Monoamine Oxidase-Catalyzed Oxidative Rearrangement of *trans,trans*-1-(Aminomethyl)-2-methoxy-3-phenylcyclopropane

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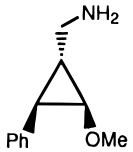
Received August 7, 1996[⊗]

trans,trans-1-(Aminomethyl)-2-methoxy-3-phenylcyclopropane (**3**) was synthesized in three steps from (*Z*)-*β*-methoxystyrene and ethyl diazoacetate. Compound **3** was shown to be a substrate and inactivator of mitochondrial beef liver monoamine oxidase (MAO) with a partition ratio of 1428. MAO-catalyzed oxidation of **3** produces one major metabolite, isolated and identified by GCOSY, GHMQC, and GHMBC NMR techniques to be *trans,trans*-2-methoxy-3-phenyl-1-*N*-[(3-phenyl-*N*-pyrrolyl)methyl]cyclopropane (**7**). A mechanism, supported by a model reaction, is proposed for the formation of this metabolite.

Monoamine oxidase (MAO, EC 1.4.3.4) catalyzes the oxidation of a variety of amine neurotransmitters to the corresponding imine, which becomes hydrolyzed to the aldehyde upon release into the aqueous medium.[1] There is much evidence to support a radical mechanism for MAO.[2] Newcomb and Chestney[3] have reported a clever approach to the differentiation of a radical from a carbocation intermediate in which the incipient high-energy site is generated adjacent to a *trans,trans*-2-methoxy-3-phenylcyclopropyl moiety (Scheme 1). It was shown that when a radical was generated (route A), the cyclopropyl C−C bond to which the phenyl group was attached was the one that broke exclusively, leading to the formation of the benzylic radical (**1**), whereas when a carbocation was generated (route B), the cyclopropyl C−C bond producing the methoxyl-stabilized cation (**2**) broke. We attempted to utilize this approach to gain further evidence for the formation of an α-radical during MAO-catalyzed amine oxidations. Consequently, *trans, trans*-1-(aminomethyl)-2-methoxy-3-phenylcyclopropane (**3**) was synthesized, and the major product generated by incubation with MAO was isolated and its structure determined by heteronuclear NMR techniques using gradient inverse detection methods. Evidence for an intermediate radical was not obtained; however, the product that formed appears to be the result of oxidation of **3** to the corresponding imine, followed by ring cleavage, leading to an unusual rearrangement following transimination with a second molecule of **3**. This paper describes the NMR structure determination of this product and provides evidence for the mechanism by which it forms.



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Results

Synthesis of *trans,trans*-1-(Aminomethyl)-2-methoxy-3-phenylcyclopropane. The route to the synthesis of *trans,trans*-1-(aminomethyl)-2-methoxy-3-phenylcyclopropane (**3**) is shown in Scheme 2. The reaction of (*Z*)-2-methoxystyrene with ethyl diazoacetate in the presence of copper sulfate produced two diastereomers of ethyl 2-methoxy-3-phenylcyclopropanecarboxylate (**4a** and **4b**). The diastereomers were separated, but it was found that treatment with base in the next step converted the *cis,cis*-isomer **4b** to the *trans,trans*-isomer **4a**. Consequently, **4a** was used for the remainder of the synthesis. Two routes to **3** were found to be successful. The shortest route was conversion of the ester to the amide **5** followed by lithium aluminum hydride reduction. Alternatively, the ester was saponified to the carboxylic acid **6**, which was converted to the amide **5** by dicyclohexylcarbodiimide coupling to ammonia. Lithium aluminum hydride reduction of the amide gave **3**.

Monoamine Oxidase B-Catalyzed Oxidation of 3. Compound **3** was a substrate and inactivator of MAO. The K_m and k_{cat} values were determined to be 10.8 mM and 1090 min^{-1}, respectively. As determined by the method of Kitz and Wilson[4] the K_I and k_{inact} values for inactivation are 48.5 mM and 1.31 min^{-1}, respectively. The partition ratio, the number of substrate molecules converted to product per inactivation event, is k_{cat}/k_{inact} = 1428. Because of the high partition ratio, it was thought that it would not be necessary to synthesize **3** in a radiolabeled form; sufficient product could be obtained for NMR structural studies with the unlabeled compound. Incubation of **3** with MAO led to the formation of a precipitate, which was removed by centrifugation. HPLC and TLC analysis of the supernatant indicated that the major component was **3**. Centrifugation and extraction of the precipitate with chloroform gave an insoluble precipitate shown to be denatured protein ($NaDodSO_4$−PAGE electrophoresis showed that it had a mass of 60−70 kDa, consistent with MAO subunits). The chloroform extract was analyzed by reversed phase HPLC and by TLC, and only one metabolite was detected.

