Conclusions

It seems probable to the authors that the reaction products expressed by the provisional formulas V and VI are two representatives of a progressive series of chemical changes produced by interaction of chlorine with the thiocyanopyrimidine IV and resulting in complete destruction of the pyrimidine ring. Chlorination of 2-chloro-6-thiocyanopyrimidine is not productive of a pyrimidine sulfonchloride and apparently leads to a welter of related chemical substances difficult to separate and purify. It is not improbable that we are dealing here with a type of chemical change closely related to the reactions taking place in the well-known transformation of alloxan hydrate VII into alloxanic acid[13] VIII and hydantoin IX, respectively.

Further work will be undertaken as soon as possible to determine whether such a postulation is tenable.

(13) Biltz, Hahn and Bergius, *Ann.*, **413**, 69 (1917); Schliepper, *ibid.*, **56**, 1 (1845); Baeyer, *ibid.*, **119**, 126 (1861).

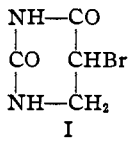
NEW HAVEN, CONNECTICUT RECEIVED AUGUST 26, 1940

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

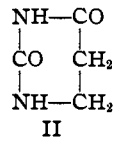
Dehydrogenation of Hydrouracil[1]

BY TREAT B. JOHNSON

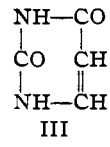
During the progress of some new pyrimidine research receiving attention in this Laboratory, experimental conditions arose calling for a modification of the present procedure employed for the dehydrogenation of hydrouracil II to uracil III.



I



II



III

The reverse change can easily be accomplished by catalytic reduction of uracil in the presence of platinum.[2]

For successful results in biochemical experimentation dealing with sensitive oxidation changes, it was necessary to use a hydrogen acceptor which would be selective in its action and active in aqueous solution, thereby permitting of the direct oxidation of hydrouracil II in neutral solution. Two experimental techniques are available at present for the successful dehydrogenation of this pyrimidine, but both methods are indirect, involving first bromination of hydrouracil with formation of 5-bromohydrouracil I. Fischer and Roëder[3] accomplished this dehydrogenation change in their original procedure for the synthesis of uracil III by heating this brominated pyrimidine with alkali or pyridine. Gabriel[4] later succeeded in improving this technique by making the discovery that the brominated pyrimidine I dissociates quantitatively into uracil III and hydrobromic acid when heated above its melting point temperature (195°). From a biochemical point of view both of these operations are impractical, and could not be considered for the experimental program receiving our attention.

It is a well-known fact that alloxan can serve as a hydrogen acceptor under mild experimental conditions. In consequence of its easy reducibility to *alloxantine* it interacts, for example, with absolute alcohol, in the presence of sunlight, to form acetaldehyde[5]; converts hydrazobenzene to azobenzene and indigo-white to indigo blue.[6] Strecker also showed as early as 1862 that α-aminoacids are oxidized by alloxan to their corresponding aldehyde derivatives.[7] It seemed

$$CH_3CH(NH_2)COOH \longrightarrow CH_3CHO$$

probable to the author, in the light of these observations, that alloxan would meet the conditions required in our experiments and serve as a suitable oxidizing reagent for the dehydrogenation of hydrouracil II.

The author now finds that hydrouracil II is oxidized quantitatively to uracil III by digestion in aqueous solution with commercial alloxan hydrate. Partial destruction of the alloxan molecule takes place as is evidenced by the fact that both oxalic acid and urea are formed during the digestion. This pyrimidine reagent fully

(1) Researches on Pyrimidines, CLXVII.

(2) Johnson and Brown, *Proc. Natl. Acad. Sci.*, **7**, 75 (1921); Brown and Johnson, THIS JOURNAL, **45**, 2702 (1923).

(3) Fischer and Roëder, *Ber.*, **34**, 3751 (1901).

(4) Gabriel, *ibid.*, **38**, 637, 1690 (1905).

(5) Ciamician and Silber, *ibid.*, **36**, 1581 (1903).

(6) Pellizzari, *Gazz. chim. ital.*, **17**, 256 (1888).

(7) Strecker, *Ann.*, **123**, 364 (1862).

meets the experimental conditions called for in the study of new oxidation changes of biochemical interest. It is interesting to note here that attempts to dehydrogenate hydrouracil II to uracil III by the action of hydrogen peroxide or methyldichloroxyhydrouracil[8] were unsuccessful.

