

and small amounts of powdered zinc were suspended in a mixture of 20 cc. of acetic acid and 15 cc. of water. The mixture was heated in an open flask on a steam bath for 11 days. More zinc was added from time to time, also small amounts of acetic acid and water. At the end of the reaction, the clear, hot solution was filtered. Cold water was added, and a heavy precipitate immediately formed. This was filtered and washed with a little cold water. The precipitate was extracted with hot water several times to remove all traces of zinc acetate. It was then extracted with alcohol. It was only slightly soluble in water, and was recrystallized from large amounts of water.

Calc. for  $C_{13}H_{11}O_6N_2Zn$ : N, 7.84. Found: 7.87.

The presence of zinc in the compound was confirmed by qualitative tests for the metal. A small amount of the salt was placed in a porcelain crucible, moistened with a few drops of nitric acid, and heated carefully to avoid spattering. The residue was yellow and insoluble in water. This was treated with hydrochloric acid and evaporated to dryness. The white residue was soluble in water. When ammonium sulfide was added to the aqueous solution, zinc sulfide was formed as a white precipitate.

The salt was much more soluble in aqueous ammonia than in pure water. Its ammoniacal solution when acidified precipitated a substance melting at  $271^\circ$  which was identified as the original acid.

#### Conclusion.

The high-melting products which are formed when 4-anisalhydantoin 1-acetic acid is treated with sodium or zinc in reducing media have been identified as the respective salts of this acid. The result disproves conclusively our previous supposition that these substances in both cases might be the geometrical isomeride of the unsaturated acid.

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[CONTRIBUTION FROM THE LABORATORIES OF THE OFFICE OF SOIL FERTILITY, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.]

### THE IDENTITY OF CYANURIC ACID WITH SO-CALLED "TETRACARBONIMID."

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In a recent publication dealing with the isolation of cyanuric acid from soil, the authors<sup>1</sup> pointed out the striking similarity between the properties of cyanuric acid and those of the so-called "tetracarbonimid" obtained by Scholtz<sup>2</sup> as a product of the oxidation of uric acid with alkaline hydrogen

<sup>1</sup> Louis E. Wise and E. H. Walters, "Isolation of Cyanuric Acid from Soil," *J. Agr. Res.*, 10, 85-92 (1917).

<sup>2</sup> M. Scholtz, "Ueber ein neues Oxydationsprodukt der Harnsäure," *Ber.*, 34, 4130 (1901).

peroxide. In an earlier publication from this laboratory "tetracarbonimid" was reported as a soil constituent,<sup>1</sup> the identification of the compounds resting directly on the researches of Scholtz and of Schittenhelm and Wiener,<sup>2</sup> whose work at that time had not been questioned. In the course of some recent investigations however, we definitely identified as cyanuric acid the substance which had been previously isolated from a soil and reported as "tetracarbonimid." This led us to suspect that cyanuric acid and the so-called "tetracarbonimid" might be one and the same substance.

With the purpose of throwing some light on the true nature of "tetracarbonimid," the authors undertook to carefully repeat Scholtz's work, and their experiments were in progress when they learned through the courtesy of Moore and Venable that the peroxide oxidation of uric acid had for some time been the subject of an extended investigation in the laboratories of the Massachusetts Institute of Technology. Since then a preliminary paper by Venable and Moore<sup>3</sup> on the probable identity of cyanuric acid and the so-called "tetracarbonimid" has appeared in this journal, in which these authors have conclusively proved that cyanuric acid is a product of the oxidation of uric acid.

The conclusions reached by Venable and Moore fully agree with the results of our own experiments carried out independently, and furthermore our results appear to us to strengthen the evidence which points to the identity of Scholtz's "tetracarbonimid" and cyanuric acid.

We have carried out the oxidation of uric acid in alkaline solution with hydrogen peroxide, carefully following the directions given by Scholtz. Only one minor change was made in the final separation of the reaction products; the conditions of oxidation and the proportions of the reacting substances used were the same as those described by Scholtz. A product of this oxidation was cyanuric acid in very small quantity contaminated with a large amount of unchanged uric acid.

