Anal. Calcd. for $C_{12}H_{20}N_2O_3$: C, 59.96; H, 8.39; N, 11.66. Found: C, 59.71; H, 8.36; N, 11.00.

Summary

1. The behavior of some 2-keto-4-alkyl-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidines on catalytic reduction has been investigated.

2. It has been shown that the 5,6-double bond in such pyrimidine combinations is very resistant to change.

3. In all cases examined the 4-aryl groups were attacked by hydrogen in the presence of the catalyst and reduced to the corresponding hexahydro or saturated structure.

4. In only one case examined, namely, 2-keto-4-methyl-5-carbethoxy-6-phenyl-1,2,3,4-pyrimidine, where phenyl is substituted on carbon adjacent to the 5,6-double bond, did we succeed in reducing the 5,6-double bond in the pyrimidine ring.

5. The pharmacological behavior of these highly reduced structures is now under investigation.

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

A Study of the Toxicity of Rotenone Hydrochloride, Acetylrotenone and Rotenolone Using the Goldfish as the Test Animal[1]

BY W. A. GERSDORFF

The toxicological examination of derivatives of rotenone and related compounds with the use of the goldfish as the test animal has been continued in this Laboratory with the threefold hope of discovering a material more toxic and more stable than rotenone, and at the same time securing data by which a correlation may be made between the toxicity and chemical structure. The method used by the author has been described in a previous paper[2] and studies by that method of some of the compounds have also been published.[3,4] This paper presents the results of a similar examination of rotenone hydrochloride, acetylrotenone and rotenolone prepared in the Insecticide Division of the Bureau of Chemistry and Soils.

Rotenone hydrochloride (m. p. 193°) was prepared from rotenone by H. L. Haller according to the method of S. Takei.[5] The compound is formed by the addition of hydrochloric acid at the double bond of the

(1) Presented before the Division of Agricultural and Food Chemistry at the Meeting of the American Chemical Society, Denver, Colo., August 22–26, 1932.

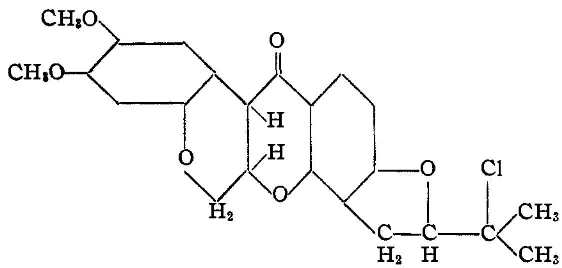
(2) Gersdorff, THIS JOURNAL, **52**, 3440–3445 (1930).

(3) Gersdorff, *ibid.*, **52**, 5051–5056 (1930).

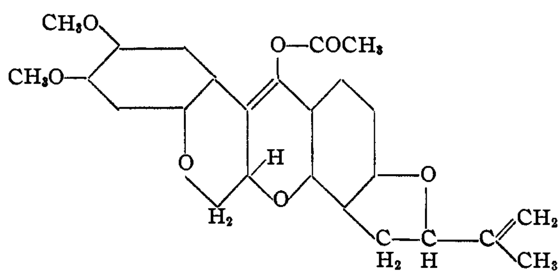
(4) Gersdorff, *ibid.*, **53**, 1897–1901 (1931).

(5) Takei, *Ber.*, **61B**, 1003–1007 (1928).

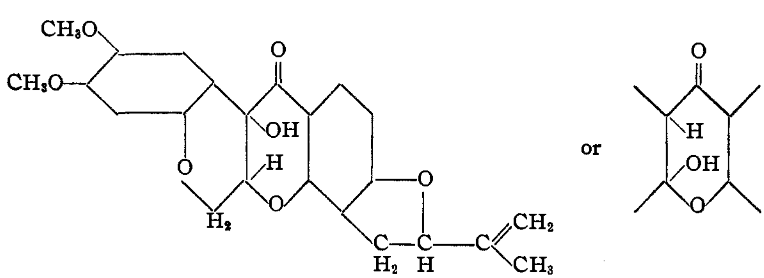
tubaic acid side chain. Its structure, now that the formula for rotenone has been established,[6] is shown by the formula



Acetylrotenone (m. p. 135°) was prepared[7] by the treatment of rotenone with acetic anhydride. The compound is an enol acetate as shown by its formula



Rotenolone (m. p. 140–141°) was prepared[8] by the saponification of the acetate obtained as one of the products of the reaction of iodine on a hot alcoholic solution of rotenone and potassium acetate. The hydroxyl group replaces the hydrogen attached to one of the adjacent asymmetric carbon atoms of rotenone, as



Which of these two formulas is correct has not yet been established.

