[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

RESEARCHES ON PYRIMIDINES. CXXII. IMPROVED METHODS FOR THE SYNTHESIS OF OROTIC ACID[1]

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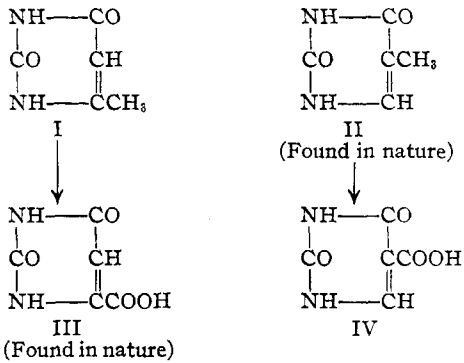
Uracil-4-carboxylic acid (orotic acid) III is the only carboxylated derivative of the pyrimidine cycle which has thus far been shown to occur in nature. It was discovered in milk by Biscaro and Belloni[3] in 1905. Regarding its origin or its function in the animal organism we have at present no information. Chemically speaking, it is an oxidation product of 4-methyl-uracil I, bearing the same structural relationship to this pyrimidine as uracil-5-carboxylic[4] acid IV does to thymine II. Behrend showed in 1910 that 4-methyluracil can be oxidized to the amide of uracil-4-carboxylic acid by potassium ferricyanide, but no one has succeeded in oxidizing thymine to the corresponding uracil-5-carboxylic acid. Furthermore, no one has, thus far, been successful in showing that 4-methyluracil occurs in nature.



The first investigator to prepare a derivative of uracil-4-carboxylic acid III was Müller,[5] who synthesized its ethyl ester by condensation of urea with diethyl oxaloacetate in acid solution. Müller did not prove, however, that he was dealing with a pyrimidine condensation product and it remained for Wheeler[6] of the Yale Laboratory to show, in 1907, that this ester of Müller's yields on saponification a true pyrimidine acid, namely, uracil-4-carboxylic acid III. Behrend and Struve[7] later found

[1] This work was reviewed in a preliminary paper presented at the Spring Meeting of the American Chemical Society held in Indianapolis, Indiana, in April, 1931.

[2] Sterling Research Fellow in Chemistry, 1930–1931.

[3] Biscaro and Belloni, *Estratto Annuario Soc. Chimica di Milano*, **11**, 1 (1905); *Chem. Zentr.*, II, 63 (1905).

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

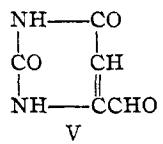
[5] Müller, *J. prakt. Chem.*, **56**, 488 (1897).

[6] Wheeler, *Am. Chem. J.*, **38**, 358 (1907).

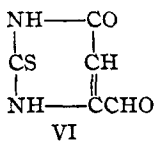
[7] Behrend and Struve, *Ann.*, **378**, 153 (1910).

that the amide of this pyrimidine acid is formed by oxidation of 4-methyluracil with potassium ferricyanide in ammoniacal solution, and showed that this is converted into the acid III by the action of alkali. The last investigator to describe the synthesis of uracil-4-carboxylic acid III was Bachstez,[8] who first prepared the ester by Müller's method and then transformed this into its acid III according to the procedure of Wheeler. Bachstez was the first one to prove that this pyrimidine acid III is identical with *orotic acid* previously separated from milk by Biscaro and Belloni. Behrend and Struve's oxidation method is of no practical value for the synthesis of uracil-4-carboxylic acid and to obtain it in quantity by condensation according to the Müller reaction is difficult on account of the low yield of pyrimidine formed. This is only about 15 to 20% of the theoretical amount.

