

NH<sub>4</sub>OH (6:2:2:1) as developing solvent, authentic 6 had  $R_f$  0.60 and authentic 1 had  $R_f$  0.44. Under these conditions the product of the DTT reaction had the same  $R_f$  as 1. With *i*-PrOH-H<sub>2</sub>O-NH<sub>4</sub>OH (7:2:1) as developing solvent, authentic 6 had  $R_f$  0.69 and authentic 1 had  $R_f$  0.38. Again in this system the material isolated from the DTT reduction had  $R_f$  identical with authentic 1. In both systems above, a trace impurity visible under uv light was noted at  $R_f \sim 0.9$ .

**Method II. Reduction by Sodium Dithionite-2-Mercaptoethanol.** To a stirred solution of K<sub>2</sub>CO<sub>3</sub> (172 mg, 1.72 mmol) and sodium dithionite (16.7 mg, 0.096 mmol) in H<sub>2</sub>O (0.65 ml) was added 2-mercaptoethanol (0.35 ml) followed immediately by 6 (2.4 mg, 0.08 mmol). After 30 min at room temperature, the reaction mixture was applied to a preparative tlc plate (1 mm, silica gel GF) and developed with acetone-EtOAc-MeOH (5:5:1). Two major zones were seen under uv light; one ( $R_f$  0.64) was eluted with MeOH, and the other ( $R_f$  0.0-0.1) was eluted with MeOH-H<sub>2</sub>O (9:1). The slower moving zone had uv spectral properties identical with authentic 1. The faster running zone had  $\lambda_{\max}$  256 nm in 10<sup>-3</sup> M DTT-0.1 M EDTA (pH 7.8) or 0.1 M EDTA (pH 7.8). 1-(2'-Deoxy- $\beta$ -D-ribofuranosyl)-5-mercaptopuracil (2) and 1-( $\beta$ -D-arabinofuranosyl)-5-mercaptopuracil (14) were not isolated as such; however, when 10<sup>-4</sup> M solution of either 10 or 11 in 0.1 M EDTA (pH 7.5) was made 10<sup>-3</sup> M in DTT, in a reaction which was instantaneous, the  $\lambda_{\max}$  335 nm appeared, characteristic of the 5-thiolate anion. In addition, 10 [10<sup>-4</sup> M in 0.1 M EDTA (pH 7.5)] was instantaneously reduced to 2 by 10<sup>-3</sup> M glutathione.

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## Synthesis and Biological Activity of Some 8-Substituted Seleno Cyclic Nucleotides and Related Compounds

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8-Bromoadenosine 3',5'-cyclic monophosphate and 8-bromoadenosine served as an intermediate for the chemical synthesis of a series of 8-substituted seleno cyclic nucleotides and their nucleosides. In an *in vitro* test using murine leukemic cells (L-5178Y) these 8-substituted derivatives of c-AMP showed some antitumor activities. A nucleic acid analyzer was used to study the hydrolysis of these cyclic nucleotides by phosphodiesterase. It was found that lengthening the side chain increased resistance to hydrolysis.

Adenosine 3',5'-cyclic phosphate (cAMP) has been recognized as an intracellular second messenger of hormone action. At least some of its actions can be explained through the stimulation of a cAMP-dependent protein kinase. Recently, a large number of new cAMP analogs have been synthesized with modification of the purine,<sup>1-9</sup> carbohydrate,<sup>10-12</sup> and phosphate moieties.<sup>13-16</sup> Some of them were more active than the parent nucleotide in sev-

eral biological systems. For example, all 8-substituted cAMPs except 8-amino were more resistant to phosphodiesterase than cAMP; 8-MeS-cAMP and 8-BzlS-cAMP were found to be inhibitors of cAMP phosphodiesterase.<sup>6</sup>

