

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

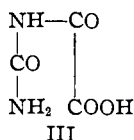
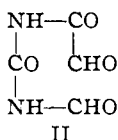
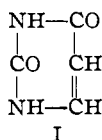
RESEARCHES ON PYRIMIDINES. CXXI. THE ACTION OF OZONE ON SOME DERIVATIVES OF URACIL

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In the previous paper from this Laboratory dealing with pyrimidine chemistry,² the authors described the behavior of uracil I toward ozone. It was shown that this pyrimidine cycle can be broken down by the action of ozone without destruction of the ureide structure, and with formation of definite oxidation products characteristic of the constitution of uracil. The characteristic ureides produced by ozonization were identified as oxaluric acid III and formyl-glyoxyl-urea II. Besides these two ureides



practically the only other products of reaction were urea, formic and oxalic acids. The definite course of the reaction, its ease of application, and the character of the products formed by decomposition of the pyrimidine were so promising that it seemed desirable to study the action of ozone on other derivatives of uracil. Any transformations of this character, which will serve for determination of the constitution of uracil derivatives, should prove of immediate practical value in many of our problems dealing with cell chemistry, and now calling for solution. In this paper we describe new applications of this reaction, and report on the action of ozone on 4-methyluracil, 4-phenyluracil, thymine, 5-bromouracil, 5-nitrouracil and 1,3-dimethyl-5-bromouracil.

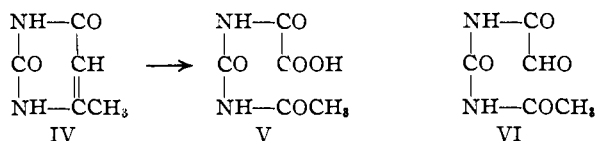
As in the case of uracil, all these pyrimidines interact with ozone in glacial acetic acid at ordinary temperature with destruction of the pyrimidine ring, but without complete degradation of the molecule. The ureide configuration of the original pyrimidine is preserved in some form in every case. As already known, 4-methyluracil is attacked by potassium permanganate in acid solution with formation of trihydroxydihydro-methyluracil which Behrend and Osten³ showed is transformed by more intensive oxidation into acetyloxaluric acid V and finally acetylurea and oxalic acid. With ozone 4-methyluracil reacts also with ring cleavage giving a 67% yield of acetyl-glyoxyl-urea represented by formula VI.

¹ Constructed from a dissertation presented by Robert Barnett Flint to the Graduate School of Yale University in June, 1930, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² Johnson and Flint, *THIS JOURNAL*, **53**, 1077 (1931).

³ Behrend and Osten, *Ann.*, **343**, 133 (1905).

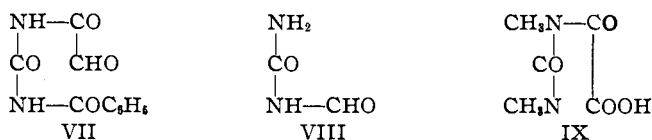
No evidence was obtained of the formation of oxaluric acid, as was observed in the case of uracil, or any acetyl-oxaluric acid V.



4-Phenyluracil reacts with ozone to give the corresponding benzoyl-glyoxylurea VII, while thymine underwent a more intensive degradation under similar experimental conditions, giving an excellent yield of formyl-urea VIII. In this respect the thymine degradation corresponds to that of 4-methyluracil, which has been shown to break down by intensive oxidation with potassium permanganate, giving acetyl-urea. Pyruvic acid, $\text{CH}_3\text{COCO}_2\text{H}$, if formed in the ozonization of thymine, was apparently destroyed by oxidation, as delicate tests for this ketone acid did not reveal its presence in the reaction mixture. Urea was formed to some extent in all cases examined and was easily detected by means of Fosse's reagent, "xanthidrol."⁴

Especially interesting was the behavior of 5-bromouracil, 5-nitrouracil, and 1,3-dimethyl-5-bromouracil toward ozone. The only one of these three derivatives which favored the formation of an acyclic ureide was 1,3-dimethyl-5-bromouracil. This gave an oxidation product having the composition and properties of dimethyloxaluric acid, IX. We did not succeed in detecting in the reaction mixture any cholestrophan or dimethyl-parabanic acid. The corresponding monomethyl-oxaluric acid has been shown to be formed by oxidation of α - and β -dimethyluracil with potassium permanganate.⁵ 5-Bromouracil, on the other hand, reacted with ozone under similar conditions giving an excellent yield of parabanic acid, while 5-nitrouracil was decomposed to form urea, oxalic acid and the two ureides, parabanic acid and oxaluric acid.

