Intramolecular Catalysis of the Hydrolysis of N-Dimethylaminomaleamic Acid

Abstract. N-*Dimethylaminomaleamic acid decomposes in aqueous solution by intramolecular reaction of the* cis-*carboxy group with the substituted carbamyl group. The reaction follows pseudo first-order kinetics and shows a dependence on* p*H. This compound retards plant growth probably because 1,1-dimethylhydrazine and 1,1-dimethylhydrazinium hydrogen maleate are products of its decomposition. Under similar conditions* N-*dimethylaminosuccinamic acid was stable.*

N-Dimethylaminomaleamic acid (CO 11) is unstable in water solutions. Recent work (*1*) showing that aqueous solutions of this compound retard plant growth has prompted us to report our preliminary results on the decomposition of this class of compounds in water solutions. The original intent in investigating this group of compounds was to determine the relative hydrogen-bond strengths of hydrogen and deuterium in the N. . .H. . .O hydrogen bond in the solvents, water and deuterium oxide. In accord with the work of Dahlgren and Long (*2*) such a bond should exist in water solutions of maleamic and substituted maleamic acids. However, if the bond does exist it apparently is not sufficiently strong to prevent decomposition.

Since the decomposition of CO11 leads to 1,1-dimethylhydrazinium hydrogen maleate (or more simply stated, maleic acid and 1,1-dimethylhydrazine), a titration of the reaction mixture with standard base should show the appearance of two distinct end points. The first end point would be equivalent to the neutralization of the unreacted CO11 (pK_a approximately 5) and the neutralization of the second hydrogen on the maleic acid product [pK_a = 6.33 (*2*)] and the second end point would be equivalent to the neutralization of the 1,1-dimethylhydrazinium cation (pK_b approximately 5).

Solutions of CO11, 0.03*M* to 0.06*M*, were made with conductivity water at 25.0°C and maintained at 25.0°C in a constant-temperature bath throughout the reaction. Time zero (t_0) was taken as the time of the initial liquid-solid contact; complete solution was attained in less than 1 minute. Considering the very long reaction time (in excess of 100 hours) the error here is quite negligible. Aliquots of the reaction mixture were titrated at 25°C with standard base on a Serfass conductance bridge; the time of sampling was taken as the run time. The conductance data were then corrected for dilution effects resulting from the added base. The corrected conductance was plotted against the volume of the base used. The results of these titrations are shown in Fig. 1.

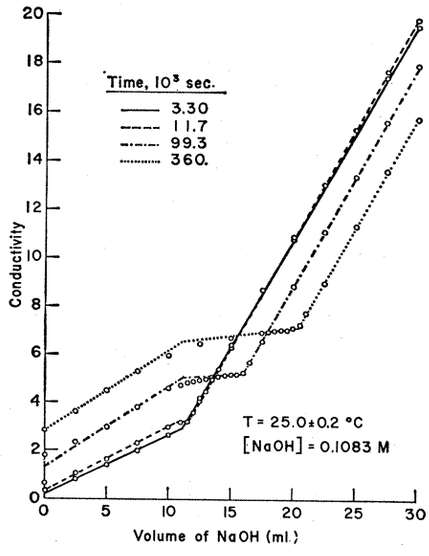

Fig. 1. Conductometric titration curves for the neutralization of aqueous solutions of *N*-dimethylaminomaleamic acid (CO11) by NaOH. Time here represents the time of sampling: the longer times show a double end point; the first corresponds to the neutralization of the product 1, CO11 and the hydrogen on the hydrogen maleate anion product, and the second to the neutralization of the product 1, 1-dimethylhydrazinium cation to 1,1-dimethylhydrazine.

