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EFFECT OF THE LOCUS OF THE OXYGEN ATOM IN AMINO ETHERS ON THE INACTIVATION OF MONOAMINE OXIDASE B

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Monoamine oxidase is a flavoenzyme that catalyzes the oxidation of a variety of primary, secondary, and tertiary amines. Although primary alkylamines, such as heptylamine, and primary arylalkyl amines, such as phenylethylamine, are excellent substrates for MAO, their analogues having an electron withdrawing group near the aminomethyl methylene group (**1**–**8**) are known to inactivate the enzyme. Inactivation has been attributed to the inductive effect of the electron-withdrawing group of these analogues. To determine the extent of the proposed inductive effect of a heteroatom on MAO B inactivation, a series of oxaheptylamine analogues (**9**–**12**) were synthesized and tested as inactivators of MAO B. The analogues in which the oxygen atom is closest to the alpha-carbon (**9** and **10**) inactivate MAO B, but activity slowly returns with time. The analogues with the oxygen atom farther from the alpha-carbon inactivate the enzyme, but activity rapidly returns. These results support the inductive effect hypothesis for inactivation.

Keywords: Monoamine oxidase B; Oxaheptylamines; Inactivation; Inductive effect; Covalent enzyme adduct

INTRODUCTION

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavin-dependent enzyme responsible for the oxidative deamination of a variety of amine neurotransmitters such as norepinephrine, serotonin, and dopamine. It exists as two distinct isozymic forms designated MAO A and MAO B based on

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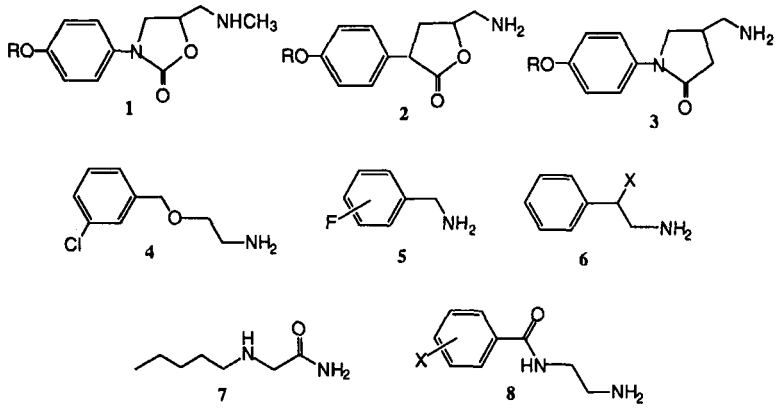
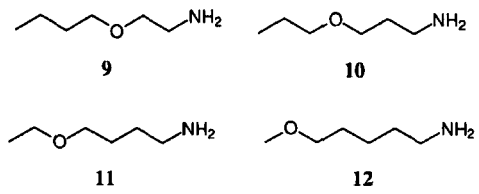
substrate and inactivator selectivity.[1] MAO A selectively oxidizes norepinephrine and serotonin and MAO B oxidizes phenylethylamine and benzylamine.[2] Compounds that selectively inhibit MAO A exhibit antidepressant activity,[3] whereas selective inhibitors of MAO B are used in the treatment of Parkinson's disease.[4] Although primary alkylamines, such as heptylamine, and primary arylalkyl amines, such as phenylethylamine, are excellent substrates for MAO,[5] their analogues having an electron withdrawing group near the aminomethyl methylene group (**1**–**8**) inactivate the enzyme.[6–13]



To determine the extent of the proposed inductive effect of a heteroatom on MAO B inactivation, a series of oxygen containing heptylamine analogues (**9**–**12**) were synthesized and tested as inactivators of MAO B. The results of that study are discussed here.



