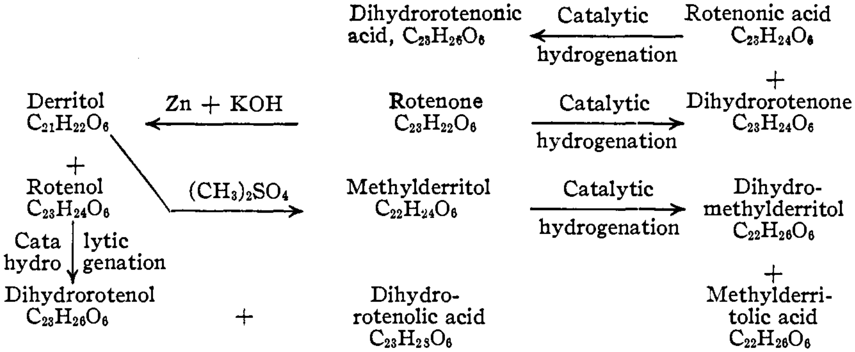


WASHINGTON, D. C.

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

ROTENONE. III. DEHYDROROTENONE[1]

BY F. B. LAFORGE AND L. E. SMITH

RECEIVED SEPTEMBER 21, 1929 PUBLISHED MARCH 6, 1930

Butenandt[2] has shown that a number of oxidizing agents, such as permanganate, potassium ferricyanide, perbenzoic acid and iodine with potassium acetate in alcoholic solution, convert rotenone into dehydrorotenone with a loss of two hydrogen atoms.

Dehydrorotenone is a yellow crystalline compound melting at about 225° and differing widely from rotenone in most of its reactions. Compounds corresponding to dehydrorotenone are easily obtainable from dihydrorotenone and isorotenone. Dehydrorotenone contains the original double bond and carbonyl group of rotenone, but unlike rotenone, it is not decomposed by alkalies, nor does it yield a compound corresponding to derritol. On treatment with alkali or with zinc in alkaline solution, it takes up two molecules of water and is converted into an acid[2] $C_{23}H_{24}O_8$ which for future reference we would like to call dehydrodihydroxyrotenonic acid.

When rotenone is subjected to treatment with stronger oxidizing agents, such as chromic or nitrous acid, the resulting product is a bright yellow compound, rotenonone, having the composition either $C_{23}H_{20}O_7$ or $C_{23}H_{18}O_7$. It is sometimes difficult to prepare rotenonone in a pure state since hydrochloric acid is likely to be added to the double bond. However, dihydrorotenone readily yields dihydrorotenonone on treatment with nitrous acid and corresponding compounds are easily obtained from isorotenone, rotenonic and dihydrorotenonic acids.

---

[1] Presented before the Division of Agricultural and Food Chemistry at the 78th Meeting of the American Chemical Society, Minneapolis, Minn., September 9 to 13, 1929.

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

Butenandt assumed that rotenonone was an α,α-diketone, because it gave an hydroxy acid on treatment with alkali. He interpreted this reaction as a benzilic acid rearrangement. Takei,[3] who first prepared rotenonone, assumed that the formation of the hydroxy acid was due to the opening of a lactone ring. This last-mentioned assumption is probably correct, because neither rotenonone nor any of the corresponding compounds are affected by strong hydrogen peroxide, as would be expected if they were α,α-diketones; moreover, they do not react with *o*-phenylenediamine. All of these diketones are derivatives of dehydrorotenone and not of rotenone itself, for the same diketone is formed from dehydrorotenone as from rotenone, and dihydrodehydrorotenone yields the same diketone as does dihydrorotenone, showing that the formation of the dehydro compounds has preceded the formation of the diketones and that they are derivatives of the dehydro series.

Although dehydrorotenone does not yield compounds corresponding to derritol, it behaves similarly to rotenone on catalytic reduction and yields an acid as the main product.

The most satisfactory method for the preparation of dehydrorotenone is by the addition of iodine to a hot alcoholic solution of rotenone and potassium acetate. Dehydrorotenone crystallizes out on cooling. The same method is used to prepare all other dehydro derivatives.

