5-Aminosalicylate, $NH_2C_6H_3(OH)CO_2.R$, m. 200.5°.
Acetylsalicylate, $CH_3COO.C_6H_4CO_2.R$, m. 90.5°.
Anisate, p-$CH_3.O.C_6H_4CO_2.R$, m. 132°.
Vanillate, $CH_3.O.C_6H_3(OH).CO_2.R$, m. 140–1°.
Piperate, $C_{12}H_9O_4.R$, m. 145°.
o-Nitrocinnamate, $NO_2.C_6H_4C_2H_2CO_2.R$, m. 132°.
m-Nitrocinnamate, $NO_2.C_6H_4C_2H_2CO_2.R$, m. 174°.
p-Nitrocinnamate, $NO_2.C_6H_4C_2H_2CO_2.R$, m. 186.5°.
Coumarate, $C_6H_4(OH).C_2H_2CO_2.R$, m. 152.5°.
β-Hydroxynaphthoate, $C_{10}H_6(OH)CO_2.R$, m. 164°.
Benzoic sulfinid ester, $C_6H_4COSO_2N.R$, m. 174.5°.
Isophthalate, $C_6H_4(CO_2.R)_2$, m. 202.5°.
Terephthalate, $C_6H_4(CO_2.R)_2$, m. 263.5°.
4,5-Dichlorophthalate, $Cl_2C_6H_2(CO_2.R)_2$, m. 164.5°.
Tetrachlorophthalate, $Cl_4C_6(CO_2.R)_2$, m. 180°.
3-Nitrophthalate, $NO_2.C_6H_3(CO_2.R)_2$, m. 189.5°.
Mellitate, $C_6(CO_2.R)_6$ (?), m. >300°.

BALTIMORE, MD.

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

CYANURIC ACID AS AN OXIDATION PRODUCT OF URIC ACID. ITS PROBABLE IDENTITY WITH TETRACARBONIMIDE.

BY C. S. VENABLE AND F. J. MOORE.

Received July 5, 1917.

Having learned through kind personal communications from other investigators that they, like ourselves, have been struck by the strong resemblance between cyanuric acid and the substance known in the literature as tetracarbonimide, it seems appropriate to publish at this time some observations of our own which speak for the identity of the two substances.

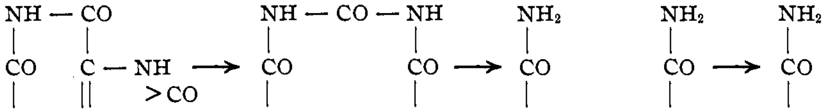
These observations form a part of an extensive study of the action of hydrogen peroxide upon uric acid and its derivatives which has been going on for some time in this laboratory. This work is now well advanced, but only such parts of its results as bear upon the identity of cyanuric acid and tetracarbonimide will be included in the present paper.

The word tetracarbonimide first appeared in the literature in 1901 when Scholtz[1] applied the name to a product which he obtained by the action of hydrogen peroxide upon an alkaline solution of uric acid. He justified his formula by an ultimate analysis, and by determinations of barium and sodium in the salts purporting to have the formulas $C_4O_4N_4Ba_2$ and $C_4H_3O_4N_4Na$.

---

[1] *Ber.*, 34, 4130 (1901).

In 1909, Schittenhelm and Wiener[1] confirmed the statement of Scholtz and further pointed out that carbonyl-di-urea is another product of the reaction. In order to isolate the latter they modified somewhat the procedure of Scholtz, purifying their product by means of the silver, rather than the barium, salt. Their experiments seemed to indicate that the yield of carbonyl-di-urea was increased by prolonging the time of oxidation, and they suggested in consequence that the oxidation of uric acid by hydrogen peroxide might take place in the following steps:



Uric acid. Tetracarbonimide. Carbonyl-di-urea. Urea.