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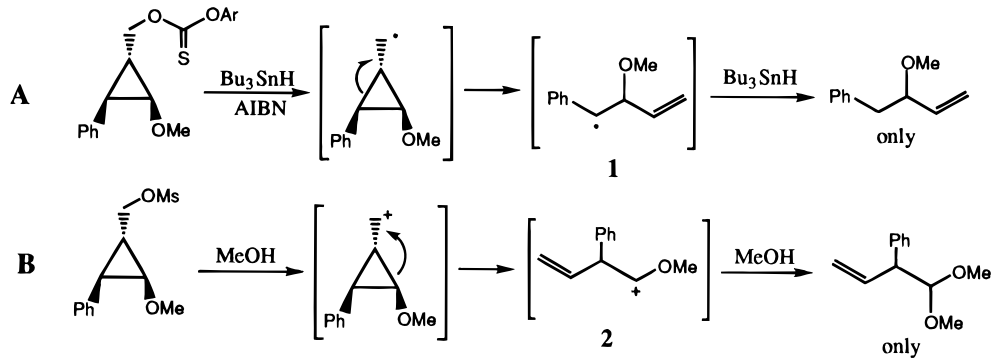
[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.

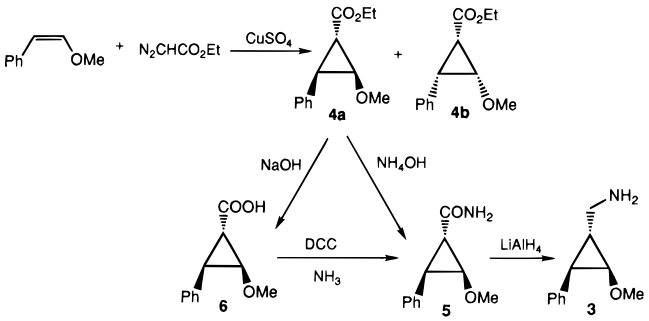
(1) Woo, J. C. G.; Silverman, R. B. *J. Am. Chem. Soc.* **1995**, *117*, 1663−1664.
(2) Silverman, R. B. *Acc. Chem. Res.* **1995**, *28*, 335−342.
(3) Newcomb, M.; Chestney, D. L. *J. Am. Chem. Soc.* **1994**, *116*, 9753−9754.
(4) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245−3249.

S0022-3263(96)01540-X CCC: $12.00 © 1996 American Chemical Society

Scheme 1



Scheme 2



On the basis of proton and ^{13}C NMR spectroscopy and high-resolution mass spectrometry, it was apparent that a dimer of some kind was formed.

NMR Analysis of the Metabolite. Because of the small amount of sample, it took about 20 h of acquisition time to obtain a reasonable ^{13}C NMR spectrum, which indicated the presence of two phenyl groups and two double-bond carbons. The low sensitivity of the spectrum, however, prevented conventional 2D C−H heteronuclear measurements. Inverse detection experiments, which measure the proton nuclear response, though, could be used to improve sensitivity. The sensitivity enhancement was further aided by the application of gradient inverse detection methods in the C−H heteronuclear experiments, which avoid long phase cycling procedures and increase sensitivity.

GCOSY in the aromatic and double-bond region shows three spin systems (Figure 1). The peaks at 7.48, 7.32, and 7.14 ppm and 7.29, 7.23, and 7.19 ppm in the ^1H NMR are from two different phenyl groups; the peaks at 7.07, 6.78, and 6.44 ppm are from double bonds. The peaks at 7.29, 7.23, and 7.19 ppm are very similar to the phenyl group protons of **3**. GCOSY in the 1−5 ppm region shows only one spin system (Table 1); the spin−spin connectivities of the peaks are very similar to those of **3**. The high-resolution mass spectrum suggests that the product is a dimeric compound with the molecular formula $C_{21}H_{21}NO$. Mass fragments indicate the presence of a cyclopropyl group with substituted phenyl and methoxyl groups. The only large chemical shift differences in the metabolite and **3** are reflected in the change in the ^{13}C spectrum (Figure 2) from 42.7 ppm in compound **3** to 51.5 ppm in the metabolite and in the ^1H spectrum (Figure 3) from 2.80 and 2.90 ppm in **3** to 3.95 and 4.10 ppm in the metabolite. Furthermore, irradiation of the proton at 3.47 ppm (on the cyclopropyl group at the carbon with the methoxyl group) gave an NOE at 2.05 ppm (cyclopropyl proton on the carbon with the phenyl group), indicating that these protons are *cis* to

