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

	% survival		
Control 100%	1.0×10^{-4} M	1.0×10^{-5} M	1.0×10^{-6} M
6-Thioguanine[a]	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

[a] 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.

Acknowledgments. This work has been supported by Grants CA 12591-02A1 and 16538-01 from the U. S. Public Health Service. The authors are indebted to Mrs. G. Shiue for carrying out the cell culture study, to Mr. F. Hanley, J. Gell, and Mrs. S. Bobick for high-pressure liquid chromatography, and to Drs. R. E. Parks, Jr., and P. Calabresi for their encouragement during the course of this investigation.

References

(1) G. Cehovic, I. Marcus, S. Vengadabady, and T. Posternak, *C. R. Seances Soc. Phys. Hist. Natur. Geneve*, **3**, 135 (1968).
(2) H. J. Thomas and J. A. Montgomery, *J. Med. Chem.*, 11, 44 (1968).
(3) A. R. Hanze, *Biochemistry*, 7, 932 (1968).
(4) T. Posternak, I. Marcus, A. Gabbai, and G. Cehovic, *C. R. Acad. Sci., Paris*, **269**, 2409 (1969).
(5) T. Posternak, I. Marcus, and G. Cehovic, *C. R. Acad. Sci., Paris*, **272**, 622 (1971).
(6) K. Muneyama, R. J. Bauer, D. A. Shuman, R. K. Robins, and L. N. Simon, *Biochemistry*, **10**, 2390 (1971).
(7) R. B. Meyer, D. A. Shuman, R. K. Robins, R. J. Bauer, M. K. Dimmitt, and L. N. Simon, *Biochemistry*, **11**, 2704 (1972).
(8) R. J. Bauer, K. R. Swiatek, R. K. Robins, and L. N. Simon, *Biochem. Biophys. Res. Commun.*, **45**, 526 (1971).
(9) C. I. Hong, G. L. Tritsch, P. Hebborn, and G. B. Chheda, Abstracts, 166th National Meeting of the American Chemical Society, Chicago, Ill., August 1973, MEDI 10.
(10) A. M. Mian, R. K. Robins, and T. A. Khwaja, ref 9, MEDI 5.
(11) T. A. Khwaja, R. Harris, and R. K. Robins, *Tetrahedron Lett.*, 4681 (1972).
(12) A. K. M. Anisuzzaman, W. C. Lake, and R. L. Whistler, *Biochemistry*, **12**, 2041 (1973).
(13) A. Murayama, B. Jastorff, F. Cramer, and H. Hettler, *J. Org. Chem.*, **36**, 3029 (1971).
(14) G. H. Jones, H. P. Albrecht, N. P. Damodaran, and J. G. Moffatt, *J. Amer. Chem. Soc.*, **92**, 5510 (1970).
(15) F. Eckstein, *J. Amer. Chem. Soc.*, **92**, 4718 (1970).
(16) R. B. Meyer, D. A. Shuman, and R. K. Robins, *Tetrahedron Lett.*, 269 (1973).
(17) C. Collard-Charon and M. Renson, *Bull. Soc. Chim. Belg.*, **71**, 563 (1962).
(18) P. R. Brown, *J. Chromatogr.*, **52**, 257 (1970).
(19) G. Michal, M. Nelböck, and G. Weimann, *Z. Anal. Chem.*, **252**, 189 (1970).
(20) G. Cehovic, T. Posternak, and E. Charollais in "Advances in Cyclic Nucleotide Research," Vol. 1, Raven Press, New York, N. Y., 1972, p 530.
(21) J. P. Miller, D. A. Shuman, M. B. Scholten, M. K. Dimmitt, C. M. Stewart, T. A. Khwaja, R. K. Robins, and L. N. Simon, *Biochemistry*, **12**, 1010 (1973).
(22) M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.*, **17**, 753 (1968).

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

Yvonne C. Martin,* James B. Holland, Charles H. Jarboe, and Nicholas Plotnikoff

Experimental Therapy Division, Abbott Laboratories, North Chicago, Illinois 60064. *Received October 3, 1973*

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.[1] 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

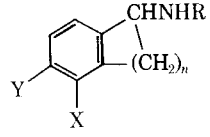
Biological Tests. The *in vitro* and *in vivo* properties of these drugs are summarized in Table I. Several are potent MAO inhibitors *in vitro;* four of the twenty are quite potent *in vivo*. Those tested are not substrates of MAO.

