

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.

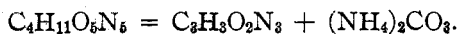
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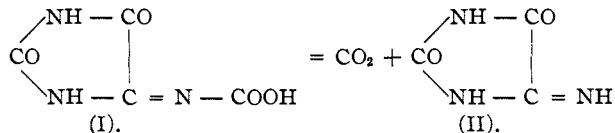
In the preceding paper Dr. Venable has shown that when uric acid is oxidized by hydrogen peroxide at room temperature and in a solution whose excess alkalinity is approximately normal, an acid of the empirical formula $C_4H_5O_5N_3$ is produced, and that this substance on further oxidation by hydrogen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

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

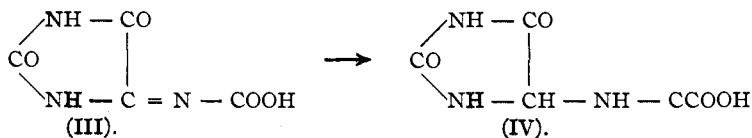


At the same time, the barium salt, when decomposed by a slight excess of sulfuric acid, yielded on evaporation the *same substance*. It was not difficult to identify this as allantoxaidin (II) which had heretofore only been prepared by acidifying the salts of allantoxanic acid (I).



It will be noticed that the formula of allantoxanic acid differs from that assigned by Venable to his compound by only a molecule of water, and that it has exactly the same carbon and nitrogen skeleton which he had already suggested. If, therefore, it were permissible to assume that ammonium allantoxanate contained an additional molecule of water then the complete mechanism of the oxidation of uric acid which leads to cyanuric acid might be considered established. As a matter of fact, however, an unexpectedly large amount of work was necessary before we felt justified in making this simple assumption.

In the first place, ammonium allantoxanate had already been prepared and analyzed by Ponomarew¹ who evidently considered it free from water of crystallization and who gave it a formula corresponding to that cited above for the free acid. It is true that he miscalculated the theoretical percentage of nitrogen for his own formula (37.43 instead of 36.64) and obtained experimental values to agree with the former (37.52 and 37.62), nevertheless a hydrated formula for allantoxanic acid in most of its derivatives was clearly out of the question, for a very considerable number of salts of the acid are known and have been analyzed by various investigators who all assign to the acid the anhydrous formula (III), and this also is the only one which harmonizes satisfactorily with its addition of two hydrogens to form hydroxonic (or dihydro-allantoxanic) acid (IV) as described by Ponomarew and confirmed by Biltz and Giesler.²



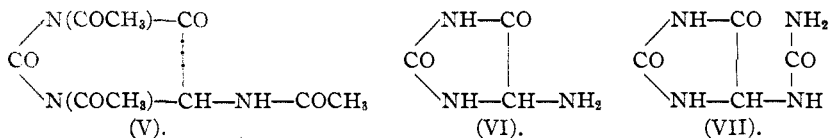
On the other hand Venable's drying experiments (which we could fully confirm) made it difficult to believe that his ammonium salt contained water of crystallization.

Another consideration which deterred us from making any assumptions which might later prove unjustified was the uncertainty which still exists concerning the constitution of allantoxaidin. The constitution of

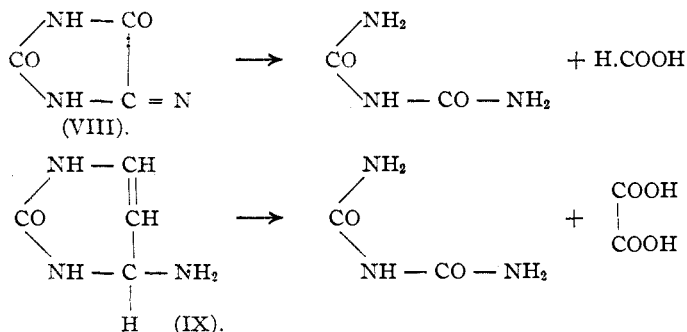
¹ Ber., 11, 2156 (1878); J. Russ. Phys. Chem. Soc., 11, 46 (1879).

