

band. This is interpreted as evidence that the *intense red color* of the alkali salts of indicators of this series does not come from the *nonionized quinone-phenol group* but arises from the *quinone-phenolate anion*.

2. In accordance with this conception we find that the introduction of negative bromo- and nitro-groups into the phenol group increases the ionization of the phenol group, increases the conductivity, lowers the  $P_H$  value and gives to the solution a greater concentration of quinone-phenolate anions and hence increases the *deep red color* and changes the position of the absorption band so as to cut out part of the yellow. The addition of hydrochloric acid suppresses the ionization of the phenol group and changes the *deep red color* into the yellow of the quinone and hence shifts the absorption band so as to include less of the yellow.

3. The similarity of the absorption spectra of the alkaline solutions of sulfonphthaleins, phenolphthaleins, aurine, fluorescein and related substances gives evidence that the deep red color in all these cases arises from the presence of a *quinone-phenolate anion*.

MADISON, WISCONSIN.

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[CONTRIBUTION FROM THE ORGANIC LABORATORY OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY.]

## THE ACTION OF HYDROGEN PEROXIDE UPON URIC ACID. SECOND PAPER ON HYDROGEN PEROXIDE AS A REAGENT IN THE PURIN GROUP.

By C. S. VENABLE.

Received April 17, 1918.

It has recently been shown by Venable and Moore<sup>1</sup> that when uric acid is treated with hydrogen peroxide at room temperature in an approximately half-normal alkaline solution, cyanuric acid is produced, with a yield about 50% that of the theory. Evidence was also produced to show that "tetracarbonimide" which other investigators<sup>2</sup> had described as a product of the reaction was itself only cyanuric acid.

Since that paper was published these views have received welcome and efficient confirmation by the independent work of Walters and Wise<sup>3</sup> who have also added valuable experimental evidence of their own. The identity of cyanuric acid with tetracarbonimide can, therefore, now be regarded as definitely established. In consequence the present paper will discuss the action of hydrogen peroxide upon uric acid from a more general point of view—dealing, first, with the influence of temperature and alkalinity, and second, with the mechanism of the reaction.

<sup>1</sup> THIS JOURNAL, 39, 1750 (1917).

<sup>2</sup> Scholtz, *Ber.*, 34, 4130 (1901); Schittenhelm and Weiner, *Z. physiol. Chem.*, 62, 100 (1909).

<sup>3</sup> THIS JOURNAL, 39, 2472 (1917).

In addition to carbonic acid, ammonia, and occasional small quantities of urea and oxalic acid, 3 organic products had hitherto been observed when hydrogen peroxide acted upon uric acid. They were cyanuric acid, carbonyl-di-urea and allantoin. The formation of carbonyl-di-urea was noted by Schittenhelm and Weiner, and also by Walters and Wise. Allantoin seems not to have been mentioned by others who have studied this reaction, though its formation by the action of other oxidizing agents upon uric acid is, of course, familiar.

Uric acid was oxidized with hydrogen peroxide in solutions of varying alkalinity and at different temperatures. Samples were withdrawn at definite time intervals and the unoxidized uric acid was precipitated by acidifying with hydrochloric acid. The slightly acid filtrate from this uric acid was evaporated at room temperature to a small volume, and the resulting liquid examined for crystals. In this manner a qualitative analysis of the oxidation products formed through a temperature range of 20-90° and an excess alkali concentration of from 0 to 0.5 *N* was obtained. As the relative quantities of these products could only be roughly estimated under the microscope, for stated typical conditions special quantitative runs were made on a larger scale.

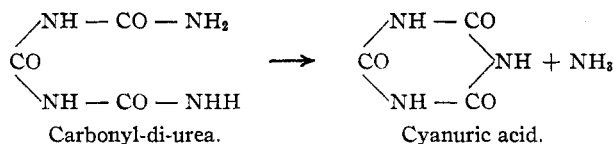
In general, it was found that in neutral or slightly alkaline solutions and at temperatures near that of boiling water, allantoin is first formed in large quantities, together with smaller quantities of carbonyl-di-urea. As the oxidation proceeds, the yield of allantoin rapidly diminishes, with the appearance of increasing quantities of cyanuric acid up to a maximum of a 10% yield of the latter. The yield of carbonyl-di-urea remained fairly constant. Increasing the alkalinity at this high temperature rapidly diminished the yields in all 3 cases, until at an excess alkalinity of 0.1 *N*, only very small amounts of cyanuric acid were observed. At lower temperatures and in weakly alkaline solutions, the same phenomenon was observed, the disappearance of the allantoin being much slower. On increasing the alkalinity, the yield of cyanuric acid rapidly increased with a corresponding decrease in the yields of both allantoin and carbonyl-di-urea. At 20° and with an excess alkalinity of 0.5 *N*, no allantoin or carbonyl-di-urea were observed, whereas the yield of cyanuric acid had risen as high as 50% of the theoretical. Further increase of alkalinity or lowering of the temperature was apparently without effect.

To trace a possible connection between the 3 products, pure carbonyl-di-urea and allantoin were prepared and the effect of hydrogen peroxide upon them under varying conditions of time, temperature, and alkalinity was studied.

It was found that allantoin in weakly alkaline solution is oxidized by hydrogen peroxide at 80-90° to give cyanuric acid in yields as high as 30% of the theoretical. On the other hand, on increasing the alkalinity

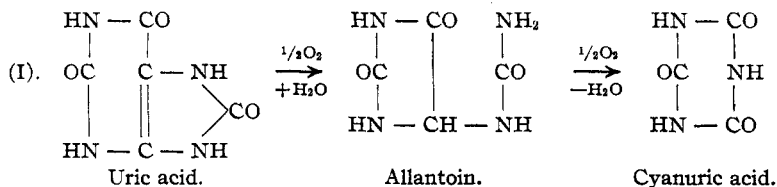
of the solution, the allantoin disappears rapidly at both low and high temperatures, but no cyanuric acid is produced. The same phenomenon took place even when the allantoin was added very slowly to an excess of the oxidizing mixture. In no case was any trace of carbonyl-di-urea to be observed. In neutral or acid solutions allantoin is stable toward hydrogen peroxide.

Likewise, it was found that carbonyl-di-urea upon standing at room temperature in 0.5 *N* sodium hydroxide, with or without hydrogen peroxide, is transformed very slowly to cyanuric acid. In more weakly alkaline solutions this transformation takes place more slowly even though the temperature be raised to 80°. A strong odor of escaping ammonia was always noticeable. This transformation is to be expected from observations made by Schmidt<sup>1</sup> on the conversion of carbonyl-di-urea to cyanuric acid when heated with alkali or alkali carbonates. The reaction involved may be expressed as follows:



The work of Behrend<sup>2</sup> and Sundwik<sup>3</sup> has shown that uroxic acid is always found with allantoin when uric acid in alkaline solution is oxidized by potassium permanganate, but since the free uroxic acid is unstable in dilute acid solution, its appearance among the oxidation products isolated from the acidified oxidation mixture could not be expected, even if it were an intermediate product of the reaction. Therefore, the effect of hydrogen peroxide on sodium uroxic acid under varying conditions was investigated. It was found that this compound in alkaline solutions of different concentrations is quite stable toward hydrogen peroxide. The free acid is unstable even in water alone, and the addition of hydrogen peroxide does not seem to affect the decomposition.

