Dehydrodihydroxyrotenonic acid of formula $C_{23}H_{24}O_8$, prepared from dehydrorotenone by the action of zinc and alkali or alkali alone, according to the directions of Butenandt, was oxidized with hydrogen peroxide in alkaline solution, and yielded a dibasic acid of formula $C_{12}H_{14}O_7$, which represents that half of the rotenone molecule which carries the original methoxyl groups as well as the carboxyl that in rotenone is coupled with the other half of the molecule to form the lactone group. The second carboxyl is formed by oxidation of the original carbonyl group.

The dibasic acid has been called "derric acid."

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[CONTRIBUTION FROM THE RESEARCH AND BIOLOGICAL LABORATORIES OF E. R. SQUIBB AND SONS]

THE RATE OF THERMAL DECOMPOSITION OF THE OXYTOCIC PRINCIPLE OF THE POSTERIOR LOBE OF THE PITUITARY GLAND. II. THE EFFECT OF TEMPERATURE

BY TILLMAN D. GERLOUGH AND ROBERT W. BATES

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This paper deals with the effect of temperature on the rate of thermal decomposition of the oxytocic principle in pituitary extract and of the highly purified oxytocic principle. Such a study has important bearing on problems concerned with its manufacture, and is of additional interest in determining whether or not further purification alters the rate of destruction of the oxytocic principle.

Experimental Methods

The oxytocic solutions and methods employed were much the same as those described in Paper I.[1] Pituitary solution No. 10122 was prepared by extracting the acetone defatted posterior lobe with acetic acid. This unpurified solution contains, in addition to the oxytocic principle, the pressor principle in nearly the same unitage as the oxytocic, inert proteins and a trace of salts. The purified oxytocic principle was prepared by the method of Kamm and co-workers.[2] Its composition is largely oxytocic protein with a small quantity of pressor principle and a very small trace of ammonium salt. Nitrogen determinations showed that the purified solution contained 0.0075 mg. of protein nitrogen per 10 international units, while the pituitary solution No. 10122 contained 0.29 mg. of nitrogen per 10 units.

The solution of the purified oxytocic principle, which had been previously assayed, was divided into two parts, (A) and (B). (A) was made by diluting with 0.2% acetic acid and (B) was made by diluting with

[1] Gerlough, THIS JOURNAL, **52**, 824 (1930).

[2] Kamm, Aldrich, Grote, Rowe and Bugbee, *ibid.*, **50**, 573 (1928).

0.1% acetic acid. The reaction of (A) was set at P_H 3.35 and that of (B) was P_H 3.55. Before starting the destruction work all solutions were placed in hard glass ampules, sealed and sterilized in streaming steam. The oxytocic activity of each solution, (A), (B) and No. 10122, was now 10 international units per cc. or 100% U. S. P. X. Some of the sterilized ampules were withheld as controls and kept at 2°. The remaining ampules were then heated at the temperatures shown in Table I and also held at 2° prior to actual test. Since the rate of destruction is so extremely slow at this temperature, no appreciable change would occur which might influence the results during the period of testing. The P_H of all heated solutions did not increase more than 0.05 from their controls. The temperatures recorded are accurate within 0.5°.

TABLE I

RATE OF THERMAL DECOMPOSITION OF THE OXYTOCIC PRINCIPLE AT VARIOUS TEMPERATURES

T, °.	t, hours	$a - x$	K	Log $1/k$	u
Pituitary Solution, No. 10122, P_H 3.25					
...	0.0	1.00	.....	..	....
308.8	1699.0	0.83	0.000109	3.96	20,500
325.6	696.0	.70	.000512	3.29	21,300
337.6	262.0	.70	.00140	2.85	22,500
351.6	95.0	.63	.00486	2.31	23,400
361.1	43.0	.66	.00965	2.01	25,500
373.1	....	..	.0333[a]	1.48	....
...	....	..	.....	..	24,000
394.1	0.75	.87	.185	0.732	
					Av. 22,900
(A) Purified Oxytocic Principle, P_H 3.35					
...	0.0	1.00	.....	..	....
309.1	2568.0	0.84	0.0000677	4.16	22,200
342.1	120.0	.77	.00217	2.66	22,300
358.6	48.5	.65	.00887	2.05	24,000
373.1	8.0	.77	.0326	1.49	
					Av. 22,800
(B) Purified Oxytocic Principle, P_H 3.55					
...	0.0	1.00	.....	..	....
309.1	2568.0	0.73	0.000122	3.91	22,100
342.1	120.0	.69	.00309	2.51	23,800
358.6	48.5	.52	.0134	1.87	25,600
373.1	8.0	.64	.0557	1.25	
					Av. 23,800

