TABLE I

PHENYLBORIC ACID + SALT = 0.005 MOLAL

Salt present, %	PH	$K\alpha \times 10^9$	Average $K\alpha \times 10^9$
25	8.16	2.31	
40	8.51	2.06	
50	8.70	2.00	2.00
60	8.89	1.94	
75	9.21	1.85	

TABLE II

PHENYLBORIC ACID + SALT = 0.01 MOLAL

Salt present, %	PH	$K\alpha \times 10^9$	Average $K\alpha \times 10^9$
25	8.38	1.39	
40	8.58	1.75	
50	8.76	1.74	1.78
60	8.91	1.84	
75	9.21	1.85	

Summary

It has been shown that the interaction of trivalent boron with adjacent groups may lead to certain anomalies which are not explainable on the basis of negativities alone. These discrepancies are all readily explained by the use of combination formulas which have been justified by Pauling from quantum mechanical considerations. The apparent anomalies in the addition compounds of boron, in the dissociation constants of boric acid and phenylboric acid, and in the orientation of phenylboric acid have been discussed. The dissociation constant of phenylboric acid was measured and found to be three times as great as that of boric acid.

BERKELEY, CALIFORNIA

RECEIVED FEBRUARY 15, 1933
PUBLISHED JULY 6, 1933

[INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

A Comparison of the Toxicity of Nicotine and Anabasine[1]

BY W. A. GERSDORFF

In a study of the lethal action of toxic substances a proper comparison of the relationship between concentration and time of death may be obtained only by determining the complete curves which are the graphic expression of this action. These curves are obtained by a series of toxicological tests at a number of concentrations of the substances. Such series of tests were made with nicotine and anabasine, the goldfish being used as the test animal.

Freshly distilled samples of nicotine and anabasine were obtained from C. R. Smith of this Laboratory. The anabasine had been separated by him from an imported sample of anabasine sulfate obtained in the extraction of the alkaloids from *Anabasis aphylla* L.[2] and found to be chemically identical with neonicotine (β-pyridyl-α'-piperidine),[3] differing only in being levorotatory, whereas synthetic neonicotine is inactive. Neo-

(1) Presented before the Division of Biological Chemistry at the spring meeting, March 26–29, 1933, of the American Chemical Society at Washington, D. C.

(2) Orechoff and Menschikoff, *Ber.*, **64**, 266–274 (1931).

(3) Smith, THIS JOURNAL, **54**, 397–399 (1932).

nicotine has been found to be of value as a contact insecticide,[4] its toxicity to aphids being of the order of that of nicotine. More recently, anabasine has been found to be about 40% as toxic as nicotine to mosquito larvae at concentrations killing 50% in eight hours.[5]

The method used in the tests reported here was that described by the author in a previous paper,[6] and used in similar studies with rotenone,[7] and related compounds.[8] There were, however, several departures from the general method as described. Because of the solubility of nicotine and anabasine in water, it was not necessary to use acetone at any stage, as was the case with the rotenone compounds. Freshly prepared solutions were always used. In making the tests the ratio of one liter of solution to each fish was maintained only in the very dilute solutions, when the survival times were so long as to require the fishes to remain in the test solution overnight. At the shorter survival times the ratio of one liter of solution to two fishes was adopted. The tests were always made on fishes from the same group.

Because the mode of toxic action of these two substances is apparently different from that of the rotenone series of compounds, the observations necessary to decide on the death point were made somewhat differently. The paralyzing action on the nervous system is such that the fishes have a tendency to locking of the jaws. This makes the death point exceedingly difficult to determine. It was found necessary to observe the fishes much more frequently toward the end of a test than in the tests with the rotenone compounds since the approach to death was not nearly so gradual. In the cases having short survival times, observations were practically continuous. It was found necessary to increase the final period of observation for lack of movement to two minutes. It was also found that a fish taken out of the test solution as dead and dipped into hydrochloric acid (about 1:3) might give no responsive action but would react upon being dipped into fresh water. Since dipping into the acid, therefore, was unreliable as a method of confirming death, this method was discontinued. Instead, after being removed from the test solutions as dead, the fishes were placed in fresh water, and a record was made of their reaction, that is, whether there was no reaction, temporary revival or complete recovery. There were only a few recoveries in the tests given.