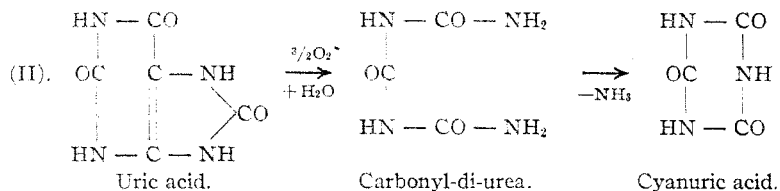
From these results, it is to be concluded that when uric acid in weakly alkaline solutions is oxidized by an excess of hydrogen peroxide at least two independent reactions are possible.



<sup>1</sup> *J. prakt. Chem.*, **5**, 39 (1872).

<sup>2</sup> *Ann.*, **333**, 141 (1904); **355**, 21 (1909); **410**, 337 (1915).

<sup>3</sup> *Z. physiol. Chem.*, **41**, 343 (1904).

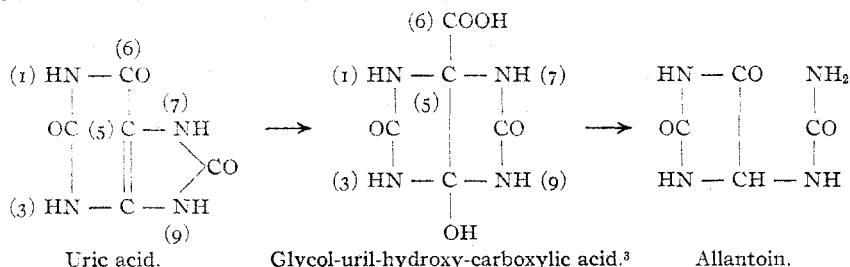


At different stages in the oxidation all 3 products are observed, but since in low alkali concentration reaction (II) takes place with difficulty, and furthermore since a nearly constant quantity of carbonyl-di-urea was obtained throughout the course of the oxidation whereas the allantoin shows a tendency to disappear, it seems probable that the major portion of the cyanuric acid observed in oxidations in weakly alkaline solution is due to a secondary decomposition of allantoin according to reaction (I).

Allantoin can be obtained from uric acid by the action of permanganate and this raised the interesting question whether a parallelism exists between the hydrogen peroxide and permanganate oxidations.

The mechanism of the latter has been a troublesome question for many years. An interesting light was thrown upon it by the observations of Emil Fischer and Ach<sup>1</sup> who showed that, when treated with either permanganate or lead peroxide, both 1-methyl uric acid and 7-methyl uric acid yield  $\beta$ -methyl allantoin, while those methyl uric acids which have the alkyl groups in Positions 3 and 9 both yield  $\alpha$ -methyl allantoin. This can hardly be accounted for save by the assumption of a symmetrically constituted intermediate product.

Not long after, Behrend<sup>2</sup> therefore suggested that a hypothetical glycol-uril-hydroxy-carboxylic acid might be the intermediate in question.



He never succeeded in isolating the intermediate product but he was able to show that, when uric acid is oxidized by alkaline permanganate under certain fixed conditions, a solution is produced which when treated with

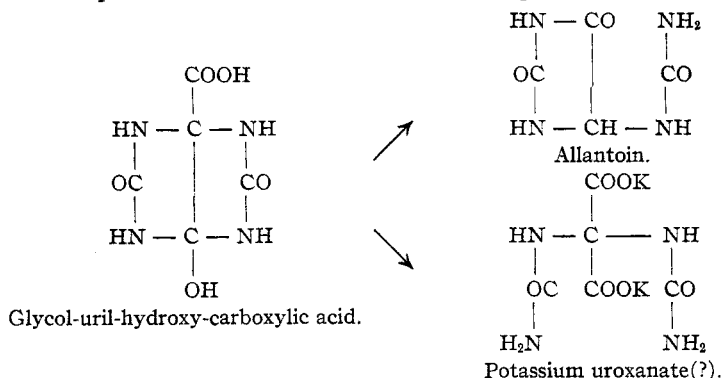
<sup>1</sup> *Ber.*, 32, 2723 (1899).

<sup>2</sup> *Ann.*, 333, 141 (1904).

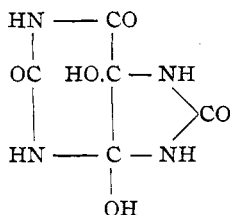
<sup>3</sup> The numbers in parentheses are added simply to show how the assumption of the intermediate product explains the fact that the 1,7 and 3,9 positions in uric acid become equivalent during the oxidation.

acetic acid yielded allantoin but when evaporated with strong alkali yielded potassium uroxanate.

It will be seen that the formula of glycol-uril-hydroxy-carboxylic acid is well adapted to account for both these changes.



In the intermediate stage between uric acid and this hypothetical glycol-uril-hydroxy-carboxylic acid Behrend<sup>1</sup> originally assumed the formation of uric acid glycol,



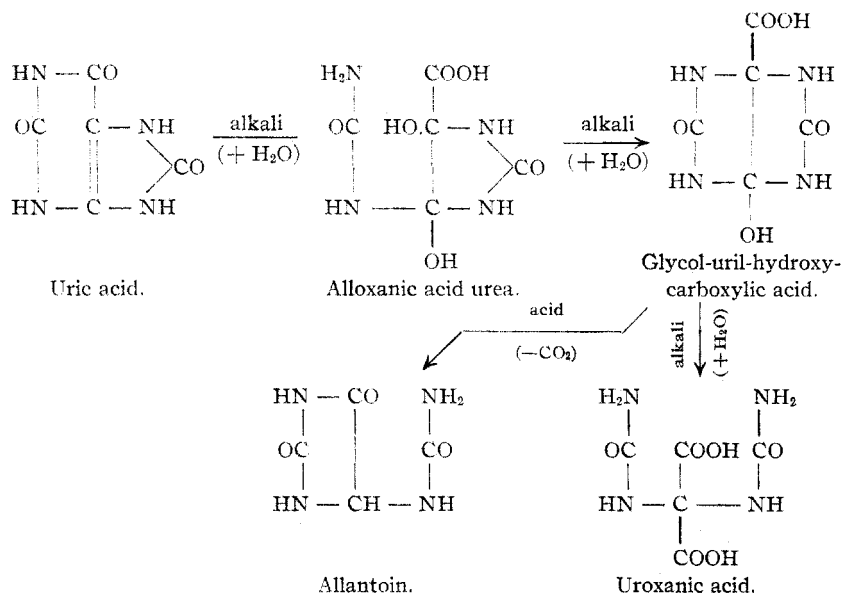
though this assumption was based on no experimental evidence. The preparation by Biltz<sup>2</sup> of uric acid glycol and the pronounced instability of this compound toward alkalis, together with the impossibility of obtaining any allantoin from it by oxidation, led Behrend<sup>3</sup> in a later publication to propose the following scheme for this oxidation of uric acid:

Behrend's attempts to prepare alloxanic acid urea and to obtain allantoin from it under conditions in any way parallel to those observed in the actual oxidation of uric acid failed to give conclusive results. Furthermore, Behrend himself, in his last publication,<sup>1</sup> seems somewhat sceptical as to the exact nature of this intermediate solution, since the ratio of carbon dioxide in this alkaline solution (as determined by precipitation with barium hydroxide) to allantoin (obtained on subsequent acidification and boiling) was found to be very nearly one to one. In other words, Behrend seems to believe that in this alkaline solution the splitting off of

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Ber.*, **43**, 1511 (1910); **45**, 1677 (1912).