² Ber., 46, 3410 (1913).

allantoxanic acid has been established by Biltz and Giesler in the following elegant way: When dihydroallantoxanic acid (IV) is treated with acetic anhydride it loses carbon dioxide and is acylated on all 3 nitrogens. By progressive hydrolysis the product (V) loses its acetyl groups to form 5-amino-hydantoin (VI), which when treated with potassium cyanate easily again yields allantoin (VII),



thus giving assurance that no rearrangement can have taken place throughout the cycle. Certain difficulties, however, still surround the formula of allantoxaidin. That already given (VIII) admirably explains its formation from allantoxanic acid, but although this formula represents it as the mono-imide of parabanic acid no genetic relationship as yet connects it with the latter substance. On hydrolysis it yields no oxalic acid derivative, but formic acid and biuret. On this account Biltz and Giesler suggest that it might have an open chain formula, perhaps that of formyl-cyan-urea CHO.NH.CO.NH.CN . The ring formula is, however, perhaps no more difficult to reconcile with the results of hydrolysis than is the oxidation of cytosin (IX) by permanganate, which yields oxalic acid and biuret.



It is clear from the above that the formation of allantoxaidin from Venable's acid was not conclusive evidence that it had a structure like that of allantoxanic acid. Unless its *identity* with the latter could be proved other formulas for it might have to be considered which were less in harmony with its formation from uric acid, unless, indeed, some new connection could be established between the oxaidin and parabanic acid. Therefore, after an unsuccessful attempt to obtain parabanic acid from the oxaidin by the action of nitrous acid, we fell back upon a more rigid comparison of the salts. Other investigators had prepared these by the oxidation of allantoin with permanganate. In consequence the acid

potassium salt (which crystallizes without water) had been analyzed by no less than 5 different persons¹ whose percentages for potassium all fell within narrow limits (19.5 to 20.1), the theory for the anhydrous formula being 20.07 and that for Venable's formula 18.31. This salt, therefore, seemed admirably adapted for comparison. It was accordingly prepared from allantoin, and, for comparison, uric acid was oxidized with hydrogen peroxide in the presence of *potassium* hydroxide. Under the microscope the two products showed identical properties, but the results of analysis were still discouraging. Our analyses of the product from allantoin agreed with those of previous investigators (potassium, 19.75-19.94) while the material from uric acid gave intermediate values (18.68-19.17).

A comparison of the acid barium salts revealed somewhat similar discrepancies, but we attach little importance to these, since the barium salt contains water very loosely held, and it is difficult in any case to determine what degree of dryness represents material suitable for analysis. The neutral ammonium salts gave a more satisfactory result. One was prepared from the acid barium salt which had been derived from allantoin, the other in the usual way from the oxidation products of uric acid. Both not only showed identical properties under the microscope, but also gave identical percentages for nitrogen and these agreed with *Venable's formula* for the salt. We therefore conclude that Ponomarew was in error in his analysis of the ammonium salt, and that this contains one more molecule of water than has previously been recognized. Additional evidence that Venable's acid is allantoxanic acid was found by reducing it to the dihydro compound already referred to. This acid and its ammonium salt showed identical crystalline habit, whether prepared from allantoin or from uric acid.

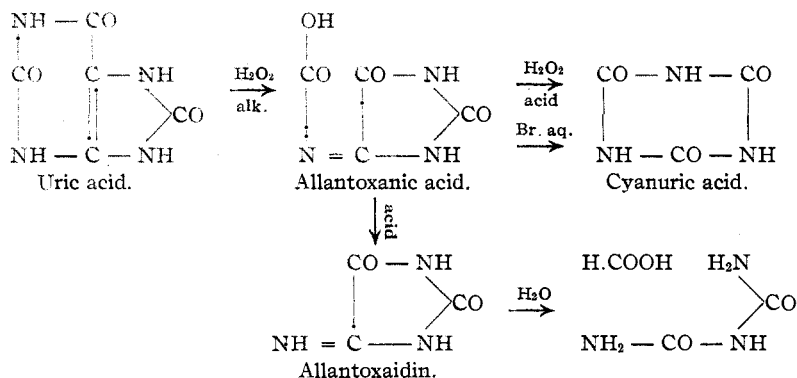
The additional molecule of water in ammonium allantoxanate might easily be called water of crystallization, rather firmly held. All things considered, however, we are inclined to assume that under certain circumstances water adds to the double bond in allantoxanic acid, this tendency being more pronounced in some salts than others. An imperfect equilibrium between the two forms might then account for the discrepancies in the analyses of certain salts.