The two lots of fishes used in these tests were slightly larger than the fishes used for the determination of the toxicity curves for rotenone and some of its derivatives in 1930,[3] and apparently somewhat more resistant

(6) LaForge and Haller, THIS JOURNAL, **54**, 810–818 (1932).
(7) Smith and LaForge, *ibid.*, **54**, 2996–3000 (1932).
(8) LaForge and Smith, *ibid.*, **52**, 1091–1098 (1930).

as shown in Table I. In this, comparisons are made of the three lots of fishes at two concentrations of rotenone.

TABLE I

RESISTANCE OF THREE LOTS OF GOLDFISHES TO ROTENONE AT 27°

Lot no. of fishes	Concn., mg. per liter	No. of fishes used	Mean wt. of fishes, g.	Mean survival time, min.
1	0.10	12	2.3	95
2	.10	5	2.4	114
3	.10	8	2.4	123
1	.05	11	2.2	150
2	.05	10	2.7	180
3	.05	12	2.3	177

TABLE II

TOXICITY OF ROTENONE HYDROCHLORIDE TO GOLDFISH 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 $\frac{100}{\text{surv. time}}$
0.50	9	41	2.2	128	0.83
.33	12	41	2.2	125	.89
.20	9	40	2.1	124	.84
.17	12	41	2.2	127	.87
.10	13	41	2.2	130	.82
.050	11	41	2.2	138	.74
.033	9	42	2.3	181	.60
.025	11	43	2.4	212	.51
.020	18	42	2.3	273	.46
.015	7	42	2.3	386	.28
.0050	10	[b]	[b]	Eight fishes, 980[c], two still active after 72 hrs.	.12[c]
.0025	11	[b]	[b]	Apparently unaffected, 72 hrs.	

[a] Estimated from length. [b] Fishes not measured, but of same approximate size. [c] These figures are only approximate since a large number of fishes would be required to give an accurate mean value. The reciprocal of the survival time of a fish surviving the test is taken as zero, since the reciprocal of any survival time longer than the test would be negligibly small.

The rotenone curves were obtained from tests made with fishes from lot number 1, the acetylrotenone and rotenolone curves from lot number 2, and the rotenone hydrochloride curves from lot number 3. Lots 2 and 3 are considered identical since the differences fall within experimental error.

The toxicity data are given in Tables II to IV. The survival time curves and the velocity of fatality curves, which were plotted from these data, are given in Fig. 1 and 2.

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

TABLE III

TOXICITY OF ACETYLROTENONE TO GOLDFISH 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 $\frac{100}{\text{surv. time}}$
0.50	12	44	2.6	98	1.08
.25	12	42	2.3	96	1.06
.15	8	47	3.1	120	0.87
.10	20	43	2.4	180	.60
.075	19	42	2.3	229	.49
.050	16	44	2.6	362	.31
.025	14	43	2.4	Ten fishes, 365[c]. Four fishes still active after 48 hrs.	.20[c]
.020	8	37 (5)	1.6	Five fishes, 904[c]. Three fishes still active after 30 hrs.	.13[c]
.015	6	41	2.2	One small fish, 670[c]. five fishes still active after 49 hrs.	.02[c]
.010	7	[b]	[b]	Two small fishes affected at first but apparently recovered. All active after 27 hrs.	

a, *b* and *c* as in Table II.

TABLE IV

TOXICITY OF ROTENOLONE TO GOLDFISH 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 $\frac{100}{\text{surv. time}}$
3.0	8	41	2.2	118	0.89
2.0	13	43	2.4	122	.84
1.5	24	42	2.3	136	.80
1.0	23	43	2.4	143	.72
0.75	18	44	2.6	178	.59
.50	7	45	2.7	199	.52
.40	13	42	2.3	250	.42
.30	7	40	2.1	358	.28
.25	15	41	2.2	448	.26
.10	6	43 (4)	2.4	856[c]	.12[c]
.050	10	[b]	[b]	Apparently unaffected in 52 hrs.	

a, *b* and *c* as in Table II.

TABLE V

COMPARATIVE TOXICITY AT 27° OF ROTENONE HYDROCHLORIDE, ACETYLROTENONE AND ROTENOLONE TO GOLDFISH

Substance	a[a], mg. per liter	TanΘ [b] liters per mg. per min.	Minimum surv. time, min.
Rotenone hydrochloride	<0.002	0.22	125
Acetylrotenone	< .002	.067	95
Rotenolone	.020	.011	115

[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 express the volume of water which must be added, throughout this portion of the curve, to any solution containing one milligram of the toxic substance in order to increase the survival time one minute.