<sup>3</sup> *Ann.*, **410**, 337 (1915).



carbon dioxide had already taken place, and no intermediate of a 5-carbon structure was present which could be considered as the source of the allantoin. The fact that some uroxic acid, however, could still be obtained from such a solution seems to show that some 5-carbon intermediate is still present in it. Whether Behrend's hypothesis is correct or not, the fact that solutions can be prepared, from which different final products can be obtained at will, offered an interesting opportunity to test whether there is any parallelism between the permanganate and hydrogen peroxide oxidations, for if so, such a solution when treated with hydrogen peroxide ought to yield at least one of the typical products of the peroxide action.

On preparing this solution according to the directions given by Behrend, and then treating it with hydrogen peroxide under varying conditions, it was found possible to obtain as high as a 10% yield of cyanuric acid (calculated on the basis of uric acid oxidized), but only when high temperatures and low alkali concentrations were observed in the peroxide part of the oxidation. At low temperatures in strong alkali concentration no cyanuric acid was obtained, though a 50% yield of allantoin was still obtainable from this solution. Experiments previously referred to have shown that allantoin as such under similar conditions should have been completely decomposed.

Therefore, these experiments neither confirm the unusual instability of this solution as observed by Behrend, nor do they confirm a connection between the peroxide and permanganate oxidation courses. Only a 10%

yield of cyanuric acid was observed, a yield theoretically possible either through the action of hydrogen peroxide on a hypothetical intermediate in the permanganate oxidation, or by the transformation of this intermediate to allantoin under the conditions of the experiment and the subsequent oxidation of this allantoin to cyanuric acid.

#### Hydrogen Peroxide Oxidation in Strongly Alkaline Solution.

The oxidation of uric acid by hydrogen peroxide was next investigated for conditions of low temperature and high excess alkalinity, *viz.*, 20°, with excess alkalinity of 0.5 *N*. From observations already noted, it is apparent that neither of the two reactions whose equations appear on pages 1101-2 can account for the results obtained under such conditions, that is, a 50% yield of cyanuric acid only. The first is excluded because allantoin, dissolved in a solution 0.5 *N* in excess alkali, is rapidly decomposed by hydrogen peroxide without producing cyanuric acid. With regard to the second, the largest yield of carbonyl-di-urea obtainable under the most favorable conditions was 15% of the theoretical. Assuming a quantitative transformation of the carbonyl-di-urea to cyanuric acid, and adding the 10% of cyanuric acid already found present with the carbonyl-di-urea, the total yield of cyanuric acid could be no larger than 30%. Furthermore, the transformation of one mol of carbonyl-di-urea to one mol of cyanuric acid requires the liberation of one mol of ammonia. In a special quantitative oxidation run at 20° and with the large excess alkali of 1 *N*, the molal ratio of ammonia formed per mol cyanuric acid recovered was found to be less than 1 : 3. Also, in all oxidations at low temperature and high excess alkali, no allantoin or carbonyl-di-urea were observed throughout the entire course (24 hours) of a complete oxidation, whereas when carbonyl-di-urea was placed under similar conditions its conversion to cyanuric acid was still incomplete at the end of 48 hours.

These facts would indicate that the oxidation of uric acid by hydrogen peroxide at low temperatures and high excess alkalinity follows a course fundamentally different from that followed when the oxidation takes place at more elevated temperatures and at a low alkaline concentration. In the case of the former, since microscopic examination had revealed no intermediates after the acidification of the oxidation mixture, such intermediates, if formed, must have existed in the alkaline solution to be decomposed upon acidification, either by the acid alone, or by the combination of acid and hydrogen peroxide.

To test this point, the excess hydrogen peroxide existing in this alkaline solution was removed before acidification by stirring in powdered manganese dioxide. Then, upon weakly acidifying the solution and cooling, a 60% yield of a hitherto unnoticed salt was obtained.

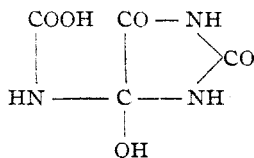
That this salt is the intermediate sought is shown by the fact that, when dissolved in warm water and neutralized with an excess of hydrochloric acid in the presence of hydrogen peroxide, it gave an 80% yield of cyanuric acid.

In addition, experiments indicated that this product, thus established to be intermediate in the oxidation of uric acid to cyanuric acid, was formed only in strongly alkaline solutions. Microscopic examination of the oxidation solution, after removal of the intermediate product and acidification, gave no evidence of allantoin or carbonyl-di-urea. Furthermore, it was found impossible to convert the intermediate into allantoin, carbonyl-di-urea or uroxic acid. These results indicate that the formation of this intermediate product is characteristic of the oxidation of uric acid by hydrogen peroxide in strongly alkaline solutions only, and that under these conditions the mechanism of the reaction differs from that characterizing the oxidation in neutral or weakly alkaline solutions through allantoin to cyanuric acid. This difference in mechanism thus fulfills the conclusions previously drawn.

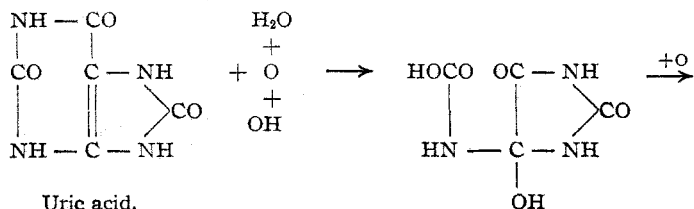
The intermediate product above mentioned proved inhomogeneous under the microscope, and the conditions of its formation made it probable that it represented the mixed acid and neutral salts of a dibasic acid. This was confirmed by the preparation of two barium salts of different solubility. Attempts to analyze the acid barium salt led to results not entirely conclusive, on account of the ease with which the salt loses a certain part of its water.

By treating the sodium salt with ammonium chloride and ammonia, however, it was possible to prepare a neutral ammonium salt, for which analysis justified the formula  $(\text{NH}_4)_2\text{C}_4\text{H}_5\text{O}_5\text{N}_3$ , an empirical composition which was entirely in accord with the proportion of barium, carbon and nitrogen in the acid barium salt.

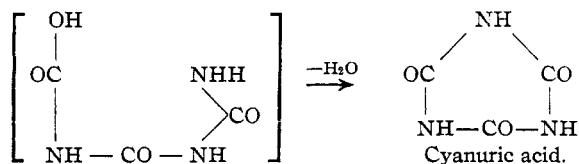
The empirical formula  $\text{C}_4\text{H}_5\text{O}_5\text{N}_3$  suggests the structure



and the following mechanism for the oxidation of uric to cyanuric acid.