None of the new compounds is active in the antioxotremorine or reserpine-reversal tests used earlier to evaluate anti-Parkinsonism activity.[1]

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.[2] In using this

Table I. Structure, Physical Properties, and MAO Inhibitory Potency



No.	Structure				Physical properties				Biological activity				
									MAO *in vitro* % *I*,	*In vivo* po potency (100 mg/kg)			
	n	R	X	Y	II	E_s[c]	*I*	*X*	10^{-4} *M*	Obsd	Calcd[a]	Calcd[b]	Calcd[d]
1	2	CH_3	H	OCH_3	1.3	0.00	1	0	92	3	3	1	1[e]
2	3	H	OCH_3	H	1.2	0.32	0	1	95	3	3	1	0[e]
3	3	H	H	OCH_3	1.3	0.32	0	0	82	3	3	1	1
4	3	CH_2CH_3	H	OCH_3	2.2	−0.07	0	0		3	3	1	1
5	3	CH_3	H	OCH_3	1.7	0.00	0	0	90	2	3	1	1[e]
6	3	CH_3	H	OH	1.7	0.00	0	0	94	2	3	1	1
7	2	H	H	OCH_3	0.8	0.32	1	0	91	2	2	1	1[e]
8	3	CH_3	OCH_3	H	1.7	0.00	0	1	82	1	1	0	0[e]
9	3	$(CH_2)_2OCH_3$	H	OCH_3	1.7	−0.66[c]	0	0		1	1	0	0[e]
10	3	$(CH_2)_2CH_3$	H	OCH_3	2.7	−0.66	0	0		1	1	0	0[e]
11	3	$(CH_2)_5CH_3$	H	OCH_3	4.2	−0.68[c]	0	0		1	1	0	0
12	3	$CH_2C_6H_5$	OCH_3	H	3.5	−0.68	0	1	68	1	0	0	0
13	3	$(CH_2)_2OH$	H	OCH_3	1.0	−0.66[c]	0	0		1	0	0	0
14	3	CH_3	OH	H	1.7	0.00	0	1	59	0	1	0	0[e]
15	3	$CH(CH_3)_2$	OCH_3	H	2.6	−1.08	0	1	81	0	0	0	0
16	3	$CH(CH_3)_2$	H	OCH_3	2.6	−1.08	0	0	79	0	0	0	0[e]
17	2	$CH(CH_3)_2$	H	OCH_3	2.1	−1.08	1	0	35	0	0	0	0
18	2	H	OCH_3	H	0.8	0.32	1	1	80	0	0	1	0[e]
19	3	$(CH_2)CH_3$	H	OCH_3	1.4	−0.66[c]	0	0		0	0	0	0[e]
20	3	$(CH_2)_6CH_3$	H	OCH_3	4.7	−0.68[c]	0	0		0	0	0	0
Pargyline									100	3 (100 mg/kg) 1 (25 mg/kg)			

[a]Four-group analysis. [b]Two-group analysis. 1 = former groups 2 and 3; 0 = former groups 0 and 1. [c]Estimated on the basis that E_s: n-C_3H_7 = −0.67; n-C_4H_9 = −0.69; n-C_5H_{11} = −0.70. [d]Two-group analysis with only half of the molecules included in the establishment of the discrimination criterion. [e]Included in the calculation of the discrimination criterion.

Table II. Physical Properties of the Potency Groups (Mean ± Standard Deviation)

a. Four-Group Analysis

	Group 0 (inactive)	Group 1 (slightly active)	Group 2 (moderately potent)	Group 3 (most potent)
N (no. of compds)	7	6	3	4
Prior probability	0.35	0.30	0.15	0.20
Π	2.27 ± 1.25	2.47 ± 1.22	1.73 ± 0.06	1.50 ± 0.47
E_s^c	−0.608 ± 0.564	−0.56 ± 0.273	0.107 ± 0.185	0.142 ± 0.207
X	0.428 ± 0.534	0.333 ± 0.516	0.0 ± 0.0	0.250 ± 0.500
I	0.285 ± 0.488	0.0 ± 0.0	0.333 ± 0.578	0.250 ± 0.500

b. Two-Group Analysis

	Group 0,1 (inactive or slightly active)	Group 2,3 (moderately or most potent)
N	13	7
Prior probability	0.65	0.35
Π	2.36 ± 1.19	1.60 ± 0.36
E_s^c	−0.58 ± 0.44	0.13 ± 0.18
X	0.38 ± 0.51	0.14 ± 0.38
I	0.15 ± 0.38	0.29 ± 0.49

procedure one divides the compounds into groups on the basis of their score on the Dopa test (the dependent variable). The various physical or structural properties (independent variables) which correspond to each compound are then analyzed to find which combination of these variables allows one to classify the molecules into the observed groups. A successful analysis classifies each molecule into the group to which it was observed to belong. By the successive examination of the discriminatory value of various combinations of variables one can determine which physical properties are related to potency and which are not.

