

Mol. wt. Subs., 0.2638, 0.3018, 0.2833; benzene, 21.96, 21.96, 21.96; freezing point lowering, 0.144, 0.169, 0.164°. *Mol. wt.*, 407, 401, 392.

Summary

New syntheses of phenylethynyl phenyl ketone (benzoylphenylacetylene) and of ethyl phenylpropiolate have been developed.

A homolog of propargyl alcohol, $RC\equiv C-CH_2OH$, and four trisubstituted analogs, $RC\equiv C-CR_2OH$, were synthesized. Three of these were converted into the corresponding propargyl chlorides and then to the phenyl ethers. These ethers were subjected to pyrolysis.

Triphenylpropargyl phenyl ether pyrolyzed into phenol and 9-phenylethynylfluorene, which was synthesized for purposes of identification from fluorene. Both trimethylpropargyl alcohol and trimethylpropargyl phenyl ether decomposed on heating into phenol and a conjugated "ene-ine" hydrocarbon, namely, methyl-2-pentene-1-ine-3. When refluxed, γ -methylpropargyl phenyl ether changed primarily into a tar, the apparent molecular weight of which was 400. No simple allenes were found although they may have been the precursors of the tars. Should propargyl aryl ethers have been found to rearrange like allyl aryl ethers, allenes would have been expected.

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RESEARCHES ON PYRIMIDINES. CXX. THE ACTION OF OZONE ON URACIL

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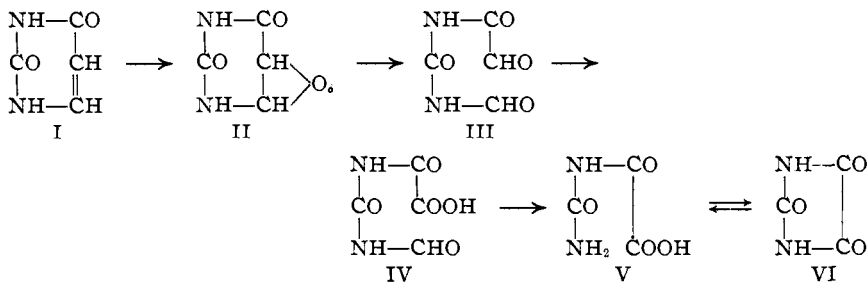
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In order to develop a new technique for determining the constitution of nucleic acids, and also known glucosidic combinations containing pyrimidine and purine cycles, the authors decided to investigate the action of ozone on pyrimidines of the uracil type, and to follow this up later with a study of the behavior of certain purines of biochemical interest toward this same reagent. So far as the authors are aware, the behavior of heterocycles of these two types toward ozone has never been investigated. Since both cycles are characterized structurally by the presence of ethylene or unsaturated groupings, it might be predicted that pyrimidines and purines would be very reactive toward ozone, and be broken down at the double bond by this reagent with formation of unique acyclic, ureide constructions possessing immediate biochemical interest. In this paper we now describe the behavior of the pyrimidine "uracil" I when allowed to interact with ozone in glacial acetic acid at ordinary temperature.

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.

Uracil I is not readily attacked by ozone in glacial acetic acid or 85% formic acid at ordinary temperature. If, however, the pyrimidine is suspended in either solvent, and washed ozone generated by passing oxygen gas through an ozonizing unit is bubbled into such a suspension for several hours, the uracil is finally completely destroyed and several products are formed as a result of the ozone action. The two major compounds produced are the ureides oxaluric acid V, and formyl-glyoxyl-urea III. Other degradation products formed along with these are urea, oxalic and formic acids and an unknown pyrimidine. The data already obtained indicate that uracil interacts with ozone in a normal manner to form first a true ozonide as expressed by formula II. This addition product is, however, very unstable and all attempts to isolate it were unsuccessful. In both acetic and formic acid solutions it undergoes decomposition rapidly under the experimental conditions employed, giving as the first product of reaction the new ureide *formyl-glyoxyl-urea* as represented by formula III. This represents the chief product of reaction and is the first member of its type to be described. The corresponding ureide acid, formyloxaluric acid IV, has been described by Gorski,² who prepared it by interaction of formylurea with oxalic acid.