The yields of the dehydro compounds are generally about 35% of the theoretical. Butenandt made only a very small quantity of dehydrorotenone by the iodine method and failed to notice that it was not the main product of the reaction. The mother liquors from the dehydrorotenone crystallization yield a crystalline residue on evaporation, which after washing with water and recrystallization amounts to more than half of the weight of rotenone employed.

The compound is the acetate of an alcohol of formula $C_{23}H_{22}O_7$. The alcohol itself, which will be called rotenolone, is easily isolated by saponification of the acetate.

Either the acetate or the alcohol is readily converted into dehydrorotenone by boiling with about 5% alcoholic sulfuric acid. It seems very likely that the first step in the reaction is the substitution of a hydrogen atom by iodine, the resulting compound then reacting with potassium acetate. Since the acetate or the alcohol which is isolated is converted into dehydrorotenone only with some difficulty, it seems likely that two isomeric hydroxy compounds are produced with the formation of a new asymmetric carbon atom and that the hydroxyl of one is more favorably situated for reaction with another hydrogen atom to form the dehydro compounds.

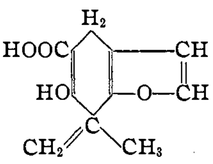
Takei in his most recent publication[4] has proposed a formula for tubaic

---

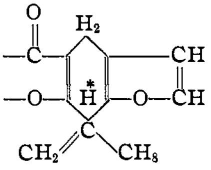
[3] Takei, *Biochem. Z.*, **157**, 14 (1925).

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

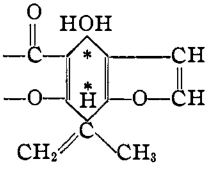
acid which incidentally offers an explanation for the formation and properties of the dehydro compounds



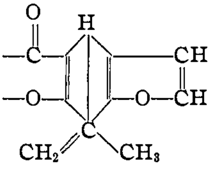
Tubaic acid



Rotenone



Rotenolone



Dehydrorotenone

Rotenolone yields ordinary rotenol and derritol on treatment with zinc in alkaline solution.

As previously stated, dehydrorotenone yields an acid of formula $C_{23}H_{24}O_8$. This acid is probably formed by the hydrolysis of both the lactone and ether linkages which are very likely present in the molecule.

The probable connection between the grouping contained in tubaic acid and the rest of the molecule is represented by a chain containing the carbonyl group.

Cleavage of this chain can be effected by oxidation with hydrogen peroxide in alkaline solution. By this treatment a dibasic acid is obtained which corresponds to the portion of the rotenone molecule containing the original methoxyl groups of rotenone and is represented by the formula $C_{12}H_{14}O_7$. It will be named "derric acid." As this formula contains one more oxygen atom than would be expected, it is probable that one is in the form of an hydroxyl group which is not present in the acid from which it is prepared.

Dehydrorotenone.[2]—Twelve grams of rotenone and 30 g. of potassium acetate are dissolved in about 300 cc. of hot absolute alcohol and about 10 g. of iodine in hot alcoholic solution is added in small portions. The iodine is absorbed rapidly until toward the end of the reaction when dehydrorotenone begins to crystallize at once from the hot solution. After cooling and filtering, the alcoholic solution is evaporated to a very small volume in an open dish on the steam-bath. The residue crystallizes in a short time and is washed with water to remove the salts.

Acetylrotenolone.—The crystalline mass insoluble in water may be dissolved in ether, generally leaving a small quantity of dehydrorotenone. The solution is dried and evaporated, or it may be directly recrystallized from absolute alcohol, or benzene and petroleum ether, from which it crystallizes in thin prisms. The yield amounts to about 6 g. of pure material, melting sharply at 184°.

Anal. Subs., 0.0693: CO_2, 0.1684; H_2O, 0.0338. Subs., 0.0213, 0.0214: AgI, 0.0230, 0.0214. Subs., 0.7182: 3.1 cc. of $N/2$ alkali. Calcd. for $C_{25}H_{24}O_8$: C, 66.37; H, 5.31; $2CH_3O$, 13.73; acetyl, 9.5. Found: C, 66.12; H, 5.42; CH_3O, 14.25; acetyl, 9.3.

The substance is the acetyl derivative of a compound which contains one oxygen more than rotenone and which will be named rotenolone.