At this point the writer was obliged to give up the work and leave the determination of the constitution of the acid  $\text{C}_4\text{H}_5\text{O}_6\text{N}_3$  to others. An account of this work will be found in the following paper.

### Experimental Part.

The comparative simplicity of the relationships set forth in the foregoing introduction was first realized when the investigation was well advanced. In consequence many series of experiments which were carried out in the earlier stages now possess only cumulative significance. Those included here will be only selected cases which fix limiting conditions.

### The Optical Method.

Throughout the investigation continual use was made of the polarizing microscope, and though the usefulness of this important piece of apparatus has been urged upon the attention of chemists by others,<sup>1</sup> yet it seems not inappropriate to add one more testimony here.

With a little practice it is not difficult to acquaint oneself with the crystalline habit of an individual product found in a chemical mixture, to determine at least the most essential of the optical properties, and usually to determine with adequate accuracy the index of refraction of at least one "ray" in the crystal by the immersion method.

Once this has been done it becomes a simple matter to trace qualitatively the course of a given reaction even if extremely complex without the necessity of isolating the individual products except in those cases where quantitative measurements are imperative. Here also the microscope furnishes the best criteria of the purity and homogeneity of the product. This holds especially true in the purin group, where sharp melting points are the exception, the only solvent water, and distillation out of the question.

### Oxidation Experiments.

More than a hundred of these were carried out in all, but, for reasons already given, only such will be described below which throw light upon the essential conditions of the reaction.

The first systematic runs were merely qualitative and were carried out essentially as follows:

5 g. uric acid was dissolved in 300 cc. of water, to which definite volumes

<sup>1</sup> See especially F. E. Wright, "The Petrographic Microscope in Analysis," *THIS JOURNAL*, **38**, 1647 (1916).

of 6 *N* sodium hydroxide had been added. The solution was brought to the desired temperature and the hydrogen peroxide added. In most of the experiments 125 cc. of the 3% solution was employed. The temperature was kept essentially constant by immersion in a large volume of water of the desired temperature, and at definite intervals 50 cc. samples were pipeted out, and the unoxidized uric acid precipitated with a slight excess of hydrochloric acid. The precipitate, recrystallized from hot water, was examined under the microscope for possible oxidation products. The filtrate from the uric acid was allowed to stand, and any crystals which separated were examined. The filtrate was then evaporated in a current of air at room temperature till sodium chloride began to crystallize out, any crystals which formed earlier being filtered off and examined under the microscope as they appeared.

These experiments gave a good idea of the general course of the reaction, and paved the way for a more nearly quantitative study by fixing the point where each product appeared least contaminated by others.

The quantitative experiments which followed never, of course, attained the accuracy of an analytical separation. They rather followed the course indicated by the qualitative experiments above described. Experience had shown, however, that when after oxidation the solution was acidified and evaporated, the allantoin was more soluble in the slightly acid solution than cyanuric acid, and the latter than carbonyl-di-urea. It was, therefore, possible to filter off the precipitates at the point when they appeared most nearly homogeneous, dry and weigh them. The weight, therefore, never really represents strictly homogeneous material. The errors thus introduced are not of a magnitude to affect the conclusions drawn in the introduction.

Of the many experiments tried the following examples are typical and are selected because of their relation to limiting conditions:

**Expt. 1.**—Temp., 100°. Excess alkalinity, 0.9 *N*. Time, 3 minutes. No oxidation product recovered.

**Expt. 2.**—Temp., 90°. Excess alkalinity, 0.02 *N*. Time, 10 minutes. Uric acid recovered, 10%. Carbonyl-di-urea, 18%. Cyanuric acid (with a little allantoin), 8%. Allantoin (with a little cyanuric acid), 5%.

**Expt. 3.**—Temp., 90°. Excess alkalinity, 0.2 *N*. Time, 1 minute. Maximum yield of allantoin 30%, with a little cyanuric acid and carbonyl-di-urea.

**Expt. 4.**—Temp., 40°. Excess alkalinity, 0.05 *N*. Time, 5 hours. Cyanuric acid (with a trace of allantoin), 12%. Carbonyl-di-urea—not weighed.

**Expt. 5.**—Temp., 30°. Excess alkalinity, 0.9 *N*. Time, 30 minutes. Uric acid recovered, 30%. Cyanuric acid, 10%.

**Expt. 6.**—Temp., 20°. Excess alkalinity, 0.33 *N*. Time, 24 hours. Cyanuric acid only, 44%.

**Expt. 7.**—Temp., 18°. Excess alkalinity, 0.5 *N*. Time, 18 hours. Cyanuric acid only, 50%.

#### Identification of Allantoin.

Allantoin had not previously been described as a product of this reaction, so some pains were taken in its identification. As produced here, it was difficult to obtain a sample which appeared entirely pure under the microscope, though the material melted at 230°. Pure allantoin melts at 234°. Six nitrogen determinations by the Kjeldahl method gave results between 34.24% and 35.91% (theory 35.52%).

Allantoin was therefore prepared by the method of Behrend<sup>1</sup> and a complete comparison made. This established perfectly the identity of the material from the two sources and tallies with the description of allantoin in Groth's *Chemische Krystallographie*, 3, 583 (1910).

#### Action of Hydrogen Peroxide upon Allantoin, Carbonyl-di-urea and Uroxic Acid.

Since in some of the experiments cyanuric acid had seemed to appear among the products of the reaction as allantoin disappeared, it became necessary to test whether cyanuric acid could be formed from allantoin under the conditions of the experiment. Pure allantoin was, therefore, prepared and oxidized in the manner already described for uric acid.

**Expt. 8.**—Temp., 20°. Excess alkalinity, 0.3 *N*. Time, 4 days. Reduced excess alkalinity to 0.06 *N* and evaporated to 0.2 original volume. No crystals obtained either in alkaline or acid solution.

**Expt. 9.**—Temp., 20°. Excess alkalinity, 0.06 *N*. Time, 4 days. Removed excess hydrogen peroxide by stirring in manganese dioxide, and evaporated in alkaline solution to  $\frac{1}{3}$  original volume.

A needle-like precipitate of sodium cyanurate appeared. Yield, 10%.

**Expt. 10.**—Temp., 80°. Excess alkalinity, 0.07 *N*. Time, 10 minutes. Evaporated in 0.03 *N* alkali to  $\frac{1}{4}$  original volume.

No crystals separated either from alkaline or acid solution.

**Expt. 11.**—Temp., 80°. No excess alkalinity. Time, 1½ hours. The hydrogen peroxide was added gradually and during the oxidation the solution had evaporated to  $\frac{1}{4}$  its original volume. On cooling sodium cyanurate separated out. Acidification of the filtrate yielded no further crystals.

Product (as cyanuric acid), 29%.

**Expt. 12.**—Allantoin (not the sodium salt) was warmed for 10 minutes at 60–80° with 1 cc. of hydrogen peroxide in *N* hydrochloric acid.

All the allantoin was recovered.