We examined Π, Π^2, E_s^c, and two dummy variables as predictors of *in vivo* potency. The first dummy variable (X) was set equal to 1.0 if the substituent is at the X position in the generic formula (Table I) and 0.0 if it is at Y. The second dummy variable (I) was set equal to 1.0 if the derivative is an indan and 0.0 if it is a tetralin. For the analysis the Statistical Analysis System (SAS) discriminant analysis program was used.[3] The calculations in this program develop the classification criteria on the basis of the generalized square distance between means. The prior probability that any compound is a member of a group was set equal to the proportional membership in that group (see Table II) for these analyses.

The mean value of each of the discriminant variables for each potency group is listed in Table IIa. It can be seen that no single physical property is a statistically significant predictor of potency. However, slight differences between groups are noted for all properties. E_s^c is apparently the best predictor.

The criterion of relative success of the discriminant analysis was the number of molecules correctly assigned. To classify a molecule its generalized square distance from each group centroid was computed from eq 1. $D_J^2(X)$ is the generalized square distance of the molecule from group J; $(X - \bar{X}_J)$ is the vector of deviations of the physical properties associated with the molecule from the mean of these properties of group J; cov^{-1} is the inverse pooled covariance matrix of all physical properties; and P_J is the prior probability that any molecule is a member of group J (not considering physical properties, just the "odds" that any one molecule will be active or inactive, for example). The first term to the right of the equal sign is the matrix notation for the sum of squares of $(X - \bar{X}_J)$ divided by the variance in order to standardize units. P_J is important when the physical properties of one molecule place it between two groups. In that case it would be assigned to the group of highest prior probability. The molecule is classified into the group from which $D_J^2(X)$ is smallest.

Table III. Generalized Squared Distances between Groups

a. Four Potency Group Analysis: Π, E_s^c, and X as Discriminatory Variables

Calcd potency	Obsd potency 0	1	2	3
0	2.1	2.5	10.4	8.6
1	2.8	2.4	9.1	9.3
2	12.1	10.5	3.8	4.1
3	9.7	8.6	3.6	3.2

b. Two Potency Group Analysis: E_s^c and X as Discriminatory Variables

Calcd potency	Obsd potency 0,1	2,3
0,1	0.86	7.87
2,3	9.10	2.10

$$D_J^2(X) = (X - \bar{X}_J)' \text{ cov}^{-1}(X - \bar{X}_J) - 2 \ln P_J \quad (1)$$

All combinations of the physical properties were tested as discriminant variables. The combination Π, E_s^c, and X assigned the largest number of molecules correctly in this case. When any of these three variables is omitted, or when I is added, several additional molecules are misclassified. The calculated classifications from the best combination are included in Table I. Three of the twenty molecules are misclassified into a neighboring group; none is misclassified by a larger distance. In this respect Table IIIa is of interest. It tabulates the mean square distance between the groups. The distance between a group and itself is a measure of the area or "spread" of that group. A successful discriminant analysis minimizes this while maximizing the between group distances. It can be seen that potency groups 0 and 1 are very close as are potency groups 2 and 3 whereas 0 and 1 are fairly distant from 2 and 3. Thus, it is not unexpected that certain compounds are misclassified into near groups.