Scholtz prepared his tetracarbonimide as follows: "Twenty grams uric acid (1 mol) and 10 g. sodium hydroxide (2 mols) are dissolved in 1200 cc. water and to the filtered solution are added 500 g. of a 3% solution of hydrogen peroxide. From time to time this solution is tested for unoxidized uric acid by acidifying small samples. If the mixture is allowed to stand at room temperature the action requires twenty-four hours. By heating on a water bath the action is hastened and should be complete in one-half hour. By saturating the resulting solution with barium chloride a dense, white precipitate is obtained which is a mixture of barium carbonate and $C_4O_4N_4Ba_2$. The well-washed precipitate is suspended in a liter of water, decomposed with sulfuric acid, warmed, filtered, and the filtrate evaporated. The tetracarbonimide remains behind as a crystalline powder that may be recrystallized from water in the form of colorless shining prismatic crystals. Yield, 2 g."

It has already been pointed out that Schittenhelm and Wiener modified this process by purification through the silver salt, which enabled them to isolate carbonyl-di-urea but did not increase the yield of tetracarbonimide.

In our hands neither the procedure of Scholtz nor that of Schittenhelm and Wiener, though faithfully repeated, yielded a product of sufficient purity to permit conclusions as to identity in a case of close resemblance, so a systematic study of the reaction was undertaken in which the temperature, time of reaction, and proportion of reagents were varied within wide limits. In this way it was found possible to control results in a very interesting way, but the details must be reserved for a later paper. We found in general that in order to prepare the product most closely resembling tetracarbonimide elevation of temperature and isolation through salts were superfluous and detrimental. The most advantageous details were the following:

---

[1] *Z. physiol. Chem.*, **62**, 100 (1909).

Five grams uric acid were dissolved in 200 cc. water containing 9 g. of sodium hydroxide. This solution was cooled to 20°, 130 cc. of a 3% solution of hydrogen peroxide were added, and the reaction allowed to proceed at room temperature for twenty-four hours. The filtered solution was then neutralized with a slight excess of hydrochloric acid and on cooling with ice water a heavy crystalline precipitate was deposited. Upon filtering and concentrating the filtrate on the water bath to a volume of 100 cc. an additional quantity may be obtained. It is easily purified by crystallization from hot water. Yield, 2 g.

This product, we are constrained to believe, is not tetracarbonimide but cyanuric acid. It crystallizes in transparent monoclinic prisms, that show a strong tendency toward twinning. These rapidly lose water on exposure to the air and become opaque. They are sparingly soluble in cold water, but readily in hot. The aqueous solution reacts neutral to litmus. The compound is not soluble in hot or cold methyl or ethyl alcohol, ether, acetone, benzene, chloroform, or other organic solvents. It dissolves without decomposition in concentrated sulfuric acid. It is easily soluble in caustic soda and can readily be precipitated from such a solution by carbon dioxide. On heating in a closed tube it does not melt but blackens at a high temperature, giving a white sublimate reacting acid to litmus. The aqueous solution yields precipitates with the soluble salts of barium, mercury, lead and silver.

Analysis.

The crystals were allowed to stand in a vacuum desiccator over sulfuric acid till weight was constant. Carbon and hydrogen were determined by combustion and nitrogen by Kjeldahl.

0.1528 g. gave 0.1554 g. CO_2 and 0.0344 g. H_2O.
0.2309 g. gave 0.2354 g. CO_2 and 0.0499 g. H_2O.
The ammonia from 0.1152 g. neutralized 23.36 cc. H_2SO_4 (0.1145 *N*).
The ammonia from 0.1420 g. neutralized 28.72 cc. H_2SO_4 (0.1145 *N*).
Calculated for $(CHON)_n$: C, 27.90; H. 2.32; N, 32.56.
Found: C, 27.74, 27.81; H, 2.56, 2.46; N, 32.50, 32.42.

Molecular Weight.