<sup>1</sup> *Ann.*, 333, 151 (1904).

**Expt. 13.**—Allantoin was warmed to 90° in an aqueous solution of hydrogen peroxide for a half-hour.

On cooling all the allantoin was recovered.

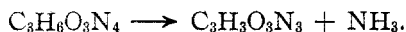
From the above it may be concluded that allantoin (as the neutral sodium salt) is oxidized by hydrogen peroxide, rapidly at high temperatures, and much more slowly at low temperatures, to produce cyanuric acid.

At high temperatures in a slight excess of alkali allantoin is decomposed by hydrogen peroxide without the accumulation of cyanuric acid in the solution. At low temperatures this decomposition takes place, but requires a large excess alkalinity.

In water alone, and in acid solution, allantoin is not affected by hydrogen peroxide.

#### Cyanuric Acid from Carbonyl-di-urea.

According to Schmidt<sup>1</sup> alkali and alkali carbonates dissolve carbonyl-di-urea undecomposed, and change it on heating into ammonia and cyanuric acid.



To test the probability that a similar reaction takes place under the experimental conditions observed in the hydrogen peroxide oxidation of uric acid, pure carbonyl-di-urea was exposed to the action of different concentrations of alkali, with and without hydrogen peroxide, the temperature being varied as indicated.

**Expt. 14.**—Temp., 20°. Excess alkalinity, 0.6 N. Time, 7 days. On neutralizing with hydrochloric acid and evaporating, a relatively large amount of carbonyl-di-urea was recovered, together with a small amount of cyanuric acid.

**Expt. 15.**—Temp., 20°. Excess alkalinity, 1.0 N. Time, 2 days. No hydrogen peroxide.

Cyanuric acid, 56%. Undecomposed carbonyl-di-urea—small.

**Expt. 16.**—Temp., 20°. Excess alkalinity, 1.0 N. Time, 2 days. Excess hydrogen peroxide.

Cyanuric acid, 50%. Undecomposed carbonyl-di-urea, approximately 5%.

**Expt. 17.**—Temp., 85–90°. Excess alkalinity, 0.06 N. Time, 45 minutes. Excess hydrogen peroxide (added gradually).

Cyanuric acid, 25%. Undecomposed carbonyl-di-urea, 10%.

**Conclusions from Expts. 14–17.**—1. At high temperature carbonyl-di-urea is slowly acted upon by even a large excess alkali to form cyanuric acid. Such a reaction (Expt. 17) could only partially account for the 8% yield of cyanuric acid obtained in Expt. 2.

<sup>1</sup> *Loc. cit.*

2. At low temperature a large excess alkali and an exposure of over two days is required for complete conversion of carbonyl-di-urea to cyanuric acid. This excess alkali and time of exposure both exceed the conditions of Expt. 7 where the maximum yield of cyanuric acid was obtained in 18 hours, with no evidence of carbonyl-di-urea formation.

3. In this conversion of carbonyl-di-urea to cyanuric acid the hydrogen peroxide is an unnecessary factor.

Since the salts of uroxic acid not infrequently appear as oxidation products of uric acid, sodium uroxanate was prepared and treated with hydrogen peroxide under varying conditions. In none of them was any carbonyl-di-urea or cyanuric acid produced.

As the results were all negative it would be superfluous to give the details of the experiments. Since, however, the optical properties of sodium uroxanate have not been described, the following details may be of interest:

**Optical Properties,  $\text{Na}_2\text{C}_5\text{H}_6\text{O}_8\text{N}_4 \cdot 8\text{H}_2\text{O}$ .**—Crystals dried on filter paper.

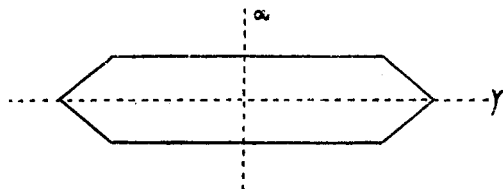


Fig. 1.—Sodium uroxanate.

Habit: Thin hexagonal tables.

Optical Orientation: Axial plane parallel to tabulation. Parallel extinction. Elongation parallel to  $\gamma$ .

Indices:  $\alpha = 1.51$ .  $\gamma = 1.52-1.53$ .

On heating these crystals at  $110^\circ$ , they become opaque but the general habit remains the same. The indices however, vary.

$\alpha = 1.53-1.54$ .  $\gamma = 1.54-1.55$ .

#### Effect of Hydrogen Peroxide on Behrend's Intermediate Solution.

By the action of alkaline permanganate upon uric acid Behrend was able to prepare an intermediate solution from which he could obtain allantoin or uroxic acid at will. In order to trace any possible connection between the oxidation by permanganate and that by hydrogen peroxide such a solution was prepared and subjected to secondary oxidation by hydrogen peroxide. The crystalline products were identified by their optical constants.

**Expt. 18.**—10 g. of uric acid was suspended in 200 cc. of water containing 6.6 g. sodium hydroxide (excess alkalinity = 0.22 N). On warming the uric acid went into solution. This solution was then cooled to  $10^\circ$ , a large precipitate of sodium urate separating out, and 6.3 g. potassium permanganate were added slowly, the mixture being shaken vigor-

ously. The temperature of the solution was kept below  $15^{\circ}$  by cooling with ice water. After 3 hours, the precipitated manganese dioxide was filtered off to leave a clear filtrate, volume 200 cc. The murexid test on a sample of this filtrate gave a negative test for uric acid.

I.—It was then divided into two parts: To the first half were added 100 cc. hydrogen peroxide and 13 cc. sodium hydroxide (6 *N*), excess alkalinity 0.5 *N*. This solution was allowed to stand 3 days at room temperature. 125 cc. of it were then neutralized with hydrochloric acid and evaporated to a volume of 20 cc. On cooling 1.4 g. of allantoin was obtained—50% of the theory.

II.—To the other half were added 80 cc. hydrogen peroxide and 3 cc. hydrochloric acid (6 *N*). Excess alkalinity, 0.0 *N*. Reaction of the solution was alkaline to litmus. Heated to  $80-90^{\circ}$  for 30 minutes, two later additions of 20 cc. hydrogen peroxide each being made. On cooling overnight, no precipitate of carbonyl-di-urea was observed. The solution was then evaporated on a water bath to a volume of 30 cc. On cooling, a large crystalline needle-like precipitate of sodium cyanurate was obtained. On filtering off this precipitate, and recrystallizing from hot dil. hydrochloric acid crystals of cyanuric acid were recovered. Weight 0.2 g., yield 5%.

The alkaline filtrate from the sodium cyanurate gave no further deposition of crystals on neutralization with hydrochloric acid.