In the course of another experiment in which Scholtz's method was somewhat modified, we isolated, besides cyanuric acid, a compound whose percentage composition closely approximated that of carbonyldiurea,  $(\text{H}_2\text{NCONH})_2\text{CO}$ , as described by Schmidt,<sup>4</sup> and by a number of other investigators. Its properties were also similar to those given in the literature for carbonyldiurea with one exception. We found that the

<sup>1</sup> E. C. Shorey and E. H. Walters, "A Nitrogenous Soil Constituent: Tetracarbonimid," *J. Agr. Res.*, **6**, 1043-1046 (1914).

<sup>2</sup> A. Schittenhelm and K. Wiener, "Carbonyldiharnstoff als Oxydationsprodukt der Harnsäure," *Z. physiol. Chem.*, **62**, 100-106 (1909).

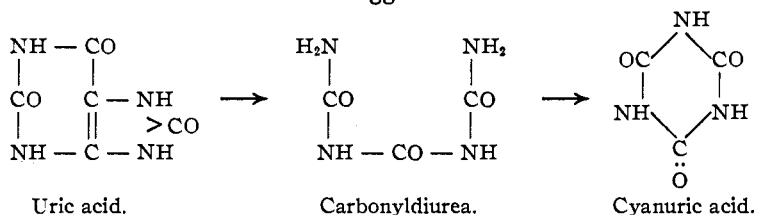
<sup>3</sup> C. S. Venable and F. J. Moore, "Cyanuric Acid as an Oxidation Product of Uric Acid. Its Probable Identity with Tetracarbonimide," *THIS JOURNAL*, **39**, 1750 (1917).

<sup>4</sup> E. Schmidt, "Ueber die Einwirkung von flüssigem Phosgen auf einige Amide," *J. prakt. Chem.*, **5**, 35-66 (1872).

substance decomposed without melting at about  $227^{\circ}$  giving off ammonia, whereas some investigators assign a rather definite melting point to the substance. Schmidt makes no mention of a melting point for carbonyldiurea and states that it decomposes on heating into cyanuric acid and ammonia. Schiff<sup>1</sup> reported that carbonyldiurea melts at  $231-232^{\circ}$  and Schittenhelm and Wiener claimed that it melted at  $233-234^{\circ}$ . We are inclined to believe, however, that a decomposition point may have been mistaken for a melting point.

The compound isolated by us was converted into cyanuric acid and ammonia by warming with alkali, a property also possessed by carbonyldiurea.

Assuming that the compound is carbonyldiurea, the following possible course of the uric acid oxidation is suggested:



The hypothesis of Schittenhelm and Wiener that uric acid is oxidized to "tetracarboximid" which is then converted into carbonyldiurea and finally to urea appears to rest on no reliable experimental data. Our own suggestion is, however, made with the greatest reservation since we have not carried out an extensive study of the mechanism of the uric acid oxidation.

Our results have also an important bearing on the distribution of cyanuric acid or its precursor in soils since it now becomes evident that a variety of soils from which "tetracarboximid" was isolated contain cyanuric acid or some precursor of this compound.

It appears advisable therefore to record briefly the results of our work and also to state that we do not intend to investigate further the peroxide oxidation of uric acid, our chief interest being the accurate identification of substances isolated from soils.

### Experimental.

Scholtz's reaction was carried out as follows: 5 g. of sodium hydroxide (2 mols) and 10 g. of purified uric acid (1 mol) were dissolved in 600 cc. of water and the clear filtered solution heated on a steam bath with 250 cc. of 3% hydrogen peroxide until a test portion of the solution failed to give a precipitate on acidification.<sup>2</sup> The solution was then cooled and

<sup>1</sup> Hugo Schiff, "Benzalbiuret und verwandte Verbindungen," *Ann.*, 291, 367-377 (1896).

<sup>2</sup> This test has not proved to be sensitive enough to show the absence of uric acid.

treated with an excess of an aqueous solution of barium chloride. The barium precipitate was filtered off, washed with cold water, suspended in hot water and treated with a slight excess of sulfuric acid. The barium sulfate thus formed was repeatedly extracted with hot water and the filtered extracts combined and concentrated.

The first crop of crystals which separated consisted of almost pure uric acid, which was identified by the murexide reaction and by a nitrogen determination.