By reconsideration and extension of our researches on pyrimidine aldehydes, which were discontinued in 1915, we have now been successful in developing a procedure for synthesizing orotic acid which is a great improvement over the methods of preparation described above. Our method is based on the discovery that the 4-aldehydic derivatives of uracil and 2-thiouracil,[9] which can be derived easily from ethyl-γ,γ-diethoxyacetoacetate by condensation with thiourea, are oxidized smoothly to orotic acid by chromic acid without destruction of the pyrimidine ring. The methods of synthesizing 2-thiouracil-4-aldehyde VI and uracil-4-aldehyde V first



V



VI

described by Johnson and Cretcher have been so greatly improved for practical application that orotic acid III can now be made in quantity without any difficulty. Various modifications in technique have been introduced for preparing the acid from different derivatives and these will be discussed in the experimental part of this paper. In every case the pyrimidine acid obtained has been found to be identical with the natural orotic acid. We are greatly indebted to Dr. Bachstez for sending us a generous supply of natural orotic acid obtained from milk, and also its potassium salt, for comparison with our synthetical product.

Experimental Part

Ethyl Diethoxyacetate, $(C_2H_5O)_2CHCOOC_2H_5$.—This ester was prepared by the method of Johnson and Cretcher.[9] The experience of former workers in this Laboratory

[8] Bachstez, *Ber.*, **63**, 1000 (1930); *Giornale di Chimica Industriale, Ed. Applicata*, **12**, 174 (1930).

[9] Johnson and Cretcher, THIS JOURNAL, **37**, 2144 (1915); *J. Biol. Chem.*, **26**, 99 (1916).

on the failure of commercial dichloroacetic acid to give satisfactory yields was verified. A very pure acid, best prepared from chloral hydrate according to the directions of Pucher,[10] is necessary. Attempts made to alkylate the moist silver salt of diethoxyacetic acid were not successful. The silver salt must be carefully dried before alkylation by spreading it out in thin layers and allowing it to stand in a vacuum desiccator over sulfuric acid in a dark place for several days. Due to the ease of decomposition, it cannot be dried by heating in a vacuum oven. Working in lots of 200 g. of dichloroacetic acid, 150 g. of the pure ester having a boiling point of 94–98° at 18 mm. pressure is easily obtained. This represents a yield of 55% of the theoretical.

Ethyl-γ,γ-diethoxyacetoacetate, $(C_2H_5O)_2CHCOCH_2COOC_2H_5$.—This β-ketone ester was prepared according to the directions of Johnson and Mikeska.[11] It was found very advantageous to wash the ethereal solution of the β-ketone ester with dilute sodium carbonate until free from acid, then to dry with sodium sulfate for ten to twelve hours. By this operation, a large part of the objectionable coloring matter is removed, resulting in a purer pyrimidine condensation product. One hundred and fifty grams of ethyl diethoxyacetate yield 150 g. of ethyl-γ,γ-diethoxyacetoacetate, or 80% of the theoretical amount.

Diethylacetal of 2-Thiouracil-4-aldehyde.—This pyrimidine was prepared by condensation of the above β-ketone ester with thiourea according to the method of Johnson and Cretcher.[9] One hundred and fifty grams of the β-ketone ester gave 110 g. of the pyrimidine, corresponding to 70% of the theoretical. It was recrystallized from 95% alcohol, separating in the form of thick, rhombic blocks, colorless or slightly yellowish and sparingly soluble in water. It melted at 160°.

2-Thiouracil-4-aldehyde. VI.—Twenty-five grams of recrystallized diethylacetal of 2-thiouracil-4-aldehyde is dissolved in 500 cc. of dilute hydrochloric acid (1:1). The solution is then heated to boiling and rapidly filtered through glass wool. On cooling, this pyrimidine aldehyde separates in the form of golden-yellow plates containing one molecule of water of crystallization. The yield is 18.0 g. or practically quantitative, and it melts with decomposition at 250°.

Diethylacetal of 2-Ethylmercaptouracil-4-aldehyde.—A solution of 2.5 g. of sodium in 250 cc. of absolute alcohol is prepared and cooled to room temperature. Twenty-five grams of the diethylacetal of 2-thiouracil-4-aldehyde is dissolved in the resulting solution and 15 g. of ethyl bromide is then added and the whole refluxed for two hours on the steam-bath. At the end of this period the reaction mixture is cooled, the precipitated sodium bromide filtered off and the filtrate evaporated to an oil on the steam-bath. On the addition of water, the oil solidifies to a white solid. It is recrystallized from a 40% alcohol–water solution and separates in the form of needles; yield, 24 g. or 85% of the theoretical. It melts at 128°.

