[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

The Reaction of Thiourea with 5,5-Dibromoxyhydrouracil and 5,5-Dibromobarbituric Acid[1]

BY TREAT B. JOHNSON[2]

Among the many heterocyclic compounds of the pyrimidine type, which have played an important part in expanding our knowledge of organic reactions of biochemical interest, is the halogenated hydropyrimidine—5,5-dibromoxyhydrouracil[3]—represented by Formula I. This is formed in quantitative yield by the action of bromine on uracil or cytosine in aqueous solution; and serves as an intermediate in the practical application of the Wheeler and Johnson well-known, qualitative color reaction for the detection of these two naturally occurring pyrimidines.

Outside the application of this useful color test, and a study of its mechanism of reaction, very little attention has been paid to the chemistry of the pyrimidine I, or any other representative of its type. In fact, the lack of chemical knowledge which exists has delayed the investigation and solution of several chemical problems that promise to lead to experimental results of immediate biochemical interest and importance. We are now engaged in carrying out a series of investigations dealing with the newer chemistry of this type of pyrimidine compounds. In this preliminary paper we will report on the characteristic behavior of the pyrimidine I toward thiourea. It is also instructive to compare at this time the behavior of 5,5-dibromobarbituric acid toward this same sulfur reagent. In order to conserve space, we have recorded the essential facts revealed to date in Table I.

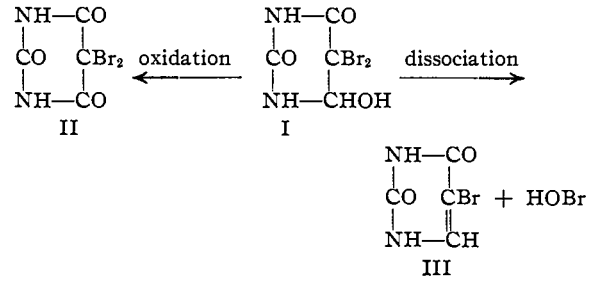


TABLE I

COMPARATIVE BEHAVIOR OF 5,5-DIBROMOXYHYDROURACIL AND 5,5-DIBROMOBARBITURIC ACID

NH—CO / CO CBr2 / NH—CHOH (Dibromoxyhydrouracil)	NH—CO / CO CBr2 / NH—CO (Dibromobarbituric Acid)
A. *Action of potassium thiocyanate:*	A. *Action of potassium thiocyanate:*
1. No formation of a thiocyanate by interaction in water or alcohol.	1. Formation of 5-thiocyanbarbituric acid.[a]
	2. Thiodialuric acid is formed by the action of sodium hydroxide on this thiocyanate.
B. *Action of thiourea:*	B. *Action of thiourea:*
1. Interact immediately when warmed in alcohol or aqueous solution in molecular proportions giving the following products: 5-bromouracil, free sulfur, hydrobromic acid and cyanamide.	1. Interacts with 5,5-dibromobarbituric acid in water or alcohol to give 5-thiouramino barbituric acid[b] (α-thiopseudouric acid).
2. Obtained no evidence of the formation of uracil-5-pseudo-thiourea.	2. Interacts with bromobarbituric yielding the same 5-thiouraminobarbituric acid.[c]
	3. 5-Thiouraminobarbituric acid is condensed by the action of concentrated sulfuric acid at 150–160° to form *"urosulfinic acid".*[d]
C. Hydrouracil-5-sulfonic acid, and uracil-5-sulfonic acid are both unknown.	C. Barbituric-5-sulfonic acid is unknown.

[a] Trzcinski, *Ber.*, **16**, 1058 (1883). [b] Trzcinski, *ibid.*, **16**, 1057 (1883); A. E. Dixon, *J. Chem Soc.*, **63**, 816 (1893), has shown that dichloroacetic acid and thiourea interact in aqueous solution in a similar manner giving pseudothiohydantoin instead of a chlorinated derivative. [c] Mulder, *Ber.*, **12**, 2309 (1879). [d] Nencki, *ibid.*, **4**, 724 (1871); **5**, 45 (1872).

(1) Researches on Pyrimidines, CLXV.

(2) This research supported in part by a grant from the George Sheffield Research Fund.