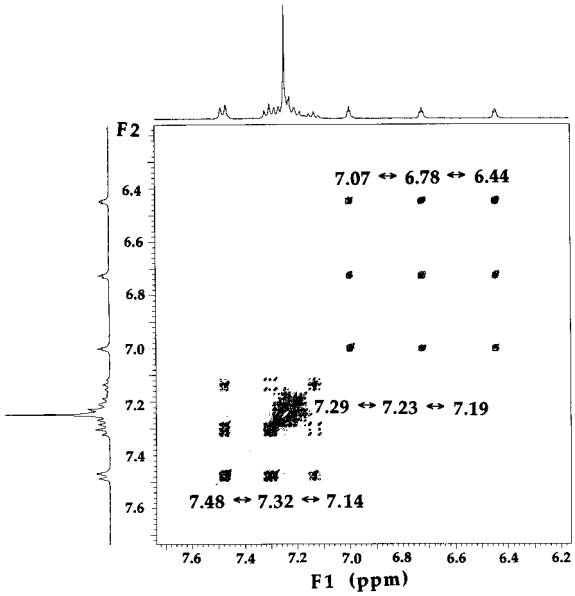


Figure 1. Partial GCOSY spectrum of the metabolite from MAO-catalyzed oxidation of **3**.

Table 1. GCOSY Data (H−H Correlations) for the Metabolite from MAO-Catalyzed Oxidation of 3 (in ppm)[a]

7.48 ↔ 7.32 ↔ 7.14
7.29 ↔ 7.23 ↔ 7.19
7.07 ↔ 6.78 ↔ 6.44
4.10 ↔ 3.95 ↔ 1.89
3.47 ↔ 2.05 ↔ 1.89

[a] The symbol ↔ denotes which protons correlate. See Figure 1.

one another (Table 2). Irradiation at 1.89 ppm produced an NOE at 7.23 ppm, indicating that the cyclopropyl proton on the carbon with the methylene group is *cis* to the phenyl group on the cyclopropyl group. This suggests a similarity of part of the metabolite structure to that of **3** and that the major difference is in the attachment of another moiety at the nitrogen of **3** (Figure 4).

GHMQC experiments were performed to establish C−H connections (Figure 5 and Table 3). Contrary to the one-dimensional ^{13}C NMR spectrum, the GHMQC spectra exhibit an excellent signal-to-noise ratio with an acquisition time of 30 h. To establish links between the separate spin systems, GHMBC experiments were performed (Figure 6 and Table 4). From these studies it is apparent that the proton at 2.05 ppm correlates with the carbon of the phenyl at 128.3 ppm, with the carbon of the aminomethyl group at 51.5 ppm, and with the carbon of the cyclopropyl group at 27.3 ppm. The methoxyl protons at 3.12 ppm correlate with the cyclopropyl carbon

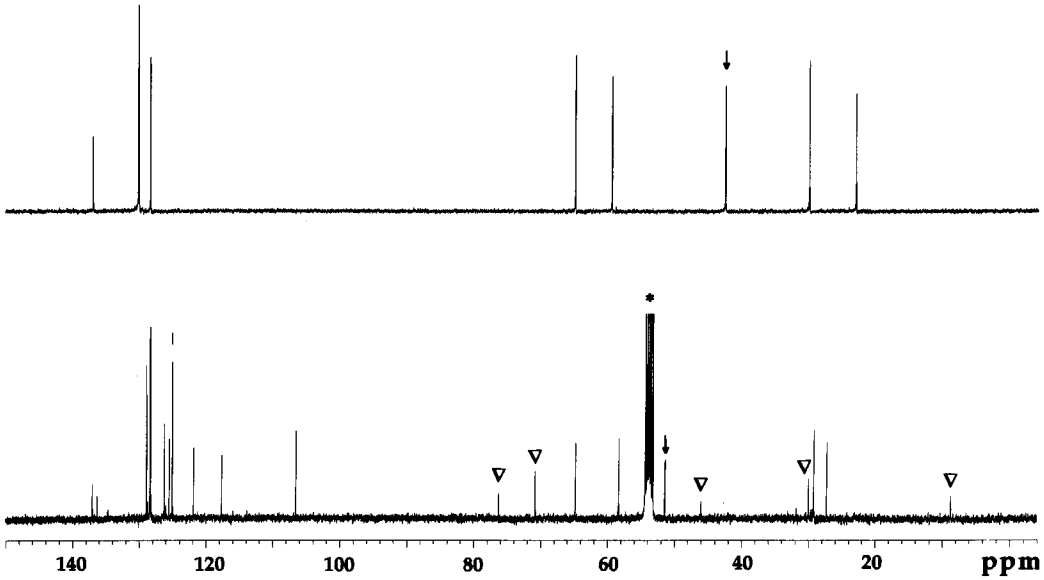


Figure 2. ^{13}C NMR spectra of **3** (top) and the metabolite from MAO-catalyzed oxidation of **3** (bottom). The down arrows indicate the shift in the spectra from 42.7 ppm in **3** to 51.5 ppm in the metabolite; the inverted triangles indicate impurities; the asterisk marks the CD_2Cl_2 carbons.

