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COMMUNICATION

(Aminoalkyl)trimethylgermanes, the First Organogermanium Mechanism-Based Enzyme Inactivators: A New Class of Monoamine Oxidase Inactivators

(Aminoalkyl)trimethylgermanes, $Me_3Ge(CH_2)_nNH_3^+$ (n = 1–3), are synthesized and shown to be mechanism-based irreversible inactivators of mitochondrial monoamine oxidase. At least two different adducts are formed by the three compounds. These are the first mechanism-based organogermanium inactivators of any specific enzyme.

Recently we reported (*1*) the first organosilicon mechanism-based inactivators (*2*) of monoamine oxidase (MAO). The design of those inactivators was based on a radical mechanism for the enzyme (*3–5*). Relative to the other members of the group IV elements, little is known about the chemistry of germanium (*6*); sometimes the inherent properties of organogermanes are different from those of the corresponding organosilanes and organostannanes (*7*). Therefore, we have synthesized the corresponding series of (aminoalkyl)trimethylgermanes

$$Me_3Ge(CH_2)_nNH_3^+Cl^-$$

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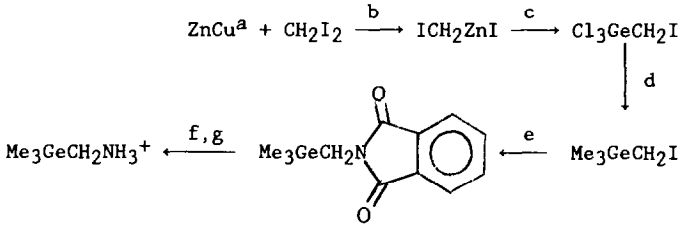
(**1**, n = 1–3) as potential mechanism-based inactivators of MAO, and have found that all three of these compounds inactivate this enzyme. These compounds (**1**) represent the first organogermanium mechanism-based enzyme inactivators of any enzyme.

(Aminomethyl)trimethylgermane hydrochloride (**1**, n = 1) was synthesized[1] by the route shown in Scheme 1. The synthesis of (aminoethyl)trimethylgermane



SCHEME 1. Synthesis of (aminomethyl)trimethylgermane hydrochloride. (a) Prepared by the method of LeGoff (*8*); (b) 45°C for 2.5 h; (c) $GeCl_4$ using the method of Seyferth and Andrews (*9*); (d) excess MeMgBr; (e) phthalimide, anhydrous K_2CO_3 in DMF at reflux for 4 h; (f) $NH_2NH_2 \cdot H_2O$ in EtOH/ reflux for 4 h; (g) HCl/EtOH, reflux for 6 h.

[1] The product was obtained as white crystals from ethanol–ether: mp 230–232°C, NMR (D_2O) δ 0.08 (s, 9H), 2.30 (s, 2H), 4.50 (HDO). *Anal.* Calcd for $C_4H_{14}ClGeN$: C, 26.08, H, 7.66, Cl, 19.25, N, 7.60. Found: C, 26.04, H, 7.72, Cl, 19.54, N, 7.69.

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$$Me_3GeBr + BrCH_2CN \xrightarrow{a} Me_3GeCH_2CN \xrightarrow{b,c} Me_3GeCH_2CH_2NH_3^+$$

SCHEME 2. Synthesis of (aminoethyl)trimethylgermane hydrochloride. (a) Two equivalents activated zinc (*10*) by the method of Matsuda *et al.* (*11*); (b) $NaBH_4/CoCl_2$ by the method of Suzuki *et al.* (*12*); (c) HCl.

hydrochloride (**1**, $n = 2$)[2] was carried out as shown in Scheme 2. (Aminopropyl)trimethylgermane was purchased from K & K Laboratories and converted into its hydrochloride salt (**1**, $n = 3$)[3] by precipitation from an ethereal solution with HCl gas.

