PYRIMIDINES: THEIR AMINO AND AMINOÖXY DERIVATIVES

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Received February 24, 1933

INTRODUCTION

The chemistry of the pyrimidines has never been completely systematized, and since a knowledge of this class of cyclic substances has become increasingly important as a result of recent developments in biochemistry, an attempt to organize this material is now being made. The field to be covered is so very extensive, however, that only one particular section of it can be reviewed at this time. A group, which includes the pyrimidine hydrocarbon cycle,[1] together with the amino and aminoöxy derivatives, has been selected because of the fact that it includes pivotal substances such as cytosine, 5-methylcytosine, and divicine, which have been shown to occur in different combinations in both plant and animal tissues. In order to show the position which these and certain other derivatives of pyrimidine occupy with respect to some of the fundamental problems of biochemistry, it seems desirable by way of an introduction to review briefly the relationships which have now been established between pyrimidines and their precursors in biochemistry,—the nucleic acids. This material is so very voluminous that it can be treated merely in outline and only so far as it serves as a necessary prelude to any systematic study of pyrimidine chemistry.

[1] Benzene being $(CH)_6$, pyrimidine is $(CH)_4N_2$.

The fact that the fundamental units of growth and reproduction are localized in the nucleus of the cell has long been recognized by biologists. It has also become of constantly increasing interest to chemists with the general acceptance of the idea that the characteristic features which serve to differentiate the cells of the different organs and also the cells of bacteria inimical to man, are to be found in the typical hydrolytic products which result from cell degradation. Of these the class of substances now generally known as "nucleic acids" is perhaps the most important. Developments in the investigation of substances belonging to this class have been exceedingly rapid in recent years, so that a number of fairly clearly defined individuals have been isolated and identified. Typical examples of substances of this kind are yeast nucleic acid, thymus nucleic acid, tuberculinic acid, etc. While it frequently happens that the name of the acid immediately suggests its origin, this is of course not always as obvious as in the case of the illustrations just mentioned.

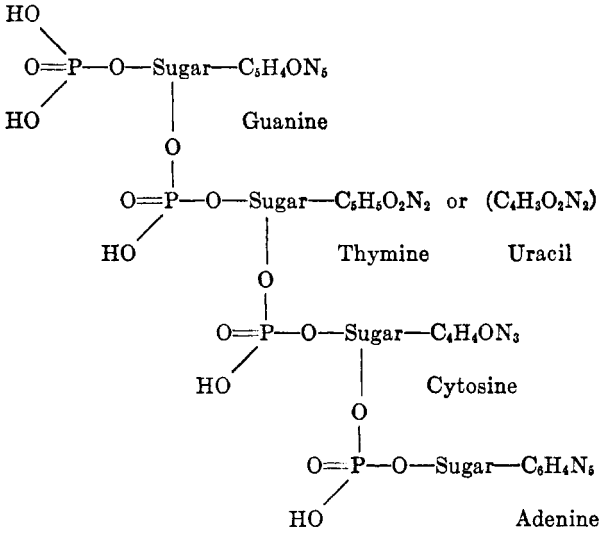
A study of nucleic acids has led to the discovery that while they are extremely complex substances, a certain regularity is to be observed in the processes by means of which they suffer degradation into simpler units. The generally accepted structure of the nucleic acid molecule has been developed as a result of intensive research in many widely separated fields, but nevertheless embodies certain fairly simple fundamental conceptions. For example, all individuals belonging to this class may be assumed to result from the association of somewhat simpler aggregates known as *nucleotides*. This term is applied to substances which are formed by the association of phosphoric acid with certain still less complex organic aggregates known as *nucleosides*. The latter are glucosidic in character and are composed of sugars linked to nitrogenous substances spoken of as nuclein bases. Since the physical and chemical properties of any given nucleoside depend upon the nature of the particular sugar and nuclein base which enter into its composition, it has been found convenient to classify them as "purine-riboses," "pyrimidine-hexoses," etc., according to the character of their components.

Such a classification accounts for the structure of the nucleoside

only in the most superficial way, since any given sugar or base (purine or pyrimidine) is known to be capable of existing in a great variety of different modifications. Moreover, since the union between any given sugar and purine or pyrimidine is severed under the action of hydrolytic agents, and since both the sugar and the cyclic base thus separated are subject to a number of different structural transformations under the experimental conditions, it follows that the particular individuals which are isolated as a result of the resolution of such glucosidic combinations may not always correctly represent the particular sugar and base actually in union in the nucleoside. Furthermore, the exact nature of this union is still far from clear. For example, if the nucleoside is found to consist of a definite sugar linked to a pyrimidine the question as to the exact point of union in the case of each molecule has still to be answered. And up to the present time none of the answers to this particular question is sufficiently supported by experimental evidence to be completely satisfactory.

