[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

RESEARCHES ON PYRIMIDINES. CXXIX. THE SYNTHESIS OF 2-THIO-OROTIC ACID

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The two sulfur analogs of uracil-4-carboxylic acid (orotic acid)[2] and uracil-5-carboxylic acid,[3] respectively, have been described in the chemical literature. The pyrimidine acid represented by formula II was synthesized

NHCSNHCOCHC(COOH) NHCSNHCOC(COOH)CH

I II

by Johnson and Ambler[4] in 1911, and the isomeric acid I by Bachstez[5] in 1930. Both of these pyrimidines have a biochemical interest, and the presence of sulfur in the molecule increases greatly the possible application of these two compounds for future synthetic operations.

Bachstez's method of preparing 2-thio-orotic acid involves the condensation of thiourea with ethyl oxaloacetate to form the ethyl ester of 2-thio-orotic acid, which is then converted into the acid I by saponification. In other words, practically the same technique is employed here for its preparation as was originally applied by Müller for the synthesis of the ethyl ester of uracil-4-carboxylic acid. Neither condensation reaction is productive of pyrimidines in good yield, that reported by Bachstez for 2-thio-orotic acid averaging, for example, less than 21% of the theoretical.

In the progress of our researches on pyrimidines we have sought for an improved method of preparing 2-thio-orotic acid, and find that it can be prepared easily by application of a Cannizzaro reaction to the corresponding pyrimidine aldehyde, 2-thiouracil-4-aldehyde. This aldehyde and also the corresponding uracil-4-aldehyde have been described previously in papers by Johnson and Cretcher[6] and by Johnson and Schroeder.[7] Both pyrimidines undergo smoothly a Cannizzaro transformation, giving practically quantitative yields of the corresponding pyrimidine acids and primary alcohols as is expressed below

1. 2-Thiouracil-4-aldehyde ⟶ 2-thio-orotic acid and 2-thio-4-hydroxymethyluracil.
2. Uracil-4-aldehyde ⟶ orotic acid and 4-hydroxymethyluracil.

These transformations (1 and 2) not only illustrate the importance of our original pyrimidine aldehyde syntheses, but they also introduce

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[2] Wheeler, *Am. Chem. J.*, **38**, 358 (1908); Müller, *J. prakt. Chem.*, **56**, 488 (1897).

[3] Wheeler, Johnson and Johns, *Am. Chem. J.*, **37**, 392 (1907).

[4] Johnson and Ambler, THIS JOURNAL, **33**, 982 (1911).

[5] Bachstez, *Ber.*, **64**, 322 (1930).

[6] Johnson and Cretcher, THIS JOURNAL, **37**, 2144 (1915).

[7] Johnson and Schroeder, *ibid.*, **53**, 1989 (1931).

workers in this field to a method for synthesizing primary alcohol derivatives of uracil, which is an improvement over the method previously developed in this Laboratory. Both 2-thio-orotic acid and the corresponding 2-thio-4-hydroxymethyluracil can be converted quantitatively into orotic acid and 4-hydroxymethyluracil, respectively, by desulfurization.

In his last publication on the chemistry of 2-thio-orotic acid, Bachstez discusses the behavior of the sodium salt of this acid toward benzyl chloride, and describes the reaction product, melting at 264°, as a N-benzyl derivative. It has been our experience that the sodium salt of 2-thio-orotic acid and also 2-thio-4-hydroxymethyluracil interact with alkyl halides to form smoothly alkyl derivatives with the substituent group attached to sulfur. Several representatives of this type of derivative have been prepared by us, and we have observed that they undergo hydrolysis by acids, in every case examined, with formation of mercaptans and the corresponding sulfur-free pyrimidines. In other words, no abnormality in behavior is revealed in this series of compounds.