The distances between groups in Table III also suggest that rather than four potency groups there are two: active (original groups 3 and 2) and inactive (original groups 1 and 0). The pharmacology also supports this: on retest compounds sometimes shift between groups 0 and 1 or 2 and 3 but rarely does a 0 or 1 become a 2 or 3 or *vice versa*. Since it seemed likely that four potency groups are overinterpretations of the data, the discriminant analysis

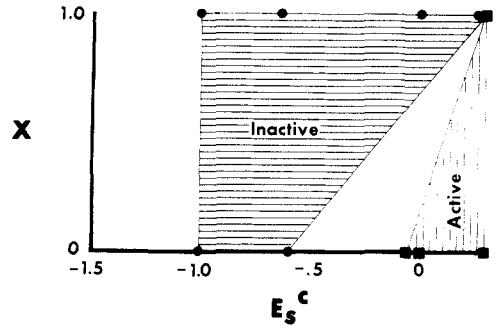


Figure 1. Region in $E_s^c - X$ space occupied by active and inactive compounds.

was repeated using two potency groups rather than four. The best results are indicated in Tables IIb and IIIb. In this case only the E_s^c and X factors are of discriminatory value. One molecule (5% of the data set) is misclassified.

Figure 1 is intended to aid in the visualization of the relationships between potency and E_s^c and X. On it is plotted the region of $E_s^c - X$ space occupied by active (group 3,2) and by inactive (group 0,1) molecules. The position of ambiguity is clearly seen, as are the unexplored regions of space.

In order to evaluate the above analysis of the MAO data a simulated prediction was performed.[4] Half of the compounds in each activity group were chosen by random number methods. The discriminant analysis was repeated on these 11 molecules which are indicated in Table I. Again, only the parameters X and E_s^c are statistically significant. Both are necessary. The predictions are also indicated in Table I. Of the 11 molecules included in this analysis, 10 (91%) are classified correctly. Of the nine molecules not included in the analysis, all were predicted properly!

A different molecule (no. 2) is misclassified in this analysis than in the analysis of all the molecules (no. 18). These compounds have identical E_s^c and X values and no. 2 is active whereas no. 18 is inactive. Figure 1 indicates that they lie at the point in $E_s^c - X$ space at which the active and inactive molecules meet.

In only the two-group case, the discriminant analysis problem can be solved by multiple regression calculations by using as the dependent variable a dummy. We chose 1.0 as active, 0.0 as inactive to study the relationship between activity and physical properties. Equations 2 and 3 are the only ones statistically justified. The figures in parentheses are the 95% confidence limits of the coefficients, n is the number of compounds, R is the multiple correlation coefficient, and s is the standard deviation of the estimates. Equation 2 misclassifies compounds no. 8, 14, and 18; eq 3 misclassifies only no. 18. Its calculated activity is 0.55 which places it in the active group; however, it is nearly as close to the correct group. This is, as it should be, the same compound misclassified by the discriminant analysis. Equation 2 may be used in place of the more cumbersome squared distance equations to classify molecules; the results are identical.

Discussion

The MAO inhibitory properties of this series were not anticipated before their synthesis. Since the molecules are benzylamine derivatives the lack of oxidation by MAO was also unexpected. However, it has been reported that α-methylbenzylamine is only $\frac{1}{10}$ as potent as benzylamine as a competitive inhibitor of 3,4-dimethoxybenzylamine oxidation.[5] In addition it was shown that 0.01 M 2-methylbenzylamine was oxidized by beef liver MAO at one-half the rate of benzylamine.[6] Both observations suggest that the two atoms of benzylamine which are substituted in the conversion to the 1-aminoindans and 1-aminotetralins are atoms which influence interaction with the enzyme.

Huebner, *et al.*,[7] reported that N-methyl-N-2-propynyl-1-indanamine and N-methyl-N-2-propynyl-1-tetralinamine are more potent *in vivo* MAO inhibitors than pargyline. On the basis of the current study it seems reasonable that a least part of the MAO inhibitory potency of Huebner's molecules may be due to the fused ring system.

The technique of discriminant analysis should be useful in the analysis of the structure–activity relationships of other series. One important use is as the above example in which the potency of compounds is rather roughly assigned. A second major use would be in series in which certain compounds are agonists, others are antagonists, and still others are without effect. A third use of discriminant analysis would be to compare active with inactive analogs. (The properties of inactive molecules are not considered in regression techniques.) Once one knew what makes a molecule active, he could use regression analysis to design the most potent analog. Finally, since biological data are most often of a qualitative nature, discriminant functions could be calculated for several activities of the same set of molecules. These data could be used to design the "best" compound in terms of all activities.

As in regression analysis, in using discriminant analysis one must be aware of such problems as multicollinearity of the physical properties and examination of too many variables as possible predictors. In addition, each group should probably contain at least five members. The discriminant function technically applies only to the data set from which it was calculated; predictions based on the function are reliable only to the extent to which the predicted molecules resemble those of the original data set.