In order to present these general relationships in a somewhat more concrete form it is necessary to consider two typical nucleic acids in somewhat greater detail. Thymus nucleic and yeast nucleic acids have been the most thoroughly investigated, and most of the fundamental knowledge of nucleic acid structure has been acquired by the study of their degradation products. The discovery of this group of organic substances was first made by Miescher (152) in 1871, and the term "nucleic acid" was furnished by Altmann (1). The general method of preparation applied with modification by later workers was also furnished by this later investigator. The first fundamental contributions regarding the structure of nucleic acids were made by Kossel and his school of workers. These developments practically began with the year 1879 when Kossel began his work on nucleins in the laboratory of Hoppe-Seyler. His first publication dealt with the nucleic acid of yeast (120). The first important work on the chemistry of thymus nucleic acid was reported by Kossel and Neumann in 1894 (122), and through the investigations of Kossel, Steudel, and other workers in Europe since that date the partial elucidation of the structure of the highly complex nucleic

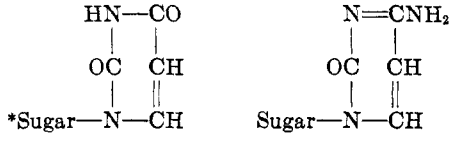
acid molecule has been accomplished. It remained, however, for Levene and his many collaborators in this country to show that nucleic acids (thymus and yeast) are actually composed of nucleosides united with one another as complex esters of phosphoric acid. On hydrolysis the molecule is finally broken down into phosphoric acid and four different nucleosides representing combinations of a sugar with cytosine, thymine (or uracil), adenine, and guanine, respectively. Although neither the exact configuration of the sugar nor its point of linkage with any of these nitrogenous cycles has as yet been determined, the following diagram showing the general arrangement of these components is today commonly accepted by biochemists as representing roughly the configuration of a nucleic acid molecule.



The outstanding difference in the constitutions of thymus and yeast nucleic acids revealed by recent research is to be found in the structure of the sugar group functioning in these two complex substances. The sugar component of thymus nucleic acid was concluded by Kossel and Neumann to be hexose, in view of its ready conversion on hydrolysis into levulinic acid. All workers on thymus nucleic acid held for years to this conception

of the hexose nature of the sugar, until Feulgen (36) called attention to errors in the analytical figures of earlier work, and questioned the conclusions of the hexose structure of the sugar. It remained for Levene and London (139) to give support to Feulgen's conclusions. They took up again the study of nitrogen-free degradation products of thymus nucleic acid and actually succeeded in isolating the sugar complex and in proving it to be a desoxypentose. The irregularity of the behavior of thymus nucleic acid, as compared to that of yeast nucleic acid, was thus shown to be due to the peculiar chemical behavior of this sugar.

It has further been demonstrated conclusively by Levene and his coworkers that yeast nucleic acid may be resolved into four nucleosides which represent combinations of the pentose sugar *d*-ribose with cytosine, uracil, adenine, and guanine, respectively, and that these combinations are present in equimolecular quantities in the yeast nucleic acid molecule. It was observed, for example, that in the hydrolysis of this acid only a part of its phosphoric acid was split off, leaving a mixture of two phosphorated substances which were named *uridylic* and *cytidylic acids* (127, 136b, 137). These represent the first degradation units of the nucleic acid molecule and are called nucleotides. They break down on further hydrolysis into the nucleosides and phosphoric acid. In the case of the two examples cited the configuration of the two nucleosides may be represented respectively in the following way:



Uridine Cytidine

*Sugar = *d*-ribose

For a complete account of the history and chemistry of nucleic acids the reader is referred to Monograph No. 56, entitled "Nucleic Acids", recently published under the auspices of the

American Chemical Society (135). The experimental evidence upon which the allocation of the sugar to the *N*-3-position in the pyrimidine ring depends will be reviewed later in connection with a somewhat detailed consideration of the various tests which may be applied in the detection of cytosine and its derivatives (136a, 138, 66d).

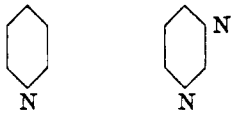
In this connection it may be noted that certain fundamental differences appear to exist in the sugar content of nucleic acids of animal or plant origin, the former yielding desoxypentose (*d*-ribodesose) (139, 142, 141) and the latter *pentose* sugars on hydrolysis. Fundamental differences have also been observed in the pyrimidine content of nucleic acids obtained from these two sources, since 5-methylcytosine and its deaminized product, thymine, are found among the degradation products of animal cells, while cytosine and its deaminized product, uracil, (but no thymine) are obtained along with other substances on intensive hydrolysis of plant tissues. Recently evidence has been obtained indicating that there may be exceptions to these observations leading to new generalizations of great significance.[2]

If, as has now been demonstrated, nucleic acids consist in part of pyrimidines in different forms of combination, any attempts to synthesize these extremely complicated aggregates must be based upon an exact and complete knowledge of the chemistry of the pyrimidines. This subject is, of course, too extensive for exhaustive treatment, but by confining the discussion in this paper to a limited section of this field, namely, to the reduced pyrimidines and their amino and aminoöxy derivatives, it will be possible to review the various types of syntheses and transformations which are typical of pyrimidines as a group, while avoiding many of the complicated problems which a complete survey of pyrimidine chemistry would involve.

Various individuals belonging to the general class of pyrimidines had been known for fifty years before Pinner in 1885 (161, 162a) called attention to the fact that all such substances could be

[2] Unpublished results.

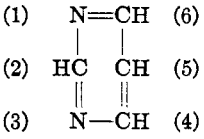
regarded as derivatives of a ring structure closely analogous to pyridine:



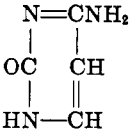
Pyridine Pyrimidine

In other words, pyrimidine consists of a heterocyclic carbon–nitrogen ring, and as such possesses strongly basic properties. Like pyridine it reacts with mineral acids to form stable salts, and its hydrochloride combines with the chlorides of gold and platinum to form relatively insoluble complex salts. It and many of its derivatives enter into stable molecular combinations with picric and picrolonic acids, and since the products which are formed in this way are relatively insoluble, crystallize well, and possess in most cases sharp melting points, they frequently serve for purpose of identification.

