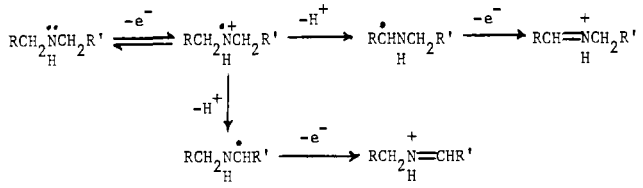
[a] [^{14}C]Pargyline titration was 1.11; these values were obtained by setting the pargyline ratio to 1.00 (Chuang et al., 1974). [b] [^{14}C]Pargyline titration was 1.04; these values were obtained by setting the pargyline ratio to 1.00 (Chuang et al., 1974). [c] Amount of *N*-[1-^{3}H]-cyclopropylamine formed. [d] Amount of radioactive non-amines generated. [e] Amount of [^{3}H]acrolein plus enzyme-bound radioactivity. [f] Amount of α-methyl[^{14}C]benzylamine generated.

Change in Optical Spectrum during Inactivation of MAO by N-CααDMBA, during Reactivation by Benzylamine, and after Urea Treatment. A time-dependent change of the flavin spectrum from oxidized to reduced was observed (Figure 2); the 2-, 8-, 15-, and 105-min time points correspond to 61, 17, 7, and 0% enzyme activity. Incubation with benzylamine resulted in partial conversion of reduced to oxidized flavin; treatment with 8 M urea resulted in no further oxidation of the flavin (Figure 2).

Inactivation of MAO by N-[1-^{3}H]CαMBA and N-[1-^{3}H]-CBA. The results are summarized in Table I. Benzyl oxidation, determined by the amount of cyclopropylamine produced, was 300 times greater for N-[1-^{3}H]CBA than for N-[1-^{3}H]CαMBA. Cyclopropyl oxidation, determined by the sum of [^{3}H]acrolein and enzyme-bound radioactivity, was 3.6 times greater for N-[1-^{3}H]CBA than for N-[1-^{3}H]CαMBA. Radioactivity from nonenzyme controls was subtracted from enzyme-generated radioactivity when amounts of metabolites formed were calculated.

*Inactivation of MAO by N-Cα*M*[*phenyl*-^{14}C]BA and N-C[7-^{14}C]BA.* The results are summarized in Table I. Benzyl oxidation, as measured by the amount of radioactive non-amines generated, was 711 times greater for N-C[7-^{14}C]BA

Scheme II: Mechanism of Secondary Amine Electrochemical Oxidation



than for N-CαM[*phenyl*-^{14}C]BA. The total amount of α-methylbenzylamine generated during inactivation of MAO by N-CαM[*phenyl*-^{14}C]BA was 1.44 equiv per enzyme inactivated. Radioactivity from nonenzyme controls was subtracted from the amount of enzyme-generated radioactivity.

Reduction of N-CαMBA-Inactivated MAO by Sodium Cyanoborohydride. When the reaction was carried out at pH 9.0, sodium cyanoborohydride-treated inactivated enzyme resulted in 0.16 equiv of radioactivity bound compared to 0.04 equiv for unreduced. The pH 9.5 reaction gave 0.07 equiv for reduced and 0.05 equiv for unreduced.