The substance is soluble with difficulty in cold water and in organic solvents. A solvent suitable for the determination of molecular weight was, however, found in sulfuric acid monohydrate, $H_2SO_4.H_2O$. This solvent has already been employed by Lespieau[1] for determining the molecular weight of uric acid.

The molecular lowering for the solvent used was first determined using a sample of Kahlbaum's cyanuric acid. The value obtained was 4.77° per 1000 g. $H_2SO_4.H_2O$, as against 4.8° as given in Landolt-Börnstein's tables.

0.1937 g. in 28.4 g. $H_2SO_3.H_2O$ gave lowering 0.244°.
0.2987 g. in 33.2 g. $H_2SO_4.H_2O$ gave lowering 0.325°.
0.2128 g. in 32.0 g. $H_2SO_4.H_2O$ gave lowering 0.229°.
Molecular weight calculated for $(CHON)_3$ = 129; $(CHON)_4$ = 172.
Found: 133, 132, 137.

The acid sodium salt described by Lemoult[2] was also prepared and analyzed for sodium. Two grams of recrystallized substance were dissolved in 65 cc. water containing 0.6 g. sodium hydroxide. The hot solution was filtered and cooled, and a crystalline product separated out. An examination under the microscope showed that the product consisted of crystalline needles with no trace of the original substance. These needles were recrystallized from 30 cc. hot water and were then dried to constant weight at 100°.

Analysis: 0.2027 g. gave 0.0837 g. Na_2SO_4. 0.2605 g. gave 0.1086 g. Na_2SO_4.
Calculated for $C_3H_2O_3N_3Na.H_2O$: Na = 13.61.
Found: 13.42, 13.52.

It is obvious that the composition of this salt is an indirect check upon the molecular weight.

A commonly accepted test for cyanuric acid is that suggested by Wöhler of treatment with ammoniacal copper sulfate and is carried out as follows: One gram of substance is dissolved in 5 cc. hot water containing 6 drops of concentrated ammonia. To the hot solution a solution of copper sulfate is added drop by drop, boiling a few seconds after each addition, until a distinct and permanent blue color makes its appearance. On cooling a compact, glistening precipitate said to have the composition $C_3O_3N_3Cu.2NH_3$, of a peculiar amethyst color, is deposited. When treated in this way our oxidation product and a sample of known cyanuric acid gave crystals of the same characteristic form under the microscope.

Both substances were also used for the preparation of a chloro-imino derivative, by dissolving them in alkali and passing in chlorine gas. Both behaved alike under these conditions, but neither gave a product whose properties quite tallied with the description of Chattaway and Wardmore.[3] As a matter of fact, this derivative decomposes rapidly in any case, and is therefore not particularly suited for purposes of identification.

---

[1] *Bull. soc. chim.*, [3] **11**, 74 (1894).

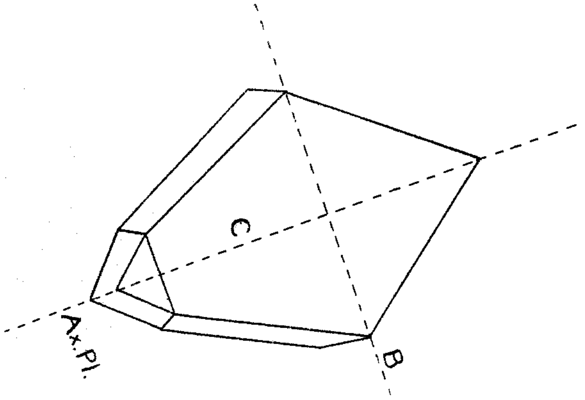
[2] *Ann. chim. phys.*, [7] **16**, 382 (1899).

[3] *J. Chem. Soc.*, **81**, 200 (1902).

Optical Properties.

The crystalline hydrates were next subjected to a parallel test of their optical properties with the following results:

Habit: Monoclinic. In general, strongly tabular parallel to *c* (001) showing a development of the prism 110 and small *a* (100) face. The crystals appear with a rhombic outline, one acute end being truncated by the *a* (100) face. Contact twinning common parallel to 101. From some preparations, crystals in a prismatic elongation are obtained.