The structural formula in common usage at the present time is not the hexagonal form that was suggested by Pinner, but the following expression:



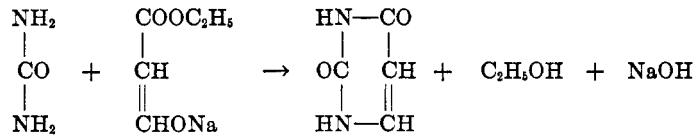
in which the position of the component atoms is indicated by numerals. According to this system cytosine would be referred to as 2-oxy-6-aminopyrimidine.[3]



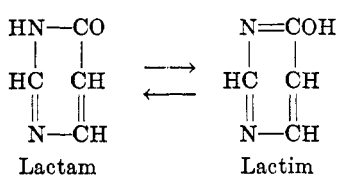
This arrangement possesses an obvious advantage over the hexagonal form because of the fact that it serves to emphasize

[3] According to the Geneva nomenclature, however, cytosine would be described as 4-amino-2-pyrimidone.

the existence of the urea grouping in the pyrimidine molecule. Since many derivatives of pyrimidine (such as, for example, uracil) may be synthesized by condensing ureas, sulfur ureas, or guanidines with different β-ketonic esters, this type of formula also presents a graphic method for representing such reactions:



Pyrimidine, like pyridine, yields derivatives (as for example, halogen, nitro, and amino substitution products) which result from direct or indirect replacement of the hydrogen atoms of the nucleus. A large number of these derivatives are, however, obtained as the result of special syntheses. So long as no oxygen or sulfur atoms are in direct union with the atoms of the ring, the strongly basic character of the pyrimidine nucleus remains unchanged. With the substitution of even one oxygen atom, however, an amphoteric substance is formed which possesses weakly basic and weakly acidic properties, the latter being due probably to the ionization of hydrogen in the lactim modification:



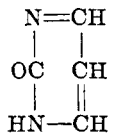
With the introduction of each additional oxygen atom in such positions the acidic properties of the pyrimidine become more pronounced, while its basic properties recede. These changes are so striking that the term "pyrimidine base" becomes exceedingly misleading when applied to these derivatives. It is in fact incorrectly used in referring to the different classes of so-called oxypyrimidines or pyrimidones (Geneva nomenclature).

In classifying the pyrimidines it has seemed desirable, in

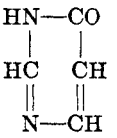
recognition of these fundamental differences in their properties, to consider special groups in the following order:

I. True pyrimidine bases. This group includes pyrimidine and its homologues together with their respective halogen, amino, and nitro derivatives.

II. Monoöxypyrimidines. Substances belonging to this group include 2-oxypyrimidine and 6-oxypyrimidine, together with their homologues and other derivatives, any one of which is capable of existing in tautomeric modifications.

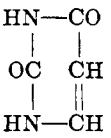


2-Oxypyrimidine



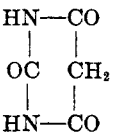
6-Oxypyrimidine

Groups III, IV, and V represent different types of more highly oxygenated pyrimidines:



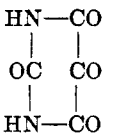
Uracil

(Group III)



Barbituric acid

(Group IV)



Alloxan

(Group V)

These substances, together with their homologues and other derivatives, are also capable of existing in lactam–lactim modifications.

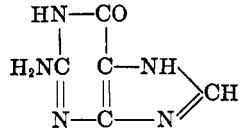
It will be noticed in comparing the above formulas that substances belonging to groups IV and V differ from those in groups I, II, and III, not only as to the position and number of oxygen atoms present in their molecules, but also in the fact that they possess completely saturated ring structures,—the ethylene linkage in the 4,5-position having been eliminated.

Pyrimidines occurring in nucleic acid combinations

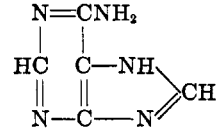
Before undertaking a systematic survey of these different groups of pyrimidines in the order mentioned above, it seems

desirable to consider briefly at this point the distinguishing characteristics and also the various tests which have been used in identifying those particular members of the pyrimidine series which occur repeatedly among the degradation products of nucleic acids. Of these cytosine, uracil, 5-methylcytosine, and thymine may be regarded as the most important. Other pyrimidine constructions which may possibly be expected to occur in the mixtures obtained under the experimental conditions of intense hydrolysis in the presence of sulfuric acid are isocytosine and 6-aminopyrimidine and its deaminized derivative, 6-oxypyrimidine.

Of the four just mentioned, cytosine, uracil, and thymine occur very widely distributed in nature. Cytosine and uracil are found among the hydrolytic products of both animal and vegetable tissues, while thymine reveals itself in animal tissue only. The question as to whether these substances are to be regarded as primary products actually present as such in the different nucleic acids, or whether they are formed as secondary products, resulting from the hydrolysis of purines, remained an open question for many years. In an attempt to answer it, Richard Burian (27) boiled guanine and adenine,

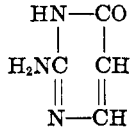


Guanine

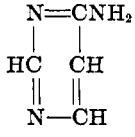


Adenine

in the presence of different carbohydrates with a 30 to 40 per cent solution of sulfuric acid. His results seemed to show that under the conditions of his experiments these purines hydrolyzed to give isocytosine and 6-aminopyrimidine, respectively.