Rotenolone.—Three grams of acetyl rotenolone was boiled for two hours with about 25 cc. of $N/2$ alcoholic potassium hydroxide solution. Addition of dilute hydrochloric acid or water gave a white semi-crystalline product, which was dissolved in ether. After drying, the ether was evaporated, leaving a glassy mass which, however, began to crystallize in a few days. On being stirred with methyl alcohol, it all became crystalline. The compound was washed with a very little methyl alcohol and recrystallized from about 8 parts of the same solvent. The yield was 2.25 g. of recrystallized material—82% of the theoretical. The compound consists of short, very heavy prisms of unusually perfect form, m. p. 140–141°.

Anal. Subs., 0.0681: CO_2, 0.1677; H_2O, 0.0334. Subs., 0.0202: AgI, 0.0229. Calcd. for $C_{23}H_{22}O_7$: C, 67.31; H, 5.36; $2CH_3O$, 15.1. Found: C, 67.15; H, 5.44; CH_3O, 14.9.

Both rotenolone and its acetate are converted quantitatively into dehydrorotenone by boiling with about 5% alcoholic sulfuric acid.

For the preparation of dehydrorotenone the crude acetate may be employed, and a total yield of about 75% of the rotenone taken may be obtained.

Dehydrodihydrorotenone.—One gram of dihydrorotenone together with 2.5 g. of potassium acetate was dissolved in 25 to 30 cc. of alcohol, and 0.7 g. of iodine in alcoholic solution was added to the hot solution in small portions. Dehydrodihydrorotenone separated out even from the hot solution. The yield was 0.35 g. It was recrystallized from absolute alcohol and dried at 110°. It forms thin pale yellow prisms which melt at 228–230°.

Anal. Subs., 0.0701, 0.0700: CO_2, 0.1790, 0.1785; H_2O, 0.0362, 0.0364. Subs., 0.0193: AgI, 0.0219. Calcd. for $C_{23}H_{22}O_6$: C, 70.05; H, 5.60; $2CH_3O$, 15.80. Found: C, 69.83, 69.70; H, 5.74, 5.78; CH_3O, 15.75.

The alcoholic mother liquor gave a flocculent precipitate when diluted with water. It was taken up in ether, separated from water and washed to remove alcohol. On evaporation the dried ethereal solution yielded a colorless sirup which crystallized on being rubbed with methyl alcohol. It was recrystallized once from methyl alcohol and then from benzene and petroleum ether. It is the acetate of dihydrorotenolone and forms colorless plates, melting at 189°.

Anal. Subs., 0.0700: CO_2, 0.1693; H_2O, 0.0365. Subs., 0.0206, 0.0216: AgI, 0.0219, 0.0226. Calcd. for $C_{25}H_{26}O_8$: C, 66.10; H, 5.73; $2CH_3O$, 13.66. Found: C, 65.90; H, 5.79; CH_3O, 13.95, 13.85.

The substance gives dehydrodihydrorotenone on boiling with alcoholic sulfuric acid.

Dehydroisorotenone.—One-half gram of isorotenone was treated with iodine and potassium acetate in exactly the same manner as dihydrorotenone and yielded 0.1 g. of yellow prisms. Recrystallized from alcohol it melted at 195°. On evaporation of the alcoholic solution a semi-crystalline mass was obtained which gave dehydroisorotenone on boiling with alcoholic sulfuric acid.

Anal. Subs., 0.0710: CO_2, 0.1840; H_2O, 0.0347. Calcd. for $C_{23}H_{20}O_6$: C, 70.37; H, 5.15. Found: C, 70.68; H, 5.43.

Dehydrodihydrorotenonic Acid.—Two grams of dehydrorotenone dissolved in 40 cc. of ethyl acetate was reduced with hydrogen and platinum catalyst in the usual manner. Two hundred and twenty-five cc. of hydrogen, which is about the theoretical volume corresponding to 2 molecules, was absorbed in one and one-half hours. The filtered solution yielded a crystalline product on evaporation which was agitated with 2% potassium hydroxide solution and filtered from a small residue. The aqueous solutions yielded a voluminous crystalline precipitate on acidification which was filtered off and recrystallized by dissolving in 25 cc. of hot alcohol and adding an equal volume of hot water. The compound consists of yellow needles, melting at 223°. The yield was 1.8 g.