Mitochondrial MAO B was purified from beef liver and assayed as previously described (*13*). The rates of inactivation of MAO by **1** ($n = 1$ or 2) at pH 9.0 were too fast to measure conveniently; consequently, the pH was lowered to 7.2 and

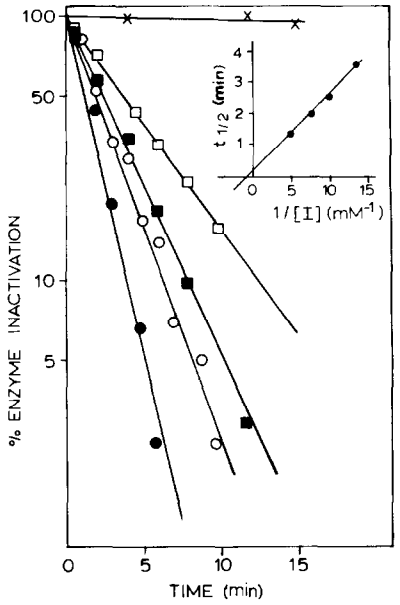


FIG. 1. Time-dependent inactivation of monoamine oxidase by (aminomethyl)trimethylgermane. MAO (10 μM), containing 200 (●), 128 (○), 100 (■), and 75 (□) μM aminomethyltrimethylgermane hydrochloride in 40 μM potassium phosphate, pH 7.2, buffer (total volume 200 μl) was incubated at 25°C. Aliquots (5 μl) were removed at given times and assayed (*5*) in a total volume of 500 μl for enzyme activity relative to a control (X) containing no inactivator. A control containing 500 μM inactivator plus 10 mM benzylamine (*X*) also was run. The insert is a Kitz and Wilson (*14*) replot of the data.

[2] The product was obtained as white crystals from methanol–ether: mp 244–245°C, NMR (D_2O) δ 0.01 (s, 9H), 0.87 (m, 2H), 2.89 (m, 2H), 4.50 (HDO). *Anal.* Calcd for $C_5H_{16}ClGeN$: C, 30.30, H, 8.14, Cl, 17.88, N, 7.07. Found: C, 29.98, H, 8.13, Cl, 17.90, N, 6.99.

[3] The product was obtained as white crystals by trituration with ethyl acetate: mp 188–189°C, NMR (D_2O) δ 0.04 (s, 9H), 0.52 (m, 2H), 1.55 (m, 2H), 2.73 (m, 2H), 4.50 (HDO). *Anal.* Calcd for $C_6H_{18}ClGeN$: C, 33.95, H, 8.55, Cl, 16.70, N, 6.60. Found: C, 33.76. H, 8.59, Cl, 16.89, N, 6.45.

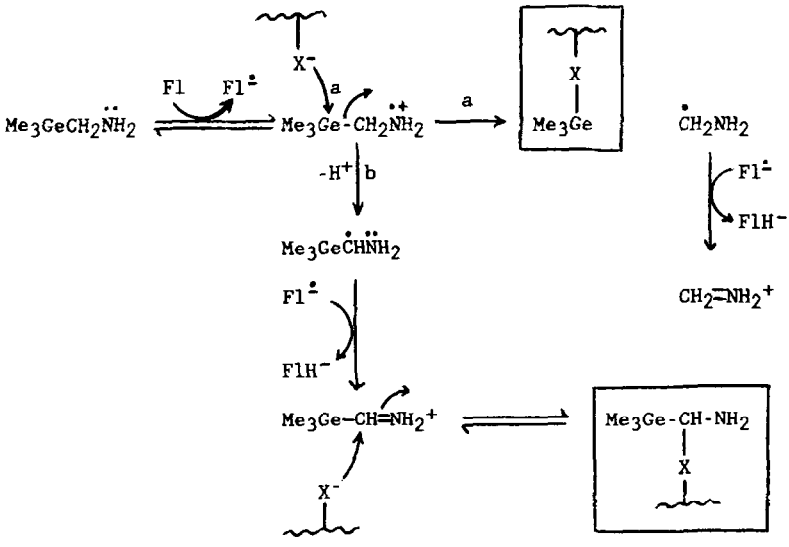
TABLE 1

Kinetic Constants [a] for $Me_3Ge(CH_2)_nNH_3^+$

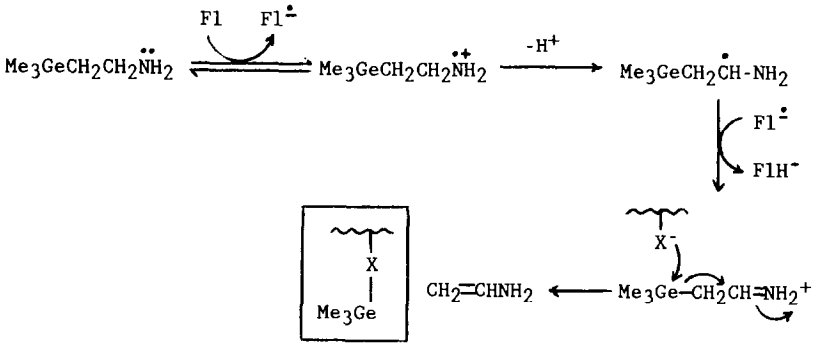
$Me_3Ge(CH_2)_nNH_3^+$ (n)	K_I (mM)	k_{inact} (min^{-1})	k_{inact}/K_I (min^{-1} mM^{-1})
1	1.4	4.33	3.09
2	9.2	2.02	0.22
3	83.8	0.53	0.006