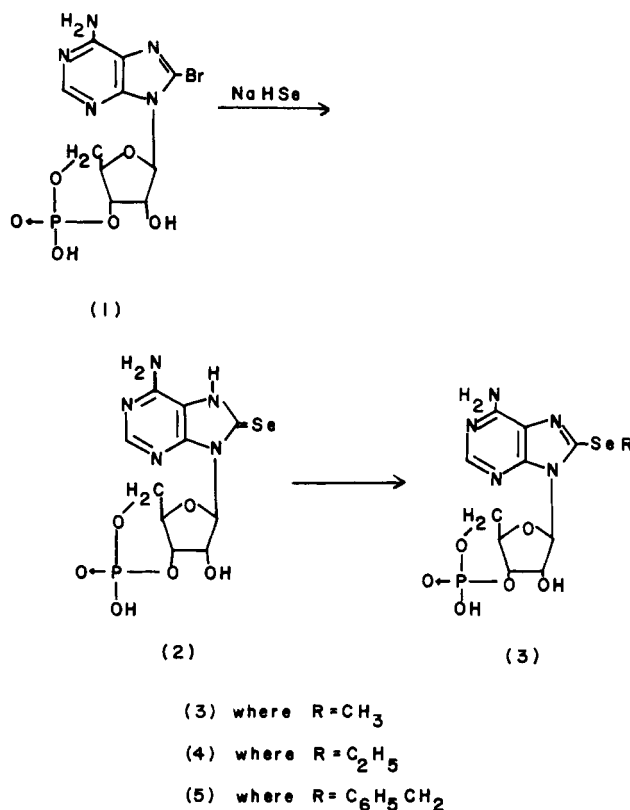
In view of these considerable changes in biological activity observed when 8-hydrogen of cAMP was replaced by sulfur, it is of interest to investigate the replacement of the 8-hydrogen by selenium as well. We have synthesized

a series of 8-seleno-cAMP analogs with the hope that they may have properties favoring an increase of their biological activities by either a better penetration to the interior of the cells or a greater resistance to the action of the phosphodiesterase.

8-Bromo-cAMP (1) was obtained by direct bromination of cAMP. Treatment of 8-bromo-cAMP with sodium hydrogen selenide in refluxing methanol gave 8-seleno-cAMP (2) and cAMP with a ratio of 1:2. However, compound 2 could not be obtained by treatment of 1 with selenourea under the same conditions. The formation of cAMP in this reaction may arise from the reduction of 8-bromo-cAMP by hydrogen selenide.<sup>17</sup>

Treatment of compound 2 with methyl iodide, ethyl bromide, or benzyl bromide yielded 8-methylseleno- (3), 8-ethylseleno- (4), and 8-benzylseleno-cAMP (5), respectively (Scheme I). The 8-substituted adenosine 3',5'-cyclic phosphates were readily purified by crystallization from water at pH 3. The physical properties of the nucleotides are shown in Table I. A comparison of the ultraviolet spectra of these 8-substituted seleno cyclic nucleotides with known 8-substituted thio cyclic nucleotides<sup>6</sup> confirmed the position of substitution. Furthermore, the structures of these 8-substituted seleno cyclic nucleotides were verified by enzymatic studies.

Scheme I



For the identification of 8-substituted seleno cyclic nucleotides several 8-substituted selenoadenosines were synthesized. Treatment of 8-bromoadenosine with sodium hydrogen selenide in refluxing ethanol gave 8-selenoadenosine (6) and adenosine with a ratio of 1:0.6. Alkylation of compound 6 with methyl iodide, ethyl bromide, or benzyl bromide yielded 8-methylseleno- (7), 8-ethylseleno- (8), and 8-benzylselenoadenosine (9), respectively. 8-Alkylselenoadenosines were purified by recrystallization from water. The physical properties of these compounds are shown in Table I.

Table I. Physical Properties of the 8-Substituted Seleno Nucleotides and Related Compounds

Compound	$\lambda_{\text{max}}, \text{m}\mu (\epsilon \times 10^{-3})$			$R_f^a$
	pH 1	pH 11	H <sub>2</sub> O	
cAMP				0.55
8-Br-cAMP (1)				0.48
8-Se-cAMP (2)	232 (7.8)	233 (16.7)	246 (14.2)	0.20
	325 (22.5)	306 (15.2)	315 (21.6)	
8-MeSe-cAMP (3)	291 (14.6)	225 (13.7)	285 (16.5)	0.40
8-EtSe-cAMP (4)	291 (14.9)	215 (15.8)	287 (13.9)	0.43
		283 (17.6)		
8-BzlSe-cAMP (5)	267 (9.1)	269 (9.6)	267 (9.5)	0.25
	290 (8.2)	290 (8.9)	290 (8.3)	
8-Se-Ado (6)	231 (12.3)	235 (21.3)	243 (14.4)	0.32
	325 (20.4)	307 (21.3)	314 (20.8)	
8-MeSe-Ado (7)	290 (17.9)	225 (15.6)	285 (16.4)	0.41
8-EtSe-Ado (8)	293 (16.2)	216 (15.2)	287 (14.1)	0.46
		287 (13.1)		
8-BzlSe-Ado (9)	294 (12.6)	271 (7.9)	271 (8.0)	0.24
		291 (7.8)	291 (7.7)	
8-Br-AMP				0.32
8-MeSe-AMP (10)				0.31
8-EtSe-AMP (11)				0.33
8-BzlSe-AMP (12)				0.07

<sup>a</sup>Thin-layer chromatography was run on a polygram CEL 300 PEI and developed with 1 M LiCl.

