Summary

1. Five new iodine derivatives of *p*-hydroxydiphenyl-sulfide have been described, namely: 4-hydroxy-2,6-di-iododiphenyl-sulfide, 4-hydroxy-2,6-di-iodo-4′-methyl-diphenyl-sulfide, 4-hydroxy-3,5-di-iododiphenyl-sulfide, 4-hydroxy-3,5-di-iodo-4′-methyldiphenyl-sulfide and 4-hydroxy-4′-iododiphenyl-sulfide.

2. All of these phenols are characterized by their extreme insolubility in water; consequently they do not exhibit any bactericidal activity when tested in the usual way.

3. The study of phenolic derivatives of diphenylsulfide will be continued.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

RESEARCHES ON PYRIMIDINES. CXIII. AN IMPROVED METHOD FOR THE SYNTHESIS OF CYTOSINE[1]

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The pyrimidine—*cytosine*—was first isolated from a nucleic acid by Kossel and Neumann[3] in 1894. It was subsequently synthesized by Wheeler and Johnson[4] and the structure of this naturally occurring compound thereby definitely established.

The successful synthesis developed by Wheeler and Johnson involves the condensation of a pseudothiourea with the sodium salt of ethyl formylacetate, resulting in the formation of a 2-mercapto-6-oxypyrimidine, I. By interaction of this cycle with phosphorus pentachloride, the corresponding 2-mercapto-6-chloropyrimidine, II, is formed, which is finally converted into the corresponding aminopyrimidine, III, by the action of ammonia. This latter pyrimidine, when digested with hydrochloric acid, is changed almost quantitatively into the hydrochloride of cytosine, IV. These various changes are expressed by the following formulas

$$RSC(NH_2)=NH + NaOCH=CHCOOC_2H_5 \longrightarrow$$

$$\underset{\text{I}}{NHC(SR)=NCH=CHCO} \longrightarrow \underset{\text{II}}{NC(SR)=NCH=CHCCl}$$

$$\underset{\text{III}}{NC(SR)=NCH=CHCNH_2} \longrightarrow \underset{\text{IV}}{NCONHCH=CHCNH_2}$$

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[1] A preliminary report of this research was given at the Spring Meeting of the American Chemical Society held in Columbus, Ohio, in April, 1929.

[2] Sterling Research Fellow 1928–1929.

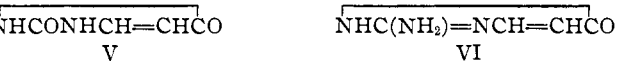
[3] Kossel and Neumann, *Ber.*, **27**, 2215 (1894).

[4] Wheeler and Johnson, *Am. Chem. J.*, **29**, 492, 505 (1903).

Notwithstanding the fact that the technique of this method of synthesis is difficult for an inexperienced worker to apply, it is the only one of practical interest recorded in the literature that has served to make this pyrimidine available in quantity for experimental work. A serious objection to the procedure, however, is the evolution of mercaptan, which is necessarily formed during the last hydrolysis change with acids when cytosine IV is formed. We have now developed a new method of operating which not only eliminates the use of sulfur compounds but also simplifies the technique of the synthesis.

The improved method for obtaining uracil, V, synthetically which was developed by Davidson and Baudisch[5] has opened up a new approach to many important derivatives of this oxypyrimidine. They have shown that uracil can be obtained in good yield by condensing urea with malic acid in the presence of fuming sulfuric acid. By applying this excellent method of synthesis and using uracil V as the starting point of our work, we have now succeeded in developing a new and practical procedure for obtaining cytosine IV easily. In fact, our method of operating permits of the preparation of both cytosine IV and isocytosine VI directly from uracil V.

$$HOOCCH_2CH(OH)COOH + NH_2CONH_2$$



V VI

By allowing 2,6-dichloropyrimidine, VII, to interact with ammonia in alcohol solution at 100°, Gabriel[6] obtained a mixture of the two isomeric pyrimidines represented by formulas VIII and IX, respectively. A partial separation of these two compounds was effected by fractional crystallization from ethyl acetate, but a yield of 15% only of 2-chloro-6-aminopyrimidine (m. p. 206–207°) IX was obtained. The remaining fraction was designated as 2-amino-6-chloropyrimidine, VIII, and was obviously highly impure, being contaminated with its isomer. Wheeler[7] later prepared these two isomers in an impure state and examined the behavior of a mixture on reduction. By digestion in water with zinc dust he found 2-amino-6-chloropyrimidine, VIII, to be reduced to 2-aminopyrimidine, while 2-chloro-6-aminopyrimidine, IX, was not changed by the treatment.

