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

A STUDY OF THE TOXICITY OF TOXICAROL, DEGUELIN AND TEPHROSIN USING THE GOLDFISH AS THE TEST ANIMAL

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The goldfish (*Carassius auratus*), having proved to be a good test animal in the study of toxicity, has been used in the toxicological examination of various substances that is now in progress in this Laboratory. The substances tested have been constituents of fish-poisoning plants and their derivatives. The method used by the author has been described in a previous paper,[1] and a study by that method of the toxicity of rotenone and two of its derivatives, isorotenone and dihydrorotenone, has also been published.[2] This paper presents the results of a similar examination of toxicarol, deguelin and tephrosin, toxic constituents of two fish poisoning plants of the genus Cracca (*Tephrosia*).

Toxicarol (m. p. 219°) and deguelin[3] (m. p. 171°) were isolated from the roots of *C. toxicaria* by E. P. Clark of this Laboratory.[4] It was shown by him that both substances are found also in the roots of *Deguelia* (*Derris*) *elliptica*, and in addition deguelin occurs in the roots of *Lonchocarpus nicou* ("cubé") and the leaves of *C. vogelii*.

Tephrosin (m. p. 198°) was isolated from the leaves of *C. vogelii*.[5] It may also be obtained, however, from derris and cubé roots.

The chemical structure of these three substances is not yet fully known. Toxicarol is an optically inactive compound of the molecular formula $C_{23}H_{22}O_7$, possessing two hydroxyl and two methoxyl groups. Deguelin is an optically inactive dimethoxy lactone having the molecular formula $C_{23}H_{22}O_6$. Tephrosin is a dimethoxy lactone with the molecular formula $C_{23}H_{22}O_7$. It is suggested by Clark that tephrosin is an hydroxydeguelin. It is to be noted that the molecular formulas for toxicarol and tephrosin differ from that of rotenone by having one more atom of oxygen, whereas the formula for deguelin is identical with that of rotenone.

On account of the slight solubility of the compounds in water, acetone was used as the solvent. It was found necessary early in these toxicological studies with rotenone and some of its derivatives to work with freshly prepared stock solutions of the compounds in acetone, since such solutions when allowed to stand underwent appreciable chemical change, as evidenced by a change in the color of the solution, with a concomitant loss in

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

[2] W. A. Gersdorff, *ibid.*, **52**, 5051–5056 (1930).

[3] Deguelin was not named until later and is referred to in the reference as "the second compound."

[4] E. P. Clark, THIS JOURNAL, **52**, 2461–2464 (1930); **53**, 313–317 (1931).

[5] E. P. Clark, *ibid.*, **53**, 729–732 (1931).

toxicity. The loss of toxicity of solutions of rotenone has also been noted by Jones and Davidson, who used insects as the test animals.[6] That the solutions change on standing was observed in the present study especially with toxicarol and deguelin. Acetone solutions of toxicarol suffer a rapid loss in toxicity to goldfish; acetone solutions of deguelin, however, undergo an appreciable increase in toxicity. Acetone solutions of tephrosin either do not undergo a change in toxicity or the change is slow as compared to that of the other two compounds.

The data are given in Tables I to III. The survival time curves and the velocity of fatality curves which were plotted from these data are given in Figs. 1 and 2. In the former curves the ordinates are survival times in minutes; in the latter, they are the reciprocals of the survival times multi-

TABLE I

TOXICITY OF TOXICAROL TO GOLDFISH AT 27.0 ± 0.3°

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	17	38	1.8	91	1.12
0.5	17	39	1.9	112	0.90
.25	15	39	1.9	138	.74
.10	14	39	1.9	158	.64
.075	11	39	1.9	185	.58
.060	12	39	1.9	239	.43
.050	24	40	2.0	310	.34
.025	11	38	1.8	960[b]	.11[b]

[a] Estimated from length. [b] One additional fish was apparently unaffected after forty-four hours in the test solution.