The investigation of pyrimidine oxidizing agents, and their practical utilization will be continued.

Experimental Part

The Oxidation of Hydrouracil to Uracil by Alloxan.—Molecular proportions of alloxan hydrate[9] (1.2 g.) and hydrouracil (0.9 g.) were dissolved in hot water (18 cc.) and the solution digested at its boiling temperature for four and one-half hours. The solution turned pink immediately on warming, but at the end of two hours this color had practically disappeared, and the solution assumed a turbid appearance. At the end of the digestion period the solution was allowed to stand for several hours when a crystalline substance deposited which was identified as uracil III. The weight was 0.6 g. and it crystallized from hot water in the characteristic, corpuscular crystals of this pyrimidine. It responded also to the Wheeler and Johnson color test[10] for this pyrimidine.

The aqueous filtrate above was concentrated to a volume of 10 cc. and cooled when 0.2 g. more of uracil separated giving a total yield of this pyrimidine III corresponding to 90%. On continued evaporation to dryness we recovered a colorless residue weighing 1.5 g. This residue was treated as follows. It was dissolved in warm water and combined with a solution of barium hydroxide. An immediate precipitation of a barium salt resulted and the odor of ammonia was noticeable. The weight of this salt, dried at 100°, was 1.0 g. and it was identified as barium oxalate. After quantitative decomposition with sulfuric acid and evaporation of the aqueous solution we recovered pure crystalline oxalic acid melting at 103–104°.

The aqueous filtrate obtained after filtration of the barium oxalate was saturated with carbon dioxide gas to precipitate the excess of barium as carbonate, and then concentrated to a small volume. This still contained a trace of oxalic acid, and when warmed with alkali evolved ammonia. The latter resulted from decomposition of urea which was identified in the form of its xanthyhydryl derivative.[11]

5-Nitrouracil.—A small quantity of the uracil formed in this experiment was dissolved in fuming nitric acid and the solution evaporated to dryness at 100°. The nitrouracil residue was then purified by crystallization from boiling water, and separated in prismatic crystals, on cooling, which melted at 280–285°.

Anal. Calcd. for $C_4H_3O_4N_3$: N, 26.75. Found: N, 26.50 and 26.65.

Attempts to Oxidize Hydrouracil.—a. An aqueous solution of hydrouracil (0.5 g. in 5 cc. water) was combined with 10 cc. of superoxol (30% hydrogen peroxide) and the solution warmed on a steam-bath for six hours. The pyrimidine was not changed by this treatment and was recovered melting at 274–275°.

Anal. Calcd. for $C_4H_6O_2N_2$: N, 24.56. Found: N, 24.45.

It is well to note here that this pyrimidine II is not attacked by potassium permanganate at ordinary temperature except on long standing in acid solution.[12]

b. Molecular proportions of hydrouracil (1 g.) and methyldichloroxyhydrouracil[8] (2 g.) were dissolved in 15 cc. of water and the mixture then heated at 100° for four hours. On cooling, the hydrouracil was recovered unaltered. The author did not succeed in isolating any material which responded to Wheeler and Johnson's color test for uracil.[10] The hydrouracil crystallized in prisms melting at 274–275°.

Attempt to Oxidize Hydrocinnamic Acid with Alloxan.—Dakin[13] has shown that the ammonium salt of this acid undergoes oxidation with hydrogen peroxide to form benzoylacetic acid (β-oxidation). It was the author's experience that digestion with alloxan in aqueous solution is not productive of either cinnamic acid or benzoylacetic acid. The hydrocinnamic acid used was recovered completely (m. p. 48°) after digestion with alloxan in aqueous solution for six hours.

Summary

Hydrouracil is converted quantitatively into uracil by digestion in aqueous solution with alloxan hydrate.

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(8) Behrend, *Ann.*, **236**, 59 (1886).

(9) Eastman Kodak Co. reagent.

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

(11) Fosse, *Compt. rend.*, **145**, 813 (1907).

(12) Gabriel, *Ber.*, **33**, 3385 (1900).

(13) Dakin, *Am. Chem. J.*, **44**, 47 (1910); *J. Biol. Chem.*, **4**, 419 (1907).