Discussion

The approach taken to alter the metabolic pathway for reaction of N-CBA by MAO (Scheme I, R = H) was based on the known mechanism of electrochemical (Mann & Barnes, 1970; Masui & Sayo, 1971; Lindsay Smith & Masheder, 1976), photochemical (Lewis & Ho, 1980), and chemical (Lindsay Smith & Mead, 1973; Smith & Loeppky, 1967) oxidation of amines (Scheme II). For electrochemical oxidation of secondary amines, the ratio of α-proton removal from the two substituents generally depends upon the kinetic acidities of the protons adjacent to the incipient amine radical cation. It also is known that as a carbon atom becomes more alkyl substituted, the pK_a of a proton attached to the central carbon atom generally increases. For example, the pK_a of ethyl acetoacetate is 10.7, but that of ethyl methylacetoacetate is 12.7 (Jones, 1973). Therefore, in order to diminish the rate of benzyl methylene proton removal (pathway b, Scheme I) from N-CBA relative to cyclopropyl ring opening (pathway a, Scheme I) during inactivation of MAO by N-CBA (and thereby minimize the partition ratio), it seemed reasonable that increasing the alkyl substitution on the benzyl methylene carbon (and thereby decreasing the kinetic acidity of the proton attached to the benzyl methylene) would be auspicious. However, it has been reported (Alles & Heegaard, 1943; Blaschko, 1952) that α-methylbenzylamine is not a substrate for MAO. More recently, the design of a new mechanism-based inhibitor of ornithine decarboxylase was based on the premise that α-substituted amines are not substrates for MAO (Danzin et al., 1983). Since a mechanism-based inhibitor must initially act as a substrate for the target enzyme, it would be difficult to rationalize the design of a mechanism-based inhibitor of MAO that was an analogue of a molecule that was *not* a substrate for MAO. Previous workers who have claimed that α-substituted amines were not substrates for MAO based that notion on results of oxygen uptake experiments (Alles & Heegaard, 1943). I also have attempted, unsuccessfully, to detect the oxidation of α-methylbenzylamine by monitoring the spectrophotometric increase in absorbance at 243 nm (λ_{max} for acetophenone), but these assays would not be sensitive enough to measure a slow rate of oxidation of α-methylbenzylamine to acetophenone. Oxidation of α-methylbenzylamine by any reasonable mechanism would result in the breakage of the α-C–H bond and the release of the α-proton into solution. Consequently, α-methyl[α-^{3}H]benzylamine was prepared and incubated with MAO. Tritium was released into solution at a time-dependent linear rate for at least 180 min (Figure 1). In order to show that oxidation of the substrate was occurring, the anaerobic reduction of the covalently bound FAD cofactor was monitored; the optical spectrum of the FAD cofactor slowly changed from that of the oxidized to that of the reduced cofactor. This indicates that the α-proton release is associated with oxidation of the substrate and that α-methylbenzylamine *is* a substrate for MAO. The turnover number for α-methylbenzylamine, assuming 1.37 mM is saturating (vide infra), was calculated from Figure 1 to be 6.73×10^{-4} min^{-1}; that for benzylamine[2] is 270 min^{-1}. Therefore, α-methylbenzylamine is oxidized at a rate that is 2.49×10^{-6} that of benzylamine. On the basis of the amine oxidation mechanism shown in Scheme II, this slow oxidation rate may be the result of the inability of α-methylbenzylamine to approach the FAD cofactor close enough for faster electron transfer [it is likely that this is the rate-determining step (Mann & Barnes, 1970)] or the difficulty of the enzyme to remove the α-proton following electron transfer as a result of steric hindrance by the methyl group (or both). It has been suggested that the electron-transfer step to the FAD is reversible (Simpson et al., 1982). Since α-methylbenzylamine is a chiral molecule, possibly only one enantiomer is capable of binding to the active site; consequently, the inhibition constants for (*R*)-(+)-, (*S*)-(−)-, and (±)-α-methylbenzylamine were determined. Stereochemistry, apparently, is not an issue with MAO since the K_i values are 103 μM and 154 μM for the (+) and (−) isomers, respectively. It is not known, however, if only one isomer is oxidized since the tritium release experiment was carried out on a racemic mixture. The α-methyl substituent, apparently, contributes to binding since the K_m for benzylamine is 380 μM.[2]

Since Mills et al. (1968) had reported that *N*-cyclopropyl-α-(methylaryl)alkylamines inhibited kynuramine oxidation (the type of inhibition was not noted) and α-methylbenzylamine was shown here to be a substrate for MAO, N-CαMBA was synthesized and tested as a mechanism-based inhibitor for MAO. N-CαMBA was found to be a time-dependent inactivator of MAO, and its K_i (44 μM) was lower than that for N-CBA (280 μM), consistent with the lower K_i of α-methylbenzylamine relative to the K_m for benzylamine. All of the criteria for mechanism-based inhibition (Silverman & Hoffman, 1984) were met; the properties of N-CαMBA were very similar to those of N-CBA (Silverman & Hoffman, 1980a; R. B. Silverman and M. L. Vazquez, unpublished results). Thus, in addition to time-dependent inhibition, which exhibits saturation, the rate of inactivation was slower in the presence of substrate than in its absence; after inactivation, dialysis did not regenerate enzyme activity; N-[1-^{3}H]CαMBA gave a 1:1 adduct with MAO; inactivation was concomitant with flavin reduction; and low concentrations of β-mercaptoethanol had no effect on inactivation kinetics.