The distribution of these individual observations is very irregular in contrast with that obtained with the rotenone compounds. With the latter, the distribution is so regular that the average survival time of the middle third closely approximates that of the whole. The sometimes large individual variations from the mean in the tests with the two alka-

(4) Smith, Richardson and Shepard, *J. Econ. Entomol.*, **23**, 863–867 (1930).
(5) Campbell, Sullivan and Smith, *ibid.*, **26**, 500 (1933).
(6) Gersdorff, THIS JOURNAL, **52**, 3440–3445 (1930).
(7) Gersdorff, *ibid.*, **52**, 5051–5056 (1930).
(8) Gersdorff, *ibid.*, **55**, 1147 (1933).

loids are probably due in part to the initial paralysis of the nervous system and in part to a marked tendency of goldfish to develop a tolerance for them. However, after a study of the data, no better criterion could be found than the usual one of taking the average of the whole.

The toxicity data are summarized in Tables I and II. The survival time curves and the velocity of fatality curves, which were plotted from these

TABLE I

TOXICITY OF NICOTINE TO GOLDFISH AT 27.0 ± 0.2°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean wt. of fishes, g.[a]	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
30.0	5	..[b]	...[b]	Two fishes, 3. Three fishes, >3	..
25.0	12	44	2.6	20	5.4
20.0	14	43	2.4	20	5.1
16.7	6	43	2.4	22	4.5
13.3	11	44	2.6	45	3.1
11.4	14	45	2.7	Thirteen fishes, 98. One fish >52 hrs.	1.8
10.0	14	44	2.6	Twelve fishes, 200. Two fishes, 1440	1.2
8.0	13	45	2.7	Nine fishes, 380. Four fishes, >1440	0.29
7.0	20	42	2.3	Eleven fishes, 39 hrs. Nine fishes, >52 hrs.	.04

[a] Estimated from length. [b] Fishes not measured but of same approximate size.

TABLE II

TOXICITY OF ANABASINE TO GOLDFISH AT 27.0 ± 0.2°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean wt. of fishes, g.[a]	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
30.9	7	46	2.8	>14	<7.5
25.8	13	45	2.7	22	5.1
20.6	8	46	2.8	22	4.7
16.5	16	46	2.8	30	3.7
14.4	16	46	2.8	74	2.3
12.7	22	44	2.6	Nineteen fishes, 122. Three fishes, >48 hrs.	1.8
10.3	22	46	2.8	Fourteen fishes, 310. Eight fishes, >48 hrs.	0.59
7.7	14	..[b]	..[b]	Five fishes, 500. Nine fishes, >48 hrs.	.07
6.2	17	..[b]	..[b]	Four fishes, 590. Thirteen fishes, >53 hrs.	.04
5.9	17	..[b]	..[b]	One fish, 960. Sixteen fishes, >53 hrs.	.01

[a] Estimated from length. [b] Fishes not measured but of same approximate size.

data, are given in Figs. 1 and 2. Because of the peculiar action of these compounds previously mentioned, true averages of the long survival times could not be calculated; however, minimal values could be obtained by considering the survival time of a fish surviving a test as equal to the elapsed time of the test. Since the portion of the curve on which the true values would fall approaches the vertical, accurate values are not necessary to determine the shape of this portion of the curve with fair approximation. A minimal average is indicated with an arrow, and the adjacent portion of the curve by a broken line in Fig. 1. In calculating the mean of the reciprocals of the survival times, in those cases in which fishes were not

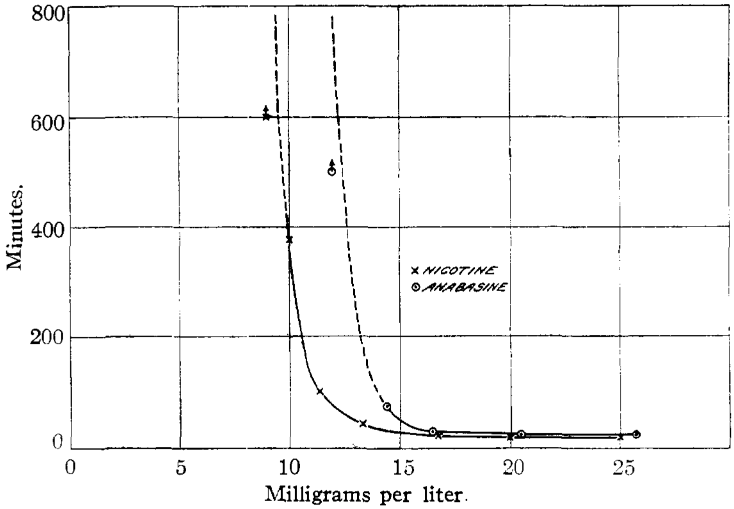

Fig. 1.—Survival time curves.

killed during the test (lasting from two to three days), these individual reciprocals were considered as zero, since the reciprocal of any value more than two days would be negligibly small in comparison with the other reciprocals.