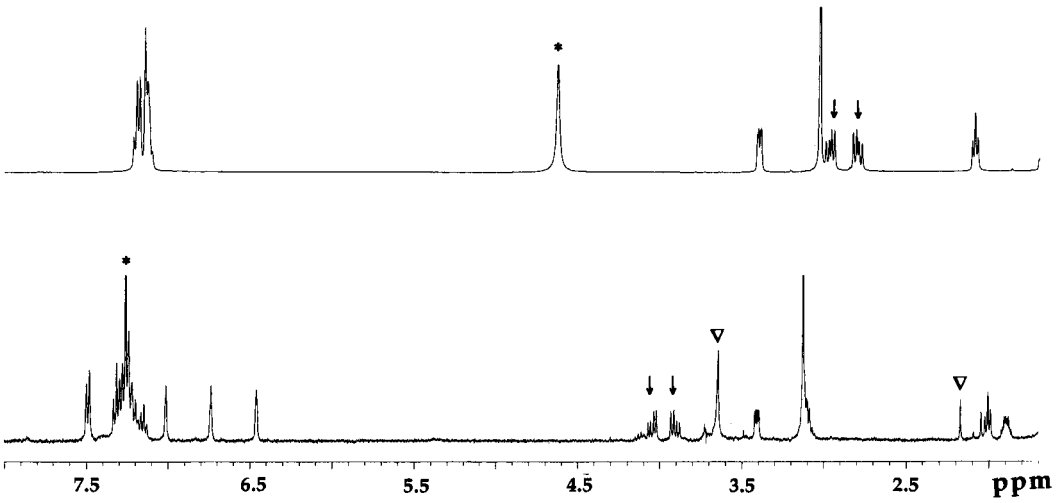


Figure 3. ^1H NMR spectra of **3** (top) and the metabolite from MAO-catalyzed oxidation of **3** (bottom). The down arrows indicate the shift in the spectra from 2.80 and 2.90 ppm in **3** to 3.95 and 4.10 ppm in the metabolite; the inverted triangles indicate impurities; the asterisk in the top spectrum marks the water peak and in the bottom spectrum marks the $CDCl_3$ proton.

Table 2. NOE Data (H−H Space Correlations) for the Metabolite from MAO-Catalyzed Oxidation of 3 (in ppm)

irradiate on 7.48, NOE on 7.32 (8.6%), 7.07 (4.3%), 6.44 (4.9%)
irradiate on 7.07, NOE on 7.48 (12.4%)
irradiate on 6.78, NOE on 6.44 (7.8%)
irradiate on 6.44, NOE on 7.48 (9.4%), 6.78 (8.3%)
irradiate on 3.95, NOE on 7.07 (3.4%), 6.78 (3.2%), 2.05(5.3%)
irradiate on 4.10, NOE on 7.07 (3.2%), 6.78 (4.1%), 3.47 (2.3%), 1.89 (3.4%)
irradiate on 3.47, NOE on 3.15 (3.4%), 2.05 (7.8%)
irradiate on 2.05, NOE on 7.23 (11.8%), 3.47 (10.5%)
irradiate on 1.89, NOE on 7.23 (14.6%)
irradiate on 7.19, NOE on 7.29 (4.9%)

to which the methoxyl group is attached (65.2 ppm). The aminomethylene protons at 3.95 and 4.10 ppm both correlate with the cyclopropyl carbon atoms at 65.2 and 27.3 ppm, but only the 3.95 ppm proton correlates with the 29.3 ppm cyclopropyl carbon. These results support the existence of the starting material structure within the full metabolite structure. Further examination of the spectrum indicates that the protons at 3.95 and 4.10 ppm also are long-range coupled to the two double-bond

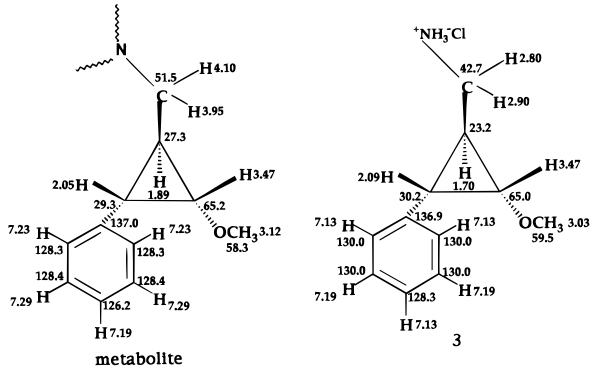
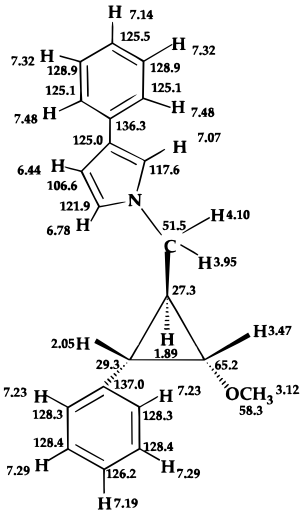