The SAS program which was used in this study is not the only discriminant analysis program available. It is easy to use and to run in parallel with other SAS programs. The BMD-UCLA Biomedical Program BMD07M, Stepwise Discriminant Analysis, is also useful.[8] This program has two advantages: (1) it provides an approximate statistical test for each independent variable included in the model, and (2) the discriminant function for each group is explicitly stated. The generalized mean square distances between groups are not provided. Both programs have provisions to calculate the expected group of an unclassified molecule.

Experimental Section

Synthesis. 6-Methoxy-1-tetralone was purchased from Aldrich Chemical Co., Milwaukee.

The Schiff base intermediates for compounds **13**, **11**, and **20** were prepared by refluxing a benzene solution of the tetralone, excess amine, and a catalytic amount of *p*-toluenesulfonic acid until 1 molar equiv of water was collected. The Schiff base intermediate for compounds **10** and **15** was prepared as above except that a 10:1 w/w ratio of 3A molecular sieve was used to collect the water and the reaction was refluxed for 24 hr. The Schiff base intermediate for compound no. 4 was prepared as for those of no. **15**

	F	n	R	s	
act. = 0.58 (±0.21) + 0.68 (±0.35) E_s^c	16.6	20	0.69	0.36	(2)
act. = 0.73 (±0.21) + 0.75 (±0.30) E_s^c − 0.42 (±0.33) X	14.2	20	0.80	0.31	(3)

Table IV. New Aminotetralins Prepared for This Study

No.	Formula[a]	$n^{25}D$	Bp, °C (mm)	% yield
13	$C_{13}H_{19}NO_2$[b]	1.5593	183 (1.5)	**23**
15	$C_{14}H_{21}NO_2$[c]	1.5320	160–161 (1.5)	**26**
10	$C_{14}H_{21}NO$	1.5301	124 (0.75)	**43**
19	$C_{14}H_{18}N_2O$	1.547	168–170 (0.4)	**27**
11	$C_{17}H_{27}NO$	1.5203	161 (1.3)	**48**
20	$C_{18}H_{29}NO$	1.5182	171–174 (1.2–1.4)	**48**
4	$C_{13}H_{19}NO$	1.5375	124 (1.2)	**64**

[a]Analysis for C, H, and N within ±0.4% except where noted. [b]H, N; C: calcd, 70.55; found, 69.99. [c]H; N: calcd, 13.6; found, 14.05.

and 10 except that the reaction was accomplished in an autoclave (to prevent loss of low boiling amines) at 100° for 24 hr. The above Schiff bases were isolated by removal of the solvent and reduced with a 10–20% ratio of Raney nickel to compound in 100–200 ml of ethanol at 3 atm of H_2 until 1 equiv was absorbed.

Compound 19 was prepared from 6-methoxy-1-aminotetralin[1] and acrylonitrile. A 1:2 mixture of the compounds was refluxed neat with Triton B as a catalyst.

In all cases the solvent was removed by evaporation and the products were purified by distillation.

The analytical data for these compounds are summarized in Table IV.

MAO Inhibition *in Vitro*. Inhibition of mouse brain MAO was measured after preincubation of the enzyme with 10^{-4} *M* inhibitor for 10 min. Tryptamine (2×10^{-5} *M*) was used as substrate.[9]

Lack of Oxidation by MAO. The ability of compounds **2**, **3**, and **7** to serve as substrates for MAO was studied by monitoring the production of the ketone which would result from the oxidative deamination. A 1:10 homogenate of mouse liver in cold 0.15 *M* Na phosphate buffer, pH 7.4, was contrifuged at 0°, 2000g, for 10 min. The supernatant suspension (0.1 ml) was added to 2.9 ml of a 1×10^{-4} *M* solution of the amine equilibrated at 38°. The absorbance of the resulting suspension was followed in a Gilford multiple sample absorbance changer at 38° for 10 min. The wavelength was that of the potential ketone product. To assure activity in the MAO preparation the oxidation of benzylamine was assayed at 250 nm. All determinations were in triplicate.