When each of the 8-substituted seleno cyclic nucleotides was incubated with 3',5'-cyclic nucleotide phosphodiesterase in Tris buffer (pH 7.5), the corresponding 5'-nucleotide was obtained. When the enzymatically prepared 8-substituted seleno 5'-nucleotides were incubated with 5'-nucleotidase, partially purified from snake venom (Sigma product), the corresponding nucleosides were formed. The nucleosides released after 5'-nucleotidase treatment migrated on PEI-cellulose (1 M LiCl) and high-pressure liquid chromatography identically with those of authentic 8-substituted selenoadenosines (see Tables I and II).

Figure 1 shows the behavior of 8-EtSe-cAMP on high-pressure liquid chromatography on a Varian LCS-1000 under the conditions described by Brown.<sup>18</sup> A single peak with retention time of 46 min was obtained. When the 8-EtSe-cAMP was treated with phosphodiesterase and chromatographed on the LCS-1000, a new peak with retention

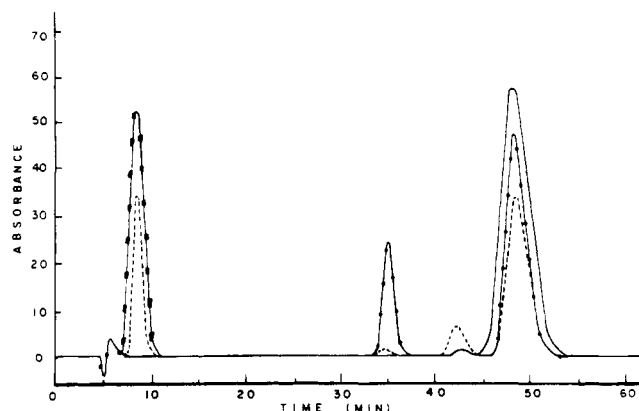


Figure 1. High-pressure liquid chromatographs of 8-EtSe-cAMP, 8-EtSe-Ado, phosphodiesterase treated 8-EtSe-cAMP, and 5'-nucleotidase-treated 8-EtSe-AMP. (—) 8-EtSe-cAMP untreated; (---) authentic 8-EtSe-Ado; (· · · · ·) the reaction products of 8-EtSe-cAMP after treatment with 5'-nucleotidase; and (— · — · —) the reaction products of 8-EtSe-cAMP after treatment with phosphodiesterase.

**Table II.** Action of 3',5'-Cyclic Nucleotide Phosphodiesterase on 8-Substituted Seleno Cyclic Nucleotides

Compound	Retention time, min	Rel rate <sup>a</sup> of hydrolysis
cAMP	25	100
8-Br-cAMP (1)	43	70
8-Se-cAMP (2)	106	0
8-MeSe-cAMP (3)	48	31
8-EtSe-cAMP (4)	46	19
8-BzlSe-cAMP (5)	104	6
8-Se-Ado (6)	43	
8-MeSe-Ado (7)	10	
8-EtSe-Ado (8)	10	
8-BzlSe-Ado (9)	24	
8-Br-AMP	33	
8-MeSe-AMP (10)	34	
8-EtSe-AMP (11)	33	
8-BzlSe-AMP (12)	66	

<sup>a</sup>Relative rates of hydrolysis are determined by high-pressure liquid chromatography.

time of 33 min was observed. Subsequently when enzymatically prepared 8-EtSe-AMP was treated with 5'-nucleotidase, a new peak with retention time of 10 min was observed. The extent of the hydrolysis of those seleno cyclic nucleotides by phosphodiesterase was calculated from the peak areas and shown in Table II.