Anal. Subs., 0.0790, 0.0687: CO_2, 0.2013, 0.1749; H_2O, 0.0414, 0.0372. Calcd. for $C_{23}H_{24}O_6$: C, 69.69; H. 6.06. Found: C, 69.46, 69.57; H, 5.82, 6.01.

The product insoluble in alkali formed flat pointed prisms from alcohol, but the quantity was too small for further study.

Dihydrorotenonone (Dehydrodihydrorotenonone).—Three grams of dihydrorotenone was dissolved in glacial acetic acid and to the solution, which was cooled with ice, 9 g. of amyl nitrite was added. To this mixture 6 cc. of concentrated hydrochloric acid in 6 cc. of acetic acid was slowly added. On warming to room temperature, the dihydrorotenone crystallized out. It was recrystallized from acetic acid, m. p., 272–275°, with decomposition; yield, 1.95 g.

Anal. Subs., 0.0589: CO_2, 0.1457; H_2O, 0.0277. Subs., 0.0211: AgI, 0.0232. Calcd. for $C_{23}H_{22}O_7$: C, 67.31; H, 5.36; $2CH_3O$, 15.12; for $C_{23}H_{20}O_7$: C, 67.61; H, 4.93; $2CH_3O$, 15.19. Found: C, 67.44; H, 5.22; CH_3O, 14.52.

Dihydrodehydrohydroxyrotenononic Acid.—One gram of dihydrorotenonone was placed in a flask with 1 g. of solid potassium hydroxide. Enough water to dissolve the potassium hydroxide was put in and an equal volume of alcohol was then added. The mixture was boiled for a few minutes until the dihydrorotenonone had dissolved. The acid was precipitated from the solution with hydrochloric acid. The yield was 0.57 g. Recrystallized from dilute acetic acid, it melted at 257°.

Anal. Subs., 0.0568: CO_2, 0.1346; H_2O, 0.0280. Subs., 0.0210: AgI, 0.0228. Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.60; $2CH_3O$, 14.48; for $C_{23}H_{22}O_8$: C, 64.79; H, 5.17; $2CH_3O$, 14.54. Found: C, 64.61; H, 5.48; CH_3O, 14.34.

Dehydrodihydrorotenonone.—The same procedure was employed for this preparation as the one described above. The proportions employed were: 0.6 g. of dehydrodihydrorotenone, 1.2 cc. of hydrochloric acid and 1.8 g. of amyl nitrite. The compound was recrystallized from a large volume of alcohol. The yield was 0.5 g. melting at 273°.

Anal. Subs., 0.0715: CO_2, 0.1774; H_2O, 0.0325. Subs., 0.0200: AgI, 0.0233. Calcd. for $C_{23}H_{20}O_7$: C, 67.61; H, 4.93; $2CH_3O$, 15.20. Found: C, 67.65; H, 5.06; CH_3O, 15.38.

Dehydrodihydrorotenonone is identical with the corresponding compound obtained from dihydrorotenone. Both have the same melting point and a mixed melting point determination showed no depression. Their identity was established beyond doubt by a crystallographic examination by George L. Keenan, of the Food, Drug and Insecticide Administration of the Department of Agriculture.

"Dihydrorotenonone and dehydrodihydrorotenonone were found to have identical optical properties. Both samples consist of fine needles with a yellowish tinge when examined in ordinary light under the microscope. In parallel polarized light (crossed Nicols) the extinction is straight and the sign of elongation +. The refractive indices are: n = 1.476 (always lengthwise); n = indetd.; n = 1.733. An intermediate index value, n_i = 1.733 occurs frequently on needles crosswise. All indices ±0.003."

Isorotenonone (Dehydroisorotenonone).—The reaction with isorotenone was carried out identically as in the preparation of dihydrorotenonone. With 2.0 g. of isorotenone, 1.2 g. of isorotenonone was obtained in the form of yellow silky needles.