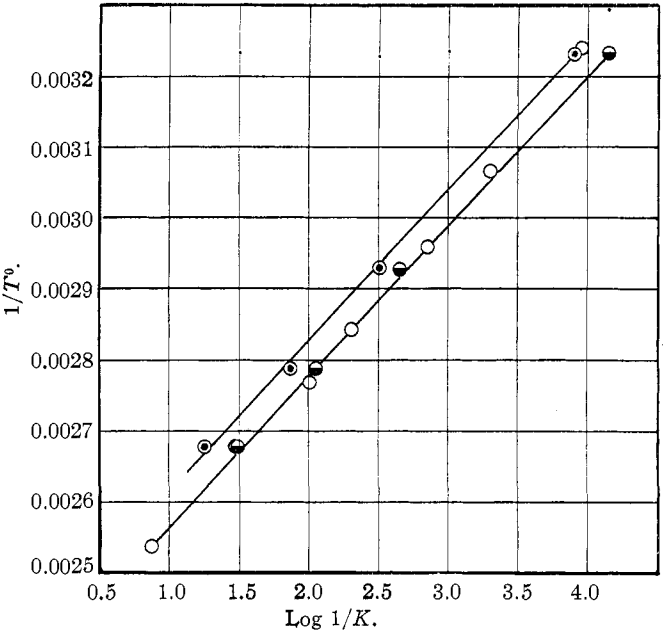
[a] The value recorded in Paper I averaged with those found by later experiments.

Assay of the oxytocic activity remaining in the solution after destruction was made according to the method of Dale and Laidlaw,[3] employing

[3] Dale and Laidlaw, *J. Pharmacol. Exptl. Therap.*, **4**, 75 (1912).

the Locke solution recommended by the U. S. P. X. One of us (R. W. B.) assayed and interpreted the results on the decomposition of Pituitary Solution No. 10122 while the other (T. D. G.) purified and assayed Solutions (A) and (B) a number of months later and made his interpretations independently.

The average number of uterus muscles used in arriving at the value of the amount destroyed at each temperature for each type of pituitary solution was as follows: Solution No. 10122, three; Solution (A), five; Solution (B), four.



○, Pituitary soln. 10122, P_H 3.25; ◒, purified oxytocic principle, P_H, 3.35; ◉, purified oxytocic principle, P_H, 3.55.

Fig. 1.—Effect of temperature on the rate of destruction of oxytocic principle.

Experimental Results

All of the experimental results are given in Table I and expressed graphically in Fig. 1. In Col. 3, $a - x$ represents the amount of oxytocic activity remaining after thermal destruction had occurred. Time, t, is expressed in hours and the rate of destruction, K, calculated from the equation, $K = 1/t\ 2.3 \log a/(a - x)$. The justification for this method of calculating the rate constants has been given in a previous paper. For convenience in plotting, log $1/k$ is used instead of k, and is recorded in Col. 5. The influence of temperature on the rate of change of the reaction velocity as represented by the constant u (see Col. 6) has been computed from the

Arrhenius empirical equation,[4] $u = 2\left(\frac{T_0T_1}{T_1 - T_0}\right) 2.3 \log \frac{k_1}{k_0}$, which has been shown to hold for many types of irreversible reactions within a comparatively short range of temperature. These values were calculated for all temperature differences between 35.7 and 100°, and between 100 and 121°.

Discussion

Purification did not noticeably alter the stability of the oxytocic principle in the temperature ranges employed, although Solution (A) had 1/30 to 1/40 of the nitrogen content of Solution No. 10122. Both solutions had, as nearly as we could determine, the same rate of destruction. The difference in the P_H of these two solutions is of no real consequence, since they are both within the range of maximum stability, P_H 3.0 to 3.4. Too much emphasis should not be placed on any particular value, due to difficulties involved in the assay. Nevertheless, we believe that the data, taken as a whole, represent fairly accurately the thermal decomposition of the oxytocic principle (see Fig. 1).