The fact that allantoxanic acid had hitherto always been formed by the oxidation of allantoin again raised the old question whether the latter might not be an intermediate in the uric acid oxidation. Venable's experiments had, however, already shown that under the circumstances there prevailing, no cyanuric acid or allantoxanate is produced from allantoin. These experiments were, however, once more repeated and verified. A further question arose with reference to allantoxidin. Since

¹ Van Embden, *Ann.*, 167, 39 (1873); Mulder, *Ber.*, 8, 1292 (1875); Ponomarew, *Loc. cit.*; Biltz and Giesler, *Loc. cit.*; Denicke, *Ann.*, 349, 269 (1906).

this is formed by acidifying the allantoxanate, might not the transition from the latter substance to cyanuric acid pass through the oxadin as an intermediate. This substance however proved entirely stable toward acid hydrogen peroxide.

So far as understood therefore, the oxidation scheme of uric acid with hydrogen peroxide at room temperature and in normal alkali assumes the following form:



No intermediates have yet been found between uric acid and allantoxanic acid. It seemed worth while, however, to test in this respect those compounds which Behrend had suggested as possible intermediates in the permanganate oxidation. Uric acid glycol and alloxanic acid urea were therefore prepared and treated with hydrogen peroxide, but no allantoxanate or cyanuric acid was obtained.

It will further be recalled that Behrend at one time assumed the existence in one of his intermediate solutions of a glycoluril-hydroxy-carboxylic acid, because from such a solution he could obtain allantoin or uroxanic acid at will. This acid is still hypothetical, but it occurred to us that so far as allantoin or allantoxanic acid are concerned glycoluril would answer as well. This compound was therefore also prepared and treated with hydrogen peroxide but without success.

Experimental Part.¹

Venable's method of oxidation of uric acid with hydrogen peroxide with a large excess of sodium hydroxide was followed successfully. The alkaline solution was allowed to stand as a rule somewhat longer than Venable suggests (48 rather than 36 hours) without lessening the yield of mixed sodium salts (6.0, 6.1, 7.1, 5.7 g.).

Several attempts were made to prepare an ester from the sodium salts, either by distillation with potassium ethyl sulfate, or by Fischer's method of esterification, by shaking an alkaline solution with methyl iodide in a sealed tube at 100° for one hour. Neither method was successful.

¹ By Ruth M. Thomas.

The mixed sodium salts gave cyanuric acid when heated with bromine water for 10 minutes. On cooling with ice, a 46% yield of cyanuric acid was obtained.

When reduced with sodium amalgam the sodium salts yielded hydroxonic (dihydroallantoxanic) acid.

Di-Ammonium Salt.

The ammonium salt was prepared without difficulty according to Venable's directions from the mixed sodium salts by means of ammonium chloride and ammonia. The crude potassium compound (to be described later) could be substituted for the sodium salts.

The crude material always appeared perfectly uniform under the microscope. It was recrystallized from hot water, cooling immediately with ice to lessen possible decomposition. Certain samples for analysis were recrystallized from quite strong ammonia.

The optical properties were as follows:

Habit: very fine needles or slender blades, usually clustered, axial plane parallel to tabulation, parallel to elongation. Parallel extinction. Birefringence very strong.

Analyses on the material checked well with Venable's results.

I. 0.1805 g. gave 0.1525 g. CO₂, 0.0865 g. H₂O.

0.0819 g. gave 24.7 cc. nitrogen at 25°, 762 mm.