[a] MAO (17 μg) was incubated at 25°C in 40 mM potassium phosphate buffer, pH 7.2, with 0.075–0.2 mM **1** (n = 1), and 0.3–3.0 mM **1** (n = 2), or in 100 mM Tris–HCl buffer, pH 7.0, with 6.6–51.8 mM **1** (n = 3). Aliquots (5 μl) were removed periodically and assayed for enzyme activity after dilution into 495 μl of 20 mM Tris–HCl, pH 9.0, containing 1 mM benzylamine.

pseudo first-order time-dependent inactivation was observed. Figure 1 shows the time-dependent loss of MAO activity by **1** (n = 1); the insert is a Kitz and Wilson (*14*). replot of the data. Time-dependent inactivation of MAO by **1** (n = 3) could be followed at both pH 9.0 and 7.2. Table 1 summarizes the kinetic constants for the three compounds. MAO was protected from inactivation by all three compounds when the inactivation was carried out in the presence of a 10-fold excess of the substrate, benzylamine, indicating that the inactivators are active-site directed. There was no difference in the rates of inactivation of MAO by **1** (n = 1, 2, or 3) in the absence or presence of 1 mM glutathione, indicating that any reactive



SCHEME 3. Proposed mechanism of inactivation of monoamine oxidase by (aminomethyl)trimethylgermane.

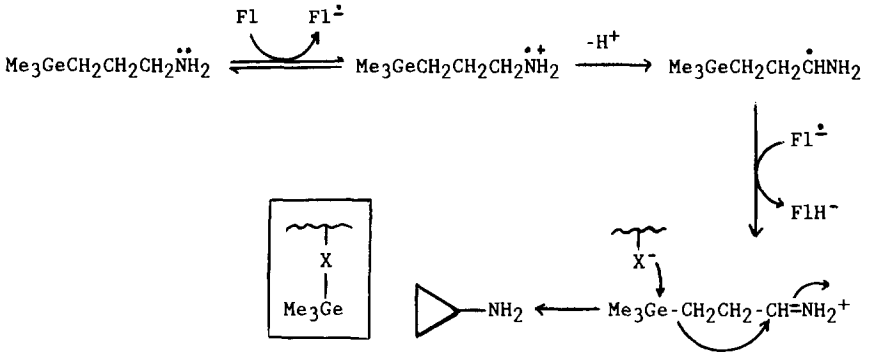


SCHEME 4. Proposed mechanism of inactivation of monoamine oxidase by (aminoethyl)trimethylgermane.

species released from the active site are not responsible for inactivation. Dialysis (20 mM Tris–HCl buffer, pH 7 or 9; 4°C) of MAO inactivated by **1** (n = 1) or **1** (n = 2) resulted in no reactivation of the enzyme. Although no reactivation of MAO inactivated by **1** (n = 3) was observed after dialysis overnight (4°C) at pH 7, partial reactivation occurred by dialysis at pH 9. These results suggest that at least two different enzyme adducts are produced by the three compounds. When dialysis was carried out at 25°C, reactivation results similar to those observed with the silane derivatives were obtained.

The inactivation mechanisms proposed for the (aminoalkyl)trimethylgermanes are the same as those suggested and discussed for inactivation of MAO by (aminoalkyl)trimethylsilanes (*1*) (Schemes 3–5). Whereas the order of potency in the silicon series ($Me_3Si(CH_2)_nNH_2$) is $n = 2 > 1 \gg 3$ (when normalized to the same temperature), in the germanium series the potency decreases with increasing n. The values of k_{inact} in the germanium series are four to six times greater than in the corresponding silicon series.

The (aminoalkyl)trimethylgermanes represent the first organogermanium mech-



SCHEME 5. Proposed mechanism of inactivation of monoamine oxidase by (aminopropyl)trimethylgermane.

anism-based enzyme inactivators and, to the best of our knowledge, are the first known organogermanium enzyme inactivators. The results described here suggest that organogermanium compounds may be a useful new addition to the armamentarium of inactivators of redox enzymes that catalyze one-electron reactions. These compounds also contribute the first compounds in a new class of MAO inactivators.

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