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

The pyrimidine I is characterized by its unique behavior when digested in water or alcohol solutions. Under these conditions it undergoes a

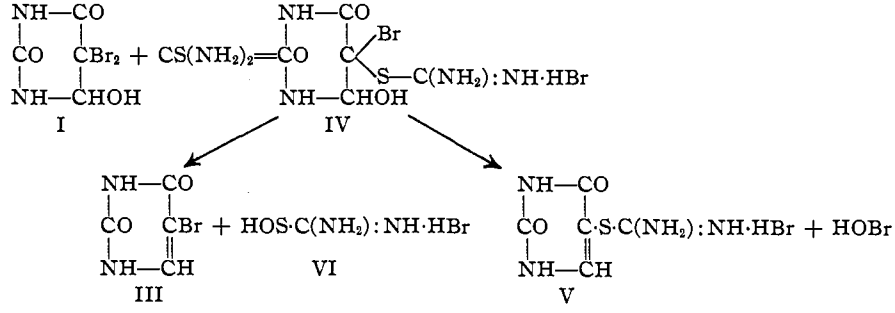
TABLE II

RESULTS OF OXIDATION

Expt.	5-Bromouracil (theoretical = 1.33 g.), g.	Sulfur (theoretical = 0.22 g.), g.	Solvent	Examination of residue
1	1.26	0.18	95% EtOH	Evolution of NH_3 by treatment with alkali in all expts.
2	1.22	.20	95% EtOH	
3	1.28	.19	95% EtOH	Sl. pptn. of $Ba{\cdot}SO_4$ on addn. of $Ba(OH)_2$.
4	1.25	.19	Water	Residue sol. in cold water, and thiourea completely destroyed.

spontaneous and quantitative dissociation into 5-bromouracil III and hypobromous acid.[3] This specific manner of degradation encourages the author to predict a new usage for this pyrimidine I, namely, as a practical oxidizing reagent for experimentation in biochemical research. The generated 5-bromouracil III is recoverable without appreciable loss, and can be reconverted quantitatively into 5,5-dibromoxyhydrouracil I by treatment with bromine. Further applications of this new oxidizing reagent will be discussed in future papers from this Laboratory.

Two reaction mechanisms are theoretically open for consideration in deciding the true course of the oxidation changes brought about by the action of thiourea on 5,5-dibromoxyhydrouracil I, namely: (1) that the pyrimidine I first undergoes dissociation with formation of 5-bromouracil III and hypobromous acid; and that it is this free acid which reacts with the thiourea leading to its complete destruction; (2) that the pyrimidine I exhibits the behavior of an alkyl halide and reacts with the thiourea yielding first an addition product of pseudothiourea structure IV. Theoretically a compound of such constitution might be expected to be very unstable and dissociate in either of two ways, namely, to form 5-bromouracil III, and a sulfoxy derivative of thiourea VI, or give hypobromous acid and uracil-5-pseudothiourea V. Thus far the author has not been able to detect the presence of such a pyrimidine derivative V among the products of reaction. Also a sulfoxy derivative of thiourea VI, if formed, would be expected to be very unstable and break down easily giving the products of reaction, water, free sulfur and cyanamide. These changes are expressed below:



The investigation of 5,5-dibromoxyhydrouracil as an oxidizing agent will be continued.

Experimental Part

Interaction of Thiourea with Dibromoxyhydrouracil.—A description of one experiment will serve to describe the technique of application of this reaction.

Proportions of reactants: 2 g. of dibromoxyhydrouracil, 0.525 g. of thiourea, 10 cc. of ethyl alcohol or water.

The two reagents are used in molecular proportions. The thiourea is first dissolved in the 10 cc. of solvent (alcohol or water) and to the warm solution is added the 2 g. of finely pulverized dibromoxyhydrouracil. The mixture is then heated on a boiling water-bath when the hydrouracil will dissolve, and on continued heating of its solution undergoes dissociation with formation of 5-bromouracil and hypobromous acid. Digestion is continued for two and one-half to three hours when the insoluble 5-bromouracil is filtered off and the aqueous or alcohol filtrate is evaporated to dryness. The 5-bromouracil is contaminated with the sulfur formed in the reaction, which is removed by trituration with carbon bisulfide and finally recovered by evaporation of the solvent. The 5-bromouracil is recovered practically pure after this treatment and on recrystallization from boiling water gives no test for sulfur and melts with decomposition at 295–298°.

In the sirupy residue left after evaporation of the alcohol or water filtrate were identified ammonium bromide, urea formed by acid hydrolysis of cyanamide, and a trace of $-SO_4$ by precipitation with barium hydroxide. No thiourea was recoverable. The analytical results obtained in four oxidation experiments are recorded in Table II.

Summary

1. 5,5-Dibromoxyhydrouracil and thiourea re-

act in alcohol or water quantitatively giving 5-bromouracil, free sulfur, cyanamide and hydrobromic acid.