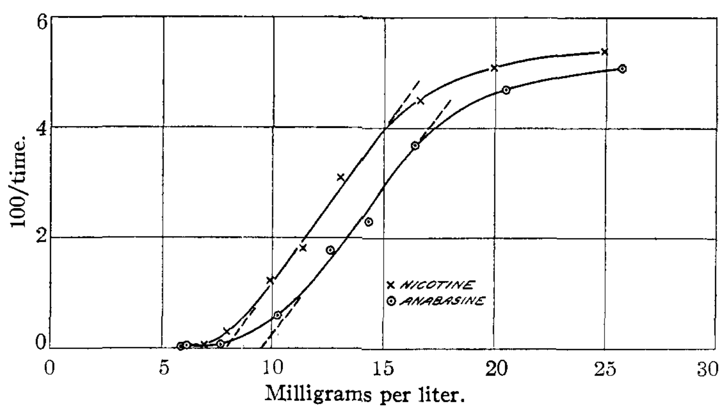

Fig. 2.—Velocity of fatality curves.

Comparative data obtained from the velocity of fatality curves are given in Table III. In each case the straight line which is an approximation of that portion of the curve corresponding to the greatest rate of

TABLE III

COMPARATIVE TOXICITY OF NICOTINE AND ANABASINE TO GOLDFISH AT 27°

Substance	a[a] mg. per liter	Tan Θ[b] cc. per mg. per min.	t_0,[c] min.
Nicotine	8.0	5.6	20
Anabasine	9.5	5.2	22

[a] The theoretical threshold of toxicity, *i. e.*, the concentration necessary to just kill.
[b] The rate of increase of the theoretical velocity of fatality with increase in concentration.
[c] The survival time corresponding to the region of constant velocity of fatality.

increase in the velocity of fatality with increase in concentration is prolonged to cut the x-axis at a point designated a; the slope of this line is designated tan θ. In this way values are obtained for the theoretical threshold of toxicity, that is, the concentration below which the substance does not cause death, and the rate of increase of the theoretical velocity of fatality with increase in concentration. Beyond this portion of the curve in the direction of higher concentrations there is a region of constant velocity, that is, the curve has become practically horizontal. The survival time for this region, designated t_0, is read directly from the data. It is not meant, however, that this is the minimum time in which fish may be killed in solutions of these materials. As a matter of fact, there are indications that death may occur more quickly in stronger solutions (as the result, probably, of a type of toxic action different from that represented in the graphs), but the limiting difficulties of experimentation preclude the obtainment of accurate data at such concentrations.

Conclusions

As compared to rotenone, solutions of nicotine and anabasine become toxic at relatively high concentrations. The theoretical thresholds of toxicity for the three compounds, against fishes from the same group, are, respectively, 0.012, 8.0 and 9.5 mg. per liter. The relatively low toxicities of the two alkaloids are also reflected in a comparison of the rate of increase of the theoretical velocity of fatality with increase in concentration. These rates, in the same order, are 90, 5.6 and 5.2 cc. per mg. per minute. According to these values, which are the more serviceable from the standpoint of practicality for use in such work as that of controlling pests, at these corresponding regions of their toxicity curves, rotenone is fifteen to twenty times as toxic as the two alkaloids. However, if the survival time alone is considered in comparison at the regions of constant velocity of toxicity, where the times are, in the same order, about one hundred and thirty, twenty and twenty-two minutes, the two alkaloids have a toxicity six to seven times as great as rotenone. Anabasine is slightly less toxic than nicotine throughout the range of concentrations used.

WASHINGTON, D. C.

RECEIVED FEBRUARY 17, 1933
PUBLISHED JULY 6, 1933