The increase in the initial conductance with time (Fig. 1) is indicative of the increase in the concentration of the ionic species 1,1-dimethylhydrazinium hydrogen maleate with time. The initial slope of each curve is characteristic of the formation of maleamate and maleate ions upon titration of maleamic acid and hydrogen maleate anion with NaOH. The second slope is the result of the conversion of 1,1-dimethlyhydrazinium ion to 1,1-dimethylhydrazine by titration with NaOH; the positive slope indicates the higher mobility of the sodium ion compared with that of the 1,1-dimethylhydrazinium ion. The last large increase in slope is the result of excess sodium hydroxide.

The pseudo first-order rate constants for the decomposition were obtained by plotting the logarithm of the CO11 concentration at time *t* against time; the slope was equal to $k_1/2.3$ (see Fig. 2). The rate constants so obtained were reproducible to within 5 percent.

During the course of the reaction no attempt was made to control the *p*H or the ionic strength of the reaction mixture. In Fig. 2 the effect of changing *p*H on the rate constant can be seen. The upper curve shows the change in rate constant (the change in slope) with time, while the lower curve gives the change in *p*H with time. The shapes of the two curves suggest that the rate increases with increase in hydrogen-ion concentration, an effect which has also been observed by Bender (*3*) for the decomposition of phthalamic acid in water solutions. It is of interest to note that the pseudo first-order rate constant for the decomposition of CO11 in water (7.4×10^{-6} sec^{-1}) is similar (1.5×10^{-5} sec^{-1}) to that for phthalamic acid in water solution at 25°C and *p*H 3.4 obtained by extrapolation of data from the literature (*4*).

If the hydrolysis is an example of intramolecular catalysis, as might be suggested by the stereochemistry of the molecule in which the attacking carboxy group is conveniently on the same side (*cis*) with the attacked carbamyl group, the reaction should be limited to *cis* compounds. To support this hypothesis

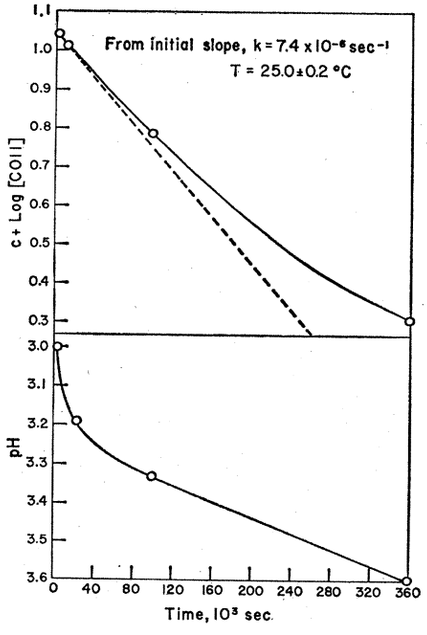

Fig. 2. (Top) First-order kinetic plot shows the change in the log of the concentration of *N*-dimethylaminomaleamic acid (CO11) with time. The initial slope is $k_1/2.3$. (Bottom) The change in *pH* with time for the same run illustrated in the top graph.

we also studied *N*-dimethylaminosuccinamic acid, where the preferred orientation should be *trans;* this *trans* compound was stable in aqueous solution at 25.0°C for several days.

The capacity of CO11 to retard plant growth is probably due to the presence of 1,1-dimethylhydrazinium hydrogen maleate in the aqueous mixture and not solely to CO11 as suggested by Riddell and coworkers (*1*).

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References and Notes

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5. We thank Naugatuck Chemical for the supply of the two acids and the American Cancer Society for support through grant P-272.

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Infant Handling: Effects on Avoidance Learning, Brain Weight, and Cholinesterase Activity

Abstract. *Infant rats were "handled" by removing them periodically from their home cages. "Non-handled" rats were left undisturbed. Half of the animals were killed at weaning, and weights and cholinesterase activity were determined on four different sections of the brain. The remaining animals were conditioned to avoid an administered shock. Handling increased ventral-cortex and subcortical weights and decreased subcortical cholinesterase. No differences in avoidance conditioning were observed.*

Many experiments demonstrate that a rat's experience early in life affects its later behavior. Several procedures have been used to manipulate early experience, all of which have in common the removal of the animal from its home cage. These preweaning experiences result in animals that are less emotional in open field tests. Thus, they show less defecation and urination and a greater degree of exploration in a strange environment (*1*), and they are better able to learn a conditioned avoidance response (*2*). Raising animals in a complex environment after weaning results in animals that are more capable of learning complex tasks, such as making fewer errors in a maze (*3*) and in a discrimination-learning situation (*4*).