TABLE II

TOXICITY OF DEGUELIN TO GOLDFISH AT 27.0 ± 0.3°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean weight of fishes,[a] g.	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
5.0	6	40	2.0	60	1.68
0.40	17	38	1.8	124	0.83
.25	16	40	2.0	158	.66
.175	27	40	2.0	186	.56
.125	13	40	2.0	208	.50
.090	20	40	2.0	261	.39
.075	16	39	1.9	278	.38
.060	13	39	1.9	312	.33
.050	4	37	1.7	400	.26
.040	15	40	2.0	478	.21
.035	21	[b]	Mean of 17 fishes, 1800. Four fishes still active after 64 hrs.		.09
.025	11	[b]	Apparently unaffected in 61 hrs.		

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

[6] H. A. Jones and W. M. Davidson, *J. Econ. Entomology*, **24**, 258–262 (1931).

TABLE III

TOXICITY OF TEPHROSIN TO GOLDFISH AT 27.0 ± 0.3°

Concn., mg. per liter	No. of fishes used	Mean length of fishes, mm.	Mean weight of fishes,[a] g.	Mean surv. time, min.	Mean $\frac{100}{\text{surv. time}}$
2.0	12	40	2.0	134	0.75
1.5	12	41	2.2	158	.64
1.0	15	43	2.4	137	.74
0.75	15	38	1.8	156	.65
.50	13	38	1.8	181	.56
.30	18	38	1.8	222	.47
.20	21	39	1.9	274	.40
.15	12	41	2.2	349	.30
.10	12	42	2.3	576	.19
.075	12	40	2.0	1360	.09
.050	9	[b]	...	Apparently unaffected in 30 hrs.	

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

plied by 100. In both types of curves the abscissas are concentrations in milligrams per liter.

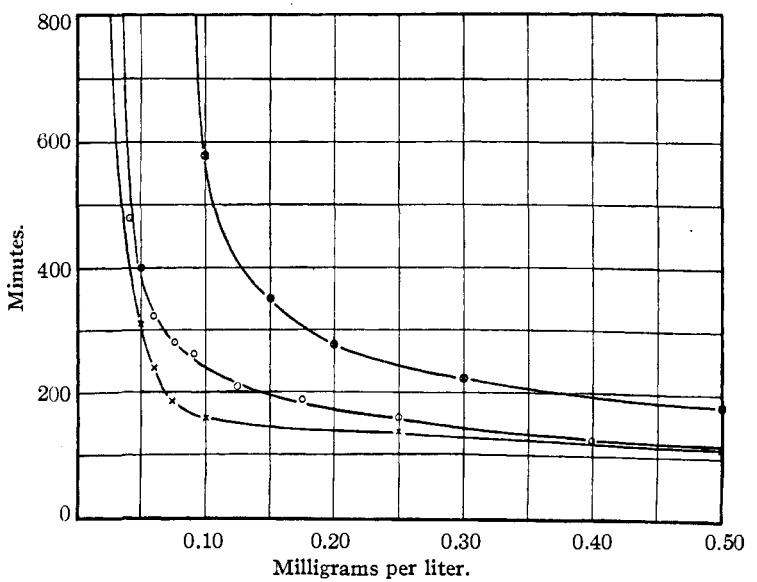

Fig. 1.—Survival time curves: X, toxicarol; ⊙, deguelin; ⊗, tephrosin.

Until an equation better expressing the course of toxic action is found acceptable, the formula used in the preceding papers, that is, Powers' formula, toxicity = $\sqrt{\tan \theta / a}$, will again be used as a measure of the relative toxicities of the three substances studied here. In this formula data obtained from the velocity of fatality curve are used. A portion of this curve approximates a straight line since its corresponding portion of the

survival time curve approximates an equilateral hyperbola. This straight line, representing the theoretical velocity of fatality, is prolonged to cut the x-axis at a point designated a; tan Θ is the slope of this line. 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. The formula expresses the opposite effect of these two variables.