2-Ethylmercaptouracil-4-aldehyde (V).—This pyrimidine aldehyde was first obtained by Johnson and Cretcher[9] by hydrolysis of the diethylacetal of 2-ethylmercaptouracil-4-aldehyde in hydrochloric acid solution. A better yield is obtained by using dilute acetic acid. Ten grams of the diethylacetal of 2-ethylmercaptouracil-4-aldehyde is dissolved in 100 cc. of dilute acetic acid (1:1) and the solution evaporated to dryness on the steam-bath. The aldehyde remains as a yellowish residue, soluble in hot water and alcohol, slightly soluble in ether and benzene. It is recrystallized from hot water, separating in the form of colorless, elongated prisms. It melted at 148–149° and the yield was 5.3 g. or 75% of the theoretical.

Oxidation of 2-Thiouracil-4-aldehyde to Orotic Acid, III.—An oxidizing solution is prepared by dissolving 25 g. of sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) and 30 g. of

[10] Pucher, THIS JOURNAL, **42**, 2251 (1920).

[11] Johnson and Mikeska, *ibid.*, **41**, 810 (1919).

concentrated sulfuric acid in 115 cc. of water. The solution is cooled to room temperature. Five grams of 2-thiouracil-4-aldehyde is now added in small portions at such a rate that the temperature does not rise above 75°. The solution is constantly stirred during the addition. A vigorous reaction occurs, which is soon completed. When all the aldehyde has been added the solution is allowed to stand for ten minutes, after which it is heated to boiling for one minute. During this time the orotic acid partially separates. After cooling, the solution is filtered with suction, and the precipitate washed with water. The crude orotic acid (4.1 g.) is obtained in quite pure form as a slightly yellowish powder. It is purified by dissolving in 350 cc. of water, decolorizing with norite, filtering and cooling the filtrate rapidly. The acid is obtained as a white crystalline powder. The yield was 3.8 g. or 76% of the theoretical. The melting point was 345° (corr.).

The essential points in this technique are the portionwise addition of the aldehyde, and the short period of boiling. If the aldehyde is added all at once, yields are decreased to about 40%. Furthermore, since orotic acid is slowly attacked by boiling chromic acid solutions, prolongation of the boiling period beyond one minute also slowly decreases the yield.

Identification of the Product.—The synthetic acid was shown to be identical in every respect with natural orotic acid sent us by Dr. Bachstez. Its melting point is 345° (corr.); that of natural orotic acid is 345–347° (corr.); a mixed melting point shows no depression, melting at 345° (corr.). It crystallizes from hot water in rhombic prisms often exhibiting a characteristic twinning habit similar to that of the natural product. It is soluble in potassium hydroxide solution, from which acetic acid precipitates, not the free acid, but the potassium salt as a mass of fine needles. Similarly, acetic acid precipitates the ammonium salt from a solution of the synthetic acid in aqueous ammonium hydroxide. The acid is soluble in cold concentrated sulfuric acid, from which it is precipitated unchanged (m. p. 344°) on addition of water. It is tasteless. It reacts neither with Fehling's solution, nor with ammoniacal silver nitrate. Silver nitrate precipitates an amorphous white solid, soluble in nitric acid, from its aqueous solution. Similarly, barium chloride precipitates a crystalline barium salt. According to the methods of Bachstez, the bromine derivative, dibromobarbituric acid and the potassium salt of 5-nitro-orotic acid were prepared from the synthetic acid. The former melted at 235° (uncorr.) and the latter showed the characteristic color change to yellow accompanying its decomposition to potassium nitro-uracil when heated to 130°.