In this way values are obtained for the theoretical threshold of toxicity, that is, the concentration below which the substance does not cause death,

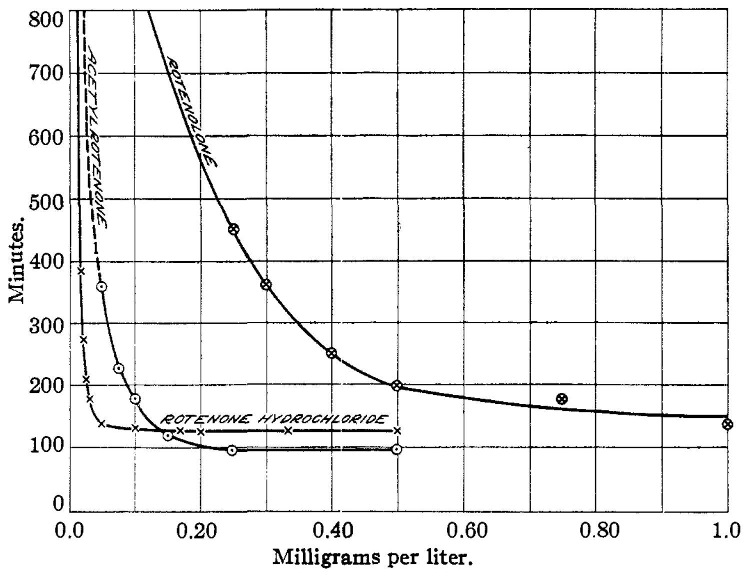


Fig. 1.—Survival time curves.

and the rate of increase of the theoretical velocity of fatality with increase in concentration. The minimum survival time is approximated from the

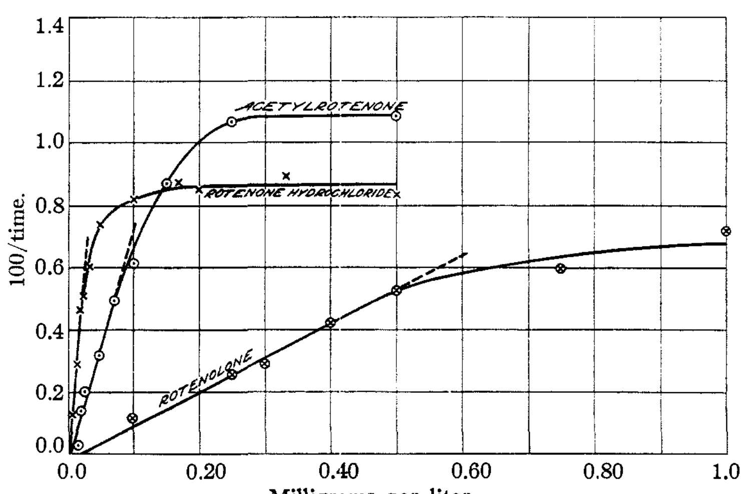


Fig. 2.—Velocity of fatality curves.

original data when the higher concentration portion of the survival time curve has become practically a horizontal line. This is assumed to occur when the survival times corresponding to concentrations, one of which is double the other, do not differ by more than 5%. The values are probably a little higher than that of the asymptote but they will serve for a practical comparison of the substances at this portion of their curves.

It was pointed out, when Powers' formula $\sqrt{\tan \Theta/a}$ was first used as a measure of the relative toxicities of substances,[3] that this formula[9] was not all that could be desired since the effect of the threshold value was thought to be too great and a third factor, the toxicity at the high concentrations as expressed by the portion of the survival time curve closely approaching the horizontal asymptote (corresponding therefore to the minimum survival time), was not considered at all. In the present study, these points are emphasized even more, since by use of this formula rotenone hydrochloride appears to have a toxicity ten times that of rotenone and even acetylrotenone appears to have a toxicity somewhat greater than rotenone. These values do not express the true relationship as shown by the curves. The former express the relationship between the toxicities of the substances only at their thresholds of toxicity and immediately beyond and may be misleading as a comparative guide for pest control work in which the toxic concentrations used are likely to be on or near the horizontal limb of the survival time curve. The use of this formula is therefore discontinued. It is still hoped that by obtaining toxicological data on other related compounds and by developing, if possible, a criterion more suitable for the expression of relative toxicity, the effect of a change in chemical structure on toxicity may be expressed arithmetically as well as graphically.

Conclusions

Solutions of rotenone hydrochloride and acetylrotenone become toxic to goldfish at very low concentrations, less than 0.002 mg. per liter, but with solutions of rotenolone the theoretical threshold of toxicity is 0.020 mg. per liter. The theoretical velocity of fatality of rotenone hydrochloride increases with increase in concentration at a higher rate than do those of acetylrotenone and rotenolone (about three and one-third and twenty times as much, respectively). The minimum survival times of these three substances, in the order, acetylrotenone, rotenolone and rotenone hydrochloride are ninety-five, one hundred and fifteen and one hundred and twenty-five minutes.

WASHINGTON, D. C.

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(9) Powers, *Ill. Biol. Mono.*, **4** (2) (1917).