Since the rate of change with temperature is reasonably constant between 36 and 121°, we feel justified in assuming that our results can be extrapolated to lower temperatures, that is, 0 to 20°, giving values indicating an exceedingly slow rate of destruction, which would harmonize with the general opinion of Smith and McClosky[5] that very little or no destruction could be detected in solutions held at temperatures below 37° over a period of six months.

The rate of destruction of Solution (B) was studied at P_H 3.55, which is slightly alkaline to the point of maximum stability of pituitary extract. It would, therefore, together with Solution (A) serve as a check as to whether or not the relationship between P_H and rate of destruction held for purified solution in the same degree as that found for the pituitary extract shown in Paper I. The rate of destruction of oxytocic principle in Solution (B) should be faster than that for (A). Such was found to be the case and the check at 100° was reasonably close to the value interpolated from Fig. 2 in Paper I at P_H 3.55. The value of log $1/k$ is 1.26 (interpolated) for the extract and 1.25 (observed) for the purified 100° solution.

Summary

1. The rate of thermal destruction of the oxytocic principle at various temperatures between 35.7 and 121° was determined.

2. Purification of pituitary extract did not alter the thermal stability of the oxytocic principle.

[4] Arrhenius, "Quantitative Laws in Biological Chemistry," Bell and Sons, London, 1915.

[5] Smith and McClosky, *U. S. P. H. Bull.*, Hyg. Lab., No. 138 (1924).

3. At P_H 3.25 to 3.35 the temperature coefficient u = 23,000, while at P_H 3.55, u = 24,000.

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

ROTENONE. IV. CONSTITUTION OF ROTENONE

BY F. B. LAFORGE

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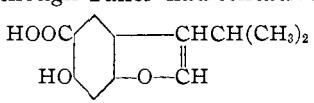
Up to the present it has not seemed advisable to propose a constitutional formula for rotenone. This has not been because data were lacking, for a very large number of rotenone derivatives have been prepared and several unusual reactions peculiar to rotenone have been observed, but until the recent publication of Takei's[1] formula for tubaic and rotenic acids, the particular key fact had been missing. About a year ago Butenandt[2] published an article in which he stated that a constitutional formula for rotenone must fulfil the following requirements.

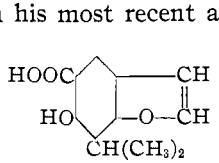
"It must explain: (1) the optical activity; (2) the indifference toward methylmagnesium iodide, ferric chloride, diazomethane and acetic anhydride; (3) the reduction of Fehling's solution; (4) the formation of two groups of structurally different oximes and hydrazones; (5) the formation of dehydrorotenone by loss of two hydrogen atoms and the conversion of this compound to a monocarboxylic acid by addition of two moles of water; (6) the cleavage of rotenone to derritol, a yellow phenol containing two atoms of carbon less than rotenone; (7) the cleavage of rotenone to tubaic acid; (8) the formation of a monocarboxylic acid $C_{23}H_{26}O_6$ by hydrogenation in ammoniacal solution."

At the time of Butenandt's publication the structure of tubaic acid had not been determined, although Takei[3] had tentatively proposed the formula

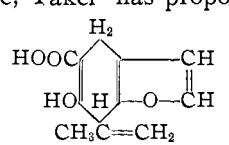


for rotenic acid. Derric acid,[4] which corresponds to the half of the rotenone molecule that carries the methoxyl groups, had not yet been isolated.

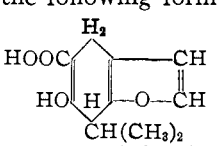
In his most recent article, Takei[1] has proposed the following formulas



Rotenic acid



Tubaic acid



Dihydrotubaic acid

[1] Takei, *Bull. Inst. Phys. Chem. Res.*, **8**, 519 (1929).

[2] Butenandt, *Ann.*, **464**, 270 (1928).

[3] Takei, *Ber.*, **61**, 2943 (1928).

[4] LaForge and Smith, THIS JOURNAL, **52**, 1091 (1930).