filtrate. Soluble mercury salts were removed by passing H_2S into the solution. The black suspension was filtered, and the filtrate was evaporated. The residue was dissolved in $CHCl_3$, washed with H_2O, and dried. Evaporation of the solvent left a residue that was chromatographed on silica gel with $CHCl_3$–10% CH_3OH as the eluant. There was obtained 500 mg (55%) of phenol **13**, mp 229–230 °C, after crystallization from ethanol. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 2.33 (s, 3 H, NCH_3), 3.78, 3.85 (2 s, 6 H, 2 OCH_3), 4.50 (s, 1 H, H-5β), 5.13 (s, 1 H, OH), 5.20, 6.10 (dd, 2 H, vinyl H), 6.40–7.16 (m, 7 H, aromatic 6 H, 1 OH). Anal. ($C_{31}H_{27}NO_5 \cdot 0.5H_2O$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(propargyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydrothebaine (14). To a solution of 400 mg (0.78 mmol) of phenol 14 and 35 mg (0.87 mmol) of NaOH in 25 mL of C_2H_5OH was added 100 mg (0.84 mmol) of propargyl bromide. The solution was refluxed for 48 h and evaporated to dryness, and the residue was chromatographed on a silica gel column with $CHCl_3$–1% CH_3OH as the eluant. There was obtained 200 mg (47%) of the propargyl ether, mp 162–163 °C, after recrystallization from heptane. NMR (R-600): δ 1.05 (s, 3 H, CH_3), 2.33 (s, 3 H, NCH_3), 2.36 (m, 1 H, C≡CH), 3.73–3.83 (2 s, 6 H, 2 OCH_3), 4.50–4.70 (m, 3 H, OCH_2, H-5β), 4.96 (s, 1 H, OH), 5.30–6.10 (dd, 2 H, vinyl H), 6.33–7.23 (m, 6 H aromatic H). IR (KBr): 3450 cm^{-1} (OH). Anal. ($C_{34}H_{39}NO_5$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(allyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydronorthebaine (8). A solution of 2.0 g (3.7 mmol) of allyl ether **7** and 1.06 g (6 mmol) of ethyl azodicarboxylate in 60 mL of dry benzene was refluxed for 5 h. The benzene was removed in vacuo, and the residue was treated with 8.0 mL of H_2O, 8.0 mL of C_2H_5OH, and 6.0 mL of saturated NH_4Cl. After being heated for 7 h, the mixture was evaporated to dryness in vacuo, neutralized with $NaHCO_3$ solution, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried, and evaporated to leave a residue that was chromatographed on silica gel with ethyl acetate as the developing solvent to furnish a crude solid, wt 1.2 g (60%). It formed a crystalline fumarate salt, mp 248–250 °C. NMR (R-600) (free base): δ 1.00 (s, 3 H, CH_3), 3.76–3.80 (2 s, 6 H, 2 OCH_3), 4.40–4.66 (d, 3 H, OCH_2, H-5β), 5.10–6.33 (m, 5 H, vinyl H and allylic H), 6.30–7.30 (m, 6 H, aromatic). Anal. ($C_{33}H_{39}NO_5 \cdot C_4H_4O_4$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(allyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydro-*N*-(cyclopropylmethyl)northebaine (9). A suspension of 970 mg (1.8 mmol) of the nor compound (**8**), 1.5 g (10 mmol) of NaI, 1.06 (0.01 m) of Na_2CO_3, and 400 mg (2.96 mmol) of cyclopropylmethyl bromide in 30 mL of 10% aqueous acetone was heated under reflux with stirring for 3 h. The mixture was poured onto H_2O, and the whole was extracted with CH_2Cl_2. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated to leave an oil that was chromatographed on silica gel with ethyl acetate–hexane (1:1) as the eluant. There was obtained 770 mg (77%) of the desired product, **9**, mp 111–112 °C. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 3.76–3.83 (2 s, 6 H, 2 OCH_3), 4.40–4.63 (d, 3 H, OCH_2, H-5β), 4.93 (s, 1 H, OH), 5.10–6.33 (m, 5 H, vinyl H and allylic H), 6.30–7.30 (m, 6 H, aromatic H). IR (KBr): 3480 cm^{-1} (OH). Anal. ($C_{37}H_{45}NO_5$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-[3-(bromomercurio)-2-methoxypropoxy]phenyl]propyl]-6,14-*endo*-ethenotetrahydro-*N*-(cyclopropylmethyl)northebaine (11). A solution of 790 mg (1.35 mmol) of allyl ether **9** in 10 mL of CH_3OH was added to a solution of 430 mg (1.35 mmol) of $Hg(OOCCH_3)_2$ in 10 mL of CH_3OH. The mixture was stirred for 3 days at room temperature and was then treated with a solution of 160 mg (1.35 mmol) of KBr in 10 mL of H_2O. The mixture was heated for 30 min, poured into H_2O, and extracted with $CHCl_3$. Chromatography (silica gel with $CHCl_3$ as the eluant) furnished the desired product: wt 350 mg (29%); mp 78–80 °C. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 3.33 (s, 3 H, OCH_3), 3.73, 3.80 (2 s, 6 H, 2 OCH_3), 3.90 (2 H, OCH_2), 4.46 (s, 1 H, H-5β), 4.87 (s, 1 H, OH), 5.26–6.00 (m, 2 H, vinyl H), 6.43–7.16 (m, 6 H aromatic). IR (KBr): 3460 cm^{-1} (OH). Anal. ($C_{38}H_{48}O_6BrHgN$) C, H, N.