Table II shows that modification of the purine nucleus substituents in the 8 position considerably increases resistance to hydrolysis. These observations are in agreement with those of Michal, *et al.*,<sup>19</sup> and Robins, *et al.*<sup>6</sup> It also shows that lengthening the side chain increases resistance to hydrolysis.

It is worthy to note that 8-Br-cAMP was hydrolyzed up to 70% in our experiments, while Cehovic, *et al.*,<sup>20</sup> reported that only 20% was hydrolyzed. A possible explanation is either that the nucleic acid analyzer is more precise than the colorimetric method or that enzymes from different sources behave differently.<sup>21</sup>

## Experimental Section†

**8-Bromoadenosine 3',5'-Cyclic Phosphate (1).** 8-Bromoadenosine 3',5'-cyclic phosphate was prepared by the method of Robins<sup>6</sup> with adenosine 3',5'-cyclic phosphate (1.6 g, 5 mmol) and Br<sub>2</sub> (0.4 ml, 15 mmol) in 60 ml of 1 M NaOAc buffer (pH 4.0) in 76% yield.

**8-Selenoadenosine 3',5'-Cyclic Phosphate (2).** Condensed H<sub>2</sub>Se† (0.5 ml, 12 mmol) was bubbled through a solution of 0.069 g (3 mmol) of Na in 90 ml of absolute MeOH. 8-Bromoadenosine 3',5'-cyclic phosphate (0.62 g, 1.5 mmol) in 10 ml of absolute MeOH was added. The suspension was refluxed for 1.5 hr. The solution was evaporated to dryness. The residue was dissolved in 10 ml of H<sub>2</sub>O and passed through a Dowex 50 column (H<sup>+</sup> form, 2.5 × 10 cm). The nucleotide was eluted with H<sub>2</sub>O. Evaporation of the appropriate fractions gave 200 mg of adenosine 3',5'-cyclic phosphate and 100 mg of 2. The analytical sample was recrystallized from H<sub>2</sub>O. *Anal.* (C<sub>10</sub>H<sub>12</sub>N<sub>5</sub>O<sub>6</sub>PS<sub>2</sub>·2H<sub>2</sub>O) C, H, N, P.

**8-Methylselenoadenosine 3',5'-Cyclic Phosphate (3).** A solution of 100 mg (0.25 mmol) of 8-selenoadenosine 3',5'-cyclic phosphate (2) and 62 mg (0.59 mmol) of Na<sub>2</sub>CO<sub>3</sub> in 5 ml of H<sub>2</sub>O was diluted with 5 ml of MeOH and 0.2 ml of MeI. The mixture was stirred at room temperature for 1 hr and evaporated to dryness.

† Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ultraviolet spectra were determined on a Perkin-Elmer Model 402 spectrophotometer. Elemental analyses were performed by Midwest Microlab, Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values. Thin-layer chromatography was run on a polygram CEL 300 PEI and developed with 1 M LiCl. High-pressure liquid chromatography was run on a Varian LCS-1000 nucleic acid analyzer. Evaporations were accomplished using a Buchler flash evaporator under reduced pressure with a bath temperature of 40°.

† 98.0% minimum purity H<sub>2</sub>Se from the Matheson Co., Inc., East Rutherford, N. J. 07073.

The residue was dissolved in 1 N NH<sub>4</sub>OH and the pH of the solution was adjusted to 3 with 1 N HCl. The white crystals were filtered and washed with H<sub>2</sub>O to give 35 mg (32%) of pure 3. *Anal.* (C<sub>11</sub>H<sub>14</sub>N<sub>5</sub>O<sub>6</sub>PS<sub>2</sub>·H<sub>2</sub>O) C, H, N, P.

**8-Ethylselenoadenosine 3',5'-Cyclic Phosphate (4).** A solution of 100 mg (0.25 mmol) of 8-selenoadenosine 3',5'-cyclic phosphate (2) and 60 mg of Na<sub>2</sub>CO<sub>3</sub> in 4 ml of H<sub>2</sub>O was diluted with 4 ml of MeOH and 30 mg (0.28 mmol) of EtBr. The mixture was stirred at room temperature overnight. Work-up as in the procedure for 3 gave 30 mg (27%) of 4. *Anal.* (C<sub>12</sub>H<sub>16</sub>N<sub>5</sub>O<sub>6</sub>PS<sub>2</sub>·H<sub>2</sub>O) C, H, N, P.

