synthesized. Through alkaline saponification it has been converted to crystalline *β-d*-glucosidoferulic acid, a glucoside which is closely related to the naturally occurring coniferin.

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[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

THE TOXICITY OF ROTENONE, ISOROTENONE AND DIHYDROROTENONE TO GOLDFISH[1]

BY W. A. GERSDORFF

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The method used by the author for the study of toxicity in which the goldfish serves as the test animal has been described in a previous paper.[2] This method was used in studying the relative toxicities of rotenone, and two of its derivatives, isorotenone and dihydrorotenone. These substances were prepared by F. B. LaForge and L. E. Smith of this Division[3] in their researches to determine the chemical structure of rotenone.

The chemical structure of these compounds is not yet known. The empirical formula of rotenone is $C_{23}H_{22}O_6$. It possesses a ketone group, two methoxyl groups, a lactone group and an oxygen atom which is probably in the form of an ether linkage. LaForge and Smith reported that dihydrorotenone was one of the products of the catalytic reduction of rotenone, the reaction involving the simple reduction of an unsaturated bond in the rotenone molecule. It is now known that the double bond reduced is that in an isopropylene group. Isorotenone differs from rotenone in the position of its double bond. The double bond of the isopropylene group in rotenone has migrated and an isopropyl group has formed. The chemical relationship of these compounds will be discussed in a forthcoming paper by H. L. Haller of this Division.

The data are given in Tables I to III. The survival time curves and velocity of fatality curves which were plotted from these data are given in Figs. 1 to 3. In the former the ordinates are survival times in minutes; in the latter, the reciprocals of the survival times multiplied by 100. In both kinds of curves the abscissas are concentrations in milligrams per liter.

These curves resemble those given by Powers[4,5] to show the general type of toxic action to goldfish and that given by Carpenter[6] to show the

[1] Presented as a part of the Insecticide Symposium before the Division of Agricultural and Food Chemistry at the 79th Meeting of the American Chemical Society, Atlanta, Ga., April 7 to 11, 1930.

[2] W. A. Gersdorff, THIS JOURNAL, **52**, 3440–3445 (1930).

[3] F. B. LaForge and L. E. Smith, *ibid.*, **51**, 2574–2581 (1929).

[4] Edwin B. Powers, *Ill. Biol. Mono.*, **4**, No. 2 (1917).

[5] Edwin B. Powers, *Ecology*, **1**, 95–112 (1920).

[6] Kathleen E. Carpenter, *Brit. J. Exptl. Biol.*, **4**, 378–390 (1927).

toxicity of sodium chloride to minnows (*Leuciscus phoxinus*). The toxic action does not, therefore, resemble that found by Powers in three anomalous cases in which cupric chloride, ferric chloride and cadmium chloride were the toxic substances nor that found by Carpenter in tests with soluble salts of heavy metals including the above three and lead, zinc and mercury. However, Carpenter found that these substances showed a similar

TABLE I

TOXICITY TO GOLDFISH OF ROTENONE AT 27.0 ± 0.2°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean weight of fishes,[a] g.	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
4.0	4	40	2.0	46	2.19
3.0	3	37	1.6	46	2.16
2.0	4	37	1.6	48	2.08
1.0	4	39	1.8	49	2.06
0.90	7	38	1.7	52	1.91
.70	7	39	1.8	60	1.71
.50	8	39	1.8	57	1.82
.30	13	39	1.8	70	1.51
.20	16	38	1.7	65	1.55
.10	12	42	2.3	95	1.08
.075	10	43	2.4	115	0.87
.050	11	41	2.2	150	.70
.035	12	41	2.2	252	.42
.025	16	43	2.4	589	.23
.015	7	..	...	2400	.04

[a] Estimated from length.

TABLE II

TOXICITY TO GOLDFISH OF ISOROTENONE AT 27.0 ± 0.2°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean weight of fishes,[a] g.	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
4.0	2	41	2.2	116	0.87
3.0	1	47	3.1	120	.83
2.0	6	41	2.2	132	.76
1.0	10	40	2.0	147	.69
0.80	8	41	2.2	153	.66
.50	11	43	2.4	155	.65
.30	7	42	2.3	148	.68
.25	10	41	2.2	162	.63
.20	13	40	2.0	197	.51
.15	12	44	2.6	226	.44
.10	17	43	2.4	396	.25
.090	1	..	...	470	.21
.070	2	43	2.4	730	.14
.060	1	..	...	1380	.07
.050	1	Still alive and apparently unaffected when taken out after 87 hours.			
.025	2				

[a] Estimated from length.