Calc. for  $C_4H_4O_4N_4$ : 33.3. Found (Kjeldahl): 33.8% N.

The filtrate from the uric acid was treated with a slight excess of mercuric sulfate<sup>1</sup> in dilute sulfuric acid. This precipitation was the only point of departure from Scholtz's method. A white flocculent precipitate was obtained which was collected, thoroughly washed with water, suspended in hot water and decomposed with hydrogen sulfide. The filtrate from the mercuric sulfide precipitate on evaporation to a small volume yielded mixed crystals which still responded to the murexide test. After rather tedious fractional crystallization we isolated 30 mg. of a product quite soluble in hot and less soluble in cold water which no longer responded to the murexide reaction, and which had the characteristic appearance of cyanuric acid under the microscope and whose optical properties were identical with those of cyanuric acid synthesized by von Walther's<sup>2</sup> method from urea.

The following optical properties identical for both preparations were determined by W. H. Fry of the Bureau of Soils to whom the authors wish to express their indebtedness:

Small glassy prisms. Optically negative. Biaxial with very small axial angles. Double refraction extremely high  $\alpha < 1.50$ ;  $\gamma > 16.4 < 1.68$ .

In spite of the small amount of material obtained from the uric acid oxidation we were able to carry out two tests which have previously been found very serviceable in the identification of cyanuric acid. Repeated experiments have shown that if a suitable indicator is used (phenolphthalein or preferably thymolphthalein) and the end points carefully controlled, a determination of the "neutralization equivalent" in the case of small amounts of cyanuric acid serves as a rapid method for obtaining the molecular weight of the compound.

The following neutralization equivalents were obtained in the case of the uric acid oxidation product and of the synthetic cyanuric acid (from urea):

<sup>1</sup> According to Schittenhelm and Wiener mercury salts should precipitate "tetracarbonimid" and they are also known to precipitate cyanuric acid.

<sup>2</sup> R. von Walther, "Eine neue Darstellungsweise von Cyanursäure aus Harnstoff," *J. prakt. Chem.*, **79**, 126-128 (1909).

28.75 mg. of the anhydrous uric acid oxidation product required 2.09 cc. of 0.1 *N* NaOH for neutralization (thymolphthalein as indicator); (a) 35.4 mg. of anhydrous synthetic cyanuric acid required 2.71 cc. of 0.1 *N* NaOH for neutralization (phenolphthalein); (b) 30.85 mg. of anhydrous synthetic cyanuric acid required 2.42 cc. of 0.1 *N* NaOH for neutralization (thymolphthalein).

Found: Molecular weight of product from uric acid.....	137
Found: Molecular weight of (a) synthetic cyanuric acid.....	131
Found: Molecular weight of (b) synthetic cyanuric acid.....	127.5
Calc. molecular weight for cyanuric acid.....	129
Calc. molecular weight for "tetracarbonimid".....	172

The product from the uric acid oxidation also yielded the typical compact amethyst-colored copper salt,  $\text{Cu}(\text{C}_3\text{H}_2\text{N}_3\text{O}_3)_2 \cdot 2\text{NH}_3$ , identical in color and microscopic appearance with that obtained from synthetic cyanuric acid.

The results of these experiments have convinced us that the product obtained by Scholtz was in fact cyanuric acid and not tetracarbonimid.

In the following experiment in which the oxidation of uric acid was carried out under slightly modified conditions, cyanuric acid was again definitely identified as one of the products of the reaction: 6.3 g. of uric acid (1 mol) and 9 g. of sodium hydroxide (6 mols) were dissolved in 1000 cc. of water. The clear filtered solution was treated with 250 cc. of 3% hydrogen peroxide and the mixture allowed to stand at room temperature for 72 hours. Under these conditions the uric acid was completely oxidized as shown by a negative murexide test. The solution was filtered to remove a slight turbidity and the clear filtrate made strongly acid with hydrochloric acid and allowed to stand at room temperature for 24 hours longer. The crystalline material which separated during this period was filtered off, washed with cold water and finally purified by repeated crystallization from hot water. 0.1421 g. of the material was obtained which appeared to be identical with carbonyldiurea.