Ring closure to form parabanic acid in the ozonization of 5-bromouracil and 5-nitrouracil represents only the end of a series of reactions. After removal of this cycle from the reaction products, both residues responded to qualitative tests indicating the presence of formyl-oxaluric acid. Formic acid, urea and oxalic acid were identified after hydrolysis, but we were unable to separate the formyl-oxaluric acid in either case.



⁴ Fosse, *Ann. chim.*, [9] 6, 13 (1916).

⁵ Behrend and Dietrich, *Ann.*, 309, 271 (1899).

Experimental Part

In studying the action of ozone on the pyrimidines incorporated in this research we have operated according to a general procedure, as follows. A definite quantity of the respective pyrimidine is suspended in a known volume of glacial acetic acid and dry ozonized oxygen is then bubbled through the suspension at ordinary temperature until the transformation of the respective pyrimidine is complete. In the majority of cases solution of the pyrimidine in the acetic acid takes place very slowly, and it is necessary to pass ozone through the acid solution for several hours before the reaction is brought to completion. Acetic acid has proved to be the best solvent for our work; no products of reaction are formed which discolor the acid, and also no resinous substances are produced which interfere with the identification of the characteristic products of reaction.

The Action of Ozone on 4-Methyluracil, IV.—The methyluracil which was used in this research was made according to the directions of Biltz and Heyn⁶ by condensing ethyl acetoacetate with urea in the presence of hydrochloric acid. The proportions used during ozonization were 5 g. of the pyrimidine and 100 cc. of glacial acetic acid. The pyrimidine suspended in the acetic acid slowly dissolves during the ozone treatment, and at the end of fourteen hours the reaction is complete and a clear colorless solution is obtained. On allowing this solution to evaporate in a current of air at 50° until the acetic acid was removed, we obtained a crystalline residue which weighed 6.0 g. Purification was effected by crystallization from absolute alcohol, when we succeeded in separating 4.2 g. of a crystalline substance which melted at 150°. Repeated crystallizations failed to raise this melting point. This was identified as *acetyl-glyoxyl-urea* and the yield obtained corresponded to 67% of the theoretical.

Anal. Calcd. for $C_6H_6O_4N_2$: N, 17.72. Found: N, 17.40, 17.52, 17.44.

Acetyl-glyoxyl-urea is soluble in water and moderately soluble in 95% alcohol and glacial acetic acid. It is insoluble in ether, benzene, chloroform and ethyl acetate. An aqueous solution of the ureide gives no precipitate of calcium oxalate when warmed in ammoniacal solution with calcium chloride. The ureide reduces Fehling's solution immediately on warming and reacts with Tollens' reagent to form a silver mirror and gives no blue color with ferric chloride. Five-tenths of a gram of the ureide was warmed in dilute hydrochloric acid solution, when it was completely decomposed with formation of urea. The latter was identified by precipitation with xanthidrol according to Fosse's⁴ method. The same experiment was repeated, but with the addition of a small amount of nitric acid. Under these conditions complete hydrolysis took place with formation of urea and also oxalic acid, which was identified by precipitation as calcium oxalate.

The alcohol filtrate from which acetyl-glyoxyl-urea was separated by crystallization was found to contain free urea and oxalic acid. We obtained no evidence in this case of the formation of oxaluric acid, as was observed when uracil was treated with ozone.²

The Action of Ozone on 4-Phenyluracil.—4-Phenyluracil was synthesized according to the directions of Johnson and Hemingway⁷ by condensation of thiourea with ethyl benzoylacetate, when 2-thio-4-phenyl-6-oxypyrimidine is formed. Desulfurization of the latter pyrimidine by the action of chloroacetic acid yields quantitatively the 4-phenyluracil melting at 270°. The proportions used for ozonization were 5 g. of the

⁶ Biltz and Heyn, *Ann.*, **413**, 109 (1917).

⁷ Johnson and Hemingway, *This Journal*, **37**, 378 (1915).

pyrimidine and 100 cc. of glacial acetic acid. The pyrimidine suspended in the acetic acid interacts readily with ozone and at the end of seven hours a colorless solution was obtained and the pyrimidine ring was completely destroyed. The acetic acid was then removed by evaporation in a current of air at 50°, when we obtained a sirupy residue which we again dissolved in 10 cc. of glacial acetic acid, and allowed to stand in an ice chest for about twelve hours. Two grams of a crystalline compound separated in the form of prisms which melted at 168°. This was identified as benzoyl-glyoxyl-urea. This ureide is insoluble in cold water, chloroform, benzene and ether, and soluble in hot water, alcohol and ethyl acetate.