Figure 4. A comparison of the general metabolite structure with that of **3**.

carbons at 117.6 and 121.9 ppm, which suggests that the aminomethyl nitrogen is directly bonded to the two double-bond carbons. Since the GCOSY spectrum shows that the three double-bond protons are spin correlated with each other, it is apparent that a five-membered

pyrrole structure is present. The C−H correlations shown in Figures 5 and 6 and listed in Tables 3 and 4 are consistent with either structure **7** or **8**. Further NOE



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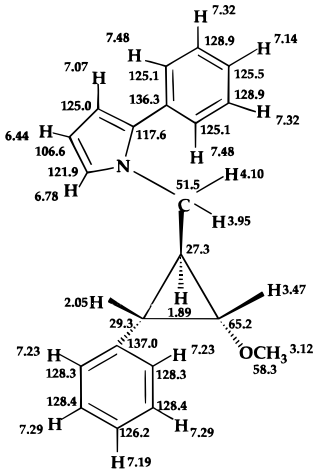

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difference experiments (Table 2) ruled out structure **8**. Irradiation of protons at 7.48 and 6.44 ppm indicates that these protons are close in space, and irradiation of the 7.48 and 7.07 ppm protons shows that they are close. These results are only consistent with structure **7**. Finally, the ^{1}H and ^{13}C chemical shifts of 3-phenylpyrrole were very similar to the chemical shifts of the moiety attached to the nitrogen of **3**.

Model Study for the Formation of 7. To test the hypothesized mechanism for the formation of **7** from the MAO-catalyzed oxidation of **3**, the reaction of 2-phenylsuccinaldehyde with **3** was carried out at −78 °C. Compound **7** was isolated as the major product.

Discussion

trans,trans-1-(Aminomethyl)-2-methoxy-3-phenylcyclopropane (**3**) was synthesized as a potential substrate for MAO to determine if radical character at the α-position could be detected. On the basis of the work of Newcomb and Chestney,[3] if an α-radical was formed and was relatively stable, then aldehyde **9** (Scheme 3) would be expected to be produced; further reaction with **3** might lead to **8**. Whereas α-cation (imine) formation would lead to 2-phenylsuccinaldehyde (**10**), reaction with **3** might give **7** (Scheme 4). It should be noted that **7** is obtained regardless of which aldehyde carbonyl is initially attacked by **3**. This product, however, does not provide direct information regarding potential intermediates in the reaction pathway because it is derived from the expected iminium product.

The molecular weight obtained by high-resolution mass spectrometry of the metabolite formed from MAO-catalyzed oxidation of **3** indicated that a dimeric material was produced. By a combination of GCOSY, GHMQC, and GHMBC homonuclear and heteronuclear NMR techniques (see Figures 1, 5, and 6), a phenyl-substituted pyrrole attached to the 2-methoxy-3-phenylcyclopropane (**7**) was identified. To test the hypothesized mechanism for the formation of **7** (Scheme 4), **3** was allowed to react with 2-phenylsuccinaldehyde (**10**), and **7** was isolated.

The results presented describe an interesting nonenzymatic rearrangement from the MAO-catalyzed oxidation of **3**. If the intermediate α-radical is formed, it does not lead to cyclopropyl ring cleavage prior to second electron oxidation to the product imine. A geometric constraint, as a result of interactions with the active site of the enzyme, theoretically could preclude ring cleavage, although Newcomb and co-workers have found no experimental evidence to support a kinetic effect by an enzyme-induced geometric constraint.[5] Previous studies,[6] however, strongly support the formation of an α-radical in the MAO-catalyzed oxidations of amines.

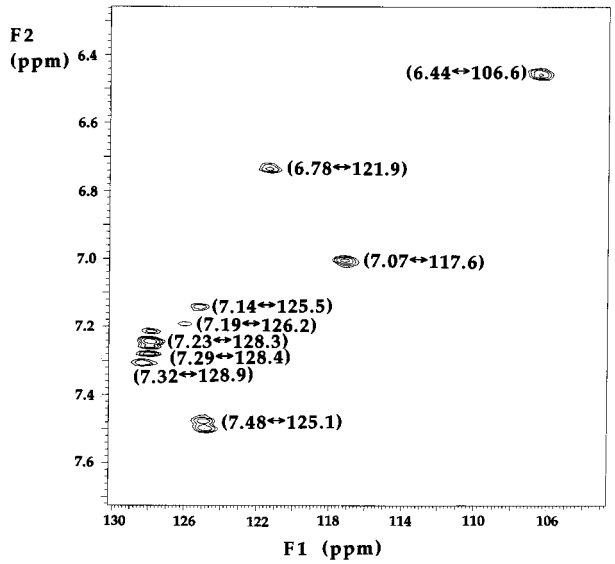


Figure 5. Partial GHMQC spectrum of the metabolite from MAO-catalyzed oxidation of **3**.

