A NEW SYNTHESIS OF CYTOSINE AND 5-METHYLCYTOSINE

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The chief difficulty in the way of a fruitful synthesis of cytosine from uracil is the essentially equal reactivities of chlorine atoms and of ethoxyl groups in the 2 and 4 positions of the pyrimidine nucleus. The reaction of 2,4-dichloropyrimidine with ammonia leads to a mixture of chloroaminopyrimidines (1) which is separable only after transformation to the methoxyaminopyrimidines. The reaction of dichloropyrimidine with sodium ethoxide has been offered as an alternative (2), since the diethoxypyrimidine so formed, on further treatment with sodium ethoxide, is converted to a mixture of ethoxyhydroxypyrimidines, the sodium salts of which are separable. Since the ethoxyl groups are replaceable by amino groups, the individual isomers can be converted to cytosine and isocytosine respectively. Both methods have been used in preference to the original method of Wheeler and Johnson (3, 4) which proceeds from 2-ethylmercapto-4-hydroxypyrimidines via chlorination, amination, and subsequent hydrolysis of the ethylmercapto grouping.

The discovery that the 4-thiol group of 2,4-dithiopyrimidines is much more reactive toward ammonia and amines than is the 2-thiol group has opened up a new route to the synthesis of 4-aminopyrimidine derivatives. The ready availability of dithiopyrimidines from thiol-, hydroxy-, and alkylmercaptopyrimidines (5) allows a considerable latitude in the choice of starting materials. From dithiouracil (I) or dithiothymine (II) the 2-

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\text{(I)} \quad R = H \\
\text{(II)} \quad R = CH_3
\]

alkylmercaptopyrimidines (5) allows a considerable latitude in the choice of starting materials. From dithiouracil (I) or dithiothymine (II) the 2-

thiol-4-aminopyrimidines (III, IV) are obtained in good yield, and the subsequent hydrolysis of the carboxymethylthiopyrimidines (V, VI) to the 2-hydroxy-4-aminopyrimidines (VII, VIII) proceeds without difficulty. An over-all yield of 60 per cent reckoned from dithiouracil or 40 per cent from thiouracil is obtainable by this method. This may be contrasted with the 20 per cent yield from uracil obtainable by the method of Hilbert and Johnson (1).

EXPERIMENTAL

2-Thiol-4-aminopyrimidine—Dithiouracil (2 gm.) was dissolved in 20 ml. of concentrated ammonium hydroxide and the solution heated in a sealed tube at 100° for 16 hours. On cooling, 2-thiol-4-aminopyrimidine (1.1 gm.) separated as long, colorless needles. Evaporation of the mother liquors to a small volume gave another 0.5 gm. (total yield, 91 per cent). The product was washed with concentrated ammonium hydroxide to remove any unchanged dithiouracil and recrystallized from 70 ml. of water. It formed long, colorless needles, m.p. 285-290° (decomposition), after darkening at about 250°.

C₄H₇N₃S. Calculated, C 37.8, H 3.9; found, C 38.2, H 4.2

2-Carboxymethylthio-4-aminopyrimidine—The above thiolaminopyrimidine (1 gm.) was refluxed with a solution of 0.7 gm. of chloroacetic acid in 7 ml. of water for 45 minutes. After cooling, the solution was neutralized with 2.5 N sodium hydroxide solution, acidified with acetic acid, and allowed to stand. Platelets separated (1.1 gm., 80 per cent) which, after recrystallization from a mixture of alcohol and ether, melted at 220° (decomposition), after darkening above 200°.

C₆H₈N₃O₂S. Calculated, C 38.9, H 3.8; found, C 39.3, H 3.8

Cytosine (2-Hydroxy-4-aminopyrimidine)—The above carboxymethylthiopyrimidine (1.05 gm.) was dissolved in 10 ml. of concentrated hydrochloric acid and refluxed gently for 2 hours. After evaporation to dryness on the steam bath, the residue was treated with dilute hydrochloric acid and evaporated to dryness again. On the addition of 5 ml. of water and 1 ml. of concentrated ammonium hydroxide shiny platelets were formed (0.5 gm., 83 per cent). After recrystallization from 10 ml. of water, the compound melted at 312° (decomposition), with darkening above 290°.

C₅H₈N₄O. Calculated, C 43.2, H 4.5, N 37.8; found, C 43.4, H 4.4, N 37.7

The substance gave a picrate decomposing at 333°.