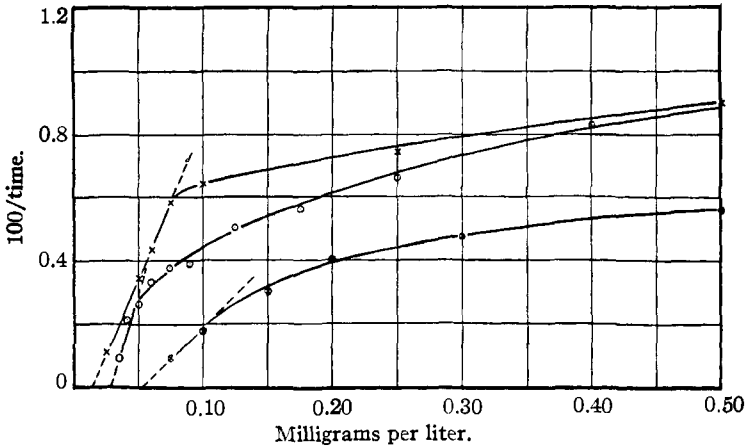

Fig. 2.—Velocity of fatality curves: ×, toxicarol; ⊙, deguelin; ⊗, tephrosin.

The comparative toxicities of the substances under consideration derived according to this equation are shown in Table IV. The values given for a and tan Θ are based on the expression of concentration in milligrams per liter and time in minutes. The values for the toxicities are compared to that for rotenone since rotenone was the first as well as the most toxic of the compounds studied.

TABLE IV

COMPARATIVE TOXICITY AT 27°C. OF TOXICAROL, DEGUELIN, AND TEPHROSIN TO GOLDFISH

Substance	a, mg. per liter	Tan Θ	$\sqrt{\frac{\tan \Theta}{a}}$	Relative toxicity with respect to rotenone
Toxicarol	0.015	0.097	2.5	0.65
Deguelin	.030	.150	2.2	.56
Tephrosin	.055	.043	0.88	.23

Conclusions

Of the three compounds studied, toxicarol has the lowest threshold of toxicity, 0.015 mg. per liter. That of deguelin is twice as great and that of tephrosin nearly four times as great. The threshold of toxicity of rotenone

is slightly lower than that of toxicarol. The velocity of fatality of deguelin increases with increase in concentration at a higher rate than that of toxicarol (half again as high), but this rate is lower for tephrosin (less than half that of toxicarol). The rate of increase for rotenone is double that of toxicarol. At higher concentrations, when the rate of increase decreases, toxicarol is slightly more toxic than deguelin and tephrosin is considerably less toxic; for example, at a concentration of 0.20 mg. per liter the survival times are, respectively, 139, 175 and 274 minutes. Rotenone at higher concentrations is considerably more toxic than toxicarol; at a concentration of 0.20 mg. per liter the survival time is sixty-five minutes. According to Powers' formula, which is an expression of relative toxicity considering the first two factors only, the three substances studied have the following decreasing order of toxicity: toxicarol, deguelin and tephrosin. Their toxicities are 65, 56 and 23%, respectively, of that of rotenone.

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CATALYTIC REDUCTION OF MIXTURES OF PARA-NITRO- AND NITROSOPHENOLS WITH ALDEHYDES AND KETONES

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In connection with an attempt to prepare certain O-arylhydroxylamines it was noted that when the product that was formed by the catalytic reduction of 2,4-dinitrophenol in solution in acetone was benzoylated by the usual method of Schotten–Baumann, the compound expected, (2,4-bis-benzaminophenyl) benzoate, m. p. 233°,[1] was not obtained. Analyses indicated that the new compound contained one C_3H_6 group more than does (2,4-bis-benzaminophenyl) benzoate. When, however, the solvent that was used was either dioxane or isopropyl alcohol instead of acetone the expected (2,4-bis-benzamino-phenyl) benzoate was formed.

These facts pointed to the probability of a condensation taking place between acetone and the phenol during the reduction.

A solution of 2,4-dinitrophenetole[2] was then catalytically reduced and benzoylated. The compound that was obtained was found to be the expected new compound (2,4-bis-benzamino)-phenetole.

Thinking that the phenol group might have something to do with the peculiar course of the reduction in the first case mentioned, ortho, meta and para nitrophenols were catalytically reduced in solution in acetone.

When *p*-nitrophenol was catalytically reduced in the presence of acetone

[1] Post and Stuckenberg, *Ann.*, **205**, 69 (1880).

[2] Willgerodt, *Ber.*, **12**, 764 (1879).