The second ureide formed in relative amount is oxaluric acid V, which probably results by oxidation and subsequent hydrolysis of formyl-glyoxyl-urea III. The urea, oxalic acid and formic acid formed in the ozone reaction may all be accounted for as resulting from oxidation and hydrolytic changes of this ureide III. The only positive experimental evidence in favor of the formation of a pyrimidine is the response to a murexide color test. The quantity formed is very small, and thus far we have secured no data which would suggest its constitution. These various transformations of uracil are expressed by the formulas



The observation that oxaluric acid V is a product of reaction by ozonization of uracil is of biochemical interest as it introduces new chemical evidence in favor of the assumption that this pyrimidine may be one source of oxaluric acid, which is known to be a product of metabolism

² Gorski, *Ber.*, 29, 2046 (1896).

and has been shown to occur in human urine.³ The source of this ureide in metabolism has generally been attributed to the destructive oxidation of purines or uric acid. At the present time we have very little information regarding the mechanism of molecular degradation when uracil is oxidized and destroyed in body metabolism.⁴

It is our purpose to determine the generality of this type of change by applying the treatment with ozone to a series of derivatives of uracil. The reaction opens up an important and practical procedure for determining the constitution of pyrimidine compounds belonging to the uracil series.

Experimental Part

Preparation of Uracil.—The uracil used in this research was prepared from urea, malic acid, and fuming sulfuric acid in accordance with a modification of the method developed by Davidson and Baudisch,⁵ which has been applied in this Laboratory with success by several workers. Four hundred cubic centimeters of 13% fuming sulfuric acid is cooled to 0° and, while the acid is stirred mechanically, 100 g. of malic acid added at such a rate that the temperature of the mixture does not rise above 5°. After the malic acid has been added, the mixture is cooled to 0° and constantly stirred while 100 g. of finely pulverized urea is rapidly added. The temperature rises rapidly to 50° and at 60° there is a vigorous evolution of carbon monoxide. The temperature of the reaction mixture is maintained at 85° for forty-five minutes, then it is cooled and finally poured over 1200 g. of crushed ice. The uracil is precipitated by this treatment and is completely deposited after twenty-four hours. It is purified by crystallization from hot water. The yield averages about 59% of the theoretical quantity.

Interaction of Uracil with Ozone in Glacial Acetic Acid.—Five grams of uracil is suspended in 100 cc. of glacial acetic acid and ozonized oxygen, previously washed with a 5% aqueous solution of sodium hydroxide and finally with concentrated sulfuric acid, is bubbled through the suspension for fourteen to sixteen hours. The uracil slowly dissolves and at the end of the reaction period only a light suspension of crystalline material remains undissolved in the acetic acid. As the reaction approaches completion, the suspended material is tested at fifteen-minute intervals for the presence of uracil by the Wheeler and Johnson⁶ method employing bromine water and barium hydroxide. When the characteristic purple precipitate no longer appears after this treatment, the ozone transformation is considered complete. Our methods of separating and identifying the products of this reaction are described below.

Identification of Oxaluric Acid, V.—The reaction product, which was insoluble in the acetic acid after completion of the ozone treatment (above), weighed 0.6 g., and it melted before purification at 183–187° with decomposition. After purification by recrystallization from 95% alcohol, in which it is difficultly soluble, or from glacial acetic acid, it decomposed when heated at 205–215°. It was difficultly soluble in water, but was soluble in alkaline solution and interacted to form insoluble barium and silver salts. Ammonia was evolved when the ureide was warmed in alkaline solution and oxalic acid was shown to be formed by hydrolysis. Urea was also identified in the solution by means

³ See Schunck, *Jahresb.*, 749 (1866).

⁴ See Cerecedo, *J. Biol. Chem.*, **75**, 661 (1927); Emerson and Cerecedo, *ibid.*, **87**, 453 (1930).

⁵ Davidson and Baudisch, *THIS JOURNAL*, **48**, 2379 (1926).

⁶ Wheeler and Johnson, *J. Biol. Chem.*, **3**, 183 (1907).

of the Fosse⁷ xanthidrol reagent. In fact, the compound agreed in all its properties with those described for oxaluric acid by Biltz and Topp.⁸

Anal. Calcd. for $C_3H_4O_4N_2$: N, 21.21. Found: N, 21.07, 20.91.

The identity of this ureide was further confirmed by the formation of its ethyl ester, which was prepared by digesting the acid in alcohol acidified with sulfuric acid. The ester separated in the form of needles and after purification by crystallization from ether it melted at 177° with decomposition.

Anal. Calcd. for $C_6H_8O_4N_2$: N, 17.50. Found: N, 17.57.

Identification of Formyl-glyoxal-urea, III.—After separation of the oxaluric acid by filtration (ozone-uracil reaction), the acetic acid was removed by heating at 50°, when we obtained 4.5 g. of a colorless crystalline substance. This was apparently a mixture of several products and all attempts at purification by crystallization from alcohol, chloroform, benzene, ethyl acetate and ether were unsuccessful. By use of hot, glacial acetic acid we succeeded in separating 2.4 g. of a definite crystalline substance which melted constantly at 162°. This was characterized by the following properties: solubility in water, giving a solution which reacted acid to litmus and in 95% alcohol and also dilute potassium carbonate solution; insoluble in ether, benzene and chloroform.