0.0847 g. gave 25.8 cc. nitrogen at 27°, 760 mm.

II. 0.2475 g. gave 75.2 cc. nitrogen at 24°, 747 mm.

Calc. for C₄H₁₁O₅N₅: C, 22.96; H, 5.31; N, 33.50. Found: C, 23.04; H, 5.36; N, 33.74, 33.62. Found, Venable: C, 23.39, 23.00; H, 5.00, 5.26; N, 33.57, 33.83.

In explanation of the nitrogen results, it should be said that the samples in I were very small, on account of the high percentage of nitrogen. In II, therefore, a large sample was used, the gas collected as usual in an azotometer, transferred at once to a gas buret, measured, and shaken in a sodium hydroxide pipet. There was no loss in volume of the nitrogen. The 1 : 1 potash solution used in the azotometer was efficient for two determinations of this kind.

The di-ammonium salt does not lose weight in a vacuum desiccator over sulfuric acid, nor when heated on an open watch glass at 80° for two hours. At 100°, however, there is a steady slow loss, the rate of which is considerably increased if the temperature is raised to 150°. The loss in weight on heating at 150° was found in 3 experiments to be 44.6, 45.7, and 45.9%. (Theory for C₄H₁₁O₅N₅ = (NH₄)₂CO₃, 45.9%.) The product obtained in this way was identified as allantoxaidin, and will be discussed later.

If, instead of heating in an open watch glass, the ammonium salt is heated in a sealed tube, slightly different results are observed. An 0.8 g. sample, sealed in a tube and heated gradually in an oil-bath, showed very little change after two hours at 100–130°, though a little liquid had con-

densed in the capillary. Another hour and a half at 140–150° produced a little more liquid, but on opening the tube there was no increased pressure and no odor of ammonia. Later heating at 150–165° produced considerable liquid, and at 190° a white sublimate appeared. Heated for an hour at 190–195°, the tube still contained some unchanged solid.

On cooling, the upper end of the tube contained a large amount of crystalline sublimate, which proved to be ammonium carbonate. On opening there was considerable pressure and a strong odor of ammonia. The semi-solid residue in the bottom of the tube apparently contained a very little biuret, soluble in alcohol, and allantoxaidin, which did not dissolve in cold absolute alcohol.

Acid Barium Salt.

The barium salt was prepared from Venable's directions from the crude sodium product, and agreed with his description as to appearance, solubility, water of crystallization, and percentage composition.

Air-dried material did not lose weight in an evacuated desiccator (without sulfuric acid) overnight. Over sulfuric acid it lost weight rapidly, taking up moisture so rapidly that weighing on a watch glass was impossible. In a tared weighing beaker which could be covered when weighing, a sample came to constant weight in 6 days, losing 3.86%. It was now exposed to the air and weighed at frequent intervals. In an hour it had come to constant weight, gaining 3.22% or almost the amount it had lost.

Material for analysis was dried for 5 hours in an evacuated desiccator without sulfuric acid, then left in the air until it came to constant weight before samples were weighed.

0.1498 g. gave 0.0651 g. BaSO₄; 0.1377 g. gave 0.0605 g. BaSO₄; 0.1388 g. gave 0.0897 g. CO₂, 0.0345 g. H₂O; 0.1145 g. gave 15.95 cc. nitrogen, at 24°, 771 mm.; 0.1217 g. gave 16.92 cc. nitrogen, at 24°, 773 mm.

Calc. for Ba(C₅H₄O₅N₃)₂·2H₂O: C, 18.41; H, 2.32; N, 16.12; Ba, 26.24. Calc. for Ba(C₄H₂O₄N₃)₂·6H₂O: C, 17.23; H, 2.87; N, 15.07; Ba, 24.59. Found: C, 17.62; H, 2.78; N, 15.77, 15.79; Ba, 25.57, 25.86. Found, Venable: C, 17.94, 17.98; H, 2.72, 2.73; N, 16.15, 16.03; Ba, 25.98, 25.83.