The ultraviolet absorption spectra of this specimen of cytosine agree with the published values of Stimson and Reuter (6), giving in 0.1 N hydrochloric acid solution a maximum at 275 mλ, \( E_m = 10,450 \); in 0.1 N sodium hydroxide solution a maximum at 281 mλ, \( E_m = 7000 \). In glycine-sodium
hydroxide buffer at pH 11, the absorption is almost identical with that in unbuffered aqueous solution (maximum at 267 mμ, E_m = 6150).

2-Thiol-4-amino-5-methylpyrimidine—Dithiothymine (2.5 gm.) was dissolved in 50 ml. of concentrated ammonium hydroxide and heated at 100° for 16 hours in a sealed tube. After cooling, the white needles were filtered off (1.5 gm.). Evaporation of the mother liquors yielded another 0.2 gm., giving a total yield of 95 per cent. The product was purified by washing the crystals with ammonium hydroxide and recrystallization from water, m.p. 273–274° (decomposition).

CsH,N$. Calculated, C 42.6, H 5.0; found, C 42.3, H 4.9

2-Carboxymethylthio-4-amino-5-methylpyrimidine—1 gm. of 2-thiol-4-amino-5-methylpyrimidine was refluxed with a solution of 0.67 gm. of chloroacetic acid in 10 ml. of water for 30 minutes. On cooling, a small amount of yellow material separated which was removed by filtration. The filtrate was neutralized with 2.5 N sodium hydroxide solution and brought back to pH 6 with acetic acid. The product separated as colorless needles (1.1 gm., 79 per cent). After recrystallization from a 1:1 mixture of alcohol and ether, it melted at 193–194°.

C₇H₉O₂N₅S. Calculated, C 42.2, H 4.5; found, C 42.4, H 4.5

5-Methylcytosine (2-Hydroxy-4-amino-5-methylpyrimidine)—The above carboxymethylthiopyrimidine (1 gm.) was refluxed for 2 hours with 5 ml. of concentrated hydrochloric acid. On neutralization of the reaction mixture with ammonium hydroxide, the basic hydrochloride of 5-methylcytosine, (CsH,0N₃)₆.3H₂O.2HCl (4), separated and was filtered off (0.6 gm., 67 per cent). This product was converted to the monohydrochloride by solution in 2.5 N hydrochloric acid and precipitation with acetone and recrystallized by the same procedure. It formed colorless platelets, m.p. 299–301° (decomposition), after sintering at about 280°.

C₇H₈O₃N₄.HCl. Calculated, C 37.2, H 4.3, N 26.0, Cl 22.0

The picarete melts at 290–291° (decomposition).

The ultraviolet absorption spectra of this 5-methylcytosine at pH 1.0 and at pH 11.0 are given in Fig. 1. These curves are identical with those given by an authentic specimen of 5-methylcytosine which was prepared by the method of Wheeler and Johnson (4).

5-Methylcytosine from 2-Thiol-4-amino-5-methylpyrimidine—In an early experiment, 5-methylcytosine was prepared from 2-thiol-4-amino-5-methylpyrimidine without attempting the isolation of the thioglycolic acid derivative. 2-Thiol-4-amino-5-methylpyrimidine (3.9 gm.) was refluxed with a solution of 4 gm. of chloroacetic acid in 50 ml. of water for 16 hours. Concentrated hydrochloric acid (10 ml.) then was added, and refluxing
was continued for an additional 4 hours. On evaporation to dryness and treatment with ammonium hydroxide, 3.05 gm. (74 per cent) of the basic hydrochloride were obtained. Later experience in the preparation of the 2-carboxymethylthio-4-amino-5-methylpyrimidine (see above) indicated that the time of refluxing with chloroacetic acid was unnecessarily protracted; however, this experiment indicates that there may be some advantage with respect to yield in omission of the isolation of the intermediate.

Fig. 1. Ultraviolet absorption spectra of 5-methylcytosine at pH 1, dash line; at pH 11, solid line.

SUMMARY

A new and relatively productive method for the synthesis of cytosine and 5-methylcytosine is described. This method is based on the conversion of the requisite 2,4-dithiopyrimidine to the 2-thiol-4-amino derivative and subsequent hydrolysis of the latter to the 2-hydroxy-4-aminopyrimidine.

BIBLIOGRAPHY

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