2. We obtained no evidence of the formation of uracil-5-pseudothiourea.

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Influence of Oxygen on the Fermentation of Maltose and Galactose[1]

BY ALFRED S. SCHULTZ, LAWRENCE ATKIN AND CHARLES N. FREY

Although bakers' yeast contains maltase, it customarily ferments maltose in pure solutions only after a rather long induction period. The addition of dextrose, in small amounts, remarkably shortens the induction period.[2,3] Further work has shown that oxygen, under appropriate conditions, shortens the induction period for maltose fermentation. The investigation was extended to galactose, the fermentation of which was aided by oxygen but not by dextrose.

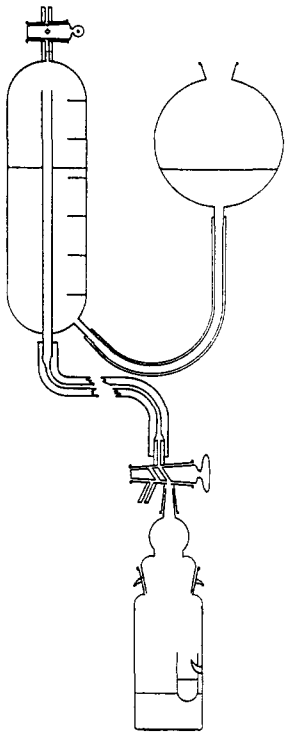

Fig. 1.—Apparatus for the measurement of fermentation under various gases.

Experimental

Apparatus.—The fermentation apparatus employed is a modification of one previously described[4] in which arrangement has been made for the use of a known atmosphere (see Fig. 1). The reaction bottles (vol. 250 ml.) were provided with glass hooks designed to hold oversize Keilin tubes containing the yeast suspension until the system had reached equilibrium. The system was filled with nitrogen or oxygen by evacuating the prepared reaction bottle and repeatedly flushing the gasometer with the gas by means of the stopcock on top and the inlet below. The evacuated reaction bottle was quickly connected with the filled gasometer, the connections flushed out by means of the three-way stopcock, and the gas admitted to the bottle. Excess gas was discharged through the top of the gasometer. All experiments were done at 30° and the bottles were shaken at 100 oscillations per minute.

Materials.—The final volume of the fermenting solution was 80 ml. and contained 0.8 g. of moist (compressed) bakers' yeast, a phosphate–citrate buffer of *p*H 5.4, 3 g. of the sugar under study, nicotinic acid (1 mg.), thiamin (0.05 mg.), and mineral salts including ammonium ions (200 mg. of ammonium sulfate).

Maltose Fermentation.—In pure nitrogen the initiation of maltose fermentation is very slow, as can be seen from Fig. 2. Air causes increased attack and oxygen still more. Dextrose in a nitrogen atmosphere causes a prompt fermentation of the maltose to occur. The initial hump in the latter curve is due to the fermentation of dextrose, the gas equivalent of the amount added being about 90 ml.

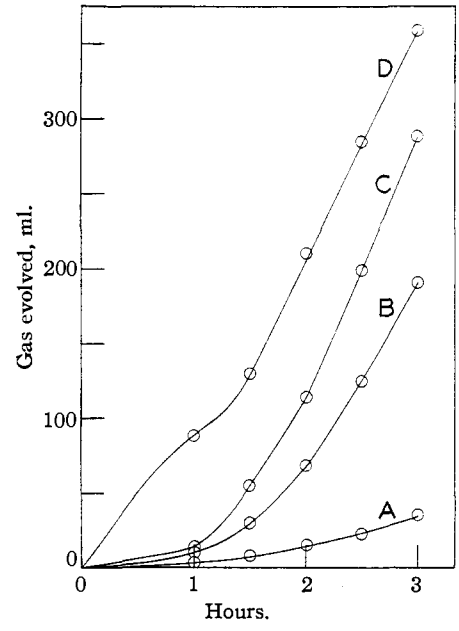

Fig. 2.—Maltose fermentation: Curve A, nitrogen atmosphere; Curve B, air atmosphere; Curve C, oxygen atmosphere; Curve D, nitrogen atmosphere plus 0.4 g. of dextrose in solution.

Dextrose Fermentation.—The fact that oxygen stimulates fermentation is contrary to the so-called Pasteur reaction wherein the reverse is supposed to occur. The explanation lies in the fact that our experimental conditions do not produce an extreme degree of aerobicity even when

(1) Presented before the Division of Biological Chemistry at the Boston meeting of the American Chemical Society, September, 1939.

(2) A. S. Schultz and L. Atkin, THIS JOURNAL, **61**, 291 (1939).

(3) J. Leibowitz and S. Hestrin, *Enzymologia*, **6**, 15 (1939).

(4) A. S. Schultz and Q. Landis, THIS JOURNAL, **54**, 211 (1932).