Table 3. GHMQC Data (C−H Correlations) for the Metabolite from MAO-Catalyzed Oxidation of 3 (in ppm)[a]

7.48 ↔ 125.1	6.44 ↔ 106.6
7.32 ↔ 128.9	4.10 ↔ 51.5
7.29 ↔ 128.4	3.95 ↔ 51.5
7.23 ↔ 128.3	3.47 ↔ 65.2
7.19 ↔ 126.2	3.15 ↔ 58.3
7.14 ↔ 125.5	2.05 ↔ 29.3
7.07 ↔ 117.6	1.89 ↔ 27.3
6.78 ↔ 121.9	

[a] The symbol ↔ denotes which protons correlate with which carbons. See Figure 5.

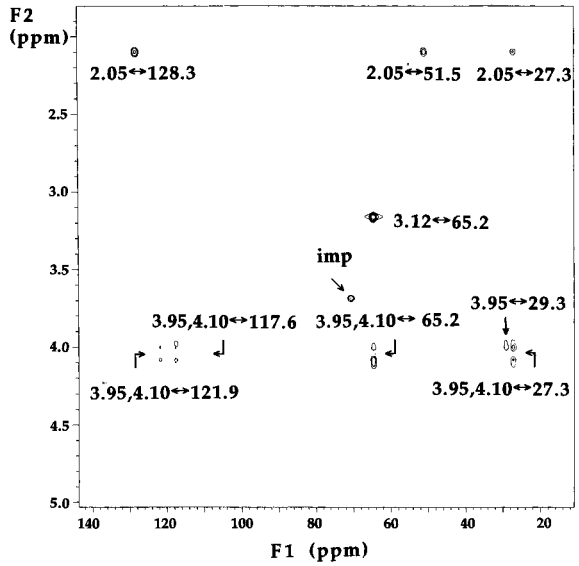


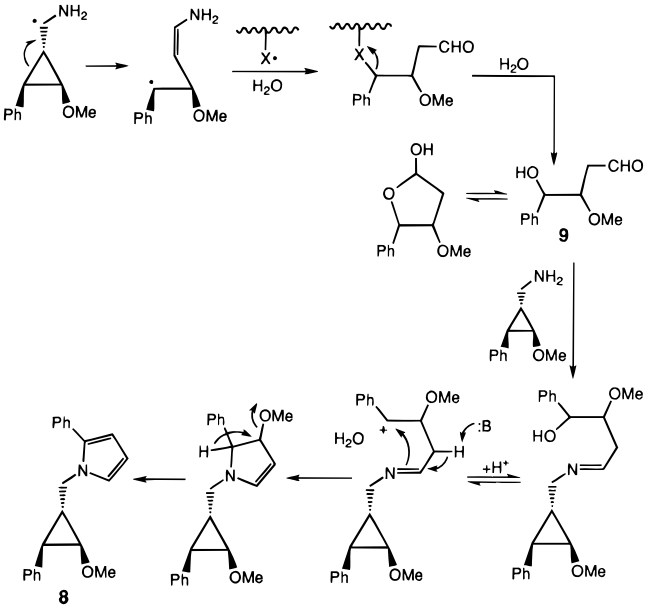
Figure 6. Partial GHMBC spectrum of the metabolite from MAO-catalyzed oxidation of **3**.

Table 4. GHMBC Data (C−H Long-Range Correlations) for the Metabolite from MAO-Catalyzed Oxidation of 3 (in ppm)[a]

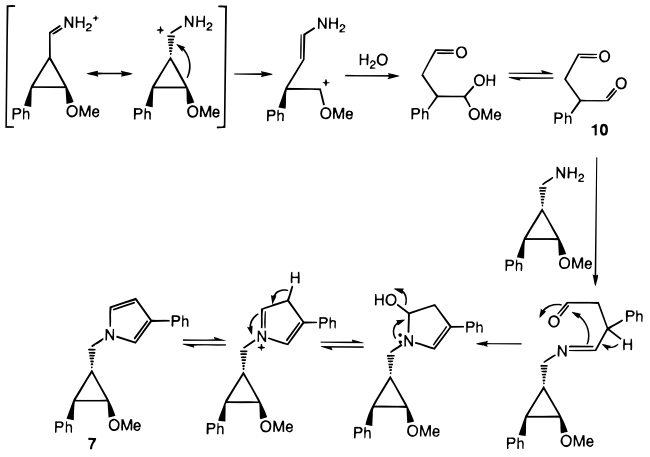
7.48 ↔ 125.0, 125.5, 128.9
7.32 ↔ 136.3, 128.9, 125.1
7.29 ↔ 126.2, 128.3, 137.0
7.23 ↔ 126.2, 128.4
7.14 ↔ 125.1
7.19 ↔ 128.3
7.07 ↔ 125.0, 121.9, 106.6
6.78 ↔ 125.0, 117.6, 106.6
6.44 ↔ 117.6, 121.9

[a] The symbol ↔ denotes which protons correlate with which carbons. See Figure 6.