**Expt. 19.**—30 g. of uric acid was dissolved by warming in a solution of 43.2 g. of sodium hydroxide in 750 cc. of water (excess alkalinity, 0.96 *N*). This solution was cooled to  $15^{\circ}$ , a small precipitate of sodium urate separating out, and 18.9 g. potassium permanganate was added. The oxidation proceeded at  $18^{\circ}$  for  $3\frac{1}{2}$  hours, and then the precipitated manganese dioxide was filtered off. A sample of the filtrate gave a negative murexid test for uric acid. The whole filtrate, which measured 700 cc., was divided into 3 equal parts and treated as follows:

I.—The first third was evaporated on the steam bath at a temperature not exceeding  $80^{\circ}$  to a volume of 40 cc. A strong odor of ammonia was observed. On cooling, a large precipitate of the characteristic crystals of sodium uroxanate was obtained. This salt was recrystallized from hot water. Weight 1.5 g., 9.6% of theory. The filtrate from the sodium uroxanate on neutralizing with hydrochloric acid gave a small precipitate of uroxic acid (star-shaped crystals), but no cyanuric acid or carbonyl-di-urea.

II.—To the second third were added 160 cc. of hydrogen peroxide and 20 cc. of sodium hydroxide (6 *N*) (excess alkalinity 0.9 *N*). This solution was allowed to stand two days at room temperature. It now measured 410 cc. and was divided into two equal parts.

IIa.—One-half was evaporated on the water bath to a volume of 35 cc. On cooling, a large precipitate of sodium uroxanate was deposited. This precipitate, filtered from the mother liquor and recrystallized from a small amount of hot water, gave crystals of habit characteristic of sodium uroxanate. Weight 1.2 g. or 15.4% of theory. This precipitate was suspended in dil. hydrochloric acid and the mixture boiled. Complete decomposition occurred, and on cooling no crystals of cyanuric acid were observed.

The mother liquor from the first precipitate of sodium uroxanate was combined with that from the recrystallization of the sodium uroxanate. On neutralization with hydrochloric acid no crystals of cyanuric acid were observed.

IIb.—To the other half was added 30 cc. of hydrochloric acid (6 *N*) (excess alkalinity 0.0 *N*). Evaporated on a steam bath to a volume of 60 cc. On cooling, a large crystalline needle-like precipitate of sodium cyanurate was obtained. This precipitate was filtered off, and the free cyanuric acid was obtained by decomposing with dil. hydrochloric acid. Weight 0.38 g. or 10% of theory.

III.—To the third portion of the original filtrate were added 40 cc. hydrochloric acid (6 *N*) and 160 cc. of hydrogen peroxide. (Excess alkalinity 0.0 *N*.) Reaction of solution alkaline to litmus. It was now divided into two parts.

IIIa.—The first part was allowed to stand one day in a refrigerator and two days at room temperature. A small needle-like crystalline precipitate deposited. This was filtered off and the filtrate was evaporated on a water bath to a volume of 60 cc. On cooling, a large additional yield of the same needle-like crystals was obtained. The two preparations, recrystallized from an excess of hot dil. hydrochloric acid, gave 0.32 g. of cyanuric acid. Yield 8.2% of theory.

On evaporation of the alkaline mother liquor to a volume of 30 cc. no sodium uroxanate was obtained on cooling.

IIIb.—The other half was heated for 10 minutes at 80°. On cooling the resulting solution overnight, a needle-like crystalline precipitate was obtained. This, on recrystallizing from an excess of hot dil. hydrochloric acid, gave 0.15 g. of cyanuric acid. The alkaline filtrate from the sodium cyanurate was neutralized with hydrochloric acid and then the solution was evaporated to a volume of 80 cc. On cooling 0.23 g. of cyanuric acid was obtained. Total yield of cyanuric acid 0.39 g. or 10 of theory.

The results of Expts. 18 and 19 are summarized as follows:

EXPT. 18.			
Int. sol. Oxid. by $\text{KMnO}_4$ at $15^\circ$ Ex. alk. 0.22 <i>N</i> Time 3 hrs.	(I) $\text{H}_2\text{O}_2$ ; (OH) = 0.5 <i>N</i>	$\xrightarrow{\text{HCl}}$	Allantoin..... 50%
	20° 3 days	Evap.	
	(II) $\text{H}_2\text{O}_2$ ; (OH) = 0.0 <i>N</i>	$\xrightarrow{\hspace{1cm}}$	Cyanuric acid..... 5%
	Evap. at $80^\circ$		Carbonyl-di-urea... 0
			Allantoin..... 0
EXPT. 19.			
Int. sol.  Oxid. by $\text{KMnO}_4$ at $15^\circ$  Ex. alk. 0.96 <i>N</i>  Time 3 1/2 hrs.	(I) Evap. at (OH) = 0.46 <i>N</i>	$\xrightarrow{\hspace{1cm}}$	Uroxanic acid..... 10%
			Cyanuric acid..... 0
	(II) $\text{H}_2\text{O}_2$ ; (OH) = 0.88 <i>N</i>	(a) Evap. $80^\circ$	Uroxanic acid..... 15%
		(OH) = 0.88 <i>N</i>	Cyanuric acid..... 0
		(b) Evap. $80^\circ$	Uroxanic acid..... 0%
		(OH) = 0.0 <i>N</i>	Cyanuric acid..... 10
	(IIIa) $\text{H}_2\text{O}_2$ ; (OH) = 0.0 <i>N</i>	$\xrightarrow{\hspace{1cm}}$	Uroxanic acid..... 0%
	20° 3 days. Evap. at (OH) = 0.0 <i>N</i>		Cyanuric acid..... 8.2
			Carbonyl-di-urea... 0
	(IIIb) $\text{H}_2\text{O}_2$ ; (OH) = 0.0 <i>N</i>	$\xrightarrow{\hspace{1cm}}$	Uroxanic acid..... 0%
	Evap. at $80^\circ$		Cyanuric acid..... 10
HAc + heat			Allantoin..... 50%

**Conclusions from Expts. 18 and 19.**—1. Hydrogen peroxide standing 3 days at room temperature in contact with an intermediate solution containing a large excess of alkali has no effect on the subsequent yield of allantoin (Expt. 18 (I)). Allantoin, present as such, would have been decomposed under these conditions.

2. Hydrogen peroxide in contact at room temperature with an intermediate solution containing a large excess of alkali has likewise no effect on the subsequent yield of uroxanic acid (Expts. 19 (I), 19 (IIa)).

3. Hydrogen peroxide in contact at  $80^\circ$  with an intermediate solution of zero excess alkalinity gives a 10% yield of cyanuric acid, and both allantoin and uroxanic acid disappear. This production of cyanuric acid was probably due to the fact that allantoin formation in the intermediate solution took place under the conditions of the experiment, and its subsequent oxidation to cyanuric acid by the hydrogen peroxide was to be expected from the results of Expt. 11.

4. When an intermediate solution containing a large excess of alkali is



evaporated with hydrogen peroxide, the allantoin is decomposed and no cyanuric acid is formed. (Expt. 19 (IIa)).

5. There is no apparent relation between the Behrend intermediate solution and the production of 50% yields of cyanuric acid under the conditions of Expt. 7.

#### Oxidation in Strongly Alkaline Solution.

Preceding experiments having failed to throw further light upon the mechanism of the reaction leading to the formation of allantoin and carbonyl-di-urea, attention was next turned to the reaction in cold conc. alkali which had yielded cyanuric acid. Quantitative runs were, therefore, made in which the quantities of all products were determined for conditions most favorable to the formation of cyanuric acid.