MATERIALS AND METHODS

General

NMR spectra were recorded on either a Varian Gemini 300-MHz or on a Varian XL-400 400-MHz spectrometer. Chemical shifts are reported as δ

values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. Thin-layer chromatography was performed on EM/UV silica gel plates with UV indicator. Amine hydrochlorides were visualized on TLC plates by spraying with a solution of ninhydrin (300 mg) and pyridine (2 mL) in acetone (100 mL) and then heating. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. All chemicals were purchased from Aldrich Chemical Co. and were used without further purification unless indicated otherwise. THF was distilled freshly from sodium benzophenone ketyl. Glassware was dried in an oven overnight when dry conditions were required. All air- and moisture-sensitive reactions were carried out in an atmosphere of inert gas (nitrogen or argon).

Syntheses

2-Benzylideneaminoethanol (**14**, **n = 1**)

A mixture of aminoethanol (11.3 g, 11.5 mL, 150 mmol) and freshly distilled benzaldehyde (15.3 mL, 160 mmol) in ethanol (150 mL) was refluxed for 10 min, then the ethanol was evaporated and the remaining residue was distilled under reduced pressure (bp 138–142°C/3 mmHg) to yield 12 g (49%) of **14** (n = 1).

N-Benzylidene-2-Butoxyethylamine (**15**, **m = 3**, **n = 1**)

A flame dried, 250-mL three-necked round-bottomed flask was allowed to cool to room temperature under a flow of argon and was charged via syringe with 60 mL of freshly distilled THF. Sodium hydride (95%) (0.6 g, 25 mmol) was added to the THF with a brief opening of the rubber septum. Benzylidene protected ethanol amine (**14**, n = 1; 3.0 g, 20 mmol) in THF was added to the flask via syringe carefully. A strong evolution of hydrogen gas was observed. After stirring the reaction mixture the flask was placed in an ice bath. 1-Bromobutane (2.74 g, 2.1 mL, 20 mmol) was added dropwise via syringe (about 10 min). The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h. The progress of the reaction was monitored by silica gel TLC using butanol, water, and acetic acid (12 : 5 : 3) as the eluent solvent. The reaction mixture was quenched with water carefully, and the THF was evaporated at reduced pressure. The remaining aqueous phase was extracted with EtOAc (3 × 50 mL), and the organic phases were combined and dried over anhydrous Na_2SO_4. The drying agent was separated by decantation, and the obtained ethyl acetate phase was used directly for the following step.

***p-Toluene Sulfonic Acid Salt of 2-butoxyethylamine* (16, m = 3, n = 1)**

The benzylidene group was deblocked by stirring the ethyl acetate phase from the previous experiment with 50 mL of 2% HCl at room temperature. The aqueous phase was separated and the organic phase was washed with 25 mL of water. The aqueous phases were combined and the water was evaporated to afford a very viscous light brown oil. Recrystallization from ethanol–ether yielded the product as white flaky crystals in the solvent, which were very hygroscopic, and turned to liquid upon filtration.

Consequently, the 2-butoxyethylamine hydrochloride (1.17 g, 7.6 mmol) was dissolved in 25 mL of water and treated with 10 mL of 20% KOH. The free base form was extracted with EtOAc (3 × 25 mL). The organic phases were combined and dried over Na_2SO_4. The drying agent was separated by decantation and was washed with a small amount of ethyl acetate. The organic phases were combined, *p*-toluenesulfonic acid (1.93 g, 10 mmol) was added, and the resulting mixture was stirred at room temperature for 15 min. All of the solvent was removed by rotary evaporation, and the product was dried under vacuum. Recrystallization from ethanol and ether (allowed to stand in the refrigerator overnight for crystallization) yielded the product as white flaky crystals (2.0 g, 88%), which were collected by suction filtration and dried under vacuum at room temperature; mp. 89–90°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.80 (3H, t), 1.30 (2H, m), 1.40 (2H, m), 2.35 (3H, s), 3.25 (2H, m), 3.30 (2H, t), 3.50 (2H, t), 7.15 (2H, d), 7.7 (2H, b s, NH_2), 7.8 (2H, d); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 13.73, 18.82, 21.20, 31.15, 39.46, 65.84, 70.77, 125.72, 125.86, 128.69, 140.13, 141.37. HRMS: Calcd for $C_6H_{16}NO$ (M + 1; amine), 118.123; Found, 118.121. Calcd for $C_7H_8O_3S$ (toluenesulfonic acid), 172.019; Found, 172.019. Anal. Calcd for $C_{13}H_{23}NO_4S$: C, 53.97; H, 7.96; N, 4.84. Found: C, 53.67; H, 8.00; N, 4.80%.