No insoluble barium salt was formed by the action of barium hydroxide, but addition of silver nitrate to an alkaline solution of the compound gave immediately a colorless, flocky precipitate of a silver salt. The warming of a solution of this silver salt in ammoniacal solution led to the production of a bright silver mirror on the walls of the glass receptacle. The silver salt was prepared by precipitation in neutral solution, washed with water, alcohol and ether and finally dried at 80°. This gave on analysis the following results for nitrogen and silver.

Anal. Calcd. for $C_4H_8O_4N_2Ag \cdot H_2O$: N, 10.41; Ag, 40.14. Found: N, 10.38; Ag, 39.98.

The compound melting at 162° reduced Fehling's solution immediately on warming and also interacted with Tollens' reagent to form a silver mirror, but there was no apparent reaction with Schiff's reagent. Application of the murexide test was negative and it produced no color when introduced into ferric chloride solution, proving that we were not dealing here with formylallanturic acid.⁹ The compound was decomposed by digestion with dilute sulfuric acid with formation of formaldehyde and urea. Some of the compound melting at 162° was warmed with dilute nitric acid and the solution then evaporated to dryness. In this residue was easily identified oxalic acid. This exhaustive study of the properties of this substance formed from uracil by the action of ozone led us to conclude that we were dealing with the hitherto unknown acyclic ureide, *viz.*, *formyl-glyoxyl-urea*, III. The identity of the compound was further established by analysis.

Anal. Calcd. for $C_4H_4O_4N_2$: C, 33.33; H, 2.8; N, 19.44. Found: C, 33.52; H, 3.0; N, 19.37, 19.33.

An attempt to prepare a phenylhydrazone of formyl-glyoxyl-urea was unsuccessful. The ureide interacted with phenylhydrazine in acetic acid solution at ordinary temperature giving a product which melted after purification at 144-145°. It crystallized from alcohol in colorless plates and was identified as formylphenylhydrazine.¹⁰

⁷ Fosse, *Ann. chim.*, [9] 6, 13 (1916).

⁸ Biltz and Topp, *Ber.*, 46, 1408 (1913).

⁹ Behrend and Grünewald, *Ann.*, 323, 201 (1902).

¹⁰ Vries, *Ber.*, 27, 1522 (1894); Bamberger, *ibid.*, 30, 1264 (1897); Claisen, *Ann.*, 287, 370 (1895).

Anal. Calcd. for $C_7H_8ON_2$: N, 20.59. Found: N, 20.49.

An attempt to prepare the phenylhydrazone by interaction in absolute alcohol was also unsuccessful. The reaction was applied with 5 cc. of phenylhydrazine, 65 cc. of alcohol and 0.5 g. of formyl-glyoxyl-urea. This solution was digested on the steam-bath for three hours, cooled and then diluted with water, when a crystalline substance separated on standing. This was purified by crystallization from absolute alcohol and separated in the form of yellow needles decomposing at 221° . A nitrogen determination by the Dumas method gave a value of 29.5%. In sodium hydroxide solution this dissolved with formation of a deep blue coloration which was discharged by acidification with hydrochloric acid. On adding alkali the blue color was regenerated. Behrend and Grünwald⁹ have described a phenylhydrazine derivative of acetylallanturic acid which possesses similar properties. The structure of this compound will be determined and discussed in a later paper.

The acetic acid filtrate from which formyl-glyoxyl-urea was separated by crystallization was examined further for secondary products of ozonization. The possibility of the presence of an ozonide here was eliminated by the fact that no iodine was liberated when the residue obtained after evaporation of the acetic acid was treated with potassium iodide. The presence of oxalic acid and urea was easily detected, and we also observed that this residue gave a pronounced murexide test, indicating the presence of an unknown pyrimidine combination. We were unable, however, to separate a definite pyrimidine combination from this residue.

Formation of Formyl-glyoxyl-urea from Uracil by the Action of Ozone in 85% Formic Acid Solution.—Five grams of uracil was suspended in 100 cc. of 85% formic acid and ozonized oxygen bubbled through the suspension for twenty-three hours. The uracil dissolved and was completely destroyed by action of the ozone. After evaporating the formic acid solution at 50° we obtained a crystalline residue containing urea, oxalic acid and formyl-glyoxyl-urea. Two grams of the latter ureide melting at 162° was obtained by fractional crystallization from glacial acetic acid. This corresponds to a yield of 31% of the theoretical. By ozonization of uracil in glacial acetic acid the yield of this ureide is raised to 38% of the theoretical.

Summary

1. Uracil and ozone interact in glacial acetic acid at ordinary temperature to form chiefly two compounds, *viz.*, formyl-glyoxyl-urea and oxaluric acid.
2. Part of the uracil molecule is completely broken down with formation of urea, oxalic and formic acids.

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