Attempts to obtain the free acid by treatment of the acid barium salt with sulfuric acid gave allantoxaidin.

Allantoxaidin.

As already mentioned, allantoxaidin is obtained in almost quantitative yield when the di-ammonium salt is heated on an open watch-glass at 100–150°.

The other method of preparation from the acid barium salt and sulfuric acid was carried out as follows: 7 g. of crude barium salt (containing 26.5% barium) was suspended in one liter of water and treated with 26.0 cc. 1.04 *N* sulfuric acid (required 26.2 cc.). There was considerable

evolution of carbon dioxide. After cooling, the solution was filtered off and allowed to evaporate in a hood window. Examination of the barium precipitate showed only a trace of organic matter. When the aqueous solution had evaporated to two-thirds its original volume considerable of the original barium salt had separated. The solution was therefore treated drop by drop with sulfuric acid till no further precipitation of barium sulfate took place and the solution was faintly acid to litmus. On complete evaporation of the filtered solution, there was left a crystalline residue of allantoxaidin. It lost water in the air or over sulfuric acid. Yield of opaque material, 2.7 g. Theoretical yield of anhydrous allantoxaidin, 3.0 g.

The crude product obtained by either method was purified for analysis by recrystallization from hot water, using from 7 to 10 cc. for one g. of material. Long heating was to be avoided on account of decomposition and the hot solution was quickly cooled with ice. Under these conditions the allantoxaidin crystallized at once in short, thick, six-sided prisms, in perfect accordance with Biltz's description.

Biltz gives the melting point at 282° with decomposition. In the present investigation no definite melting point was found. A sample from heated ammonium salt darkened very slightly at 230° , moistened and blackened at $245-250^{\circ}$, and decomposed gradually on higher heating without melting. Recrystallized, a sample moistened at 220° , darkened about 238° , and did not melt. A sample of material from the barium salt darkened at $220-230^{\circ}$ and later shrank somewhat, but did not melt up to 290° .

Biltz found one molecule of water of crystallization which was partly lost in the air, and completely at 110° . Present results indicate that the crystals contain more than one molecule of water, part of which is removed over sulfuric acid, but the last molecule only by heating at 110° . A sample lost 16.9% of its weight over sulfuric acid in 36 hours, then 13.0% when heated at 110° for one hour. These two products were analyzed and gave results corresponding to the monohydrate and anhydrous allantoxaidin.

- I. Dried over H_2SO_4 . Loss 18.4%.
- 0.0685 g. gave 20.60 cc. nitrogen at 23° , 760 mm.
 - Dried at 110° . Loss 7.0%.
 - 0.1395 g. gave 0.1615 g. CO_2 , 0.0343 g. H_2O .
 - 0.1408 g. gave 0.1639 g. CO_2 , 0.0355 g. H_2O .
 - 0.0573 g. gave 18.92 cc. nitrogen at 24° , 756 mm.
 - 0.0613 g. gave 20.02 cc. nitrogen at 22° , 757 mm.
- II. Dried at 110° . Loss 15.1%.
- 0.0578 g. gave 18.72 cc. nitrogen at 22° , 757 mm.

III. Dried over sulfuric acid, loss 16.9%.

0.1386 g. gave 0.1409 g. CO₂, 0.0478 g. H₂O.

0.1286 g. gave 0.1295 g. CO₂, 0.0442 g. H₂O.

0.0791 g. gave 22.20 cc. nitrogen at 21°, 763 mm.

0.0718 g. gave 20.12 cc. nitrogen at 21°, 763 mm.

Dried at 110°. Loss 13.0%.

0.1336 g. gave 0.1544 g. CO₂, 0.0325 g. H₂O.

0.0574 g. gave 18.55 cc. nitrogen at 21.5°, 760 mm.

Calc. for C₈H₈O₂N₃.H₂O: C, 27.48; H, 3.85; N, 32.08; H₂O, 13.7.

Calc. for C₈H₈O₂N₃: C, 31.86; H, 2.68; N, 37.19; H₂O, 0.0.