Reaction of 10 with 1,2-Dithioglycerol (BAL). A solution of 50 mg (0.0585 mmol) of **10** and 7.3 mg (0.0587 mmol) of 1,2-dithioglycerol in 2 mL of $CHCl_3$ was stirred overnight at room temperature. The suspension was filtered and the filtrate was evaporated to dryness, leaving an oily residue that was chromatographed on silica gel with ethyl acetate–MeOH (19:1) as the eluant. There was obtained 28 mg (88%) of the allyl ether, **7**, mp 159–161 °C, identical in all respects (mixed melting point IR, NMR) with an authentic sample.

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Registry No. **1**, 98704-48-4; **2**, 149-30-4; **3**, 98704-49-5; **4a**, 98704-50-8; **4b**, 98704-51-9; **5**, 15358-22-2; **7**, 95596-67-1; **8**, 98704-52-0; **8**·fumarate, 98719-88-1; **9**, 98704-53-1; **10**, 98704-59-7; **11**, 98704-60-0; **12**, 98704-54-2; **13**, 98704-55-3; **14**, 98704-56-4; 2-[4-(allyloxy)phenyl]ethyl alcohol tosylate, 98704-57-5; 2-[4-(allyloxy)phenyl]ethyl alcohol, 98704-58-6; allyl phenyl ether, 1746-13-0; allyl bromide, 106-95-6; 2-(*p*-hydroxyphenethyl) alcohol, 501-94-0; propargyl bromide, 106-96-7; cyclopropylmethyl bromide, 7051-34-5.

1-Benzylcyclopropylamine and 1-(Phenylcyclopropyl)methylamine: An Inactivator and a Substrate of Monoamine Oxidase

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1-Benzylcyclopropylamine (1) and 1-(phenylcyclopropyl)methylamine (2), cyclopropane analogues of phenethylamine, were tested as inactivators for monoamine oxidase (MAO). Compound 1 is a potent competitive reversible inhibitor of the oxidation of benzylamine and also is a mechanism-based inactivator. It requires 2.3 equiv of 1 to inactivate 1 equiv of MAO. The excess equivalents of 1 are converted into benzyl vinyl ketone. A one-electron mechanism of inactivation is proposed. Compound 2 is a substrate for MAO and is converted into 1-phenylcyclopropanecarboxaldehyde without inactivation of the enzyme. Mechanistic consequences are discussed as a result of this observation.

Mitochondrial monoamine oxidase (MAO; E.C. 1.4.3.4) is one of the enzymes responsible for the catabolism of the biogenic amines. Compounds that inhibit MAO exhibit antidepressant activity;[1-3] many substituted cyclopropyl-