The mechanism for inactivation of MAO by N-CαMBA is expected to be the same as that for N-CBA (Scheme I, R = CH_3) (R. B. Silverman and M. L. Vazquez, unpublished results); the principal differences in the overall reaction should arise as a result of the pK_a difference of the α-protons discussed above and a steric effect, both effects produced by the α-methyl group. The first obvious difference between N-CBA and N-CαMBA, as shown in Table I, is the stoichiometry of binding. As observed previously (R. B. Silverman and M. L. Vazquez, unpublished results), N-[1-^{3}H]CBA leads to the incorporation of several moles of tritium per mole of enzyme

[2] R. B. Silverman and R. B. Yamasaki, unpublished results.

because of a continuous generation of [^{3}H]acrolein during inactivation; this [^{3}H]acrolein most likely alkylates peripheral amino acid residues. In the case of N-[1-^{3}H]CαMBA, however, only 1.2 mol of tritium/mol of enzyme remains bound after inactivation. The α-methyl substituent may be sterically hindering the elimination of [^{3}H]acrolein so that inactivation can occur before much acrolein has been generated. We have found that if incubation of MAO with N-[1-^{3}H]CαMBA continues for a longer period of time, larger stoichiometries are obtained.[3] The lower stoichiometry of labeling obtained when N-CαM[*phenyl*-^{14}C]BA was used compared with that from N-C[7-^{14}C]BA (Table I) is consistent with less [*phenyl*-^{14}C]acetophenone generated from the former compound compared with the [7-^{14}C]benzaldehyde produced by the latter. These radioactive carbonyl compounds may be involved in nonspecific addition reactions with protein nucleophiles. In order to determine the effect of α-methylation on the partitioning between benzyl oxidation, which leads to the formation of cyclopropylamine and either benzaldehyde or acetophenone depending on which compound is used, and cyclopropyl oxidation, which leads to inactivation and acrolein (R. B. Silverman and M. L. Vazquez, unpublished results), parallel experiments were carried out with N-[1-^{3}H]CαMBA vs. N-[1-^{3}H]CBA and N-CαM[*phenyl*-^{14}C]BA vs. N-C[7-^{14}C]-BA. As shown in Table I, the partition ratio of 1.00 for N-CBA drops to 0.012 when it is α-methylated. Since the pK_a for methylated compounds is, in general, about 2.0 units highers than that for the corresponding parent compound (Jones, 1973), the partition ratio drop for N-CαMBA to only 1% of that for N-CBA is reasonable. The same amount of benzyl oxidation was determined when N-CαM[*phenyl*-^{14}C]BA was the inactivator as when N-[1-^{3}H]CαMBA was used, indicating the reproducibility of the reactions (each of these experiments was carried out twice). In order to obtain these values, the amount of ^{14}C-labeled non-amines (presumably acetophenone) produced from N-CαM[*phenyl*-^{14}C]BA was compared to the amount of N-[1-^{3}H]cyclopropylamine produced from N-[1-^{3}H]CαMBA. The amount of benzyl oxidation for N-CBA, however, appeared to be almost 2.5 times greater when the amount of ^{14}C-labeled non-amines (benzaldehyde) from N-C[7-^{14}C]BA was compared to the amount of N-[1-^{3}H]cyclopropylamine produced from N-[1-^{3}H]CBA. Most likely, the source of this higher [^{14}C]benzaldehyde value derives from the normal enzyme-catalyzed conversion of the [^{14}C]benzylamine (produced during cyclopropyl oxidation of N-C[7-^{14}C]BA) to [^{14}C]benzaldehyde and is anomalous. The amount of cyclopropyl oxidation, as determined from the amount of α-methyl[^{14}C]benzylamine produced from N-CαM[*phenyl*-^{14}C]BA, was similar to the amount of cyclopropyl oxidation determined from the amount of [^{3}H]acrolein plus enzyme-bound radioactivity by using N-[1-^{3}H]CαMBA. The benzyl/cyclopropyl oxidation ratio calculated from the experiments with N-CαM[*phenyl*-^{14}C]BA (0.017) is quite similar to that obtained from N-[1-^{3}H]-CαMBA (0.012). The amount of α-methyl[^{14}C]benzylamine produced from N-CαM[*phenyl*-^{14}C]BA is 1.44 times the amount of enzyme inactivated. This corresponds well to the 1.20 equiv of tritium bound to the enzyme when N-[1-^{3}H]-CαMBA is used as the inactivator. All of the results summarized in Table I suggest that α-methylation has a profound effect on the partition ratio. However, the metabolite problem was not eliminated, although it was reduced by 98.8% (based on the N-[1-^{3}H]CαMBA results). The inhibitor results with *d*- and *l*-α-methylbenzylamine suggest a total solution to the problem. Since MAO does not have a significant preference for binding the chiral α-methyl molecules (vide supra), it seemed reasonable that α,α-dimethyl analogues also should bind well to MAO. α,α-Dimethylation would eliminate any possibility for α-proton removal and, therefore, should prevent pathway b of Scheme I from occurring; the only pathway possible would be that leading to inactivation. Although the K_i at pH 9.0 for N-C$\alpha\alpha$DMBA (323 μM) was higher than that for N-CαMBA (44 μM), it was slightly lower than the K_m for benzylamine (380 μM). The k_{cat} at saturation for N-C$\alpha\alpha$DMBA (4.0 min^{-1}), however, was 16 times greater than that for N-CαMBA (0.25 min^{-1}), albeit only $^1/_{67}$ that for benzylamine oxidation. One major difference between the properties of N-C$\alpha\alpha$DMBA and N-CαMBA became obvious from experiments involving reactivation of inactivated MAO by benzylamine. N-CBA (Silverman & Hoffman, 1980a) and N-CαMBA-inactivated MAO are completely reactivated by treatment with benzylamine. However, when N-C$\alpha\alpha$DMBA-inactivated MAO is treated with benzylamine, only about two-thirds of the enzyme activity returns. Consistent with this observation, the flavin in MAO, which is completely reduced by treatment with N-C$\alpha\alpha$DMBA, is only partially reoxidized by benzylamine and urea treatment (Figure 2); complete flavin reoxidation occurs when N-CBA- or N-CαMBA-inactivated MAO is treated with benzylamine or urea. Unlike N-CBA and N-CαMBA, which were shown to become attached to a residue other than the flavin,[3] this is evidence for, at least, partial attachment of N-C$\alpha\alpha$DMBA to the flavin. This observation is similar to that found for inactivation of MAO by 1-phenylcyclopropylamine.[4] The additional methyl group of N-C$\alpha\alpha$DMBA may have an effect on the conformation of the enzyme or on the geometry of the intermediate so that attack by the purported flavin radical at the active site becomes feasible part of the time.