The following analytical data were obtained:

0.0329 g. of material yielded 0.01257 g. of nitrogen.

Calc. for carbonyldiurea,  $\text{C}_3\text{H}_6\text{N}_4\text{O}_3$ : 38.35. Found: 38.21% N.

(a) 0.01662 g. of substance gave 0.01565 g. of  $\text{CO}_2$  and 0.0066 g. of  $\text{H}_2\text{O}$  (microcombustion); (b) 0.01743 g. of substance gave 0.01588 g. of  $\text{CO}_2$  and 0.00668 g. of  $\text{H}_2\text{O}$  (microcombustion, see THIS JOURNAL, 39, 2055 (1917)).

Sample a: 25.7% C; 4.4% H; Sample b: 24.8, 4.3. Calc. for carbonyldiurea,  $\text{C}_3\text{H}_6\text{N}_4\text{O}_3$ : 24.7, 4.1.

On heating with sodium hydroxide solution the substance was converted into cyanuric acid and ammonia. The cyanuric acid which crystallized out after rendering the solution acid with hydrochloric acid and concentrating was identified by converting it into the typical amethyst-colored copper ammonium salt. The substance which is believed to be carbonyldiurea has no melting point but begins to decompose at about 227° into cyanuric acid and ammonia. The substance was apparently not affected by heating with strong hydrochloric acid.

On concentrating the filtrate from this compound on a steam bath, a second crop of crystals was obtained, which was purified by repeated crystallization from hot water and finally identified as cyanuric acid. Yield, 0.7563 g. The following analytical data were obtained:

0.1035 g. of anhydrous substance required 0.0326 g. of sodium hydroxide for neutralization (thymolphthalein), and yielded 0.03375 g. of nitrogen. The molecular weight was found to be 127. The molecular weight calculated for cyanuric acid is 129.

Calc. for  $C_3H_3N_3O_3$ : 32.60. Found: 32.63% N.

0.03318 g. of anhydrous substance yielded 0.03405 g. of  $CO_2$  and 0.00736 g. of  $H_2O$  (micro-combustion).

Calc. for  $C_3H_3N_3O_3$ : 27.90, 2.34. Found: 27.98% C; 2.48% H.

0.1020 g. of the copper ammonium salt gave 0.02255 g. of  $CuO$ .

Calc. for  $Cu(C_3H_2N_3O_3)_2 \cdot 2NH_3$ : 17.96. Found: 17.66% Cu.

These data establish the identity of this compound as cyanuric acid.

### Summary.

1. The so-called "tetracarbonimid" prepared by Scholtz by oxidizing uric acid with hydrogen peroxide in alkaline solution is in fact cyanuric acid.

2. The nitrogenous compound isolated from a number of soils and believed at first to be "tetracarbonimid" has been shown to be cyanuric acid.

3. Cyanuric acid has been isolated from the following soils: (1) 12 samples of sandy soils taken from different locations in Florida, (2) Norfolk sandy loam from Virginia, (3) lawn soil from the grounds of the Department of Agriculture, (4) Elkton silt loam from Maryland, (5) Scottsburg silt loam from Indiana, (6) Caribou loam from Maine, and (7) a Susquehanna fine sandy loam from Texas. It is apparent that cyanuric acid or its precursor is widely distributed in soils.

4. The results of Venable and Moore and our own agree in showing that the oxidation of uric acid in alkaline solution with hydrogen peroxide yields cyanuric acid as a product of this reaction.

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## ON THE ORIGIN OF THE HUMIN FORMED BY THE ACID HYDROLYSIS OF PROTEINS. III. HYDROLYSIS IN THE PRESENCE OF ALDEHYDES. II. HYDROLYSIS IN THE PRESENCE OF FORMALDEHYDE.<sup>1</sup>

BY ROSS AIKEN GORTNER AND GEORGE E. HOLM.

Received August 19, 1917.

In the preceding papers of this series,<sup>4,5\*</sup> the various hypotheses regarding the origin of the humin of protein hydrolysis have been discussed

<sup>1</sup> Published with the approval of the Director as Paper No. 84, Journal Series of the Minnesota Agricultural Experiment Station.

\* References are to a bibliography at the end of this article.