In few of these investigations have the related changes in the nervous system which result from these experiences been studied. In one promising line of research animals raised in conditions which provided a large amount of environmental stimulation during growth showed differences in brain weight and brain cholinesterase activity from littermates raised in restricted environments (*5*). These experiments have manipulated the experience of the animals after weaning. The purpose of our experiment was to determine the effects of handling before weaning on avoidance conditioning, the distribution of brain weight, and brain cholinesterase activity.

The subjects for this experiment were 58 descendants of the Tryon S_3 strain that were littered by six female rats. Before the pups were born each mother was assigned at random to either the control or the experimental group. The control litters were not disturbed until weaning. The experimental litters were handled once daily from age 2 to 10 days. The handling procedure consisted of removing the infant rats from the home cage and placing them in a metal pan partly filled with wood shavings for 5 minutes. At the end of this period the pups were returned to the home cage. After the ninth day of handling the experimental animals were not disturbed until weaning. None of the cages were changed throughout the course of the experimental treatment, and care was taken not to stimulate the animals unnecessarily.

At 25 days of age the animals were removed from the home cage, their ears were punched for identification purposes, and approximately half the animals of each sex from each litter were killed for chemical analysis. The remaining animals were kept for avoidance training and were housed by sex and by litter.

The animals designated for chemical analysis were decapitated, the brains were dissected, and samples of tissue were removed by a standardized gross dissection procedure (*6*). The tissues analyzed were from the visual and somesthetic areas of both cortical hemispheres, the remaining dorsal cortex, the ventral cortex, and the remaining brain (subcortex). Weights and cholinesterase activity of the visual and somesthetic areas were averaged and represent the sensory cortex. Immediately after dissection, the weight of each sample was determined to the nearest 0.01 mg with a direct-reading analytical balance. Samples were frozen on dry ice and stored at −20°C until they were analyzed.

Table 1. Means of cholinesterase activity and brain weights for handled (H) and nonhandled (NH) groups. Cholinesterase activity: 10^{10} moles of acetylcholine hydrolized per minute per milligram of tissue.

Brain weight (mg)		Cholinesterase activity	
H	NH	H	NH
Sensory cortex			
52	50	57	57
Remaining dorsal cortex			
259	248	56	61
Ventral cortex			
276	254*	84	90
Subcortical brain			
790	710*	170	182*

* $p < .05$.

Cholinesterase activity was assayed by the method of Rosenzweig, Krech, and Bennett (*7*). For avoidance training, a box was divided into two compartments, each 12 by 4 by 8 inches, separated by a guillotine-type door. The start compartment had a grid floor through which a 0.5-ma shock could be delivered to the animal's feet; the safe compartment had a wooden floor. The experimenter could observe the animal through Plexiglas lids on the top of each compartment.

A trial began when the center door was raised. This action closed a microswitch which simultaneously started a warning buzzer, a standard electric timer, and a time-delay relay which controlled the onset of the shock. Shocks were terminated when the animal entered the safe compartment and interrupted a photocell beam. Latency (the time between the opening of the door and the interruption of the photocell) was recorded after each trial. The interval between the onset of the buzzer and the onset of the shock was 5 seconds. A 30-second rest period was allowed in the safe compartment before the animal was returned to the shock compartment. A total of 40 seconds elapsed between the end of one trial and the beginning of the next.

The first day of avoidance training was begun when the animal had reached 30 days of age. The procedure for each of the four trials on this day was the same as that described, but no shock was delivered to the animal. A maximum latency of 60 seconds was allowed. If the subject had not crossed to the safe compartment in this time,