We have repeated the work of Gabriel and find that the reaction between 2,6-dichloropyrimidine, VII, and ammonia takes place smoothly at ordinary temperature. The yield of 2-chloro-6-aminopyrimidine, IX, and 2-amino-6-chloropyrimidine, VIII, obtained is about 40 and 60%, respectively. Attempts to separate these isomers by fractional crystallization from various solvents proved unsatisfactory. Purification was finally

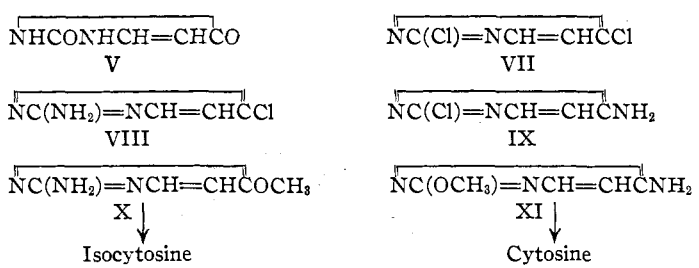
[5] Davidson and Baudisch, THIS JOURNAL, **48**, 2379 (1926).

[6] Gabriel, *Ber.*, **38**, 1689 (1905).

[7] Wheeler, *J. Biol. Chem.*, **3**, 288 (1907).

effected by means of steam distillation, 2-amino-6-chloropyrimidine, VIII, distilling easily, whereas its isomer remained behind. Unfortunately, secondary reactions take place during steam distillation which reduce the yields. The chlorine atom in 2-amino-6-chloropyrimidine, VIII, is much more reactive, as Wheeler observed, than the chlorine in the isomeric form, IX. This was shown by the difference in behavior of the two compounds toward the Wheeler and Johnson color test.[8] The former, VIII, gave a positive color test characteristic for isocytosine, while the isomer, 2-chloro-6-aminopyrimidine, IX, failed to give a corresponding reaction. The behavior on hydrolysis also revealed the difference in reactivity of the chlorine atom in these respective isomers. On heating with water 2-amino-6-chloropyrimidine, VIII, gave isocytosine, whereas the 2-chloro-6-aminopyrimidine, IX, failed to react. Hydrolysis of the latter to cytosine was brought about, however, by heating with water at 140°. The partial destruction of the chloro-aminopyrimidine with formation of uracil made it impossible, however, to obtain cytosine by this method in good yields.

A satisfactory method for preparing cytosine was finally developed by first transforming the chloro-aminopyrimidines into their corresponding methoxy derivatives by interaction with sodium methylate. The resulting isomeric methyl ethers were easily separated by fractional crystallization from hot water, in which 2-amino-6-methoxypyrimidine, X, is extremely soluble. By this method we were able to obtain consistently yields of 2-methoxy-6-aminopyrimidine, XI, from 2,6-dichloropyrimidine, VII, corresponding to 40% of the theoretical. When this pyrimidine, XI, was warmed on the steam-bath with hydrochloric acid, practically a quantitative yield of cytosine hydrochloride was obtained. The various transformations discussed above are expressed by the following formulas.



Experimental Part

Preparation of 2,6-Dichloropyrimidine, VII.—A yield of this pyrimidine corresponding to 50% of the theoretical was obtained by Johnson and Menge[9] by interaction of 2-thiouracil with phosphorus pentachloride. Gabriel[6] obtained this pyrimidine in a

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

[9] Johnson and Menge, *ibid.*, **2**, 115 (1906).

yield of 80% by heating uracil with phosphorus oxychloride under pressure at a temperature of 140°. He worked with very small quantities of uracil (2.5 g.), and his technique is not suitable for preparing the dichloropyrimidine from uracil in quantity. We recommend the following procedure for the preparation of this compound.