***3-Benzylideneaminopropanol* (14, n = 2)**

The same procedure as for **14** (n = 1) was carried out using aminopropanol (11.3 g, 11.5 mL, 150 mmol); yield: 13.5 g (55%); bp 135–140°C/3 mm Hg.

***N-Benzylidene-3-propoxypropylamine* (15, m = 2, n = 2)**

The same procedure was followed as for **15** (m = 3, n = 1) using 1-bromopropane (2.74 g, 2.1 mL, 20 mmol). The product was used directly in the next step.

***p-Toluene Sulfonic Acid Salt of 3-propoxypropylamine* (16, m = 2, n = 2)**

The same procedure was followed as for **16** (m = 3, n = 1). The yield of the product was 1.8 g (60% from the benzylidene protected amine); mp. 84–85°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.78 (3H, t), 1.45 (2H, m), 1.77 (2H, m), 2.32 (3H, s), 2.94 (2H, t), 3.19 (2H, t), 3.32 (2H, t), 6.99 (3H, s, vb), 7.11 (2H, d), 7.72 (2H, d). ^{13}C-NMR (300 MHz, $CDCl_3$) δ 13.18, 21.06, 22.43, 27.00, 38.20, 67.82, 72.43, 125.70 (2C), 128.63 (2C), 140.31, 140.70. Anal. Calcd for $C_{13}H_{23}NO_4S$: C, 53.97; H, 7.96; N, 4.84. Found: C, 53.76; H, 7.79; N, 4.76%. HRMS: Calcd for $C_{13}H_{23}NO_4S$, 289.135; Found, 289.137. Calcd for $C_6H_{16}NO$ (M + 1; amine), 118.123; Found, 118.123. Calcd for $C_7H_8O_3S$ (toluenesulfonic acid), 172.019; Found, 172.018.

***4-Benzylideneaminobutanol* (14, n = 3)**

The same procedure as for **14** (n = 1) was carried out using aminobutanol (5.0 g, 5.2 mL, 56 mmol); yield: 8.2 g (83%); bp 128–135°C/2 mm Hg.

***N-Benzylidene-4-ethoxybutylamine* (15, m = 1, n = 3)**

The same procedure was followed as for **15** (m = 3, n = 1) using 1-iodoethane (3.11 g, 1.6 mL, 20 mmol). The product was used directly in the next step.

***p-Toluene Sulfonic Acid Salt of 4-ethoxybutylamine* (16, m = 1, n = 3)**

The same procedure was followed as for **16** (m = 3, n = 1). The yield of the product was 1.92 g (37% starting from protected amine); mp. 84.7–85.0°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.13 (3H, t), 1.48 (2H, m), 1.60 (2H, m), 2.35 (3H, s), 2.82 (2H, t), 3.25 (2H, t), 3.38 (2H, q), 7.18 (2H, d), 7.64 (3H, bs), 7.73 (2H, d); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 14.93, 21.19, 24.25, 26.41, 39.72, 65.96, 69.45, 125.78 (2C), 125.87 (2C), 140.59, 140.84. HRMS: Calcd for $C_6H_{16}NO$ (M + 1; amine), 118.123; Found, 118.123. Calcd for $C_7H_8O_3S$ (toluenesulfonic acid), 172.019; Found, 172.018. Anal. Calcd for $C_{13}H_{23}NO_4S$: C, 53.97; H, 7.96; N, 4.84. Found: C, 54.09; H, 7.90; N, 4.79%.

