[Contribution from the Chemistry Department, Fordham University]

Studies on the Oxidation of Uracil in Vitro

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Observations on the metabolism of uracil and uracil derivatives in the dog have led Cerecedo[1] to assume that the biological breakdown of this pyrimidine takes place according to the scheme: uracil → isobarbituric acid → isodialuric acid → oxaluric acid → urea and an unknown carbon compound, presumably oxalic acid.

Previous studies on the oxidation of uracil suggested that such a scheme should be duplicable *in vitro*. Wheeler and Johnson[2] found that isodialuric acid is obtained from uracil on treatment with bromine water. Johnson and Flint[3] succeeded in identifying oxaluric acid, oxalic acid and urea among the products of oxidation by means of ozone. Urea has also been obtained by splitting uracil with air, oxygen or hydrogen peroxide in the presence of ferrous compounds.[4]

The present investigation was undertaken with a view to correlating the above results, and, if possible, to duplicate *in vitro* the oxidative breakdown of uracil *in vivo*.

Unsuccessful attempts with oxygen alone, oxygen in the presence of charcoal and hydrogen peroxide, led us to investigate the action of hydrogen peroxide in the presence of charcoal. Charcoal was found to control and regulate the oxidation of uracil by means of hydrogen peroxide at 95–98° so that several intermediate products could be identified. Isobarbituric acid, isodialuric acid (isolated as potassium dialurate), urea, oxalic acid and ammonia were obtained in small yields.

In order to determine whether the formation of isobarbituric acid was the result of a secondary reaction, or whether it was truly an intermediate in the formation of the other products, we also investigated its oxidation. This pyrimidine is more easily oxidized than uracil, as already noted by Davidson and Baudisch,[5] who obtained di-isobarbituric acid as the result of the oxidation with potassium ferricyanide. We found that the quantity of peroxide had to be reduced to two moles. A yield of potassium dialurate nearly double that obtained with the equivalent amount of uracil resulted. The same color reactions as with uracil were obtained on making alkaline with potassium hydroxide, ammonia also being evolved. The yield of urea was about the same, whereas oxalic acid was formed in larger amounts.

Experimental

Uracil was prepared according to the directions of Davidson and Baudisch.[6] It was recrystallized four times from distilled water. Isobarbituric acid was synthesized according to the method of Davidson and Baudisch.[5] Dialuric acid was made according to Hill;[7] isodialuric acid according to Behrend and Roosen;[8] oxaluric acid according to Biltz and Topp.[9]

The charcoal used in this research was a commercial preparation, Nuchar.[10] A 30% solution of hydrogen peroxide was used in all experiments.

Oxidation of Uracil.—Five grams of uracil, 2.50 g. of Nuchar W and 450 cc. of water were brought to boiling in an Erlenmeyer flask, which was then transferred to a steam-bath; 10 cc. of 30% hydrogen peroxide was added. The mixture was shaken by hand every five minutes. After about twenty-five minutes a drop of the suspension failed to give a blue color with starch-iodide paper. Five cc. more of peroxide was added, and when that was used up, 5 cc. more. As soon as the last portion of the oxidant had disappeared, the mixture was filtered through a Nuchar mat. The filtrate was evaporated *in vacuo* to a volume of 30 cc. at a temperature below 40°. On cooling, the concentrate gave a trace of isobarbituric acid, and, if less than 20 cc. of peroxide were used, some unchanged uracil. The solution was filtered, and the filtrate cooled on ice. Cold 30% potassium hydroxide was added until a deep purple color was evident. Three cc. more of potassium hydroxide was then added, and the solution was placed on ice for three or four hours. A strong odor of ammonia was noticed every time the oxidized uracil solution was made alkaline. The white precipitate (A), which had formed, was filtered off, and the filtrate (B) was concentrated to one-half its volume on the water-bath. Precipitate (A) weighed 0.8 g. The substance was dissolved in 1:1 hydrochloric acid at 80–90°. The solution was filtered and cooled. A crystalline precipitate formed, which was recrystallized twice from water acidified with hydrochloric acid. The purified substance reddened at 190° and melted at 216°. When mixed with pure synthetic dialuric acid no change in melting point was observed. A solution of the substance gave a deep purple precipitate with barium hydroxide. Filtrate (B) yielded, on cooling, a precipitate (C). This was filtered off. On adding xanthydrol reagent to the filtrate, a precipitate

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(1) L. R. Cerecedo, *J. Biol. Chem.*, **88**, 695 (1930); **93**, 269 (1931).