Experimental Part

The Application of Cannizzaro's Reaction to 2-Thiouracil-4-aldehyde.[6,7] The Preparation of 2-Thio-orotic Acid and 2-Thio-4-hydroxymethyluracil.—Five grams of 2-thiouracil-4-aldehyde is dissolved in a solution of 5 g. of potassium hydroxide and 12 cc. of water, previously cooled to room temperature. The aldehyde dissolves readily with rise in temperature to give a clear bright red solution. The reaction is practically complete after standing at room temperature for forty-eight hours.

The reaction mixture is then acidified with dilute hydrochloric acid and the precipitate, consisting of both reaction products, is separated by filtration, suspended in 50 cc. of water and redissolved by addition of a small amount of sodium hydroxide solution. On acidifying this solution with acetic acid the 2-thio-4-hydroxymethyluracil is precipitated in crystalline condition, and the separation is usually complete within half an hour. This alcohol is easily purified by crystallization from hot water, and separates on cooling in the form of colorless needles. The yield was 1.8 g. This pyrimidine is moderately soluble in hot alcohol, easily soluble in concentrated sulfuric acid and hot acetic acid, and insoluble in ether, benzene, ligroin and acetone. It melts with decomposition at 259°.

Anal. Calcd. for $C_5H_6O_2N_2S$: N, 17.72. Found: N, 17.66, 17.70.

The filtrate from the acetic acid precipitation is then made strongly acid with hydrochloric acid when the 2-thio-orotic acid is precipitated as a yellow powder. The yield was 2.4 g. This pyrimidine was purified by repeated reprecipitations from alkaline solution with hydrochloric acid. It does not show a sharp melting point and decomposes with strong effervescence when heated at 338–339°. The acid described by Bachstez[5] showed a similar behavior, melting at the same temperature. This pyrimidine is very insoluble in cold water, alcohol and ether, but is moderately soluble in hot water.

Anal. Calcd. for $C_5H_4O_3N_2S$: N, 16.28. Found: N, 16.30, 16.43.

Conversion of 2-Thio-orotic Acid into Orotic Acid.—The desulfurization of 2-thio-orotic acid is accomplished quantitatively by oxidation with chromic acid. Two grams of the sulfo acid is slowly added to an oxidizing mixture consisting of 5 g. of sodium dichromate, 5 cc. of sulfuric acid and 25 cc. of water. Considerable heat is evolved during the addition. After standing for a short time, the reaction mixture is heated to

boiling for about one minute. On cooling, the sulfur-free orotic acid separates out as a yellowish solid. It is decolorized with norite and recrystallized from hot water. The purified product melted at 345°. A mixed melting point with known orotic acid showed no depression; yield, 1.9 g.

The desulfurization may also be accomplished by heating for a short time with alkaline 3% hydrogen peroxide. Two grams of 2-thio-orotic acid is covered with 25 cc. of 3% hydrogen peroxide and sufficient sodium hydroxide solution is added to dissolve the acid. The solution is heated to a gentle boil for three minutes. On cooling and acidifying with hydrochloric acid, the orotic acid separates out as a colorless solid. The yield was 1.8 g. Attempts to desulfurize 2-thio-orotic acid with monochloroacetic acid were not successful.

Conversion of 2-Thio-4-hydroxymethyluracil into 4-Hydroxymethyluracil.—Two grams of 2-thio-4-hydroxymethyluracil is suspended in a solution of two grams of monochloroacetic acid in 25 cc. of water. The pyrimidine readily goes into solution on warming. The solution is heated under a reflux condenser for five hours, remaining perfectly clear during this period. On cooling, 4-hydroxymethyluracil separates out in the form of colorless elongated prisms or plates. It may be purified by recrystallization from a small quantity of hot water; yield, 1.5 g. The product darkened at 248° and then melted sharply at 255–256°. It gave no test for sulfur. It was easily soluble in hot water, acetic acid, methyl and ethyl alcohol, and insoluble in ether and benzene.

Anal. Calcd. for $C_5H_6O_2N_2$: N, 19.72. Found: N, 19.52, 19.65.