Optical Orientation: Axial plane parallel to 010. B_x acute emerges from the tabular crystals in the acute <B, the center of the figure lying just outside the field. By tilting the crystals (see sketch) show with a small optic angle, 2F = 25–30°. Optically negative.

Birefringence: Very strong.

Indices: β-ray, 1.66–1.67.

These observations were exactly duplicated in the case of a sample of Kahlbaum's cyanuric acid. The habit and optical orientation as described tally with the description given in Groth[1] for the di-hydrate of cyanuric acid.

The foregoing seems to furnish conclusive evidence that when uric acid is oxidized by hydrogen peroxide in alkaline solution under the conditions described, cyanuric acid is produced with a yield amounting to about 50% of the theory. It only remains to examine the evidence for the formation of tetracarbonimide by the same reaction.

In the first place, the properties described by Scholtz are not distinguishable from those of cyanuric acid. His analysis of tetracarbonimide would, of course, agree equally well with that of cyanuric acid, since both are polymers of cyanic acid. The same would hold true of the neutral barium salt, which he analyzed and gave the formula $C_4O_4N_4Ba_2$, although here it must be conceded that we have found no description of any neutral barium salt of cyanuric acid in the literature. Still, Scholtz worked with an excess of barium hydroxide and may have obtained one.

There remains only the sodium salt, to which Scholtz assigns the formula $C_4H_3O_4Na$, but he himself concedes that he found great difficulty

---

[1] "Chemische Krystallographie," III, 562.

in securing a salt of constant composition, and, as we know from the work of Lemoult and others, conditions must be very rigorously maintained in order to secure any given salt of cyanuric acid in a state of purity.

For these reasons we believe that the tetracarbonimide of Scholtz is only cyanuric acid.

The authors take pleasure in acknowledging their indebtedness to Professor C. H. Warren, of this Institute, for invaluable assistance in that portion of this investigation dealing with the optical characteristics of crystals.

Summary.

1. It has been shown that uric acid can be oxidized by hydrogen peroxide in such a way as to yield cyanuric acid to the extent of about 50% of the theory.

2. The cyanuric acid thus obtained has been subjected to an exceptionally thorough identification.

3. The accounts of previous investigators who have described tetracarbonimide as a product of the reaction show no conclusive evidence that they were dealing with any other compound than cyanuric acid. It is therefore concluded that the compounds are identical.

4. A systematic study of the reaction of hydrogen peroxide upon uric acid and its derivatives is already far advanced in this laboratory and we desire to reserve this field.

CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE LABORATORY OF SANITARY CHEMISTRY, CORNELL UNIVERSITY.]

STUDIES ON THE CULTURE MEDIA EMPLOYED IN THE BACTERIOLOGICAL EXAMINATION OF WATER.

IV. NEUTRAL RED LACTOSE PEPTONE MEDIA.

BY E. M. CHAMOT AND C. M. SHERWOOD.

Received June 22, 1917.

Neutral red was discovered by Witt[1] in 1879 and further investigated by Bernthsen and Sweitzer[2] in 1886, but not until 1898 was it applied to bacteriological diagnosis. In that year Rothberger[3] described the effects of various organisms on thirty-five dyes and showed that while some organisms were able to cause a change in color when inoculated into the media containing certain of these substances, others produced no change. As a result of this investigation he recommended bouillon agar containing neutral red as a medium for distinguishing between *B. coli* and *B. typhosus*. At 37° *B. coli* causes a ruby-red colored medium to change to a canary-

---

[1] *Ber.*, **12**, 931 (1879).

[2] *Ibid.*, **19**, 2604 (1886).

[3] *Centr. Bakt. Parasitenk, Abt. I*, **24**, 513 (1898); **25**, 15, 69 (1899).