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

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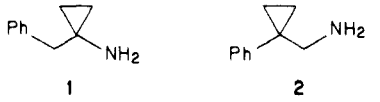
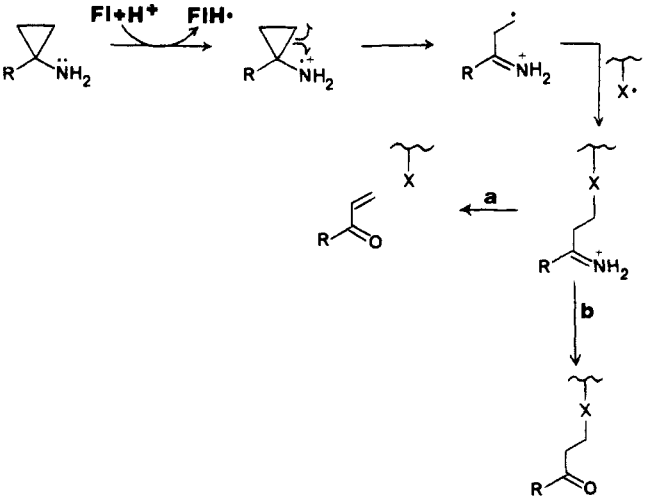
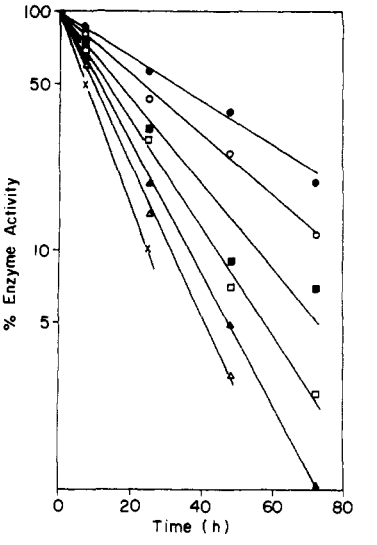
amines have been shown to be potent inhibitors of MAO.[4-13] *trans*-2-Phenylcyclopropylamine is a clinically used antidepressant agent;[1-3] a chemical mechanism for inactivation of MAO by this drug has been proposed.[10] It was shown that *trans*-2-phenylcyclopropylamine[10] and other cyclopropylamines[9,12-14] are mechanism-based inactivators of MAO. A mechanism-based inactivator[15-18] is an unreactive compound that is structurally similar to a substrate or product of the target enzyme and is converted by the target enzyme into a species that, without prior release from the active site, inactivates that enzyme. *trans*-2-Phenylcyclopropylamine is structurally similar to the MAO substrate phenethylamine, where the carbons at positions 1 and 2 comprise part of the cyclopropane ring. Two other phenethylamine derivatives containing cyclopropane rings, namely 1-benzylcyclopropylamine (1) and 1-(phenylcyclopropyl)methylamine (2), were shown indirectly to be MAO inhibitors by measuring their in vivo tryptamine potentiation.[4] The potencies of these compounds relative to *trans*-2-phenylcyclopropylamine were 4% and 2%, respectively. Recently, we found that 1-phenylcyclopropylamine was a mechanism-based inactivator of MAO.[13] In the above study of in vivo tryptamine potentiation,[4] 1-phenylcyclopropylamine had a potency relative to *trans*-2-phenylcyclopropylamine of only 2%, so it was possible that compounds 1 and 2 also may be mechanism-based inactivators of MAO. The mechanism of inactivation by 1 should be similar to that for 1-phenylcyclopropylamine,[13] which is depicted in Scheme I (R = Ph). Compound 2 has the potential to inactivate MAO by a radical mechanism (pathway a, Scheme II) or by a homo-Michael addition[19] after oxidation of the amine to an imine (pathway b, Scheme II). This paper describes our results with these two cyclopropane derivatives of phenethylamine.



1 2

Scheme I. Proposed Mechanism for Inactivation of MAO by 1-Phenylcyclopropylamine (R = Ph) and 1-Benzylcyclopropylamine (R = $PhCH_2$) (R = Ph, X = Amino Acid Residue for Pathway a, X = Reduced Flavin for Pathway b)[13]





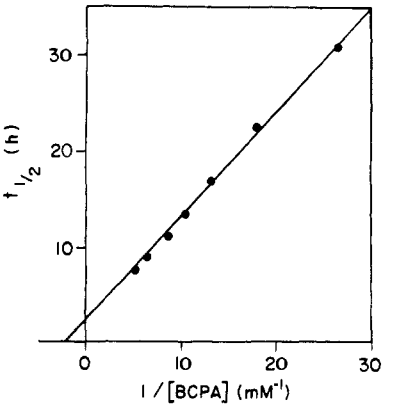


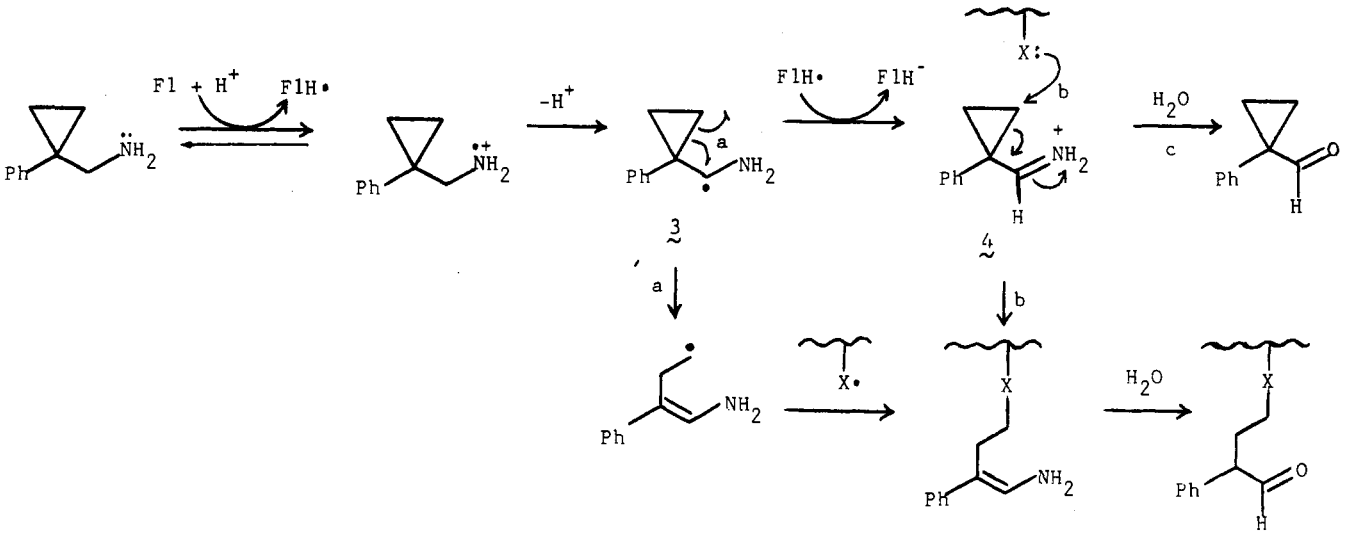
Figure 1. Time-dependent inactivation of MAO by 1. Concentrations (μM) of 1: 38 (●), 56 (○), 75 (■), 94 (□), 112 (▲), 150 (△), 190 (×). See the Experimental Section for the procedure. Below is a Kitz and Wilson[21] replot of the data.