***5-Benzylideneaminopropanol* (14, n = 4)**

The same procedure as for **14** (n = 1) was carried out using aminopentanol (5.2 g, 50 mmol); yield: 8.7 g (91%); bp 138–145°C/2 mm Hg.

***N-Benzylidene-5-methoxypentylamine* (15, m = 0, n = 4)**

The same procedure was followed as for **15** (m = 3, n = 1) using iodomethane (2.84 g, 1.25 mL, 20 mmol). The product was used directly in the next step.

***p-Toluene Sulfonic Acid Salt of 5-methoxypentylamine* (16, m = 0, n = 4)**

The same procedure was followed as for **16** (m = 3, n = 1). The yield of the product was 2.38 g (43% yield starting from protected amine); mp. 90.0–90.8°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.20 (2H, m), 1.40 (2H, m), 1.49 (2H, m), 2.35 (3H, s), 2.74 (2H, m), 3.20 (2H, t), 3.24 (3H, s), 7.17 (2H, d), 7.73 (2H, d), 7.61 (3H, bs); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 21.15, 22.74, 26.97, 28.68, 39.67, 58.27, 72.04, 125.73 (2C), 128.82 (2C), 140.62, 140.91. HRMS: Calcd for $C_{13}H_{23}NO_4S$, 289.135; Found, 289.137. Calcd for $C_6H_{16}NO$ (M + 1; amine), 118.123; Found, 118.123. Calcd for $C_7H_8O_3S$ (toluenesulfonic acid), 172.019; Found, 172.019. Anal. Calcd for $C_{13}H_{23}NO_4S$: C, 53.97; H, 7.96; N, 4.84. Found: C, 53.78; H, 7.83; N, 4.66%.

Enzymology

Enzyme and Assay

Beef liver MAO B was isolated as described previously[14] and was stored as a concentrated solution (15–25 mg/mL) in sodium phosphate buffer (50 mM, pH 7.2) at 4°C. The specific activity varied among preparations, ranging from 3.5 to 7 units per mg, where a unit of activity is the conversion of 1 mmol of benzylamine to benzaldehyde per min in Tris-HCl buffer, pH 9.0 and 30°C.

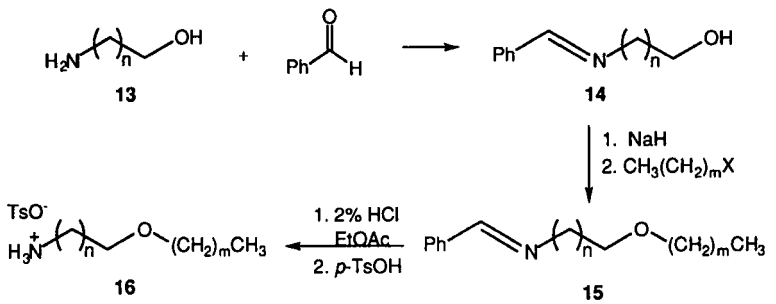
Time-Dependent Inactivation Experiments

Solutions (210 μL each) of an inactivator (1 mM) and a control containing no inactivator in potassium phosphate buffer (50 mM, pH 9.0) were incubated with MAO B (270 μg) for 30 min at 30°C. After inactivation, excess inhibitors were removed by the method of Penefsky (a total of 2 min for the separation).[15] The MAO B activity was then assayed by periodically removing 20 μL aliquots and adding to 480 μL of 1.05 mM benzylamine solution in Tris buffer, pH 9.0 according to the published procedure.[16]

RESULTS AND DISCUSSION

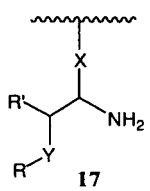
The initial attempted synthesis of compounds **9–12** was very straightforward: the appropriate alkyl halide was added to the sodium salt of the corresponding amino alcohol. However, selective alkylation of the hydroxyl group over the amino group of the corresponding amino alcohol via the literature procedure[9] was not successful. Several other conditions were tried,

including modification of the base, the temperature, and the deprotonation conditions, but little or no desired product was obtained. Protection of the amino group with Cbz also proved to be unsuccessful. The approach that worked well was protection of the amino group of the amino alcohol as the benzylidene prior its reaction with the alkyl halide (Scheme 1).[17]