Scheme 3



Scheme 4



Experimental Section

Reagents. Compounds **4a** and **4b** were synthesized as reported.[7] All reagents were purchased from Aldrich Chemical Co.

***trans,trans*-2-Methoxy-3-phenylcyclopropanecarboxamide (5).** Ammonium hydroxide (20 mL) was added to **4a** (1.0 g, 4.5 mmol), and the mixture was stirred at rt overnight. The precipitate that formed was washed with hexane to give a pale yellow solid (0.78 g, 90%): ^{1}H NMR ($CDCl_3$) δ 7.2−7.4 (m, 5 H), 5.71 (br, 1 H), 5.59 (br, 1 H), 3.86 (dd, 1 H), 3.28 (s, 3 H), 2.71 (t, 1 H), 2.04 (dd, 1 H); HRMS calcd 191.0946, found 191.0930, *m*/*z* 191 (191, 159, 147, 131, 115, 103, 91, 77, 51).

***trans,trans*-1-(Aminomethyl)-2-methoxy-3-phenylcyclopropane (3).** Compound **5** (2.0 g, 10.5 mmol) was reduced with lithium aluminum hydride (1.2 g, 37 mmol) in 25 mL of anhydrous THF at reflux under a positive pressure of nitrogen for 4 h followed by quenching with water in THF. Aqueous 20% sodium hydroxide (4 mL) was added to remove the aluminate salts. The THF solution was extracted with 2 N HCl (3 × 10 mL), the acid extracts were back-extracted with ether (10 mL), the aqueous solution was evaporated to dryness, and the residue was crystallized with ethanol−ethyl acetate to give 1.9 g (85%) of **3**: NMR ($CDCl_3$) δ 8.26 (br, 2 H), 7.2−7.3 (m, 5 H), 3.42 (dd, 1 H), 3.11 (s, 3 H), 2.6−2.8 (br, 2 H), 2.06 (t, 1 H), 1.90 (dd, 1 H). Anal. Calcd for $C_{11}H_{16}ClNO$: C, 61.82; H, 7.49; N, 6.56; Cl, 16.63. Found: C, 61.50; H, 7.49; N, 6.50; Cl, 16.83.

***trans,trans*-2-Methoxy-3-phenylcyclopropanecarboxylic Acid (6).** Compound **4a** (1.0 g, 4.5 mmol) was saponified by the addition of 1.5 g of potassium hydroxide in 10 mL of ethanol at rt with stirring for 4 h. After acidification with 15 mL of 3 N HCl, **6** (0.79 g, 90%) was obtained by extraction into ether followed by evaporation: NMR ($CDCl_3$) δ 7.2−7.4 (m, 5 H), 3.89 (dd, 1 H), 3.35 (s, 3 H), 2.78 (t, 1 H), 2.20 (dd, 1 H); HRMS calcd 192.0786, found 192.0798, *m*/*z* 192 (192, 162, 147, 131, 115, 103, 91, 77, 51).

***trans,trans*-2-Methoxy-3-phenylcyclopropanecarboxamide (5).** A second procedure for the synthesis of **5** is as follows: To a stirred mixture of the compound **6** (192 mg, 1.0 mmol) and *N*-hydroxysuccinimide (138 mg, 1.2 mmol) in methylene chloride (10 mL) at 0 °C was added *N*,*N*′-dicyclohexylcarbodiimide (247 mg, 1.2 mmol). The reaction mixture was stirred for 1.5 h under the same conditions, and 30% aqueous ammonia (166 μL) was added to the stirred solution. The reaction mixture was allowed to continue to stir for 1 h. Dicyclohexylurea, which precipitated during the reaction, was filtered off. The filtrate was evaporated under reduced pressure to dryness, giving **5** (150 mg, 78%).