Results and Discussion

1-Benzylcyclopropylamine (1) was a competitive inhibitor and an inactivator of MAO. Its K_i value in competition with oxidation of benzylamine was determined by Lineweaver–Burk analysis[20] to be 51 μM. This compound also

(2) Baldessarini, R. J. "Chemotherapy in Psychiatry"; Harvard University Press: Cambridge, 1977.
(3) Berger, P. A.; Barchas, J. D. In "Psychotherapeutic Drugs"; Usdin, E., Forrest, I. S., Eds.; Marcel Dekker: New York, 1977; Part II.
(4) Zirkle, C. L.; Kaiser, C.; Tedeschi, D. H.; Tedeschi, R. E.; Burger, A. *J. Med. Pharm. Chem.* **1962**, *5*, 1265–1284.
(5) Fuller, R. W.; Hemrick, S. K.; Mills, J. *Biochem. Pharmacol.* **1978**, *27*, 2255–2261.
(6) Long, R. F.; Mantle, T. J.; Wilson, K. *Biochem. Pharmacol.* **1976**, *25*, 247–252.
(7) Murphy, D. L.; Donnelly, C. H.; Richelson, E.; Fuller, R. W. *Biochem. Pharmacol.* **1978**, *27*, 1767–1769.
(8) Winn, M.; Horrom, B. W.; Rasmussen, R. R.; Chappell, E. G.; Plotnikoff, N. P. *J. Med. Chem.* **1975**, *18*, 437–438.
(9) Silverman, R. B.; Yamasaki, R. B. *Biochemistry* **1984**, *23*, 1322–1332.
(10) Silverman, R. B. *J. Biol. Chem.* **1983**, *258*, 14766–14769.
(11) Silverman, R. B.; Hoffman, S. J. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 1396–1401.
(12) Silverman, R. B. *Biochemistry* **1984**, *23*, 5206–5213.
(13) Silverman, R. B.; Zieske, P. A. *Biochemistry* **1985**, *24*, 2128–2138.
(14) Vazquez, M. L.; Silverman, R. B. *Biochemistry* **1985**, *24*, 6538.
(15) Silverman, R. B.; Hoffman, S. J. *Med. Res. Rev.* **1984**, *4*, 415–447.
(16) Walsh, C. *Ann. Rev. Biochem.* **1984**, *53*, 493–535.
(17) Abeles, R. H.; Maycock, A. L. *Acc. Chem. Res.* **1976**, *9*, 313–319.
(18) Rando, R. R. *Science (Washington, D.C.)* **1974**, *185*, 320–324.
(19) Danishefsky, S. *Acc. Chem. Res.* **1979**, *12*, 66–72.

Scheme II. Possible Pathways for the Reaction of MAO with 1-(Phenylcyclopropyl)methylamine (X = Amino Acid Residue or Reduced Flavin)