Found (H ₂ SO ₄):	C.	H.	N.	(110°).	C.	H.	N.
I.....			33.72		31.57	2.75	36.81
					31.75	2.82	36.82
II.....							36.52
III.....	27.71	3.86	32.14		31.52	2.72	36.72
	27.46	3.84	32.06				

A sample of allantoxaidin (containing 36.52% nitrogen) was suspended in 50 cc. water and titrated with 0.1 *N* alkali using phenolphthalein as an indicator, taking as an end-point a color permanent for one minute. The molecular weight calculated from the titrations was 121, 119. Theory for C₈H₈O₂N₃, 113.

Several unsuccessful attempts were made to oxidize allantoxaidin with hydrogen peroxide, testing especially in each case for cyanuric acid.

In the hope of obtaining parabanic acid, allantoxaidin was treated with nitrous acid, but all experiments were unsuccessful. After sodium nitrite in acid solution was found unsatisfactory the mixed gases from starch and nitric acid were used. Since the results were negative experimental details will not be given, but it may be said that no trace of parabanic acid was found, and that considerable allantoxaidin was recovered.

Allantoxaidin dissolved in fuming nitric acid with vigorous evolution of gas. On evaporation of the acid, however, the residue consists almost entirely of unchanged allantoxaidin. One g. was dissolved in fuming nitric acid, heated a few minutes on the water bath, then allowed to evaporate. After several recrystallizations the allantoxaidin recovered weighed 0.5 g. This was again dissolved in fuming nitric acid, warmed for 15 minutes on the water bath, then evaporated. The residue, air-dried, weighed 0.5 g. It gave no test for parabanic acid, and on recrystallization was found to consist almost entirely of allantoxaidin.

Oxidation of Uric Acid in Potassium Hydroxide Solution.

When potassium hydroxide was substituted for sodium hydroxide in the oxidation of uric acid with hydrogen peroxide, the reaction took place more rapidly, the yield was not so large, and the product appeared more nearly uniform than in the first case.

Ten g. of uric acid was suspended in 300 cc. of water and treated with 33.5 g. of potassium hydroxide, followed by 150 cc. of hydrogen peroxide

After standing 29 hours at room temperature a sample gave no precipitate on acidification and no murexid test on evaporation. The solution was then treated with powdered manganese dioxide, allowed to stand half an hour, filtered, cooled with ice, and acidified with glacial acetic acid. The potassium salt crystallized at once. Weight 4.0 g., 30%.

Under the microscope the crude product consisted almost entirely of very slender, colorless needles or blades, usually in radiating clusters. These were mixed with small rectangular plates, resembling uric acid, but giving no murexid test. Under the polarizing microscope it was found that the axial plane was parallel to the tabulation, and α parallel to the elongation. The crystals exhibited parallel extinction.

$$\alpha = 1.52.$$

$$\gamma = 1.66.$$

The results of potassium determinations did not agree well with the theory.

0.1557 g. gave 0.0648 g. K_2SO_4 ; 0.1870 g. gave 0.0784 g.

0.2825 g. gave 0.1188 g. K_2SO_4 ; 0.2226 g. gave 0.0942 g.

0.2439 g. gave 0.1040 g. K_2SO_4 ; 0.2166 g. gave 0.0925 g.

Calc. for $C_4H_4O_6N_3K$: K, 18.31. Calc. for $C_4H_2O_4N_3K$: K, 20.07. Found: K, 18.68, 18.82, 18.88, 19.00, 19.14, 19.17.

It is known that the salts of allantoxanic acid decompose on boiling with water. Hence it is possible that the last two analyses given, where only one recrystallization was made, and the product washed with boiling alcohol, may be more nearly correct.

Acid Potassium Allantoxanate.

This salt was prepared from allantoin and permanganate in alkaline solution, following the directions of Biltz.¹ The crude product, which appeared entirely uniform under the microscope, was recrystallized from hot water. Long heating, however, was to be avoided, as the crystals were then not uniform under the microscope.