TABLE III

TOXICITY TO GOLDFISH OF DIHYDROROTENONE AT 27.0 ± 0.2°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean weight of fishes[a], g.	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
1.0	4	44	2.6	75	1.36
0.50	8	43	2.5	74	1.38
.40	2	42	2.3	92	1.09
.25	12	42	2.3	96	1.09
.20	16	44	2.6	111	0.94
.15	13	41	2.2	121	.85
.10	14	40	2.1	128	.84
.080	13	40	2.1	132	.82
.050	21	39	1.9	158	.70
.040	12	37	1.7	174	.58
.030	14	40	2.1	280	.42
.015	6	40	2.1	720 ±	.17

[a] Estimated from length.

general type of effect which was marked by the formation of film over gills and skin and resulted in death by suffocation. When less than a certain amount of metallic ion was present the film was shed and complete recovery took place. In the tests with rotenone, isorotenone and dihydrorotenone no such film formation was apparent.

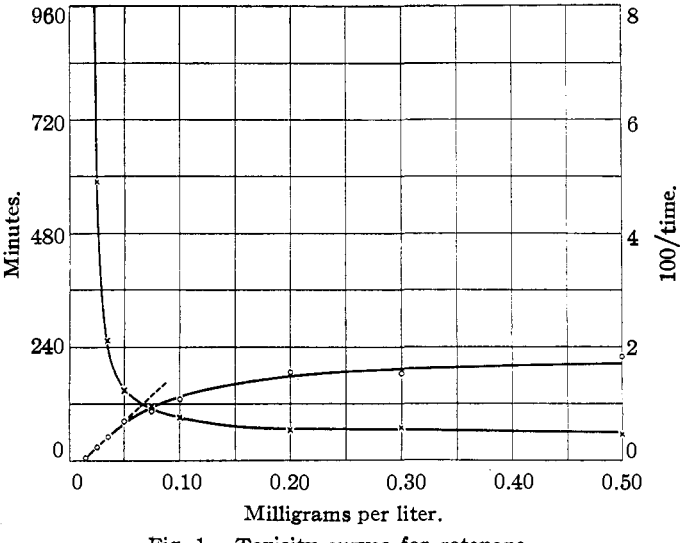

Fig. 1.—Toxicity curves for rotenone.

As Osterhout[7] pointed out, the values for the relative toxicities of substances will vary according to the criterion used. There are serious ob-

[7] W. J. V. Osterhout, "The Measurement of Toxicity," *J. Biol. Chem.*, **23**, 26–70 (1915).

jections to the comparison of the survival times at a given concentration, to the comparison of the concentrations necessary to produce death (or any other phenomenon used as the criterion) in any arbitrarily fixed time, and to the comparison of the concentrations necessary to just cause death (threshold of toxicity concentration). These are easily seen in an inspection of the survival time curves. Limiting concentrations vary so that the relative toxicities of two substances, when compared by the first method, will change, depending on the concentration used. For example, as seen in the data, at 4 mg. per liter, rotenone is apparently two and one-half times as toxic as isorotenone; at 0.20 mg. it is three times, at 0.10 mg. four times and at 0.075 mg. nearly six times as toxic, whereas at

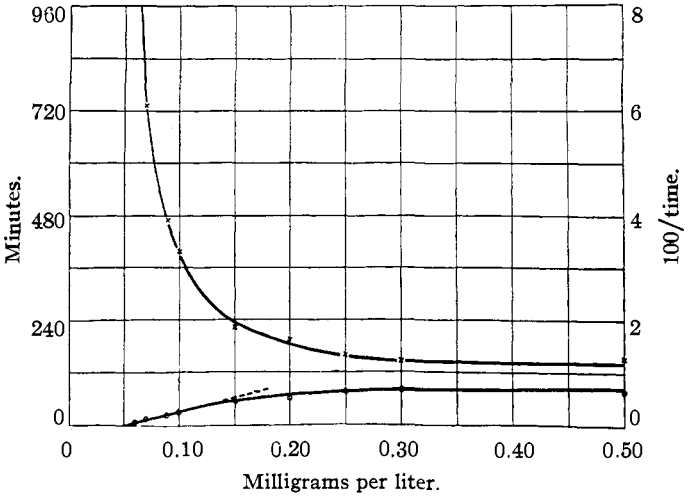

Fig. 2.—Toxicity curves for isorotenone.