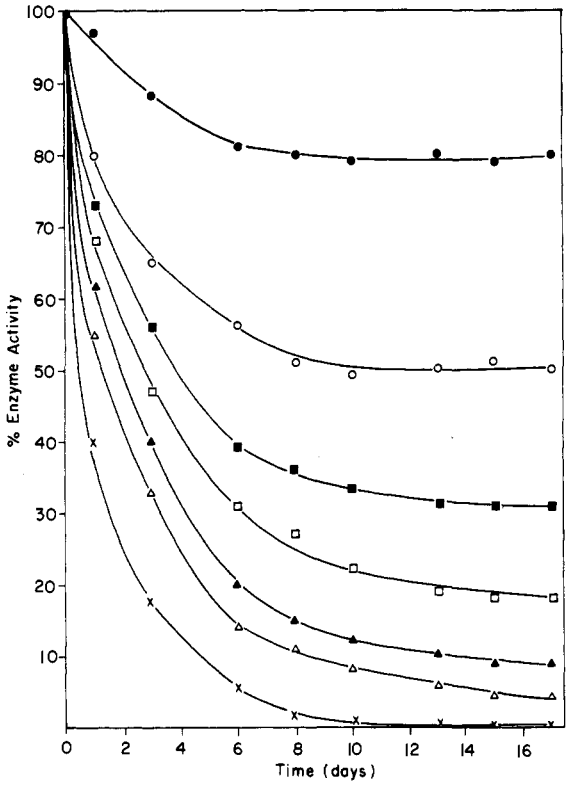


Figure 2. Extended time course for the reaction of MAO with various concentrations of 1. Inactivator/enzyme ratios: 0.5 (●), 1.0 (○), 1.5 (■), 2.0 (□), 2.5 (▲), 3.0 (△), 5.0 (×).

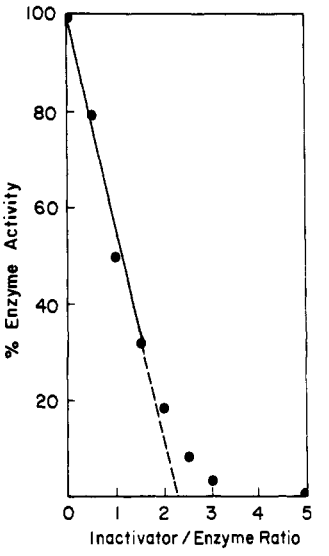


Figure 3. Determination of the turnover number during inactivation of MAO by 1. The data are plotted for enzyme activity remaining, relative to a noninactivator control, after 17 days vs. the inactivator/enzyme ratio (these values were obtained from Figure 2).

was a time-dependent inactivator of MAO (Figure 1); the enzyme was protected from inactivation by the substrate benzylamine. From a plot[21] of $t_{1/2}$ vs. 1/[I] (Figure 1, bottom) the K_i and k_{inact} values for inactivation at saturation were 480 μM and 0.005 min^{-1}, respectively. Upon dialysis, part of the enzyme activity was regenerated. This observation is reminiscent of that observed with 1-phenylcyclopropylamine, which was shown to produce a mixture of irreversible and reversible inhibition.[13] Consequently, further comparisons of the two inactivators were made. Inactivation of MAO by 1 was carried out with molar ratios of inactivator to enzyme between 0 and 5 over an extended time period (Figure 2). The gradual loss of enzyme activity reached a constant level that was dependent upon the inactivator concentration. A plot of enzyme activity at the leveled value vs. the number of equivalents of 1 (Figure 3) indicates that 2.3 equiv of 1 are required for complete irreversible inactivation of the enzyme. The deviation from linearity in Figure 3 at higher inactivator concentrations probably results from inhibition of inactivation by the metabolite. This behavior parallels that reported for 1-phenylcyclopropylamine in which case 8 equiv of inactivator were expended for each irreversible inactivation event.[13] Concomitant with inactivation, the optical spectrum of the FAD cofactor was converted to its reduced form. Depending upon the number of equivalents of 1 used and the time of incubation, dialysis or denaturation resulted in partial or no return of the spectrum to that of oxidized FAD. This suggests that irreversible inactivation leads to flavin attachment. After inactivation, a single metabolite was isolated and identified as benzyl vinyl ketone. This result, again, is similar to that observed with 1-phenylcyclopropylamine in which case acrylophenone was produced from the 7 equiv of inactivator that did not remain bound to the enzyme.[13] On the basis of

(20) Segel, I. H. "Enzyme Kinetics"; Wiley: New York, 1975.
(21) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245–3249.

these results and the results obtained with 1-phenylcyclopropylamine,[13] the mechanism proposed for inactivation of MAO by 1 is shown in Scheme I (R = $PhCH_2$; X = reduced flavin).

1-(Phenylcyclopropyl)methylamine (2) also was a potent competitive inhibitor of benzylamine oxidation. Because its general backbone structure is the same as that for 1 (both phenethylamine analogues), they apparently bind quite similarly; the K_i value for 2 was 50 μM, which is identical with that for 1. No time-dependent inactivation of MAO, however, was observed for 2 at a concentration of 1 mM for 75 h. It was an excellent substrate, though, having K_m and k_{cat} values of 40 μM and 91 min^{-1}, respectively. The corresponding kinetic values for phenethylamine were determined[22] to be 75 μM and 240 min^{-1}. A single product was isolated from the oxidation of 2 and was identified as 1-phenylcyclopropanecarboxaldehyde. This indicates that oxidation of 2 does not lead to cyclopropane ring opening and provides evidence against both pathways a and b in Scheme II and evidence for pathway c. Although the cyclopropylmethyl radical generally leads to homolytic cyclopropane ring fission,[23] this is not always the case,[24a,b] especially when the cyclopropyl methyl radical is adjacent to a heteroatom.[24c] Since the second electron transfer in amine oxidation is a rapid process,[25] it is not surprising that oxidation of 3 (Scheme II) to the imine of 1-phenylcyclopropanecarboxaldehyde (4) competes favorably with ring cleavage. Pathway b may be disfavored for kinetic reasons as well. Although nucleophiles add to cyclopropanes in homoconjugation with carbonyl substituents, this reaction is not facile unless two reasonance-stabilizing groups are attached.[19] Phenyl, apparently, is not sufficiently stabilizing to permit nucleophilic addition to compete with release from the active site. The exclusive pathway, then, becomes that of pathway c, simple amine oxidation followed by hydrolysis.