Heated at 130°, the synthetic orotic acid loses one molecule of crystal water. 0.2240 g. of substance lost 0.0233 g. of H_2O: Calcd. for $C_5H_4O_4N_2 \cdot H_2O$: H_2O, 10.3. Found: H_2O, 10.4.

The acid was titrated in hot aqueous solution using phenolphthalein as an indicator. 0.3215 g. of substance required 18.7 cc. of 0.1295 *N* NaOH. Calcd. for $C_5H_4O_4N_2 \cdot H_2O$: 18.5 cc. 0.2582 g. required 14.9 cc. of 0.1295 *N* NaOH. Calcd., 14.8 cc.

Oxidation of 2-Thiouracil-4-aldehyde-diethylacetal, 2-Ethylmercaptouracil-4-aldehyde-diethylacetal and 2-Ethylmercaptouracil-4-aldehyde to Orotic Acid.—The 2-ethylmercaptouracil-4-aldehyde was oxidized by the method previously described for the oxidation of 2-thiouracil-4-aldehyde. Three grams of the ethylmercapto-aldehyde gave 1.4 g. of pure orotic acid, corresponding to 55% of the theoretical. It was found that in the case of the acetals, portionwise addition of the solid acetal to the oxidation mixture gave very low yields. However, fairly good yields could be obtained by boiling the acetals for a few minutes in dilute sulfuric acid to effect hydrolysis of the acetal grouping, and then adding the oxidizing mixture to the resulting solution.

Three grams of 2-thiouracil-4-aldehyde-diethylacetal is suspended in a solution of 20 cc. of concentrated sulfuric acid in 50 cc. of water. The mixture is boiled for several

minutes, when most of the acetal dissolves and the solution assumes the bright yellow color of the free aldehyde. The solution is now removed from the flame, allowed to cool somewhat, and a solution of 15 g. of sodium dichromate in 30 cc. of water added at such a rate that the temperature remains between 80 and 90°. At the end of the addition, the solution is boiled for another minute. The orotic acid is recovered as previously described; yield, 1.5 g. or 68%.

Similarly, by the above procedure, 3 g. of 2-ethylmercaptouracil-4-aldehyde-diethylacetal gave 1.0 g. of pure orotic acid, corresponding to 53% of the theoretical.

Preparation of the Diethylacetal of Uracil-4-aldehyde from the Diethylacetal of 2-Thiouracil-4-aldehyde.—Two grams of 2-thiouracil-4-aldehyde diethylacetal is covered with 60 cc. of 3% hydrogen peroxide and 15 cc. of 20% sodium hydroxide. The pyrimidine readily dissolves, yielding a colorless solution. Considerable heat is developed, the temperature rising to about 50°. The solution is now heated to a gentle boil for three minutes. It is then cooled in ice water and acidified with concentrated hydrochloric acid. Large amounts of sulfur dioxide are evolved, and separation of the sulfur-free acetal usually begins spontaneously. Occasionally vigorous scratching of the sides of the beaker with a glass rod is necessary to induce crystallization. The latter appears to be favored by a considerable excess of hydrochloric acid. The reaction mixture is allowed to stand at room temperature for several hours. The crude acetal is then filtered off, washed with cold water and recrystallized from a small amount of hot water. The diethylacetal of uracil-4-aldehyde separates in the form of colorless hexagonal prisms, melting sharply to a clear oil at 179°: yield, 1.8 g., or practically quantitative. The purified product gives no test for sulfur. It is easily soluble in the cold in methyl alcohol, ethyl alcohol, acetone and acetic acid, and insoluble in ether, benzene and ligroin. By oxidation with a $Na_2Cr_2O_7$–H_2SO_4 mixture as previously described, a good yield of orotic acid is obtained.

Anal. (Kjeldahl). Calcd. for $C_9H_{14}O_4N_2$: N, 13.08. Found: N, 13.05, 13.15.