Diethyl Phenylsuccinate. To a 25-mL round bottom flask fitted with a Dean−Stark trap were added phenylsuccinic acid (1.0 g, 5.15 mmol), ethyl alcohol (5 mL), *p*-toluenesulfonic acid (50 mg), and benzene (10 mL). Following overnight reflux, the benzene solution was washed with saturated sodium bicarbonate (2 × 5 mL) and water (2 × 5 mL) and dried over sodium sulfate. Removal of the solvent resulted in a colorless oil (1.1 g, 86%): ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5 H), 4.13 (m, 5 H), 3.18 (dd,1 H), 2.68 (dd, 1 H), 1.21 (m, 6 H), agrees with the spectrum reported.[8]

Reaction of 3 with 2-Phenylsuccinaldehyde. To a stirred solution of diethyl phenylsuccinate (1.0 g, 4.02 mmol) in dry toluene (10 mL) at −78 °C was added a −78 °C solution of 1.5 M diisobutylaluminum hydride (DIBAL) in toluene (10 mL) via cannula. The rate of addition was adjusted so as to keep the internal temperature below −65 °C over the ca. 45 min addition time. The 2-phenylsuccinaldehyde produced was too unstable to isolate. The reaction mixture was stirred for an additional 3 h at −78 °C, and then the reaction was quenched by slow addition of 200 mg (1 mmol) of compound **3** in 3 mL of anhydrous methanol at −78 °C, again so as to keep the internal temperature below −65 °C. The resulting white emulsion was allowed to warm to rt and was poured into 50 mL of 1 N HCl with swirling over 15 min. The aqueous mixture was then extracted with methylene chloride (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give 50 mg of crude product as a yellow oil (4%). The

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compound was purified by flash chromatography, eluting with 10% ethyl acetate in hexane, and by TLC on silica gel, eluting with methylene chloride: ^{1}H NMR ($CDCl_3$) δ 7.48 (d, 2 H), 7.14−7.32 (m, 8 H), 7.07 (m, 1 H), 6.78 (m, 1 H), 6.44 (m, 1 H), 4.10 (m, 1 H), 3.95 (m, 1 H), 3.47 (m, 1 H), 3.12 (s, 3 H), 2.05 (m, 1 H), 1.89 (m, 1 H); ^{13}C NMR (CH_2Cl_2) δ 129.0, 128.5, 128.4, 126.1, 125.6, 125.2, 122.0, 117.5, 106.7, 65.3, 58.2, 51.5, 29.3, 27.4; HRMS calcd 303.1623, found 303.1661.

Enzyme and Assay. Mitochondrial beef liver MAO was isolated and assayed as previously reported.[9]

MAO-Catalyzed Oxidation of 3. Compound **3** (15 mg) in 1.00 mL of 100 mM sodium phosphate buffer (pH 7.2) was incubated with MAO B (100 μL, 200 μM) at rt for 2 days. The activity of the MAO B, compared with a control experiment without compound **3**, was less than 10%. During incubation the solution gradually turned cloudy; the cloudy mixture was centrifuged in a microcentrifuge to isolate the precipitate. The supernatant was analyzed by reversed phase HPLC using a 5 μm, 4.6 × 250 mm C-18 column, eluting with 40% water in acetonitrile and monitoring at 254 nm. The main component was the substrate. The same result was shown in the TLC analysis, eluting with 1-butanol/H_2O/HOAC (12/5/3) on a silica gel plate with ninhydrin detection.

The precipitate was dried with a speed vac concentrator followed by extraction with $CHCl_3$. The molecular weight of the $CHCl_3$-insoluble material was determined by $NaDodSO_4$ gel electrophoresis to be 60−70 kDa, which is denatured protein. The $CHCl_3$ extract was analyzed by reversed phase HPLC on a C-18 column as above, which showed that only one compound was formed. The extract also was analyzed by silica gel TLC, eluting with ethyl acetate/hexane (20/80) or 1-butanol/H_2O/HOAC (12/5/3), which showed that only one metabolite was present. The extract was washed with 2% HCl to remove some **3** and, after evaporation of the solvent, was analyzed by ^{1}H NMR and ^{13}C NMR: ^{1}H NMR (CD_2Cl_2) δ 7.48 (d, 2 H), 7.32 (m, 2 H), 7.29 (m, 2 H), 7.23 (m, 2 H), 7.19 (m, 1 H), 7.14 (m, 1 H), 7.07 (m, 1 H), 6.78 (m, 1 H), 6.44 (m, 1 H), 4.10 (m, 1 H), 3.95 (m, 1 H), 3.47 (m, 1 H), 3.12 (s, 3 H), 2.10 (m, 1 H), 1.89 (m, 1 H); ^{13}C NMR (CD_2Cl_2) δ 137.0, 136.3, 128.9 (2 C), 128.4 (2 C), 128.3 (2 C), 126.2, 125.5, 125.1 (2 C), 121.9, 117.6, 106.6, 65.2, 58.3, 51.5, 29.3, 27.3. In addition, GCOSY and GHMQC were used for determination of the H−H correlations and C−H correlations, respectively. The molecular ion of the metabolite was determined by HRMS (calcd 303.1623, found 303.1634) or as M + 1 (calcd 304.1681, found 304.1653); the main fragments are 272, 156, 147, 117, 91, 43, and 41.

Acknowledgment. Financial support of this research from the National Institutes of Health (GM 32634) is gratefully acknowledged.

JO961540A

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