0.050 mg. its toxicity becomes indefinitely greater since at this concentration isorotenone is no longer toxic at all. Similarly, the relative toxic values vary when the concentrations necessary to kill in an arbitrarily chosen survival time are compared, and they become worthless if they are obtained from data falling on either that portion of the survival time curve approaching the horizontal or that portion approaching the vertical. If substances are compared by this single survival time method, this survival time should fall on the middle portion of the curve, that is, the portion that approaches an equilateral hyperbola. The third method, the comparison of the concentrations necessary to just cause death, involves the determination of values very difficult to obtain with precision and in addition deals only with one factor in toxicity, the threshold of toxicity. From the velocity of fatality curves it is seen that there are at least two variables in toxicity and these apparently vary independ-

ently of each other. They are the threshold of toxicity concentration and the rate of increase of the velocity of fatality. Powers[4] adopts as a measure of toxicity a value based on the reciprocal relation of these two factors and calculated from the equation, toxicity $= \sqrt{\tan \Theta / a}$, where $\tan \Theta$ represents the slope of that portion of the velocity of fatality curve which approaches a straight line and a its point of intersection, when prolonged, with the x-axis. Powers designated this straight line as the theoretical velocity of fatality curve and the concentration represented by the point a as the theoretical threshold of toxicity concentration.

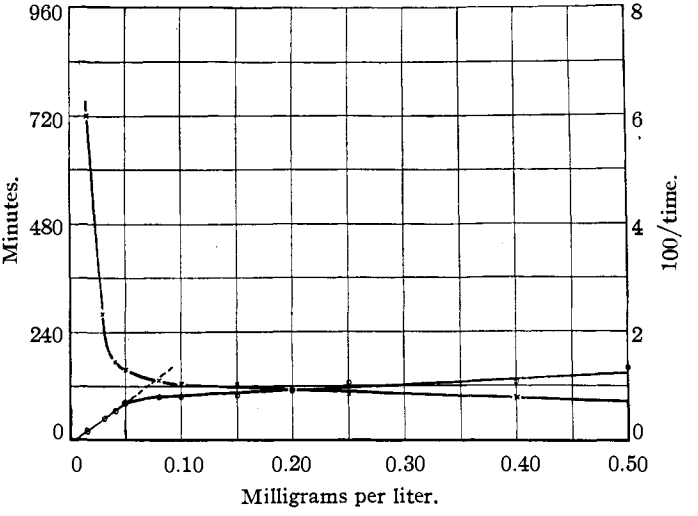

Fig. 3.—Toxicity curves for dihydrorotenone.

The comparative toxicities of the substances considered here, derived according to this equation, are shown in Table IV.

TABLE IV

COMPARATIVE TOXICITIES TO GOLDFISH OF ROTENONE, ISOROTENONE AND DIHYDROROTENONE AT 27°

Substance	a,[a] mg. per liter	Tan Θ[b]	Toxicity $\sqrt{\frac{\tan \Theta}{a}}$	Relative toxicity with respect to rotenone
Rotenone	0.0125	0.187	3.9	1.0
Isorotenone	.055	.053	1.0	0.26
Dihydrorotenone	.005	.156	5.6	1.4

[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. These values are based on the expression of concentration in milligrams per liter and time in minutes.

It would appear from this study that this formula for toxicity is not all that could be desired. Because the threshold of toxicity concentration

for dihydrorotenone is much smaller than that for rotenone, the relative toxicity of the former is nearly half again as great as that of the latter. Yet there is another variable which, if considered, would alter these relative values and bring them closer together, a relationship more credible after an inspection of the curves of the two substances. These curves show that after reaching its maximum the rate of increase of the velocity of fatality with increase in concentration decreases much more rapidly in dihydrorotenone than in rotenone. It is hoped that a formula may be developed which will include this third factor. On the other hand it may not really be significant to express the toxicity of a substance at a single value, but to define it according to the three variables, threshold of toxicity, rate of increase of the velocity of fatality and decrease of this rate.

Conclusions

The toxicity of rotenone begins at a higher concentration than that of dihydrorotenone (about twice, according to Powers' formula) and a lower concentration than that of isorotenone (about one-fourth). The toxicities of rotenone and dihydrorotenone increase with increase in concentration at about the same rate, but this rate is lower in the case of isorotenone (about one-third). At higher concentrations, rotenone is the most toxic and isorotenone the least. According to Powers' formula, which is an expression of relative toxicity based on the first two variables, these substances have the following decreasing order of toxicity: dihydrorotenone, rotenone and isorotenone.

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THE RESOLUTION OF 1-(ALPHA-1-PIPERIDYLBENZYL)-2-NAPHTHOL[1]

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In a previous article on the condensation of secondary amines with naphthols and aldehydes[2] a description was given of the preparation of 1-(α-1-piperidybenzyl)-2-naphthol. This asymmetric amine is one of a series of amines which are being prepared and tested as resolving agents. At the present time only one synthetic amine, namely, α-phenylethylamine, has been used to any great extent for this work, most basic resolving agents being alkaloids, where only one of the two possible active forms is available for use.

[1] An abstract of a portion of a thesis submitted by Joseph B. Littman in partial fulfilment of the requirements for the degree of Doctor of Philosophy at The Ohio State University.

[2] Littman and Brode, THIS JOURNAL, **52**, 1655 (1930).