Isocytosine



6-Aminopyrimidine

It therefore seems possible from such evidence that these substances, together with their deaminized derivatives, uracil and 6-oxypyrimidine, may be found along with other pyrimidines among the products formed in the energetic hydrolysis of nucleic acids under the action of sulfuric acid (196a), since guanine and adenine have been identified as occurring in these complex combinations with great frequency. Such a conclusion has, however, been refuted by Kossel and Steudel (124), by Steudel (183), and by Levene and Mandel (140). In spite of the negative experimental evidence brought forward by these investigators, the possibility still remains that with refinements in the technique of separating the pyrimidine fractions from nucleic acids after hydrolysis, and of isolating their components, Burian's conclusions may yet find confirmation. But whether or not pyrimidines will be found under certain conditions to be formed as secondary products of hydrolysis, it has now been demonstrated conclusively that the pyrimidines cytosine, uracil, and thymine are present as such coupled with sugars in nucleic acids.

Of the two aminopyrimidines cytosine and 5-methylcytosine, the former is the only representative which has been shown to be widely disseminated in nature. Cytosine was discovered and named by Kossel and Neumann (121), who separated it in impure condition from the cleavage products of thymus nucleic acid. It was not isolated in pure form until some years later, when it was obtained independently from the testicles of the sturgeon and the herring by Kossel and Steudel (123), and from various animal organs by Levene (130). Convenient methods of preparing it from the thymus, spleen, and pancreas glands have been described by Levene (132a, 133). It has also been found among the hydrolytic products from wheat embryo (158a, 203g) and from yeast nucleic acid (132b, 127).

The structure of cytosine, based on the results of its analysis and the fact that it yields uracil by hydrolysis, was first suggested by Kossel and Steudel (123) and later confirmed by Wheeler and Johnson (203a), who synthesized both cytosine and isocytosine and established the identity of the former with specimens of natural cytosine obtained from the nucleic acids of wheat embryo and from spleen.

Interest in 5-methylcytosine centers at present in the fact that Johnson and Coghill report having isolated and identified it among the products of hydrolysis of tuberculinic acid (91). Tuberculinic acid, obtained from tubercle bacilli, was first studied by Ruppel (172), who stated that it yielded thymine on hydrolysis, although he offered no analytical data to support his conclusions. It was investigated later by Levene (129), who did not, however, succeed in obtaining a pure pyrimidine compound of constant composition. Working with the small quantities of acid at his disposal, his analytical data nevertheless appeared to indicate the presence of a mixture of thymine and uracil in the pyrimidine fraction which he was able to separate from the products of hydrolysis. Johnson and Brown, in continuing this investigation at the request of the Research Committee of the National Tuberculosis Association, were able to report improved methods for preparing proteins from the tubercle bacilli (87) and a convenient method for isolating a tuberculinic acid of constant composition (85). In continuing this work they were able to report the separation and identification of thymine and cytosine from the hydrolytic products which were formed under the action of sulfuric acid. They, therefore, came to the conclusion that tuberculinic acid is a nucleic acid of animal origin; this was later confirmed by the separation of levulinic and formic acids from the sugar fraction in amounts large enough to indicate the presence of a hexose sugar in the tuberculinic acid molecule (23). Whether this sugar will be found to be *d*-desoxyribose, as in the case of thymus nucleic acid, remains to be determined by further investigation.

As a result of their continued investigations, Johnson and Brown were able to show that the purines guanine and adenine were also present along with the pyrimidines mentioned above (86) and that in the process of purifying the nucleic acid, guanine showed a tendency to be split off leaving a trinucleotide molecule. Both guanine and adenine were obtained in amounts sufficient to indicate considerable cleavage of tuberculinic acid during the process of purification.

Still later Johnson and Coghill (91) reported the separation of a

third pyrimidine which they identified as 5-methylcytosine. This substance was obtained in the form of its picrate, which was found to agree in all respects with the picrate of synthetic 5-methylcytosine (204a). This work has not been repeated and the structure of 5-methylcytosine therefore depends entirely upon the results of its analysis and its synthesis. The discovery of this pyrimidine among the hydrolytic products of tuberculinic acid requires a revised conception of the configuration of the nucleic acid molecule. It now becomes an open question as to whether thymine is present as such or whether it represents a secondary product formed as the result of the deaminization of 5-methylcytosine. The answer to this question cannot be given until much more exhaustive investigations of tuberculinic acid and other nucleic acids have been completed. Further study of the nucleic acids functioning in bacterial cells will undoubtedly contribute valuable data which will advance our knowledge of this interesting class of biological substances.

Tests for cytosine and uracil, 5-methylcytosine and thymine

Up to the year 1907, the identification of pyrimidines, which were isolated from mixtures resulting from the hydrolysis of nucleic acids, depended upon analyses of the pure substances, further confirmation being obtained as a result of the preparation of their sulfates, chloroplatinates, and picrates or picrolonates in cases where such derivatives showed characteristic features (melting points, crystal form, etc.) and were readily accessible. With the discovery of certain definite color tests for cytosine and uracil by Wheeler and Johnson (205a), a convenient method was introduced by means of which small quantities of these compounds could be detected even when they were impure or actually in mixtures with other substances.