The following is typical: Two and a half g. of uric acid was dissolved in a mixture of 6 g. of potassium hydroxide and 100 cc. of water. After cooling to 18°, 65 cc. of 3% hydrogen peroxide solution was added. The excess alkalinity was then 0.47 *N*.

The mixture was allowed to stand 24 hours at room temperature. Fifteen cc. of hydrochloric acid (12 *N*) was then added and the carbon dioxide evolved aspirated through weighed potash bulbs. After applying a correction for the carbonate previously present in the alkali, 1.22 g. of carbon dioxide was shown to have been evolved.

The acid solution was then cooled and a precipitate of cyanuric acid filtered off which weighed 0.6 g.

The filtrate from the cyanuric acid was divided into two parts for the determination of oxalic acid and ammonia.

**Portion I.**—The solution was made neutral to litmus with sodium hydroxide. 3 cc. of glacial acetic acid and 15 cc. of calcium chloride solution were then added, and the mixture allowed to stand overnight. The precipitate of calcium oxalate was filtered off. This precipitate was redissolved in 10 cc. of hydrochloric acid (6 *N*), and after filtering was reprecipitated with 10 cc. of ammonium hydroxide (6 *N*), 5 cc. of dil. acetic acid, and 3 cc. of calcium chloride solution. The resulting white crystalline precipitate of calcium oxalate was filtered off, washed with water, and ignited to calcium oxide. It weighed 0.0484 g.

**Portion II.**—In the second portion ammonia was determined by the method of Folin<sup>1</sup> as follows:

The solution was neutralized with potassium hydroxide, 5 g. of sodium carbonate added, and then aspirated rapidly, the gas stream passing from the flask through a prolong containing dry asbestos, and then through a wash bottle containing sulfuric acid (0.114 *N*). Time of aspiration, 3 hours. The excess sulfuric acid was titrated with standard potassium hydroxide. Sulfuric acid required, 3.14 cc.

<sup>1</sup> *Z. physiol. Chem.*, **37**, 161 (1903).

On combining the average results from several experiments like the above, it was found that when one formula weight of uric acid is oxidized by hydrogen peroxide in a solution whose alkalinity exceeds 0.3 *N* one-half a formula weight of cyanuric acid is produced and two of carbonic acid.

The other products are negligible in comparison, but oxalic acid and ammonia in some cases attained a value of one-tenth of a formula weight. These substances are evidently not involved in the principal reaction.

**Isolation of the Intermediate Compound Formed during the Oxidation in Strongly Alkaline Solution.**

Up to this point it had been assumed that at the point of acidification the salt present in the alkaline solution was sodium cyanurate just as is the case when carbonyl-di-urea is treated with alkali. It was possible, however, that some intermediate might be present which would yield cyanuric acid either with acid or a combination of acid and hydrogen peroxide. The latter proved to be the case.

When the oxidation was carried out in the usual way and powdered manganese dioxide stirred into the solution, subsequent addition of acetic acid produced a heavy crystalline precipitate, which could be dissolved in alkali and reprecipitated with carbon dioxide.

Microscopic examination indicated that this product was a mixture of two crystalline species.

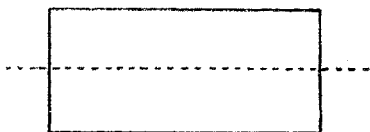


Fig. 2.—Species A.

Habit: Large, thin, transparent, rectangular plates.

Optical Orientation: Parallel extinction. Axial plane parallel to tabulation.

Interference Figure: None.

Indices:  $\alpha = 1.51$ .  $\gamma = 1.68$ .

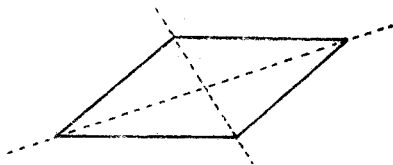


Fig. 3.—Species B.

Habit: Short, thick transparent tables.

Optical Orientation: Symmetrical extinction. Axial plane parallel to tabulation.

Indices:  $\alpha = 1.46$ .  $\gamma = 1.74-1.77$ .

Birefringence: Unusually strong, a double refraction greater than 0.3 being quite unusual.

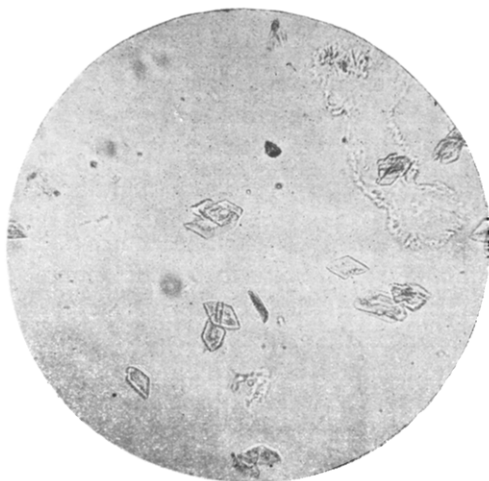


Fig. 4.—Species B.

Both compounds, A and B, are insoluble in all the usual organic solvents, but attempts were made to separate them by fractional crystallization from water, and from dilute acetic acid, and by fractional precipitation from alkaline solution with carbon dioxide—all without success.

Experiments undertaken to determine the most favorable conditions for the preparation of the new intermediate showed that an excess alkalinity below 0.3 *N* or above 1.6 *N* was distinctly unfavorable. The optimum conditions were normal alkali and room temperature. Most preparations were hereafter carried out as follows: 10 g. uric acid was suspended in 300 cc. water and then 24 g. sodium hydroxide slowly added in solid form with constant shaking and cooling in running water. When dissolved in this way the acid does not clot, and shows no tendency to precipitate on the addition of more alkali. 150 cc. of 3% hydrogen peroxide was next added, and the whole allowed to stand 36 hours. Powdered manganese dioxide was then stirred into the liquid and when oxygen evolution had ceased, the solution was filtered, and the filtrate carefully neutralized with acetic acid. Yield of mixed salts, 6.2 g.

It was next demonstrated by a series of experiments that the new product could be transformed into cyanuric acid by hydrogen peroxide in acid solution. The best conditions were low temperature and slight acidity but the reaction took place to some extent even in weakly alkaline solutions. The best yields were obtained as follows: 0.5 g. of the mixed sodium salts was dissolved in 20 cc. water to which had been added 0.5 cc. 6 *N* sodium hydroxide solution, 15 cc. of 3% hydrogen peroxide solution was then added and finally 1.5 cc. 6 *N* hydrochloric acid. There was an immediate deposition of crystals. After filtering, the solution was evaporated to 10 cc., when a second crop was obtained. These were

identified by the optical properties as cyanuric acid. The total yield was 0.3 g. It should be added that when no hydrogen peroxide is employed, hydrochloric acid causes effervescence but no cyanuric acid is obtained.