In conclusion, then, 1-benzylcyclopropylamine (1) is a mechanism-based inactivator of MAO whose mechanism of inactivation resembles that of 1-phenylcyclopropylamine. 1-(Phenylcyclopropyl)methylamine (2) is an excellent substrate which is converted to 1-phenylcyclopropanecarboxaldehyde without enzyme inactivation.

Experimental Section

Analytical Methods. MAO activity assays were carried out on a Perkin-Elmer Lambda 1 UV/vis spectrophotometer. Optical spectra were recorded with semimicro cuvettes in a Perkin-Elmer 330 UV/vis spectrophotometer equipped with a Perkin-Elmer Data Station.

Reagents. 1-Phenylcyclopropanecarbonitrile, cyclopropyl cyanide, benzyl bromide, diisobutylaluminum hydride, phenylacetaldehyde, and vinylmagnesium bromide were obtained from Aldrich Chemical Co. Benzylamine hydrochloride was prepared by bubbling hydrogen chloride gas through an anhydrous ethereal solution of benzylamine and then recrystallizing the salt from ethanol. 1-Benzylcyclopropyl amine (1) was prepared by the method of Kaiser et al.[26] starting from cyclopropyl cyanide and benzyl bromide. 1-(Phenylcyclopropyl)methylamine (2) was prepared by the lithium aluminum hydride reduction of 1-phenylcyclopropanecarbonitrile using the method of Kaiser et al.[26] 1-Phenylcyclopropanecarboxaldehyde was prepared from 1-phenylcyclopropanecarbonitrile by the method of Schuster and Roberts,[27] except using diisobutylaluminum hydride as the reducing agent instead of lithium aluminum hydride. Benzyl vinyl ketone[28] was prepared via the Grignard reaction of phenylacetaldehyde and vinylmagnesium bromide,[29] followed by oxidation of the alcohol.[30] The (2,4-dinitrophenyl)hydrazone derivatives of these compounds were prepared by the method of Shriner et al.[31]

Enzymes and Assays. These were described previously.[12]

Determination of the Competitive Inhibition Constants for 1 and 2. Aliquots (50 μL) of a solution of MAO (7.5 μM) containing various concentrations of 1 or 2 (0–300 μM) in 40 mM Tris, pH 9.0, buffer were removed and assayed with various concentrations of benzylamine (167, 200, 250, 333, 500, 667 μM) in the same buffer (total volume 500 μL). The competitive inhibition constant, K_i, was obtained from a plot of $1/v$ vs. $1/s$ for each of the inhibitor concentrations.

Determination of the Substrate Constants for 2. The Michaelis constant, K_m, and the catalytic rate constant, k_{cat}, for 2 were determined by the following procedure. MAO (0.75 mM) was incubated with several concentrations of the substrate (0.2–2.0 mM) in a total volume of 500 μL of 50 mM potassium phosphate buffer, pH 7.2, at 25 °C for 3 min. The amount of enzymatic oxidation, as measured by the production of hydrogen peroxide, was determined by the leuco crystal violet method.[32] The production of hydrogen peroxide was linear with time for the incubation period selected.

Time-Dependent Inactivation of MAO by Compounds 1 and 2. MAO (8 μM) containing various concentrations of 1 or 2 (0–1 mM) in 50 mM potassium phosphate, pH 7.2, buffer (500 μL total volume) was incubated at 25 °C in the dark. Aliquots (50 μL) were removed periodically and assayed (total volume 500 μL) for enzyme activity relative to a control containing no inactivator.

Identification of the Nonbound Oxidation Products. Following incubation of the substrates (1 or 2) with MAO (8 μM), the aqueous solutions were extracted several times with ethyl acetate. Thin-layer chromatography of the concentrated extracts were performed on silica gel 60-coated plastic plates (Merck) using two solvent systems (A, 3:1 *n*-hexane/ethyl acetate; B, 1:1 *n*-hexane/ethyl acetate) with benzyl vinyl ketone (R_f^A = 0.27, R_f^B = 0.35) or 1-phenylcyclopropanecarboxaldehyde (R_f^A = 0.47, R_f^B = 0.58) as standards. The extracts were then treated with (2,4-dinitrophenyl)hydrazine reagent,[31] extracted into ethyl acetate, and chromatographed against the (2,4-dinitrophenyl)hydrazone derivatives of benzyl vinyl ketone (R_f^A = 0.28, R_f^B = 0.55) or 1-phenylcyclopropanecarboxaldehyde (R_f^A = 0.40, R_f^B = 0.60). Chromatography of (2,4-dinitrophenyl)hydrazine gave R_f^A = 0.06 and R_f^B = 0.16.