One hundred grams of uracil, prepared according to the method of Davidson and Baudisch,[5] is suspended in 400 cc. of phosphorus oxychloride and the mixture heated at 110–120° for five hours. The uracil rapidly dissolves with evolution of hydrochloric acid, yielding a dark brown homogeneous solution. After removing the excess of phosphorus oxychloride by distillation under diminished pressure, 750 g. of crushed ice is then gradually added to the dark-colored viscous residue. The temperature of the reaction mixture was not allowed to rise above 20° during this treatment because of the ease of hydrolysis of the dichloropyrimidine to uracil. During the addition of the ice, ether was also added to facilitate the decomposition of a complex phosphorus compound which was present. The pyrimidine is slightly basic, forming a hydrochloride very soluble in ether. After removing the ether layer, the strongly acid solution was repeatedly extracted with ether and the combined extracts washed with sodium carbonate solution and finally dried over calcium chloride. After removal of the ether the dichloropyrimidine was then purified by distillation. It boiled at 203° (uncorr.) and solidified on cooling, melting at 61°. A yield of 90 g. was obtained equivalent to 68% of the theoretical. The pyrimidine is a strong lachrymator and has an irritating effect on the skin.

Interaction of 2,6-Dichloropyrimidine with Ammonia. The Formation of 2-Amino-6-chloropyrimidine, VIII, and 2-Chloro-6-aminopyrimidine, IX.—This chloride reacts with ammonia at ordinary temperature. Forty grams of the pulverized dichloropyrimidine is dissolved in 300 cc. of absolute alcohol previously saturated with ammonia gas and the solution preserved in a stoppered bottle. The reaction starts almost immediately and after standing for an hour the solution warms up appreciably and a crystalline solid commences to separate from the alcohol. After allowing to stand for about eighteen hours the solid material is filtered off and the alcohol filtrate evaporated to expel the excess of alcohol and ammonia and recover dissolved pyrimidines. The total reaction product is washed thoroughly with cold water to remove ammonium chloride and then dried. We obtained 33 g. of the mixed chloro-aminopyrimidines, corresponding to a yield of 95% of the theoretical.

Of the various methods applied for separating the two isomeric chloro-aminopyrimidines, the following was as satisfactory as any thus far reported. Thirty-three grams of the mixed pyrimidines was suspended in 200 cc. of water made alkaline with 15 cc. of concentrated ammonia and then subjected to steam distillation. The 2-amino-6-chloropyrimidine distilled over as a colorless solid and was obtained in a pure state after one crystallization from alcohol. The yield was 4.0 g. In order to obtain the isomeric 2-chloro-6-aminopyrimidine, the hot aqueous solution was filtered free from a small amount of flocculent impurity and then chilled, when the pyrimidine separated in a crystalline condition and was further purified by crystallization from hot water. The yield obtained was 10 g. As indicated by the yields obtained, over 50% of the original mixture of pyrimidines is destroyed by the treatment with steam. In fact, in one experiment a considerable proportion of the 2-chloro-6-amino-pyrimidine was converted into cytosine.

2-Amino-6-chloropyrimidine, VIII.—This crystallizes from alcohol in glistening plates which turn brown on heating and decompose at 168°. Gabriel and Colman[10] prepared the pyrimidine by treatment of isocytosine with phosphorus oxychloride. This pyrimidine responds to the Wheeler and Johnson color test for isocytosine and forms an

[10] Gabriel and Colman, *Ber.*, **36**, 3383 (1903).

insoluble picrate which decomposes at an indefinite temperature (270°), depending on the rate of heating.

Anal. Calcd. for $C_4H_4N_3Cl$: Cl, 27.39. Found: Cl, 27.27, 27.34.

This pyrimidine is formed almost exclusively when 2,6-dichloropyrimidine is warmed with concentrated aqueous ammonia at 50°.

2-Chloro-6-aminopyrimidine, IX.—This pyrimidine crystallizes from hot water in long needles melting with decomposition at 219–220°. The compound is somewhat more soluble in water and alcohol than its isomer. Unlike 2-amino-6-chloropyrimidine, it does not respond to the Wheeler and Johnson color test.

Anal. Calcd. for $C_4H_4N_3Cl$: Cl, 27.39. Found: Cl, 27.69, 27.49.

Formation of Isocytosine.—One gram of 2-amino-6-chloropyrimidine was refluxed with 50 cc. of water for twenty hours. The solution had then assumed a yellow color and was strongly acid to litmus. After adding ammonia to neutralize hydrochloric acid, the solution was concentrated to a volume of about 20 cc. and allowed to cool slowly, when stout prisms of isocytosine separated. They melted at 280° with decomposition, were free from chlorine and responded to the Wheeler and Johnson color test characteristic for isocytosine. Aqueous solutions of this base have a tendency to gel when cooled quickly. It is also of interest to mention here that a solution of this pyrimidine supports the growth of a mold when exposed to the air.