Since the sodium salts above described were obviously not homogeneous, others were sought which might be more suitable for analysis. Double decompositions carried out qualitatively in aqueous solution yielded the following:

1. A lead salt as a heavy crystalline precipitate, insoluble in hot water and in dilute acetic acid, but soluble in mineral acids.
2. An acid barium salt formed by treating the mixed sodium salts with barium chloride in dilute acetic acid solution. It formed transparent crystalline needles difficultly soluble in hot water, insoluble in cold.
3. A neutral barium salt formed by precipitation in neutral solution. It is insoluble in hot and cold water.
4. A silver salt with similar properties.
5. A neutral ammonium salt formed from the sodium salt by treatment with ammonium chloride and an excess of ammonia. It crystallizes in needles readily soluble in hot water from which it crystallizes again on cooling.

Of these the acid barium and the neutral ammonium salts seemed most suitable for analysis, since from their method of preparation they were not likely to contain a mixture of acid and neutral salts.

In preparing the ammonium salt for analysis, 5 g. of the crude sodium salts was dissolved in 100 cc. of warm water and to this solution were added 2.7 g. ammonium chloride and 20 cc. 6 *N* ammonia. After removing a small insoluble residue the filtrate was cooled with ice. A large crystalline precipitate was deposited which when examined under the microscope proved to be homogeneous and needle-like in form. After washing it was recrystallized twice more from hot water. The air-dried product left no residue on ignition and lost no weight when placed in a desiccator over sulfuric acid. Even when heated in an oven at 90° it lost only about 1% per hour, but when heated to 110° slow decomposition took place, a sample losing at this temperature 45% of its weight in 28 hours. These experiments seemed to justify the conclusion that the salt contained no water of crystallization.

Subst.	H <sub>2</sub> O.	CO <sub>2</sub> .	N (cc. at 20° and 759 mm.).
0.1319	0.059	....	...
0.1426	....	0.1223	...
0.1962	0.0907	0.1624	...
0.0581	....	....	17.15
0.0571	....	....	16.98

Calc. for  $C_4H_{11}O_5N_3$  ( $= (NH_4)_2C_4H_5O_5N_3$ ): C, 23.39; H, 5.31; N, 33.50. Found: C, 23.00, 23.39; H, 5.27, 5.00; N, 33.67, 33.83.

Ratio C : H : O : N = 4.02 : 10.75 : 4.95 : 5.02.

The acid barium salt was next prepared for analysis as follows: 5 g. of the crude sodium salts was dissolved in 150 cc. of boiling water to which 30 cc. 2 *N* barium chloride solution and 5 cc. glacial acetic acid were added. There was evidence of some decomposition but on cooling a rapid deposition of a crystalline precipitate took place. This was again dissolved in 400 cc. of water to which had been added 3 cc. glacial acetic acid and 2 cc. barium chloride solution. The product which separated was once more recrystallized from hot water, and then dried in an evacuated desiccator without a dehydrating agent. Under these circumstances it came to constant weight, but over sulfuric acid it lost steadily, the weight decreasing 10% in 4 days. When heated in an oven between 120° and 130° it lost as much as 32% in two days, becoming discolored after a loss of 25%. It is evident from the above that the line between loss of water of crystallization and further decomposition is here difficult to draw. Air-dried material was employed for analysis.

#### Analysis.

0.1268 g. gave 0.0551 g. BaSO<sub>4</sub>; 0.1991 g. gave 0.0874 g. BaSO<sub>4</sub>.  
 Ammonia from 0.1248 g. required 14.67 cc. 0.9811 *N* H<sub>2</sub>SO<sub>4</sub>.  
 Ammonia from 0.1340 g. required 15.63 cc.  
 0.1302 g. gave 0.0856 g. CO<sub>2</sub>, 0.0313 g. H<sub>2</sub>O.  
 0.1468 g. gave 0.0966 g. CO<sub>2</sub>, 0.0354 g. H<sub>2</sub>O.  
 Calc. for Ba(C<sub>4</sub>H<sub>4</sub>O<sub>5</sub>N<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O: C, 18.41; H, 2.32; N, 16.12; Ba, 26.34. Found:  
 C, 17.94, 17.98; H, 2.72, 2.73; N, 16.15, 16.03; Ba, 25.98, 25.83.  
 Ratio Ba : C : N = 1 : 7.90 : 6.06.

The theoretical conclusions drawn from these analytical data have already been discussed in the introduction. In closing the writer desires to express his thanks to Professor F. J. Moore of the Institute of Technology, at whose suggestion this investigation was undertaken, and to Professor C. H. Warren for much valued assistance in that portion of the work dealing with the optical properties of crystals.

#### Summary.

I.—It has been shown that the oxidation of uric acid with hydrogen peroxide leads to three different products according to the experimental conditions.

II.—In weakly alkaline or neutral solutions, and at a temperature just below that of boiling water, the products are allantoin and carbonyl-diurea; but though these substances are formed under similar conditions no genetic relationships connecting them have been discovered. They seem to be products of two independent reactions.

III.—At room temperature, and in an alkaline solution approximately normal, the product is an acid not previously noticed, whose empirical composition (as deduced from the analysis of two of its salts) appears to be C<sub>4</sub>H<sub>4</sub>O<sub>5</sub>N<sub>3</sub>. A structural formula has been suggested for this acid.

Its formation is in no way connected with that of allantoin or carbonyl-di-urea.

IV.—The formation of cyanuric acid is in all cases secondary. It may be formed by the alkaline oxidation of allantoin, especially at high temperature and in neutral solution; by the action of alkali alone upon carbonyl-di-urea; or finally (with the best yield) by the action of hydrogen peroxide in *acid* solution upon the compound  $C_4H_5O_5N_3$ .

V.—It has not been proved possible to trace any parallel between the action of hydrogen peroxide upon uric acid and that of permanganate.

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[CONTRIBUTION FROM THE ORGANIC LABORATORY OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY.]

## ALLANTOXANIC ACID AS AN OXIDATION PRODUCT OF URIC ACID.

### THIRD PAPER ON HYDROGEN PEROXIDE AS A REAGENT IN THE PURIN GROUP.

By F. J. MOORE AND RUTH M. THOMAS.

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In the preceding paper Dr. Venable has shown that when uric acid is oxidized by hydrogen peroxide at room temperature and in a solution whose excess alkalinity is approximately normal, an acid of the empirical formula  $C_4H_5O_5N_3$  is produced, and that this substance on further oxidation by hydrogen peroxide in acid solution goes over smoothly into cyanuric acid.

Dr. Venable was obliged to discontinue this work before he could fix with certainty the constitution of the new intermediate, but he had partially characterized the compound by the preparation of several of its salts, had analyzed two of these, and observed that the free acid readily decomposed with evolution of carbon dioxide when liberated by a mineral acid in aqueous solution. After again preparing Venable's compounds, and confirming the results of his analyses, our own first efforts were therefore directed toward the preparation of additional derivatives. Having failed to prepare esters, the attempt was next made to isolate whatever product might be formed when the free acid decomposed in aqueous solution; and also to obtain an amide by heating the ammonium salt, for Venable had already observed that this lost a large proportion of its weight even at  $110^\circ$ .

When the heating was carried out in sealed tubes it yielded a sublimate of ammonium carbonate. The reaction proved to be nearly quantitative, and from the slightly impure residue it was not difficult to isolate a compound  $C_3H_3O_2N_3$ , formed in the sense of the equation