Changes in the Optical Spectrum of MAO during Inactivation by 1. A solution of MAO (8 μM) containing 3.5 mM of 1 in 50 mM potassium phosphate, pH 7.2, buffer (total volume 1.2 mL) was prepared. Aliquots (50 μL) were removed periodically and assayed for enzyme activity (total volume 500 μL). The optical spectrum of the solution was recorded against a control containing no inactivator at these times. Half of each solution (inactivator and control) was exhaustively dialyzed against 25 mM potassium phosphate, pH 7.2, buffer and assayed for enzyme activity, and the optical spectra were recorded. Urea (8 M final concentration) was added to the other portions, and the optical spectra were recorded after 15 min.

(22) Yamasaki, R. B.; Silverman, R. B. *Biochemistry* **1985**, *24*, 6543.
(23) Nonhebel, D. C.; Walton, J. C. "Free Radical Chemistry"; Cambridge University Press: Cambridge, 1974; p 106.
(24) (a) Schuster, D. I.; Roberts, J. D. *J. Org. Chem.* **1962**, *27*, 51–53. (b) Brown, H. C.; Borkowski, M. *J. Am. Chem. Soc.* **1952**, *74*, 1894–1902. (c) van Niel, J. C. G.; Pandit, U. K. *J. Chem. Soc., Chem. Commun.* **1983**, 149–150.
(25) (a) Mann, C. K.; Barnes, K. K. "Electrochemical Reactions in Nonaqueous Systems"; Marcel Dekker: New York, 1970; Chapter 9. (b) Lindsay Smith, J. R.; Masheder, D. *J. Chem. Soc., Perkin Trans. 2* **1976**, 47.
(26) Kaiser, C.; Lester, B. M.; Zirkle, C. L.; Burger, A.; Davis, C. S.; Delia, T. J.; Zirngibl, L. *J. Med. Pharm. Chem.* **1962**, *5*, 1243–1265.
(27) Schuster, D. I.; Roberts, J. D. *J. Org. Chem.* **1962**, *27*, 51–53.
(28) Dana, G.; Thuan, S. L. T.; Gharbi-Bengrons, J. *Bull. Soc. Chim. Fr.* **1974**, *9–10*, 2089–2094.
(29) Rose, C. B.; Taylor, S. K. *J. Org. Chem.* **1974**, *39*, 578–581.
(30) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651–1660.
(31) Shriner, R. L.; Fuson, R. C.; Curtin, D. Y. "The Systematic Identification of Organic Compounds", 5th ed.; Wiley: New York, 1964; p 126.
(32) Mottola, H. A.; Simpson, B. E.; Gorin, G. *Anal. Chem.* **42**, 410–411.

Determination of the Turnover Number during Inactivation of MAO by 1. MAO (8 mM) containing various concentrations of 1 in 50 mM potassium phosphate, pH 7.2, buffer (300-μL total volume) was incubated at 25 °C in closed tubes in the dark. Aliquots (20 μL) were periodically removed for up to 17 days and assayed for enzyme activity. After 17 days, the control enzyme retained 66% of its original activity. Inactivator/enzyme ratios of 0, 0.5, 1, 1.5, 2, 2.5, 3, and 5 were used.

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Registry No. 1, 27067-03-4; 2, 935-42-2; MAO, 9001-66-5; 1-phenylcyclopropanecarboxaldehyde, 6120-95-2.

A Dipeptide Derived from Kainic and L-Glutamic Acids: A Selective Antagonist of Amino Acid Induced Neuroexcitation with Anticonvulsant Properties

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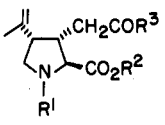
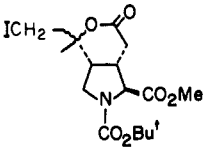
The dipeptide *N*-[[[2′*S*-(2′α,3′β,4′β)]-2′-carboxy-4′-(1″-methylethenyl)-3′-pyrrolidinyl)acetyl]-L-glutamic acid (**6**) has been synthesized by a route that involves the selective protection of the α-carboxyl function of kainic acid. This dipeptide inhibits the stimulation of Na^+ fluxes induced in brain slices by the neuroexcitant *N*-methyl-D-aspartic acid. Administered intracerebroventricularly, it is also effective in protecting mice from picrotoxin-induced convulsions with an ED_{50} of 0.17 μmol.