Anal. Subs., 0.0578: CO_2, 0.1434; H_2O, 0.0233. Subs., 0.0218: AgI, 0.0246. Calcd. for $C_{23}H_{20}O_7$: C, 67.61; H, 4.93; $2CH_3O$, 15.19; for $C_{23}H_{18}O_7$: C, 67.98; H, 4.43; $2CH_3O$, 15.27. Found: C, 67.62; H, 4.48; CH_3O, 14.91.

Rotenol and Derritol from Acetylrotenolone.—Three grams of acetylrotenolone was dissolved in 90 cc. of alcohol and 30 cc. of 15% potassium hydroxide was added. After boiling for about one minute, 6 g. of zinc dust was added, and the solution was boiled under reflux for about four hours. Dilute hydrochloric acid was added to the cooled solution and the precipitate dissolved in ether. The ethereal solution was extracted with dilute alkali and the aqueous solution acidified. The precipitated material was dissolved in ether, the solvent dried and evaporated. The sirupy residue crystallized when rubbed with methyl alcohol and the crystalline material was recrystallized from 50% alcohol. The yield was 0.9 g. It proved to be ordinary derritol, melting at 163° and showing no depression when melted with derritol from rotenone.

Anal. Subs., 0.0707: CO_2, 0.1771; H_2O, 0.0380. Calcd. for $C_{21}H_{22}O_6$: C, 68.07; H, 5.99. Found: C, 68.31; H, 5.97.

The ethereal solution containing the alkali-insoluble fraction yielded 1 g. of rotenol which on recrystallization melted at 121°, and showed no depression when mixed with rotenol from rotenone.

Anal. Subs., 0.0675: CO_2, 0.1724; H_2O, 0.038. Calcd. for $C_{23}H_{24}O_6$: C, 69.67; H, 6.06. Found: C, 69.66; H, 6.26.

Dehydrodihydroxyrotenonic Acid.—It was found that the procedure given by Butenandt for the preparation of dehydrodihydroxyrotenonic acid gave very poor yields, and owing to excessive tar the product was difficult to purify. The procedure used in the preparation of rotenol and derritol[5] gave much better results. Four grams of dehydrorotenone was refluxed with 40 cc. of 15% potassium hydroxide, 120 cc. of ethyl alcohol and 8 g. of zinc dust for two hours. The zinc was removed by filtration and the solution acidified with dilute hydrochloric acid. Water was added to the hot solution until crystallization started and then the solution was cooled in an ice-bath. This gave a fairly pure product which was recrystallized from dilute ethyl alcohol; yield, 2.5 g.; m. p. 157°.

Dehydrodihydrorotenononic Acid.—Six grams of dihydrorotenonic acid was dissolved in 50 cc. of glacial acetic acid, the solution cooled in an ice-bath and 18 g. of amyl nitrite added. A mixture of 12 cc. of concentrated hydrochloric acid and 12 cc. of acetic acid was added slowly over a period of about forty-five minutes. The flask was then allowed to come to room temperature. Crystallization began after about one hour and proceeded rapidly with gas and heat evolution and the flask soon became filled with a yellow crystalline mass. As soon as the gas evolution had ceased the product was filtered off. The yield is diminished if the product is allowed to stand for very long.

The compound is very difficultly soluble in all reagents but may be recrystallized from acetic acid. The yield was 5 g. The melting point is very high and it was not accurately determined.

Anal. Subs., 0.0575: CO_2, 0.1414; H_2O, 0.0288. Subs., 0.0189: AgI, 0.0216. Calcd. for $C_{23}H_{22}O_7$: C, 67.32; H, 5.37; $2CH_3O$, 15.06. Found: C, 67.23; H, 5.57; CH_3O, 15.08.