Formation of Cytosine.—Four grams of 2-chloro-6-aminopyrimidine was heated with 20 cc. of water at 140° for two hours. A clear solution was obtained which was acid to litmus. After making slightly alkaline with ammonia and cooling in an ice-bath, a mixture of cytosine and uracil separated. This was separated by filtration, treated with dilute hydrochloric acid to separate the uracil and the acid solution made alkaline with ammonia and cooled. The cytosine separated in crystalline condition and was further purified by crystallization from hot water. The total yield was equal to 85% of the theoretical. The base contained one molecule of water of crystallization, which was lost by heating at 100°.

Anal. Calcd. for $C_4H_5ON_3 \cdot H_2O$: H_2O, 13.95; N, 37.84 (anhydrous base). Found: H_2O, 13.96; N, 37.74 (anhydrous base).

2-Chloro-6-aminopyrimidine was recovered unchanged after refluxing a water solution for twenty hours. After heating with water for several hours at 190° a mixture of cytosine and uracil was obtained, the uracil predominating.

Conversion of the Aminochloropyrimidines, VIII and IX, into Their Corresponding Methoxypyrimidines, X and XI

2-Methoxy-6-aminopyrimidine, XI.—A solution of 57 g. of a mixture of the isomeric pyrimidines (VIII and IX) in 700 cc. of dry methyl alcohol was combined with a methyl alcohol solution containing 12 g. of sodium and the mixture refluxed for six hours. Sodium chloride separated immediately and within a short time the reaction was complete. After filtering from sodium chloride, the solution was concentrated to a volume of 100 cc. and cooled, when the above pyrimidine separated in a practically pure form. It crystallized in the form of needles which melted at 173°. The yield was 16.5 g. The alcohol filtrate was then evaporated to dryness in a blast of dry air and the residue, weighing 35 g., dissolved in boiling water; the solution was chilled after decolorizing with norite. Eight grams more of the above pyrimidine separated. On recrystallizing all our product from water, we obtained 22 g. or about 40% of a theoretical yield. This pyrimidine crystallizes in bundles of colorless prisms which shrivel in a capillary tube at 169° and melt at 174°.

Anal. Calcd. for $C_5H_7ON_3$: N, 33.60. Found: N, 33.69, 33.50.

2-Amino-6-methoxypyrimidine, X.—This pyrimidine is recovered from the above aqueous filtrates from which the 2-methoxy-6-aminopyrimidine has been separated. This compound has previously been described by Gabriel and Colman,[10] who have shown that it is converted to isocytosine by hydrolysis with hydrochloric acid.

A practically quantitative yield of 2-amino-6-methoxypyrimidine is formed by treatment of 2-amino-6-chloropyrimidine with sodium methylate. It crystallizes from water in the form of colorless prisms which melt at 125° to an oil. After sublimation the melting point was raised to 125–126°. An attempt to separate and purify the two isomeric methoxypyrimidines, X and XI, by fractional sublimation was unsuccessful.

Formation of Cytosine by Hydrolysis of 2-Methoxy-6-aminopyrimidine, XI.—Five grams of this pyrimidine was dissolved in concentrated hydrochloric acid and the solution then evaporated to dryness by heating on a steam-bath. The crude hydrochloride of cytosine was dissolved in water, the solution clarified by filtration and finally made alkaline with ammonia. The free base was thereby precipitated in a crystalline condition and was purified by crystallization from hot water, separating in large colorless plates. The product was identical in all respects with cytosine prepared from 2-chloro-6-aminopyrimidine. The yield was quantitative.

Anal. Calcd. for $C_4H_5ON_3 \cdot H_2O$: H_2O, 13.95; N, 37.84 (anhydrous base). Found: H_2O, 14.00; N, 37.61, 38.1 (anhydrous base).

Summary

1. An improved method for the preparation of 2,6-dichloropyrimidine is described.

2. A further study has been made of the chemical properties of the two isomers—2-chloro-6-amino- and 2-amino-6-chloropyrimidine.

3. The isomeric 2-methoxy-6-amino- and 2-amino-6-methoxypyrimidines have been synthesized. Both pyrimidines are transformed quantitatively into cytosine and isocytosine, respectively, when warmed in aqueous solution with hydrochloric acid.

4. The new method developed for the preparation of cytosine directly from uracil is an improvement over the older method of Wheeler and Johnson which involves the use of pseudothioureas.

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