4-Hydroxymethyluracil was first prepared by Johnson and Chernoff[8] by way of the intermediate, 4-ethoxymethyluracil. The product which they obtained did not melt sharply, showing signs of decomposition at 240° then melting at 254°.

Application of the Cannizzaro Reaction to Uracil-4-aldehyde.—Uracil-4-aldehyde undergoes a Cannizzaro reaction when allowed to stand in aqueous potassium hydroxide solution to give orotic acid and 4-hydroxymethyluracil. Five grams of the aldehyde, prepared according to the method of Johnson and Schroeder[7] is dissolved in a solution of 8 g. of potassium hydroxide and 18 cc. of water, and allowed to stand at room temperature for forty-eight hours. The solution assumes a deep yellow color and partially solidifies. At the end of the reaction 25 cc. of water is added and the solution then acidified with concentrated hydrochloric acid, cooled in ice water, and the yellowish precipitated mixture of alcohol and acid filtered off by suction.

The separation of the two reaction products depends on the much greater solubility of the pyrimidine alcohol in hot water. The precipitate obtained as described above is transferred to a beaker, covered with 50 cc. of water and heated nearly to boiling (85–90°). Practically the whole of the pyrimidine alcohol goes into solution, while if the heating is not continued too long, only negligible amounts of orotic acid dissolve. The mixture is rapidly filtered hot, and the extraction of the solid residue is repeated in the same way, using 25 cc. of water. The crude yellow residue of orotic acid is purified with norite and recrystallized from hot water, from which it separates in colorless rhombic blocks; yield, 2.0 g. It melted at 345°. A mixed melting point with known orotic acid showed no depression.

The alcohol, 4-hydroxymethyluracil, is obtained on concentrating and cooling the extraction liquors. It is purified with norite and recrystallized from a small quantity of hot water, separating in the form of colorless elongated prisms or plates, melting at 255–256°. A mixed melting point with the product previously described, obtained by desulfurization of 2-thio-4-hydroxymethyluracil, showed no depression, melting at 255°; yield, 1.6 g.

[8] Johnson and Chernoff, THIS JOURNAL, **36**, 1742 (1914).

2-Ethylmercapto-4-hydroxymethyluracil (A) and 2-Benzylmercapto-4-hydroxymethyluracil (B).—These two pyrimidines are easily prepared by alkylation of the sodium salt of 2-thio-4-hydroxymethyluracil with ethyl iodide and benzyl chloride, respectively, in alcohol solution. Practically quantitative yields of the mercapto pyrimidines are obtained. The pyrimidine (A) melts at 168°. It is easily soluble in alcohol, acetone and acetic acid, and slightly soluble in ether and benzene. When digested with hydrochloric acid ethyl mercaptan is evolved and 4-hydroxymethyluracil is formed, melting at 255°.

Anal. Calcd. for $C_7H_{10}O_2N_2S$: N, 15.05. Found: N, 15.14, 15.08.

The benzyl derivative (B) crystallizes from hot water in the form of needles melting at 156°. This pyrimidine is easily soluble in alcohol, acetone and acetic acid, and insoluble in ether and benzene. When digested with hydrochloric acid benzyl mercaptan and 4-hydroxymethyluracil are formed.

Anal. Calcd. for $C_{12}H_{12}O_2N_2S$: N, 11.29. Found: N, 11.18, 11.22.

2-Benzylmercapto-orotic Acid (A). 2-Ethylmercapto-orotic Acid (B) and 2-Methylmercapto-orotic Acid (C).—(A) This pyrimidine was prepared by the following modification of the method employed by Bachstez. Two grams of thio-orotic acid was dissolved in a solution of 1.3 g. of potassium hydroxide (two mols) in 24 cc. of an 80% alcohol–water mixture, and refluxed for three hours with 1.5 g. of benzyl chloride (one mol). At the end of this time the reaction mixture was diluted with 200 cc. of water and acidified with hydrochloric acid. The white solid separating out was recrystallized from a large quantity of hot water and came out in the form of small colorless prisms melting at 265° (corr.). Bachstez reports a melting point of 264° (corr.). The yield was 1.8 g. The product was difficultly soluble in alcohol and ether.