In optical properties the allantoxanate agreed absolutely with the potassium salt above described.

Potassium determinations agreed with the results of five previous investigators.

Subst.: 0.1617, 0.2017, 0.4153. K_2SO_4 : 0.0718, 0.0880, 0.1827.

Calc. for $C_4H_2O_4N_3K$: K, 20.07. Calc. for $C_4H_4O_6K$: K, 18.31.

Found: K, 19.94, 19.59, 19.75; Van Embden, 19.8, 19.5, 20.09, 20.1, 20.08; Mulder, 19.8; Ponomarew, 19.78; Biltz, 19.9; Denicke, 19.73.

Acid potassium allantoxanate, oxidized with hydrogen peroxide in alkaline solution, gave cyanuric acid, identified microscopically and by the Wöhler test. Reduction with sodium amalgam gave dihydroallantoxanic acid. With ammonium chloride and ammonia the potassium salt formed

¹ Ber., 46, 3410 (1913).

an ammonium salt, not to be distinguished from the one obtained from the uric acid oxidation product.

Neutral Ammonium Allantoxanate.

Neutral ammonium allantoxanate was first prepared in this investigation from the acid potassium salt by treatment with ammonium chloride and ammonia. Two g. of crude potassium salt was suspended in 50 cc. of hot water and treated with 1.25 g. ammonium chloride and 10 cc. ammonia. On cooling an immediate precipitate of the di-ammonium salt formed. It was twice recrystallized from hot water.

It agreed in solubility and optical properties with the ammonium salt obtained from the uric acid oxidation product, described on page 1125.

Nitrogen determinations were made by both the Dumas and Will and Varrentrapp methods with concordant results.

0.0738 g. gave 22.30 cc. nitrogen at 22°, 759 mm.

0.0755 g. gave 22.75 cc. nitrogen at 22°, 758 mm.

0.1849 g. required 10.37 cc. 0.4325 *N* HCl to neutralize NH₃ formed.

0.1851 g. required 10.38 cc. HCl.

Calc. for C₄H₁₁O₅N₅: N, 33.50. Calc. for C₄H₉O₅N₅: N, 36.64. Found: N, 34.17, 34.05, 33.96, 33.96.

Finally, neutral ammonium allantoxanate was also prepared according to Ponomarew's method. The acid barium salt was made by treating acid potassium allantoxanate (4.8 g.), dissolved in hot water, with barium chloride (15 cc. 2 *N* solution). The acid barium salt crystallized out on cooling with ice. This product (6.3 g.) was next suspended in water (400 cc.) and treated with ammonium sulfate. After filtering from the barium sulfate and cooling with ice, the acid ammonium salt crystallized in papery needles (3.3 g.). This acid salt was converted to the neutral salt by recrystallizing from dilute ammonia. On cooling with ice 2.4 g. of neutral ammonium allantoxanate were obtained.

The product agreed in optical properties with those already described. It was analyzed for nitrogen.

Subst.: 0.1794; 55.40 cc. N at 23°, 733 mm. Found: N, 33.54.

A comparison of this result with those obtained on the allantoxanate prepared directly from the potassium salt, and with those on the ammonium salt obtained from the uric acid oxidation product (p. 1125) indicates that the 3 are identical.

Dihydroallantoxanic Acid.

Dihydroallantoxanic acid was prepared according to Biltz's directions by the reduction of acid potassium allantoxanate with 3% sodium amalgam. It was recrystallized from boiling water, and came out on cooling in tiny, hard lumps, either spherulitic or made up of extremely microscopic crystals. The product obtained by reducing the crude sodium salts from the uric acid oxidation was exactly similar. In either case, the

product darkened about 240° and did not melt on further heating. Biltz describes the pure acid as darkening from 270 – 280° , and not changed on slow heating to 380° , but decomposed on rapid heating to 310° .

The ammonium salt was prepared from both reduction products by treating an aqueous solution with a few drops of ammonia, filtering the solution, and adding acetic acid. In both cases there slowly formed very tiny plates, which were optically identical.