**8-Benzylselenoadenosine 3',5'-Cyclic Phosphate (5).** A solution of 100 mg (0.25 mmol) of 8-selenoadenosine 3',5'-cyclic phosphate (2) and 60 mg of Na<sub>2</sub>CO<sub>3</sub> in 5 ml of H<sub>2</sub>O was diluted with 5 ml of MeOH and 50 mg (0.30 mmol) of BzlBr. The mixture was stirred at room temperature for 2 hr. Work-up as in the procedure for 3 gave 48 mg (38%) of 5. *Anal.* (C<sub>17</sub>H<sub>18</sub>N<sub>5</sub>O<sub>6</sub>PS<sub>2</sub>·0.5H<sub>2</sub>O) C, H, P; N: calcd, 13.80; found, 13.05.

**8-Selenoadenosine (6).** Condensed H<sub>2</sub>Se (1.2 ml, 30 mmol) was bubbled through a solution of 0.35 g (15 mmol) of Na in 200 ml of absolute EtOH. 8-Bromoadenosine (1.73 g, 5 mmol) in 10 ml of absolute EtOH was added. The suspension was refluxed for 4.5 hr and then cooled in ice bath. The solid was filtered by suction to give 1.1 g of crude product 6. The filtrate was evaporated to dryness. The residue was dissolved in 5 ml of H<sub>2</sub>O, acidified with HOAc, and then passed through a charcoal column (1.5 × 14 cm). The column was washed with H<sub>2</sub>O and then eluted with H<sub>2</sub>O-NH<sub>4</sub>OH-EtOH (v/v, 1:1:1) to give 290 mg of adenosine. The crude product 6 was washed with 20 ml of absolute EtOH, dissolved in 2 ml of H<sub>2</sub>O, and acidified with HOAc to pH 4. The yellow oil was collected and washed with 3 ml of ice-cooled H<sub>2</sub>O. The solid was collected and dried *in vacuo* to give 510 mg (29%) of pure 6, mp 164–166° dec. *Anal.* (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>Se·0.75H<sub>2</sub>O) C, H, N.

**8-Methylselenoadenosine (7).** A solution of 173 mg (0.5 mmol) of 8-selenoadenosine (6) and 120 mg (1.18 mmol) of Na<sub>2</sub>CO<sub>3</sub> in 4 ml of H<sub>2</sub>O was diluted with 5 ml of MeOH and 0.4 ml of MeI. The mixture was stirred at room temperature for 1.5 hr. The solvent was evaporated to a small volume. The solid was filtered by suction, washed with H<sub>2</sub>O, and dried to give 70 mg of 7. The analytical sample was recrystallized from H<sub>2</sub>O: mp 178–179°. *Anal.* (C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>4</sub>Se·0.5H<sub>2</sub>O) C, H, N.

**8-Ethylselenoadenosine (8).** A solution of 173 mg (0.5 mmol) of 8-selenoadenosine (6) and 120 mg (1.18 mmol) of Na<sub>2</sub>CO<sub>3</sub> in 5 ml of H<sub>2</sub>O was diluted with 5 ml of MeOH and 60 mg of EtBr (0.58 mmol). The mixture was stirred at room temperature overnight. The solvent was evaporated to a small volume. The oil was precipitated in Et<sub>2</sub>O to give 75 mg of 8. The analytical sample was recrystallized from H<sub>2</sub>O, mp 162.5–163.5°. *Anal.* (C<sub>12</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub>Se) C, H, N.

**8-Benzylselenoadenosine (9).** A solution of 173 mg (0.5 mmol) of 8-selenoadenosine (6) and 120 mg (1.18 mmol) of Na<sub>2</sub>CO<sub>3</sub> in 5 ml of H<sub>2</sub>O was diluted with 5 ml of MeOH and 100 mg (0.60 mmol) of BzlBr. The mixture was stirred at room temperature overnight. Work-up as in the procedure for 8 gave 131 mg of 9. The analytical sample was recrystallized from H<sub>2</sub>O: mp 167–169°. *Anal.* (C<sub>17</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>Se·H<sub>2</sub>O) C, H; N: calcd, 15.71; found, 15.17.