This test consists in treating 5 cc. of an aqueous solution of the substance to be tested with small quantities of bromine water until the red bromine color is permanent. Excess of bromine is to be avoided and may be removed by passing a current of air through the solution. If the substance to be tested is cytosine the solution should be warmed or boiled and then cooled after

adding the bromine, but prolonged boiling should be avoided as it interferes with the test. To the cold solution prepared in this way an aqueous solution of barium hydroxide is then added in excess, when a purple or violet-blue precipitate or color is immediately formed. In cases where the solution to be tested is very dilute, it may be evaporated to dryness, taken up in a small quantity of bromine water, and then treated as described above. In this way quantities as small as 0.001 g. of uracil have been found to give a bluish pink or lavender color. Since the presence of picric acid interferes with this test it should be removed before making it.

These reactions with bromine water and barium hydroxide involve the transformation of both cytosine and uracil into dibromoöxydihydrouracil and the conversion of the latter into the almost insoluble, purple barium salt of dialuric acid (14b).

A color test that can be used for the detection of either 5-methylcytosine or thymine was discovered by Johnson and Baudisch (84, 9) some fourteen years later. This is somewhat more complicated and consists in dissolving, for example, 2.5 g. of thymine in 200 cc. of hot water in a 5 l. flask and adding first 200 g. of sodium acid carbonate dissolved in 2 l. of water and then 100 g. of freshly prepared ferrous sulfate dissolved in 500 cc. of water. This mixture is shaken violently in a current of air, when a colorless precipitate of iron hydrogen carbonate immediately begins to separate. This precipitate, on shaking, gradually absorbs oxygen from the air with the formation of an unstable peroxide which acts as an oxidizing agent. During a period of one-half to three-quarters of an hour the thymine is gradually oxidized and this is accompanied by a change in the color of the precipitate from white to greenish grey, and then to a brownish color resembling that of ferric hydroxide. When this point is reached the shaking is discontinued and the mixture allowed to stand until the precipitate has completely settled. A portion of the clear supernatant liquid equal to about 100–200 cc. is then evaporated to dryness on a steam bath, when the residue is redissolved and again evaporated. The dry residue is then taken up in a small quantity of water and the indigo test for pyruvic

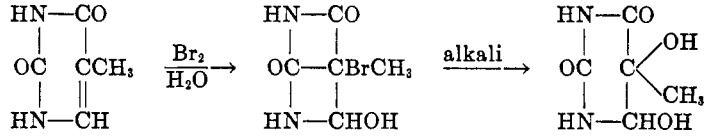
acid applied. This consists in treating 5–10 cc. of solution with a few drops of *o*-nitrobenzaldehyde, adding 1–2 cc. of concentrated potassium hydroxide, and then shaking the mixture with chloroform. The indigo which forms immediately dissolves in the chloroform, giving a characteristic blue solution. The aqueous layer may then be separated and tested for urea with xanthydrol (Fosse's reagent).

The remainder of the original thymine solution is then distilled by boiling over a free flame and the distillate treated with a few drops of *o*-aminobenzaldehyde, when it is again heated to boiling. If acetol is present here the cooled solution will show a characteristic blue fluorescence due to the formation of 3-oxyquinaldine, on being first acidified with hydrochloric acid and then neutralized with sodium acid carbonate. If desired, colorless needles of 3-oxyquinaldine may be obtained by extracting the solution with ether and then removing the ether by evaporation. In this case the Baudisch test for 3-oxyquinaldine may be applied by treating an aqueous alcohol solution of 3-oxyquinaldine with an alcohol solution of iron chloride, when a characteristic deep red complex salt is formed. The presence of sugar interferes with the above method for detecting thymine and should therefore be avoided (33b).

These two tests for cytosine and uracil, and for 5-methylcytosine and thymine, respectively, may be carried out simultaneously if the following procedure is employed (65a): A solution containing a mixture of uracil and thymine is first treated with bromine water according to the method described on p. 205. If cytosine or uracil is present a violet-blue color or precipitate will be formed immediately upon the addition of aqueous barium hydroxide. If the mixture is then subjected to distillation, the presence of thymine in the original solution will be indicated by the appearance of acetol in the distillate. This can be detected by adding sodium hydroxide to the distillate until it is strongly alkaline and then applying the Baudisch test for acetol (6). 5-Methylcytosine can be detected by precipitating as a picrate and then decomposing this salt with mineral acids and finally testing for thymine in the usual manner. As a result of this

general procedure all four of these pyrimidines may be tested for in a very few minutes.

The formation of acetol from thymine may be explained by assuming that the first stages of the reaction follow the same general course as has been indicated in the case of cytosine and uracil, giving thymine glycol (8).



Under the continued action of oxidizing agents this glycol is then oxidized and hydrolyzed in a manner similar to that described by Baudisch, with the disruption of the pyrimidine ring and the formation of urea and acetol. This may be formulated as resulting first in the formation of the intermediate aldehyde-acid $OCH \cdot C(CH_3)OH \cdot COOH$ which would then lose carbon dioxide to give the aldehyde of lactic acid. The latter on treatment with alkali would then rearrange to form acetol, CH_3COCH_2OH.

Additional data of service in the separation and identification of cytosine (203a, 196e) and 5-methylcytosine (204a) is to be found in the relative solubilities of these two bases in water along with a knowledge of their respective hydrates and characteristic salts with hydrochloric, hydrobromic, and sulfuric acids. A comparative table showing these relationships has been arranged by Johnson and Menge (110b) which may be of considerable interest. As has been stated, these substances all yield uracil and thymine, respectively, on hydrolysis.