It may be mentioned that, although, the salts of allantoxanic acid oxidize readily to cyanuric acid with hydrogen peroxide, dihydroallantoxanic acid is extremely stable toward this reagent.

Uric Acid Glycol.

Uric acid glycol was prepared according to the method of Biltz¹ from alloxan tetrahydrate and urea in the presence of a little bromine.

In alkaline solution with hydrogen peroxide, no allantoin, carbonyl-di-urea or cyanuric acid were obtained. The glycol decomposed quite rapidly in this solution.

In neutral solution the glycol was stable. 0.5 g. of glycol, 3 cc. water and 7 cc. hydrogen peroxide were left at room temperature 24 hours. The glycol did not dissolve. The mixture was then warmed at 70° for two hours, but on cooling the glycol crystallized unchanged. It was next treated with a few drops of sodium hydroxide and warmed a few minutes. On acidification there was no trace of any of the oxidation products sought, and the glycol was almost entirely destroyed.

Alloxanic Acid Urea.

Alloxanic acid urea was prepared according to Behrend² from alloxan tetrahydrate, hydrolyzed with potassium hydroxide, and urea.

No allantoin, carbonyl-di-urea or cyanuric acid were obtained when the urea was treated with hydrogen peroxide under conditions comparable with the uric acid oxidation.

With high excess alkalinity at room temperature (0.5 g. alloxanic acid urea, 0.9 g. sodium hydroxide, 15 cc. water and 13 cc. hydrogen peroxide) for 24 hours there was very little decomposition. On acidification and spontaneous evaporation, the original material was recovered.

With low excess alkalinity at 90° (0.5 g. alloxanic acid urea, 0.3 g. sodium hydroxide, 15 cc. water and 30 cc. hydrogen peroxide) for $1\frac{1}{2}$ minutes no precipitate was obtained on subsequent acidification. On evaporation the residue consisted almost entirely of sodium chloride, mixed with a very little alloxanic acid urea.

In acid solution with hydrogen peroxide at room temperature the material was unchanged.

¹ *Ber.*, **47**, 459 (1914).

² *Ann.*, **410**, 337 (1915).

Glycol-Uril.

Glycol-uril was prepared according to the directions of Rheineck¹ by the reduction of allantoin with 1% sodium amalgam.

Glycol-uril does not melt. It crystallizes in fairly large octahedra, or on rapid cooling in slender needles.

It is too stable in both alkaline and acid solution toward hydrogen peroxide to be the intermediate sought in the cyanuric acid oxidation.

Two-tenths of crude glycol-uril, 0.2 cc. 6 *N* sodium hydroxide, 5 cc. of water and 5 cc. hydrogen peroxide were warmed at 80° for one-half hour. Much of the glycol-uril was undissolved. On acidification of the solution nothing but unchanged material was obtained.

In a second experiment the amount of alkali was doubled, and the heating was continued for several hours, but the glycol-uril was still unchanged.

In acid solution glycol-uril was boiled with hydrogen peroxide without change.

Summary.

1. The acid to which Venable had tentatively assigned the formula $C_4H_5O_5N_3$ (see previous paper) is here recognized as allantoxanic acid $C_4H_3O_4N_3$ which, however, seems to exist in a hydrated condition in certain of its salts in accordance with Venable's graphic formula. With this addition all facts now known concerning the oxidation of uric acid under these special conditions are adequately accounted for.

2. In the formation of allantoxanic acid from uric acid by hydrogen peroxide neither allantoin nor glycol-uril are intermediate products. The same may be said of the other compounds which Behrend has suggested as possible intermediates in the permanganate oxidation.

3. The oxidation of allantoxanic acid to cyanuric acid by the action of hydrogen peroxide in acid solution does not pass through allantoxidin.

4. The investigation will be continued by studying the action of hydrogen peroxide upon substituted uric acids and other purin derivatives in the hope of obtaining further light upon the mechanism of the reaction.

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¹ *Ann.*, **134**, 219 (1865).