The structure of this compound is erroneously reported by Bachstez to be that of a N-benzyl-2-thio-orotic acid, the benzyl group being linked to one of the nitrogen atoms in the pyrimidine cycle. That the benzyl group is in fact linked to sulfur is shown by the ready conversion of the compound in question to orotic acid, with loss of benzyl mercaptan. A small amount of 2-benzylmercapto-orotic acid is dissolved in concentrated hydrochloric acid and refluxed for six hours. A strong mercaptan-like odor appears. On evaporating the solution to dryness and extracting the residue with hot water, a good yield of orotic acid is obtained.

(B) This pyrimidine is prepared by the action of ethyl bromide on the sodium salt of thio-orotic acid in alcohol solution. The pyrimidine is easily purified by recrystallization from hot water and separates on cooling in the form of colorless prisms melting at 248°. It is easily soluble in hot water and alcohol and insoluble in ether and benzene. When the pyrimidine was digested with hydrochloric acid ethyl mercaptan and orotic acid were formed quantitatively.

Anal. Calcd. for $C_7H_8O_3N_2S$: N. 14.00. Found: N, 13.92.

(C) This pyrimidine crystallizes from hot water in the form of prisms melting at 255°. It is converted quantitatively into orotic acid with evolution of methyl mercaptan when digested with hydrochloric acid.

Anal. Calcd. for $C_6H_6O_3N_2S$: N, 15.05. Found: N, 15.09.

Summary

1. The pyrimidine aldehyde, 2-thiouracil-4-aldehyde and uracil-4-aldehyde react normally toward alkali and undergo the Cannizzaro reaction, giving equivalent amounts of the corresponding pyrimidine acid and alcohol.

2. 2-Thio-orotic acid is desulfurized by the action of chromic acid, or with hydrogen peroxide in alkaline solution to give orotic acid quantitatively. The acid is not desulfurized by the action of chloroacetic acid.

3. 2-Thio-4-hydroxymethyluracil is desulfurized and converted quantitatively into 4-hydroxymethyluracil by digestion with chloroacetic acid in aqueous solution.

4. 2-Thio-orotic acid and 2-thio-4-hydroxymethyluracil interact with alkyl halides in alkaline solution to give alkyl derivatives with substitution on the sulfur atom.

5. The investigation of these compounds is being continued.

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SUBSTITUTED PHENYLDIHALOARSINES

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A number of substituted phenyldihaloarsines, not described hitherto, have been prepared in the hope that they might be used for the preparation of certain types of arsenicals under investigation in this Laboratory. Since many of them have been found to be unsuitable for our purpose, a brief description of them is published at this time.

Considerable difficulty was experienced in the preparation of many of the dihaloarsines in pure form. The following was found to be a very satisfactory general procedure: preparation of the arylarsonic acid, conversion of the acid into the corresponding aryldichloroarsine, purification of the latter by recrystallization from acetic acid or absolute ether, hydrolysis of the chloride into the arylarsine oxide and treatment of the latter at ordinary temperature with the desired halogen acid.

Arylarsine oxides are sometimes contaminated by small quantities of the arylarsonic acid and as a result of this contamination the preparation of the aryldihaloarsine is often very troublesome. Since the pure oxides usually possess rather indefinite melting points, the detection of the arsonic acid by means of a melting point determination cannot be relied upon. However, since the arylarsonic acid and concentrated hydriodic acid yield the corresponding aryldi-iodoarsine and free iodine, it is merely necessary to add a cubic centimeter of hydriodic acid to a fraction of a gram of the solid oxide in order to determine the presence or absence of the arsonic acid. If the oxide is pure, the mixture assumes the yellow color of the aryldi-iodoarsine; if even a slight amount of arsonic acid is present, the latter is detected by a deep red color due to the free iodine.