**Biological Studies.** (1) **Enzymatic.** Each 3',5'-cyclic nucleotide (1 μmol) was added to tubes containing Tris buffer (pH 7.5, 50 μmol), magnesium sulfate hydrate (1 μmol), and 3',5'-cyclic nucleotide phosphodiesterase (0.42 mg) in a final volume of 1 ml. The mixtures were incubated at 30° for 30 min, and the reaction was stopped by boiling in water bath for 3 min. An aliquot of each incubation mixture was subjected to tlc and to high-pressure liquid chromatography on a Varian LCS-1000 under the conditions described by Brown.<sup>18</sup> Peak areas were determined by multiplying the height of the peak by the width at half-weight.

After the release of 5'-nucleotides, 200 μg of 5'-nucleotidase was added to each tube and incubated at 37° for 14 hr. The reaction was stopped by boiling in a water bath for 15 min. The supernatants were subjected to tlc and to high-pressure liquid chromatography. The results are shown in Tables I and II.

(2) **Effects on Cultured Mouse Leukemia Cells.** The results of the cell culture experiments, using L5178Y mouse leukemic cells, are shown in Table III. The cell viability, after 2 hr exposure to cyclic nucleotide analogs, was determined by the dilute agar colony method.<sup>22</sup> For comparison the corresponding nucleosides were also investigated.

§ 3',5'-Cyclic nucleotide phosphodiesterase (from beef heart) and snake venom were obtained from Sigma Chemical Co., St. Louis, Mo.

**Table III.** Effect of 8-Seleno-cAMP, 8-Methylseleno-cAMP, 8-Ethylseleno-cAMP, 8-Benzylseleno-cAMP, and Their Nucleosides on the Growth of L5178Y

Control 100%	% survival		
	$1.0 \times 10^{-4}$ M	$1.0 \times 10^{-5}$ M	$1.0 \times 10^{-6}$ M
6-Thioguanine <sup>a</sup>	4	9	33
8-Se-cAMP (2)	59	70	75
8-MeSe-cAMP (3)	61	73	97
8-EtSe-cAMP (4)	67	68	92
8-BzlSe-cAMP (5)	49	70	83
8-Se-Ado (6)	68	74	78
8-MeSe-Ado (7)	75	76	81
8-EtSe-Ado (8)	78	81	88
8-BzlSe-Ado (9)	78	81	88

<sup>a</sup>S. H. Chu, *J. Med. Chem.*, **14**, 254 (1971).

Table III indicates that each cyclic nucleotide slightly inhibits cell growth. It also shows that cyclic nucleotides are more active than the corresponding nucleosides, although none of these compounds inhibits cell growth at very low concentrations.

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## Discriminant Analysis of the Relationship between Physical Properties and the Inhibition of Monoamine Oxidase by Aminotetralins and Aminoindans

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*N*-Methyl-5-methoxy-1-indanamine, *N*-ethyl-5-methoxy-1-tetralinamine, and 5-methoxy- and 6-methoxy-1-tetralinamine are potent inhibitors of mouse monoamine oxidase at 100 mg/kg po. Discriminant analysis of 20 analogs of these compounds suggests that the size of the amine substituent as well as the position of methoxyl substitution influences *in vivo* potency.

During the course of a search for new anti-Parkinson drugs a series of aminoindans and aminotetralins was synthesized.<sup>1</sup> When the first compounds (Table I, no. 1 and 3) were tested for dopaminergic activity, it was observed that they are potent monoamine oxidase (MAO) inhibitors, *i.e.*, they potentiate the effect of L-Dopa but produce no dopaminergic effects when administered alone. The substituent on the amine was then varied in order to examine the effect of hydrophobic and steric factors on MAO inhibitory potency.

## Results

**Biological Tests.** The *in vitro* and *in vivo* properties of these drugs are summarized in Table I. Several are potent MAO inhibitors *in vitro*; four of the twenty are quite potent *in vivo*. Those tested are not substrates of MAO.

None of the new compounds is active in the antioxotremorine or reserpine-reversal tests used earlier to evaluate anti-Parkinsonism activity.<sup>1</sup>

**Discriminant Analysis of the Relationship between Physical Properties and *in Vivo* Potency.** For this purpose it was decided to use the screening ratings of *in vivo* activities as presented in Table I rather than generating a dose-response curve for each drug. The former is the type of information which is typically available to the medicinal chemist at the point in time when he must decide which analog to make next. It is characterized by a graded response at a fixed dose.

Since the scale of potency in the Dopa test is not a linear continuous one, it is not appropriate to use multiple regression calculations. However, the multivariate technique of discriminant analysis is applicable.<sup>2</sup> In using this