(2) H. L. Wheeler and T. B. Johnson, *ibid.*, **3**, 183 (1907).

(3) T. B. Johnson and R. B. Flint, This Journal, **53**, 1077 (1931).

(4) M. H. Pfaltz and O. Baudisch, *ibid.*, **45**, 2972 (1923).

(5) D. Davidson and O. Baudisch, *J. Biol. Chem.*, **64**, 619 (1925).

(6) D. Davidson and O. Baudisch, This Journal, **48**, 2379 (1926).

(7) E. S. Hill, *J. Biol. Chem.*, **85**, 713 (1929–1930).

(8) R. Behrend and O. Roosen, *Ann.*, **251**, 235 (1889).

(9) H. Biltz and E. Topp, *Ber.*, **46**, 1404 (1913).

(10) Supplied by the Industrial Chemical Sales Co., New York.

of dixanthylurea formed. This was identified by mixed melting point. The yield of urea was 0.3 g. Substance (C) was dissolved in 1:1 hydrochloric acid. The resulting shiny white crystals melted around 160°. A saturated solution of the crystals was treated with nitron reagent. A voluminous precipitate was obtained which melted at 230–233°. A sample of the nitron derivative of commercial oxalic acid melted at 228–230°. The mixed melting point was 232°. The original crystals therefore were potassium oxalate. The yield of oxalic acid was 0.46 g.

When the amount of Nuchar was reduced to 2.0 g., maintaining otherwise the conditions as described above, a sufficient amount of isobarbituric acid was formed to permit isolation. The filtrate of the oxidized mixture was concentrated on the steam-bath to a small volume (25 cc.). On cooling, a reddish precipitate fell out. This was recrystallized from a small amount of water, then fractionated by means of 95% alcohol. The first crystals, formed almost immediately after filtering the solution saturated at the boiling point, were discarded. On standing in the ice-box, a typical scale of isobarbituric acid formed. It was filtered, washed with 5 cc. of water and dried *in vacuo*. The substance weighed 0.4 g.

Anal. Calcd. for $C_4H_4O_3N_2$: N, 21.87. Found: N, 21.93.

The oxidation of isobarbituric acid was carried out as in the case of uracil, 5.7 g. of the substance being taken, 2.5 g. of Nuchar W and 500 cc. of water. Only 10 cc. of peroxide was used, all added at once. The filtrate of the mixture gave no test for isobarbituric acid. We also failed to detect di-isobarbituric acid. After concentration *in vacuo*, the resulting solution was treated as described above: yields, potassium dialurate 1.40 g., oxalic acid 0.81 g. urea 0.20 g.

Discussion

Our statement that the isolation of dialuric acid from the reaction mixture may be interpreted as showing the presence of isodialuric acid may seem unsupported by facts. It must be remembered, however, that dialuric acid is very easily oxidized, being, in fact, autoxidizable,[7] and is, as we have found, very readily attacked by peroxide and charcoal. Moreover, a saturated solution of dialuric acid does not give a precipitate of potassium dialurate with strong potassium hydroxide solution, whereas a moderately strong solution of isodialuric acid, which is much more soluble, does give this salt.[8] Alloxantin, the presence of which might seem to be indicated by the formation of the deep bluish-purple color when the reaction mixture is treated with potassium hydroxide, does not give a precipitate of potassium dialurate even in saturated solution. Furthermore, we have found that whenever a solution of isodialuric acid is made alkaline in the presence of ammonium salts, a purple color is formed. It seems, therefore, that the substance giving rise to the dialuric acid consisted mainly, if not entirely, of isodialuric acid, which, of course, on treatment with potassium hydroxide was immediately isomerized to dialuric acid, and precipitated as the potassium salt.

One substance which we were unable to isolate or detect at any time is oxaluric acid. It is probable that oxaluric acid is an intermediate in this reaction but, as soon as formed, is hydrolyzed to urea and oxalic acid since solutions of this compound are known to be very sensitive to heat.[9]

Summary

1. Hydrogen peroxide attacks uracil to a slight extent in the cold and to a greater extent at boiling temperature, but no oxidation products (except traces of urea) could be isolated.

2. Hydrogen peroxide in the presence of charcoal oxidizes uracil to isobarbituric acid, isodialuric acid, oxalic acid and urea.

3. Isobarbituric acid, when subjected to the same oxidizing medium, gives rise to isodialuric acid, oxalic acid and urea.

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