The fact that the purines guanine and adenine yield isocytosine and 6-aminopyrimidine when hydrolyzed under certain conditions has already been mentioned (27). This fact, in conjunction with the observations of Johnson and Coghill (91) in their investigation of tuberculinic acid, suggests at least the possibility that both of these substances, together with their deaminized derivatives, uracil and 6-oxypyrimidine, may be formed as secondary products during the intensive hydrolysis of nucleic acids in the presence of sulfuric acid. All four of these substances have been

synthesized, isocytosine by Wheeler and Johnson (203d), by Gabriel and Colman (58d), by Traube (190c, 187), and more recently by Hilbert and Johnson (66b); uracil by Fischer and Roeder (46), by Wheeler and Merriam (207a), and later by Wheeler and Liddle (206), by Gabriel and Colman (58a), and by Davidson and Baudisch (32); 6-aminopyrimidine by Buttner (29h), and by Wheeler and Johnson (205d); and 6-oxypyrimidine by Wheeler and Bristol (198g) and by Gabriel (54). It has also been shown that although isocytosine may be hydrolyzed to give uracil, it differs from its isomer, cytosine, in the fact that when treated with bromine water under the same conditions as have been described in the case of cytosine and uracil, it yields a derivative which is not identical with dibromoöxydihydrouracil and which gives an intense blue color when treated with aqueous barium hydroxide. This color is readily distinguished from the purple or violet-blue coloration which is produced by cytosine and uracil under the same conditions, and can be readily identified by the fact that it immediately disappears on the addition of an excess of aqueous barium hydroxide. Because of this peculiarity the production of this color serves as a very delicate test for isocytosine (205b). 6-Aminopyrimidine gives no color reaction when treated with bromine water and aqueous barium hydroxide under the same conditions (205c). Because of the fact that isocytosine, 6-aminopyrimidine, and 6-oxypyrimidine are precipitated by the same general reagents as are used in the precipitation of cytosine and have been observed to adhere obstinately to the latter compound in such precipitates, a comparative table showing the melting points of these free bases together with those of their respective acetyl derivatives, picrates, picrolonates, and salts with hydrochloric and sulfuric acids has been compiled by Wheeler (196g). This may possibly prove of service in connection with future investigations of the nucleic acids and their degradation products.

Since alkylated, and in particular methylated, purines occur in nature, it would be reasonable to conclude that methylated pyrimidines may also occur in natural products (90a). This possibility has acquired a certain added importance through the

discovery of a supposed methylaminopyrimidine among the products obtained from the hydrolysis of Japanese *schōyu* (184), and led Johnson and Mackenzie (106b) to undertake a systematic investigation of methylated pyrimidines (97a) and other alkylated derivatives of various types.

The subject is of particular interest because of the fact that the same tests which may be applied in determining the relative position of an alkyl radical in the pyrimidine nucleus, may also be used in attempting to determine the position of the sugar linkage on pyrimidines in nucleosidic combinations. Richard Burian was the first to speculate on this point. The fact that imidazole rings were known to couple with diazonium salts to give colored derivatives led him to think that since the structure of the purine molecule indicates the presence of an imidazole ring, purines might also behave in a similar way when treated with diazobenzenesulfonic acid, except in cases where the reactive hydrogen in the purine had already been substituted. His failure to obtain colored compounds from *N*-7-substituted purines led him to assume that the reason that purine nucleosides also refused to react with diazobenzenesulfonic acid was due to the fact that the *N*-7-position was in such cases occupied by a sugar molecule. These observations of Burian, while not leading to correct conclusions, nevertheless acted as a great stimulus to later investigations involving the use of this reagent.

Thus the application made by Burian of diazobenzenesulfonic acid in the case of purines actually led to its use in attempts to allocate the sugar linkage in pyrimidine nucleosides. In addition to Burian (28), Evans (35), Steudel (182), and Pauly (159a) have contributed to developments in this field. It was not until 1908, however, that a systematic attempt was made by Johnson and Clapp (90e) to investigate the action of diazobenzenesulfonic acid upon a variety of different classes of pyrimidines. The results which they obtained and which are tabulated in a series of comparative tables by Johnson and Clapp, show that thymine, uracil, and cytosine all react with this reagent to give intensely red solutions, the color in every case being due to the formation of a diazo dye (47). Johnson and Clapp were also able to dem-

onstrate that in the case of a relatively large number of other pyrimidines, in which the hydrogen atoms in the *N*-3-positions had not been replaced by substituting groups, red diazo combinations were formed. In cases where no color was produced these investigators were able to prove that the hydrogen atoms in the *N*-3-positions had been replaced. If these observations are of general application it would seem to follow that pyrimidines which give no color reactions when treated with diazobenzene sulfonic acid[4] may now be regarded as already possessing substituting groups in the *N*-3-position.[5] Subsequent experimental evidence has been in harmony with this conclusion. For example, *N*-1-benzyluracil and *N*-1-benzylthymine react with diazobenzenesulfonic acid to give a red color, while the corresponding *N*-3-benzyl derivatives do not (92). This same characteristic behavior has also been observed in investigations dealing with the behavior of various alkyl halides towards uracil and thymine compounds during alkylation experimentations.