Uracil-4-aldehyde, V.—A quantitative yield of this interesting pyrimidine aldehyde may be obtained by the careful hydrolysis of the corresponding uracil-4-aldehyde-diethylacetal. Two grams of the latter compound is dissolved in 25 cc. of dilute hydrochloric acid (one part concentrated acid and two parts water) and the solution finally heated to boiling for one minute. The solution assumes a pinkish color, which deepens on prolonged boiling. On cooling in ice water and scratching the beaker with a glass rod, the uracil-4-aldehyde separates out as a white solid. After standing for several hours, the reaction product is filtered off, washed with cold water and recrystallized from a small amount of hot water or dilute hydrochloric acid. The aldehyde separates in the form of colorless elongated prisms arranged in rosets; yield, 1.5 g. It does not possess a sharp melting point, but turns yellow when heated to about 260° and carbonizes without melting at 273–275°. This behavior is not changed by repeated crystallization from dilute hydrochloric acid. Uracil-4-aldehyde reduces Fehling's solution on warming, and ammoniacal silver nitrate in the cold. It gives a good Schiff's aldehyde reaction. With phenylhydrazine it yields a crystalline yellow hydrazone in the cold, decomposing at 330°. The aldehyde is very sensitive to the action of hot mineral acids. When boiled for a short time with concentrated hydrochloric acid its solution assumes a deep red color. It is much less soluble in the common organic solvents than the corresponding acetal, being insoluble in ether, benzene, ligroin and acetone, and moderately soluble in hot methyl and ethyl alcohol and hot acetic acid. By means of $Na_2Cr_2O_7$–H_2SO_4 mixture, it is easily oxidized to orotic acid. The aldehyde contains a molecule of water of crystallization, which it loses when heated to 120° for one hour.

Anal. (Kjeldahl). Calcd. for $C_5H_4O_3N_2 \cdot H_2O$: N, 17.72. Found: N, 17.66, 17.80. 0.4320 g. of substance lost 0.0497 g. of H_2O. Calcd.: 0.0492 g.

Summary

1. Uracil-4-carboxylic acid (orotic acid) known to occur in milk is most easily prepared in quantity by oxidation of 2-thiouracil-4-aldehyde or uracil-4-aldehyde with chromic acid.

2. The uracil-4-carboxylic acid thus obtained is identical with the natural orotic acid from milk furnished to us for comparison by Dr. Bachstez of Italy.

3. This is the only pyrimidine carboxylic acid thus far known to occur in nature.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

ALPHA PIPERIDINO BENZALACETOPHENONE

BY E. P. KOHLER AND W. F. BRUCE

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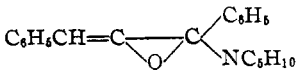
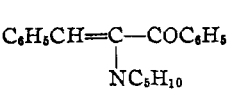
In a recent paper Kohler and Addinall[1] expressed the opinion that a peculiar red piperidine derivative which was first described by Watson[2] and later studied with care by Dufraisse and Moureu[3] is an unsaturated ethylene oxide (I). This view is erroneous; the substance is an unsaturated ketone and it has the structure assigned to it by Dufraisse and Moureu (II).

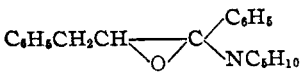


I

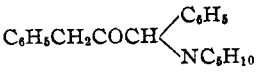


II

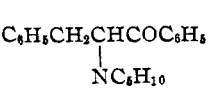
As a means of discrimination between the two formulas all methods of degradation by oxidation proved useless, but fairly convincing evidence was obtained from a series of transformations based on hydrogenation. In the presence of platinum the red compound combines with two atoms of hydrogen and forms a yellow addition product which reacts with a mole of methyl magnesium iodide without liberating gas. This compound might, therefore, be either a saturated oxide or one of two saturated ketones.



III



IV



V

The yellow reduction product can be made by the action of piperidine on α-bromo benzylacetophenone and it reacts with hydroxylamine like

[1] Kohler and Addinall, THIS JOURNAL, **52**, 3728 (1930).

[2] Watson, *J. Chem. Soc.*, **85**, 1322 (1904).

[3] Dufraisse and Moureu, *Bull. soc. chim.*, [4] **41**, 457 (1927).