Anal. Calcd. for $C_{10}H_8O_4N_2$: N, 12.73. Found: N, 12.80, 12.61.

This ureide reacted in a normal manner with Fehling's and Tollens' solutions, showing the presence of an aldehyde group in the compound. No oxalic acid was produced by hydrolysis with hydrochloric acid, but benzoic acid was easily identified, and separated on cooling in colorless crystals melting at 122°. Urea was separated and identified by means of Fosse's reagent (xanthidrol). When nitric acid was added to the hydrochloric acid and the ureide then subjected to hydrolysis, oxalic acid was formed as a product of reaction. This was identified by precipitation as calcium oxalate.

Action of Phenylhydrazine on Benzoyl-glyoxyl-urea.—One-half gram of the ureide was dissolved in a mixture of 5 cc. of phenylhydrazine, 5 cc. of 50% acetic acid and 15 cc. of water. There was a reaction at ordinary temperature and on standing a crystalline substance separated. After purification by crystallization from alcohol, this melted at 168° and was identified as benzoylphenylhydrazine.⁸ A mixture of this substance and benzoylphenylhydrazine prepared according to Fischer's directions melted at the same temperature.

Anal. Calcd. for $C_{13}H_{12}ON_2$: N, 13.21. Found: N, 13.22.

After separation of the benzoylphenylhydrazine, the reaction mixture was allowed to stand at room temperature for several days, when a yellow substance separated which was purified by crystallization from boiling absolute alcohol. It separated, on cooling, in the form of yellow needles which melted at 221°. This compound was found to be identical with the substance obtained by interaction of phenylhydrazine with formyl-glyoxyl-urea,² and also with acetyl-glyoxyl-urea described above. No change in the melting point (221°) was produced when mixtures of the three different preparations were heated in capillary tubes.

The Action of Ozone on Thymine.—The proportions used for the ozone reaction were 3.5 g. of the pyrimidine suspended in 50 cc. of glacial acetic acid. The thymine was attacked immediately by the ozone and after bubbling ozonized oxygen through the suspension for five hours, the thymine had dissolved and was completely decomposed. The acetic acid was then evaporated as usual in a stream of air at 50°, when we obtained 2.6 g. of a semi-crystalline residue. From this was separated by crystallization from absolute alcohol 1.0 g. of formylurea, corresponding to a yield of 40% of the theoretical. It melted at 168°.⁹ When this ureide was dissolved in dilute sulfuric acid, urea was formed, and when the vapor evolved on distillation was conducted into a solution of silver nitrate, there was an immediate reduction with formation of metallic silver. Urea was also produced by digesting the ureide in aqueous solution.

Anal. Calcd. for $C_2H_4O_2N_2$: N, 31.82. Found: N, 31.62, 31.73.

The alcohol filtrates obtained after purification of formylurea were combined and evaporated to dryness. In this residue was detected urea by precipitation with xan-

⁸ Fischer, *Ann.*, 190, 125 (1878).

⁹ Gorski, *Ber.*, 29, 2046 (1896).

thydroly. Baudisch's¹⁰ test for pyruvic acid failed to reveal the presence of this ketone acid, indicating that it had been destroyed by oxidation.

Action of Ozone on 5-Bromouracil.—Ozone reacted immediately with bromouracil when bubbled through a suspension of 5.0 g. of the pyrimidine in 100 cc. of glacial acetic acid. Bromine was liberated in this reaction and at the end of eleven hours the bromouracil was completely destroyed. After evaporation of the acetic acid there was obtained 3.6 g. of a crystalline residue from which we obtained by crystallization from acetic acid 1.2 g. of parabanic acid melting with decomposition at 238 to 244°.

Anal. Calcd. for $C_5H_2O_3N_2$; N, 24.56. Found: N, 24.55, 24.42.

In the filtrate left after crystallization of the parabanic acid was detected urea and oxalic acid. We did not succeed in identifying formylurea or oxaluric acid as products of the reaction.

Action of Ozone on 5-Nitrouracil.—5-Nitrouracil was prepared according to the directions of Johnson and Matsuo¹¹ and treated with ozone according to the technique already described. The action of ozone was very slow and it required twenty-eight hours before the complete destruction of 3 g. of nitrouracil was accomplished. Evaporation of the acetic acid gave 2.3 g. of a crystalline residue which yielded after washing with alcohol and water 0.5 g. of oxaluric acid decomposing at 205–215°.