The finding that γ-dipeptides of the neuroexcitants D- and L-glutamic acids with α-amino carboxylic acids are capable of inhibiting the effects of neuroexcitatory amino acids,[1-3] as well as acting as anticonvulsants,[4] led us to question whether the analogous derivatives of the excitatory dicarboxylic amino acid kainic acid (**1**), namely γ-kainyl derivatives of type **2**, would show similar or modified activities.[5] We are now reporting the synthesis and the pharmacological properties of a member of this class of compounds, γ-kainyl-L-glutamic acid (**6**).





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1 $R^1=R^2=H$, $R^3=OH$
2 $R^1=R^2=H$, $R^3=NHCH(R)CO_2H$
3 $R^1=CO_2Bu^t$, $R^2=H$, $R^3=OH$
4 $R^1=CO_2Bu^t$, $R^2=Me$, $R^3=OH$
5 $R^1=CO_2Bu^t$, $R^2=Me$, $R^3=NHCH(CH_2CH_2CO_2Me)CO_2Me$ (L)
6 $R^1=R^2=H$, $R^3=NHCH(CH_2CH_2CO_2H)CO_2H$ (L)
7 $R^1=R^2=H$, $R^3=NHCH_2CO_2H$

Chemistry. The preparation of γ-dipeptides (**2**) derived from kainic acid (**1**) required the development of a method for the exclusive protection of the α-carboxyl group of kainic acid (**1**). This was achieved by the transient incorporation of the γ-carboxyl group into a δ-lactone ring formed by iodolactonization involving the isopropenyl side chain. The synthetic sequence leading to the dipeptide γ-kainyl-L-glutamic acid (**6**) through the intermediate monoester **4** started with the iodolactonization of the *N*-*tert*-butyloxy derivative (**3**)[6] followed by esterification with diazomethane. The iodolactone **8** was obtained as a mixture of two diastereoisomers.[6] Reduction of this mixture with zinc in aqueous acetic acid regenerated both the isopropenyl side chain and the free γ-carboxyl group. The monoester **4** thus obtained was treated with dimethyl glutamate and *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to give the fully protected dipeptide **5**, which was hydrolyzed and acidolyzed to the desired γ-kainyl-L-glutamic acid (**6**).

Table I. Percent Response to Agonists in the Presence of Antagonists in a $^{22}Na^+$ Efflux Assay[a]

	agonist			
antagonist	30 μM NMDA	0.1 mM KA	0.5 mM L-Glu	0.1 mM Quis
1 mM **6**	16 ± 9**[b]	84 ± 5	90 ± 31	69 ± 11*[b]
1 mM γ-DGG	5 ± 2*[b]	47 ± 14*[b]	71 ± 11*[b]	100 ± 4

[a] For the method by which the stimulation of $^{22}Na^+$ efflux from preloaded rat striatum slices and its inhibition by antagonists were measured see ref 8 and 9. One hundred percent response is the increase in $^{22}Na^+$ efflux rate induced by the given concentration of the agonist in the absence of any antagonist. The data represent the mean response plus or minus standard deviation from at least triplicate experiments. [b] The statistical significance of the difference from control as calculated by variance analysis: *, $p < 0.01$; **, $p < 0.001$.

Biological Results and Discussion. The dipeptide **6** was tested[7] for its ability to block the increase in the permeability of the neuronal membrane to sodium ions produced in rat striatum slices by excitatory amino acids.[8] The excitants used were *N*-methyl-D-aspartic acid (NMDA), kainic acid (KA), L-glutamic acid (L-Glu), and quisqualic acid (Quis), representing four classes of recep-

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(1) Francis, A. A.; Jones, A. W.; Watkins, J. C. *J. Neurochem.* **1980**, *35*, 1458.
(2) Tal, N.; Goldberg, O.; Luini, A.; Teichberg, V. I. *J. Neurochem.* **1982**, *39*, 574.
(3) Jones, A. W.; Smith, D. A. S.; Watkins, J. C. *Neurosci.* **1984**, *13*, 573.
(4) Croucher, M. J.; Collins, J. F.; Meldrum, B. S. *Science (Washington, D.C.)* **1982**, *216*, 899. Croucher, M. J.; Meldrum, B. S.; Jones, A. W.; Watkins, J. C. *Brain Res.* **1984**, *322*, 111.
(5) Some compounds of this type have recently been reported to show anticonvulsant properties: see Collins, J. F.; Dixon, A. J.; Badman, G.; De Sarro, G.; Chapman, A. G.; Hart, G. P.; Meldrum, B. S. *Neurosci. Lett.* **1984**, *51*, 371.
(6) Goldberg, O.; Luini, A.; Teichberg, V. I. *J. Med. Chem.* **1983**, *26*, 39.
(7) For a detailed description of the assay see ref 8 and 9.
(8) Luini, A.; Goldberg, O.; Teichberg, V. I. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3250.

0022-2623/85/1828-1957$01.50/0