MAO Inhibition *in Vivo*. The potentiation of the response to Dopa was used as an *in vivo* measure of the inhibition of MAO.[10] The drug was administered po as a suspension in carboxymethylcellulose to a group of four mice at a dose of 100 mg/kg. Four hr later 200 mg/kg of *dl*-Dopa was injected ip. The mice were observed for 1 hr; the severity of symptoms was scored 0 to 3+ according to the established criteria.[10]

Physical Properties. For hydrophobic properties the Π value (change in octanol–water partition coefficient) was calculated as the sum of Π for the substituent on the nitrogen plus Π for the nonaromatic carbon atoms of the ring. The Π values were taken from the compilation of Leo, *et al.*[11] For steric properties the Taft E_s values[12] corrected for hyperconjugation[13] were used.

Acknowledgment. The *in vitro* studies of monoamine oxidase inhibition were performed by Dr. Elizabeth B. Chappell.

References

(1) Y. C. Martin, C. H. Jarboe, R. A. Krause, K. R. Lynn, D. Dunnigan, and J. B. Holland, *J. Med. Chem.*, **16**, 147 (1973).
(2) (a) D. Morrison, "Multivariate Statistical Methods," McGraw-Hill, New York, N. Y., 1967, pp 130–133; (b) D. Morrison, *J. Marketing Res.*, **6**, 156 (1969).
(3) J. Service, "A User's Guide to the Statistical Analysis System (SAS)," Student Supply Stores, North Carolina State University, Raleigh, N. C., 1972, pp 190–195.
(4) R. E. Frank, W. F. Massy, and D. G. Morrison, *J. Marketing Res.*, **2**, 250 (1965).
(5) C. M. McEwen, Jr., G. Sasaki, and D. C. Jones, *Biochemistry*, **8**, 3952 (1969).
(6) E. A. Zeller, *Ann. N. Y. Acad. Sci.*, **107**, 811 (1963).
(7) C. F. Huebner, E. M. Donoghue, A. J. Plummer, and P. A. Furness, *J. Med. Chem.*, **9**, 830 (1966).
(8) W. J. Dixon, Ed., "BMD Biomedical Computer Programs," University of California Press, Berkeley, Calif., 1971, p 214a.
(9) R. J. Wurtman and J. Axelrod, *Biochem. Pharmacol.*, **12**, 1439 (1963).
(10) G. M. Everett and R. G. Wiegand, *Proc. Int. Pharmacol. Meet., 1st*, **8**, 85 (1962).
(11) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(12) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 598.
(13) C. K. Hancock, E. A. Meyers, and B. J. Yager, *J. Amer. Chem. Soc.*, **83**, 4211 (1961).

Mouse Liver Glyoxalase I Inhibition by S-Substituted Glutathiones†

Marian V. Kester, Jon A. Reese, and Scotty J. Norton*

Department of Chemistry, North Texas State University, Denton, Texas 76203. *Received August 20, 1973*

A series of *S*-(ω-phthalimidoalkyl)glutathiones and of *S*-(ω-aminoalkyl)glutathiones has been prepared. Both of the series of compounds exhibit inhibitory activity toward the glyoxalase I of mouse liver. In general, inhibition increases with increasing length of the alkyl chain separating the phthalimido group or the amino group from the glutathione residue. The effect of chain length is quite pronounced with the aminoalkylglutathione series; an approximate 40-fold increase in inhibition activity results as the alkyl chain is lengthened from four to ten carbon atoms.

Previous studies have shown that *S*-alkyl- and *S*-arylglutathiones competitively inhibit glyoxalase I of the glyoxalase system of yeast[1,2] and that they are cytotoxic to mammalian cells in cell culture.[3] In the latter case cytotoxicity is substantially decreased by preincubating noninhibitory concentrations of methylglyoxal (pyruvaldehyde) with the cell cultures. Methylglyoxal has been shown to be carcinostatic as well as generally cytotoxic.[4,5] A consequence of the later observation has been the proposal that the widely distributed glyoxalase system is involved in cell growth regulation by controlling cellular levels of methylglyoxal.[1] Glutathione (GSH), implicated in the cell division process,[6] is a required cofactor for the enzyme system. The two-enzyme glyoxalase system catalyzes the following reaction.

$$CH_3COCHO + GSH \xrightarrow{\text{glyoxalase I}} CH_3CHOHCO{-}SG \xrightarrow{\text{glyoxalase II}} CH_3CHOHCOOH + GSH$$

Inhibitors of glyoxalase I activity have been proposed to elicit an endogenous buildup of methylglyoxal and thus give rise to a cytotoxic condition.[1]

†This investigation was supported by The Robert A. Welch Foundation of Texas, Grant No. B-133, and by Faculty Research of North Texas State University, Grant No. 35233.