Anal. Calcd. for $C_5H_4O_4N_2$; N, 21.21. Found: N, 21.10.

From the alcohol extract was separated by crystallization 1.3 g. of a crystalline substance which yielded by further purification 1.0 g. of parabanic acid melting at 238–242°. When mixed with pure synthetic parabanic acid no change in melting point was observed.

Anal. Calcd. for $C_5H_2O_3N_2$; N, 24.56. Found: N, 24.48.

Urea was also detected in the final residues by means of the Fosse reagent (xanthidrol).

The Action of Ozone on 1,3-Dimethyl-5-bromouracil.—This bromine derivative of 1,3-dimethyluracil is easily obtained by direct bromination of the dimethylpyrimidine in aqueous solution, and was obtained in the form of prisms melting at 186°. Johnson and Clapp¹² reported a melting point of 181–182°. Ozonization of this pyrimidine (2.8 g.) was not complete under twelve to thirteen hours' treatment. The liberation of bromine was noticeable during the change and after evaporation of the acetic acid a residue of 1.4 g. was obtained. The only products identified were urea, oxalic acid and the hitherto unknown ureide *dimethyl-oxaluric acid*. This was purified by crystallization from absolute alcohol and melted at 124–125°. The acid is soluble in water and glacial acetic acid.

Anal. Calcd. for $C_8H_8O_4N_2$; N, 17.50. Found: N, 17.55.

Summary

1. The behavior of 4-methyluracil, 4-phenyluracil, thymine, 5-bromouracil, 5-nitrouracil and 1,3-dimethyl-5-bromouracil toward ozone has been investigated.

2. In every case the pyrimidine ring is destroyed with formation of characteristic ureide constructions and simple oxidation products. In no case is the urea configuration of the respective pyrimidines destroyed by the ozone treatment.

¹⁰ Baudisch, *THIS JOURNAL*, 45, 2978 (1923).

¹¹ Johnson and Matsuo, *ibid.*, 41, 783 (1919).

¹² Johnson and Clapp, *J. Biol. Chem.*, 5, 49 (1909).

3. The application of ozonization makes possible an improved technique for determining the structure of uracil compounds. The advantages gained experimentally become apparent when one is called upon to separate and identify the products of oxidation.

4. The urea configuration of the pyrimidine nucleus is not destroyed by the action of ozone.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, HOLY CROSS COLLEGE]

THE EFFECT OF SUBSTITUENTS ON CERTAIN PHYSICAL PROPERTIES OF BENZENE PICRATE¹

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Only a few of the aromatic hydrocarbons may be identified from their picrates. The reason for this is that very few aromatic hydrocarbons form picrates which are stable and can be purified by crystallization from suitable solvents. Most of the aromatic hydrocarbons form picrates which are decomposed by solvents or on standing in air.

The influence of substituents on the color of benzene picrate was studied by Franzen.² From a study of the absorption spectrum of a mixture of picric acid and a number of aromatic hydrocarbons and their halogen derivatives, he found that an increase in the number of alkyl groups in general causes an increase in the depth of the color produced. He also found that *m*-derivatives are the brightest, and that *p*-derivatives are the darkest.

Compounds of picric acid with aromatic hydrocarbons were studied by Efenov³ by means of thermal analysis of the binary mixtures. He found that molecular quantities of the substances usually reacted, but diphenyl, diphenylmethane and triphenylmethane do not give picrates.

Kendall⁴ explains the existence of the picrates by the formation of oxonium salts. The existence and stability of the addition compounds were determined from the freezing-point curve. The melting point was determined by distectic points on the fusion curve, *i. e.*, the temperature at which crystallization begins. The molecular composition of the picrates was found to be of the general type 1:1.

In our study with picrates, we have prepared the picrates of several

¹ Some of the experimental results described in this paper are taken from a thesis submitted to the Faculty of Holy Cross College by E. S. Hauber in partial fulfillment of the requirements for the degree of Master of Science.

² Hartig Franzen, *J. prakt. Chem.*, **98**, 67-80 (1918).

³ N. N. Efenov, *J. Russ. Phys.-Chem. Soc.*, **50**, 1, 372-421 (1918).

⁴ Kendall, *THIS JOURNAL*, **38**, 1314 (1916).