The effect of the α-methyl group also was observed in the attempted reduction of the proposed Schiff base enzyme adduct (**1**, Scheme I, R = CH_3) by sodium cyanoborohydride. In the case of N-(1-methyl)C[7-^{14}C]BA (Silverman & Yamasaki, 1984), sodium cyanoborohydride reduction of the inactivated enzyme led to irreversible binding of 1 equiv of radioactivity. With N-CαM[*phenyl*-^{14}C]BA, however, there was no insignificant difference in the amount of radioactivity bound whether or not sodium cyanoborohydride treatment was carried out. This suggests that the Schiff base formed by N-CαMBA inactivation (**1**, Scheme I, R = CH_3) is unstable and the hydrolysis equilibrium lies on the side of aldehyde and α-methylbenzylamine (a product of the inactivation). The steric effect of the α-methyl substituent may be responsible for this change in equilibrium.

Often when designing new specific enzyme inhibitors it is necessary to add substituents to the parent inactivator molecule for purposes of increasing its binding to a specific enzyme; however, in so doing, alternate undesired enzyme-catalyzed reactions become available. The results described here suggest that, through an understanding of the enzyme mechanism and application of basic chemical principles, it may be possible to control these side reactions and increase the efficacy of an enzyme inhibitor.

References

Abeles, R. H., & Maycock, A. L. (1976) *Acc. Chem. Res. 9*, 313–319.

[3] R. B. Silverman and M. L. Vazquez, unpublished results.

[4] R. B. Silverman and P. A. Zieske, unpublished results.