This color test with diazobenzenesulfonic acid is more general in its applications than any other test which has yet been investigated and would seem to indicate with considerable accuracy the position of groups substituting on nitrogen in the pyrimidine ring. Other reagents which have been employed with the same general end in view have not proved so convenient nor have they been capable of very extended application. Diazomethane, for example, has frequently been used successfully as a reagent in various types of methylation and a study of its reaction with cytosine, uracil, and thymine was therefore undertaken with a view to applying it later to nucleic acids as a methylating agent (30). It was thought that if nucleic acids could first be methylated and then subsequently hydrolyzed, the position of the sugar linked to the pyrimidine could be determined from the structure of the methylated pyrimidine which was formed as a result of cleavage. The results of preliminary experiments showed that in the case of uracil and thymine methylation proceeded smoothly

[4] Prepared according to the method described by Pauly (159b).

[5] For different methods of procedure in applying this test see Johnson and Clapp (90e).

and resulted in the formation of *N*-1-*N*-3-dimethyl derivatives in both cases. Cytosine, on the other hand, was found to be very resistant to the action of this reagent, and in the methylation of yeast nucleic acid side reactions occurred so that no definite conclusions could be reached.

Hydrazine sulfate represents still another reagent which has been applied in attempts to allocate the position of the sugar linkage in nucleic acids. In the single case recorded satisfactory results were obtained. The fact that uracil can be hydrolyzed by this reagent to free urea and formylacetic acid, which then combines with the hydrazine to form pyrazolone (50), formed the basis for this investigation. It was thought that since both urea and pyrazolone give relatively insoluble xanthyl derivatives, it might be possible to obtain corresponding derivatives of either a substituted urea or pyrazolone as the case might be. In applying this test to uridine Levene and Bass (134) obtained the xanthyl derivative of pyrazolone as the only product. This would seem to show that the sugar linkage is on a nitrogen atom of the pyrimidine ring, a conclusion obviously depending upon indirect evidence since a urea pentoside could not be isolated.

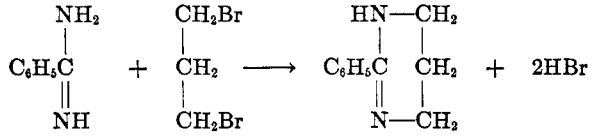
In summary, it may be said that the attachment of sugars to the *N*-3-position of the pyrimidine nucleus in nucleosides may be assumed on the basis of the following facts: nucleosides give negative color tests with diazobenzenesulfonic acid; they give negative color tests with bromine water and aqueous barium hydroxide; in one single instance they have been observed to give an unsubstituted pyrazolone under the action of hydrazine sulfate; in a single instance a hexose-pyrimidine nucleoside has been synthesized in which the sugar linkage was definitely determined to be in the *N*-3-position on the pyrimidine (66d). This synthetic nucleoside exhibited the characteristic behavior of the natural nucleoside uridine.

I. PYRIMIDINE HYDROCARBONS, THEIR HOMOLOGUES AND AMINO DERIVATIVES

Methods of preparation of pyrimidines and their homologues

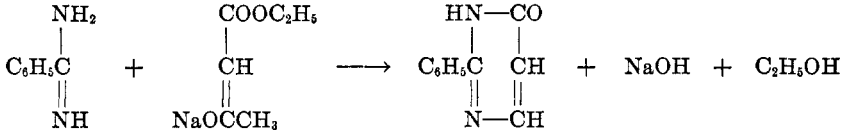
Since the reduced pyrimidines may be regarded as derived from formamidine, $NH{=}CH \cdot NH_2$, or substituted amidines having

the general formula NH=CR'NHR'', by the incorporation of these nitrogen linkages into a cyclic structure (194), the first attempts to prepare pyrimidines consisted in an effort to bring about condensations involving ring formation with the synthesis of cyclic guanidines. Only one illustration of direct action resulting in the formation of a cyclic hydrocarbon of this type is recorded in the literature, namely, 2-phenylhydropyrimidine, which was obtained by treating benzamidine with propylene bromide (164a):



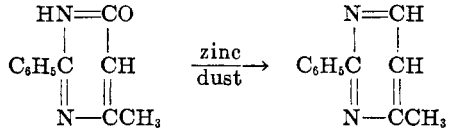
Since this method was not found to be capable of general application, two other methods of somewhat broader scope, but based upon the same general conception, were developed.

Method I. The first method consists in condensing an amidine in alkaline solution with a β-ketonic ester (161, 162b) as, for example:

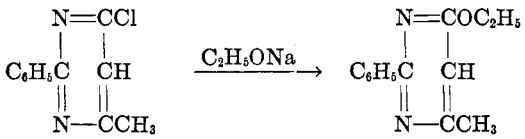


Benzamidine Ethyl acetoacetate 2-Phenyl-6-oxypyrimidine

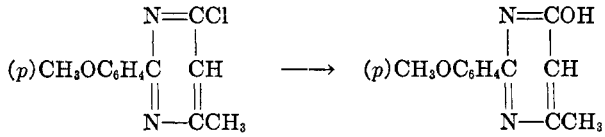
Other amidines which have been found to condense readily with this ketonic ester are $NH{=}C(CH_3)NH_2$, $NH{=}C(C_2H_5)NH_2$, $NH{=}C(C_6H_4OCH_3\text{-}p)NH_2$, and $NH{=}C(C_6H_4NO_2\text{-}p)NH_2$ (162b). The preparation of a pyrimidine hydrocarbon by this method depends for its ultimate success upon the replacement of oxygen in the 6-position of the pyrimidine cycle by hydrogen, but in investigating the condensation products which were obtained in this way Pinner (162b) found that they were extremely stable toward reducing agents and not acted upon by zinc in sodium hydroxide, nor by zinc or tin in hydrochloric acid. In only one case was he actually able to effect reduction, i.e.,