SCHEME 1

Inactivation of MAO B by **1–8** was proposed[6–13] to result from attachment of the α-carbon to an active site residue (**17**; X is an active site residue; Y is an electron-withdrawing group); it was suggested that the adducts were stabilized by the inductive effect of the electron withdrawing atom(s) nearby. The order of stability of MAO adducts for compounds **1–3** was found[7–8] to be **1** > **2** > **3**, which corresponds to the strength of the inductive effect of the nearby electronegative atom(s). Compounds **9–12** were designed to clarify the effect of a nearby heteroatom on the stability of the proposed adduct formed between the inactivator and an active site residue of MAO (**17**).



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Compounds **9–12** are all time-dependent inhibitors of MAO B. The relative stabilities of the adducts formed after inactivation of MAO were determined by measuring the rates of reactivation of the enzyme. Although no direct correlation between distance and stability can be made, it is apparent from Figure 1 that the compounds with the oxygen atom closer to the amino group (**9** and **10**) produce more stable adducts than the compounds with the oxygen atom farther from the amino group (**11** and **12**).

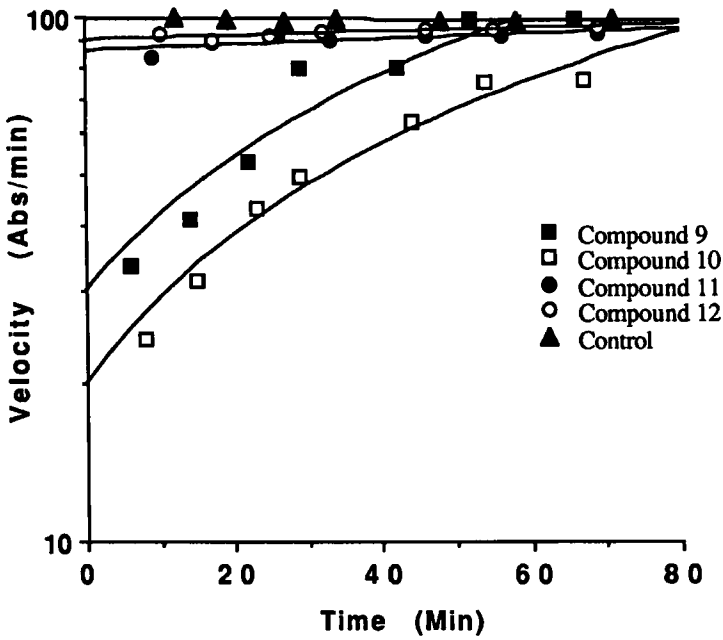
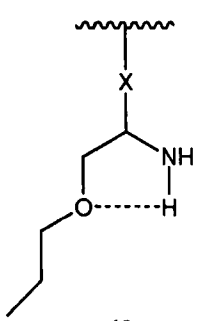


FIGURE 1 Time-dependent reactivation of MAO inactivated by **9–12**.

From this hypothesis it would be predicted that **9** should be more effective than **10**; however, the opposite was observed (Figure 1). A possible rationale for this observation is depicted by **18** and **19**. If the adduct has some flexibility in the active site, then intramolecular hydrogen bonding may be important. That being the case, then the adduct formed from **10** (i.e., **19**) would be more stable than that formed from **9** (i.e., **18**) because hydrogen bonding to give a six-membered ring is thermodynamically more stable than that to give a five-membered ring.[18]



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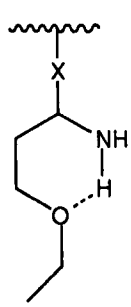

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These results are consistent with an inductive effect being important to the stability of the proposed covalent adduct. Attempts to make more stable adducts are ongoing.

Acknowledgments

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