Alles, G., & Heegaard, E. V. (1943) *J. Biol. Chem. 147*, 487–503.

Baldessarini, R. J. (1977) *Chemotherapy in Psychiatry*, Harvard University Press, Cambridge, MA.

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

Blaschko, H. (1952) *Pharmacol. Rev. 4*, 415–458.

Chuang, H. Y. K., Patek, D. R., & Hellerman, L. (1974) *J. Biol. Chem. 249*, 2381–2384.

Danzin, C., Casara, P., Claverie, N., Metcalf, B. W., & Jung, M. J. (1983) *Biochem. Biophys. Res. Commun. 116*, 237–243.

Fuller, R. W., Hemrich, S. K., & Mills, J. (1978) *Biochem. Pharmacol. 27*, 2255–2261.

Fuller, R. W., Hemrich-Luecke, S. K., & Molloy, B. B. (1983) *Biochem. Pharmacol. 32*, 1243–1249.

Jones, J. R. (1973) *The Ionisation of Carbon Acids*, p 29, Academic Press, London.

Kaiser, C., & Setler, P. E. (1981) in *Burger's Medicinal Chemistry* (Wolff, M. E., Ed.) 4th ed., Part III, pp 1000–1001, Wiley, New York.

Kitz, R., & Wilson, I. B. (1962) *J. Biol. Chem. 237*, 3245–3249.

Klinman, J. P., & Krueger, M. (1982) *Biochemistry 21*, 67–75.

Kutter, E., & Hansch, C. (1969) *J. Med. Chem. 12*, 647–652.

Lewis, F. D., & Ho, T. (1980) *J. Am. Chem. Soc. 102*, 1751–1752.

Lindsay Smith, J. R., & Mead, L. A. V. (1973) *J. Chem. Soc., Perkin Trans. 2*, 206–210.

Lindsay Smith, J. R., & Masheder, D. (1976) *J. Chem. Soc., Perkin Trans. 2*, 47–51.

Long, R. F., Mantle, T. J., & Wilson, K. (1976) *Biochem. Pharmacol. 25*, 247–252.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Mann, C. K., & Barnes, K. K. (1970) *Electrochemical Reactions in Nonaqueous Systems*, Chapter 9, Marcel Dekker, New York.

Masui, M., & Sayo, H. (1971) *J. Chem. Soc. B*, 1593–1596.

Mills, J., Kattau, R., Slater, I. H., & Fuller, R. W.(1968) *J. Med. Chem. 11*, 95–97.

Murphy, D. L., Donnelly, C. H., Richelson, E., & Fuller, R. W. (1978) *Biochem. Pharmacol. 27*, 1767–1769.

Rando, R. R. (1974) *Science (Washington, D.C.) 185*, 320–324.

Rowan, P. R., Paykal, E. S., Parker, R. R., Gatehouse, J. M., & Rao, B. M. (1981) *Monoamine Oxidase Inhibitors—The State of the Art* (Youdim, M. B. H., & Paykal, E. S., Eds.) Wiley, Chichester, England.

Salach, J. I. (1979) *Arch. Biochem. Biophys. 192*, 128–137.

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

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

Silverman, R. B., & Hoffman, S. J. (1980b) *J. Am. Chem. Soc. 102*, 7126–7128.

Silverman, R. B., & Hoffman, S. J. (1981) *J. Labelled Compd. Radiopharm. 18*, 781–790.

Silverman, R. B., & Hoffman, S. J. (1984) *Med. Res. Rev. 4*, 415–447.

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

Simpson, J. T., Krantz, A., Lewis, F. D., & Kokel, B. (1982) *J. Am. Chem. Soc. 104*, 7155–7161.

Smith, P. A. S., & Loeppky, R. N. (1967) *J. Am. Chem. Soc. 89*, 1147–1157.

Tabor, C. W., Tabor, H., & Rosenthal, S. M. (1954) *J. Biol. Chem. 208*, 645–661.

Tyrer, P. (1976) *Br. J. Psychiatry 128*, 354.

Walsh, C. (1982) *Tetrahedron 38*, 871–909.

Winn, M., Horrom, B. W., Rasmussen, R. R., Chappell, E. B., & Plotnikoff, N. P. (1975) *J. Med. Chem. 18*, 437–438.