Oxidation of Dehydrodihydroxyrotenonic Acid to Derric Acid.—One gram of acid was dissolved in 10 cc. of 5% potassium hydroxide. This solution was heated nearly to boiling in a 400-cc. beaker and 3 g. of 30% hydrogen peroxide was added slowly. The solution turned very dark and effervesced considerably. With the addition of the last of the hydrogen peroxide, the solution became somewhat lighter in color, although with some runs there was a slight amount of charring. The reaction is complete when acid produces no precipitate from a diluted test portion. The solution is acidified with hydrochloric acid, producing a precipitate in the concentrated solution. A high concentration facilitates extraction of the reaction products with ether. The ether extract from this solution is dried over sodium sulfate and on evaporation gives an oil which slowly crystallizes. As the crystallization is never complete, the crystals are separated from the oily by-products by pressing between filter papers. The small amount of remaining oil can be removed with chloroform. The compound is then

---

[5] LaForge and Smith, THIS JOURNAL, **51**, 2579 (1929).

recrystallized from *n*-butyl ether. The yields averaged 0.15 g.; m. p. 169°. The compound is soluble in water, giving an acid reaction to litmus. It is also soluble in methyl, ethyl and butyl alcohols, ethyl acetate and acetic acid, but insoluble in chloroform, carbon tetrachloride, benzene, toluene and carbon disulfide. It gives no phenol test with ferric chloride.

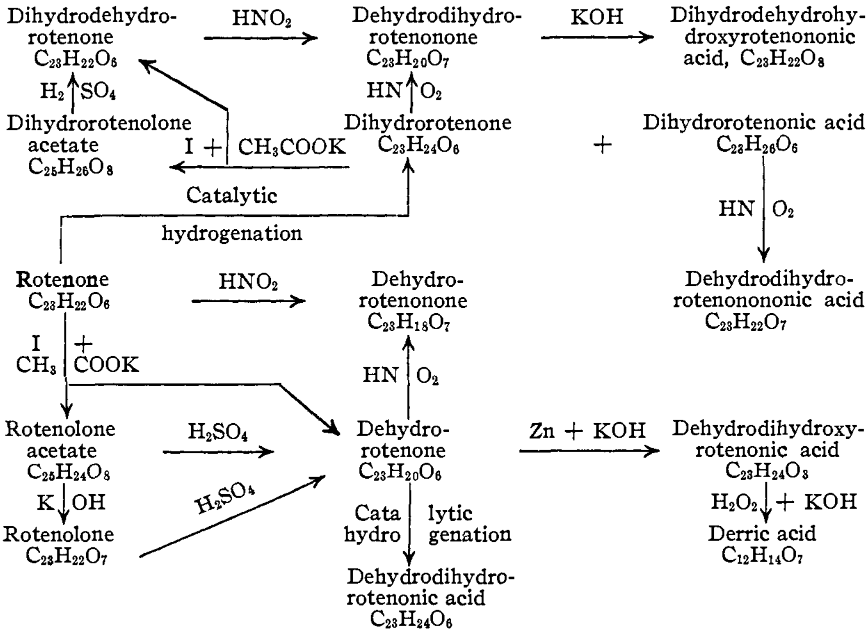
Anal. Subs., 0.0521, 0.0510: CO_2, 0.1008, 0.0996; H_2O, 0.0246, 0.0245. Subs., 0.0203, 0.0201: AgI, 0.0349, 0.0349. Calcd. for $C_{12}H_{14}O_7$: C, 53.33; H, 5.18; $2CH_3O$, 22.96. Found: C, 52.77, 53.26; H, 5.24, 5.33; CH_3O, 22.71, 22.93. Titration: 0.0202 g. used 1.5 cc. of 0.1002 *N* KOH; mol. wt. calcd., 270; found for dibasic acid, 269.

Summary

Dehydrorotenone and the corresponding compounds of the dehydro series are best prepared by the action of iodine and potassium acetate on rotenone and other of its derivatives. A portion of the dehydrorotenone derivatives is obtained directly, but the main product of the reaction is an acetyl derivative of a compound $C_{23}H_{22}O_7$ (in the case of rotenone itself), which yields an hydroxyl derivative on saponification. This compound loses water on boiling with alcoholic sulfuric acid and is converted into dehydrorotenone.

Rotenonone and other diketones prepared by oxidation from rotenone, etc., are derivatives of the dehydro series.

Rotenolone, the hydroxy derivative of rotenone, yields derritol and rotenol on treatment with zinc in alkaline solution.



REACTIONS INVOLVED

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."

WASHINGTON, D. C.

[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

RECEIVED SEPTEMBER 24, 1929 PUBLISHED MARCH 6, 1930

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).