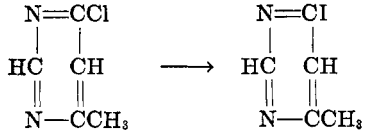
Attempts to overcome this resistance by treating the 6-oxypyrimidine with phosphorus pentachloride or phosphorus oxychloride preliminary to reduction, served to show that chlorine in this position is also very inert toward many of the common reducing agents and that when a reaction takes place it is not always in the sense desired,—sodium and alcohol, for example, reacting to give an oxygen ether:



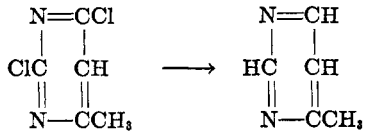
It is of interest in this connection to note that a comparison of the results obtained by treating different 6-chloropyrimidines with different reducing agents under different conditions brings out quite striking differences in behavior. For example, the action of hydrogen iodide and red phosphorus and also of zinc dust and water may result in hydrolysis (55a, 55g, 173c):



The action of hydrogen iodide and red phosphorus may bring about replacement of the chlorine by iodide, as, for example (55x, 29e):

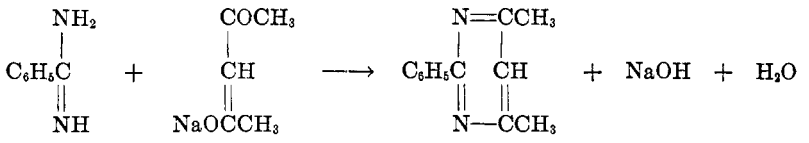


On the other hand, hydrogen iodide and red phosphorus may in certain cases result in replacement by hydrogen (55h):

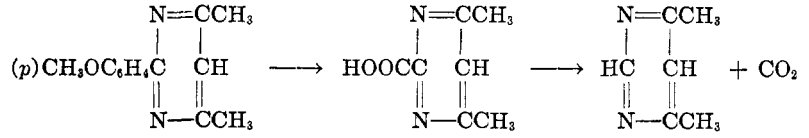


The most dependable results have been obtained by the use of zinc, since reductions with zinc dust and boiling water can almost invariably be relied upon to take place smoothly and to give excellent yields. This applies not only to reductions of chlorine in the 6-position but also in positions 2 and 4 (175c, 55j, 173d). They, therefore, afford in most cases a practical solution of the difficulties that have been experienced in reactions of this type.

Method II. The second condensation referred to above consists in condensing an amidine in alkaline solution with a β-dike-



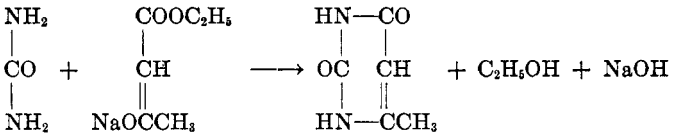
tone (164c). This condensation also takes place readily with other amidines, as for example, *p*-tolylamidine, $NH{=}C(C_6H_4CH_3)$-NH_2, benzenylamidine, $NH{=}C(CH_2C_6H_5)NH_2$, β-naphthylamidine, furylamidine (164b), and *p*-methoxybenzamidine, $NH{=}C$-$(C_6H_4OCH_3)NH_2$ (55b). In the last case the reaction product was successfully transformed into 2,6-dimethylpyrimidine on oxidation with potassium permanganate and subsequent elimination of a carboxyl group on heating (55c). This method of synthesizing the homologues of the pyrimidine cycle has not received much attention since the researches of the German investigators.



It should be noted, however, in this connection that the action of potassium permanganate has also been observed to result in a cleavage of the pyrimidine ring structure as, for example, in the case of 2-phenyl-4-methyl-6-oxypyrimidine which under these conditions yields benzamidine (162b).

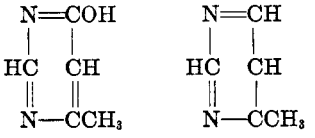
The substitution of the urea grouping for the guanidine grouping in condensations with β-ketonic esters and β-diketones

has further extended the scope of this general method for the synthesis of pyrimidines (10b). For example,



4-Methyluracil

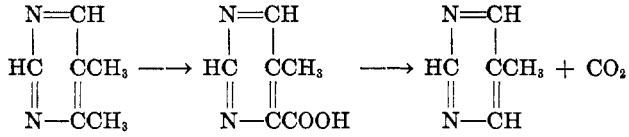
While condensations of this type take place readily they also depend for their ultimate success in the synthesis of reduced pyrimidines upon the subsequent replacement of oxygen by hydrogen in the condensation products. Here again replacement by chlorine is always preliminary to reduction, and is brought about in the usual way under the action of phosphorus pentachloride or the oxychloride. These chlorination reactions take place readily and in excellent yields and a study of the behavior of the resulting 2,6-dichloropyrimidines towards reducing agents has opened up a practical approach to the synthesis of reduced pyrimidines. The investigation of these 2,6-dihalogenated pyrimidines has revealed in several cases a remarkable variation in the behavior of the chlorine atoms occupying these two positions toward reagents other than zinc dust and water. For example, when 2,6-dichloro-4-methylpyrimidine is treated with hydrogen iodide (sp. gr. 1.7) and red phosphorus it reacts to give 4-methyl-6-oxypyrimidine (I); while on the other hand, when treated with fuming hydrogen iodide and red phosphorus or with zinc dust and boiling water it is completely reduced to 4-methylpyrimidine (II) (55e).



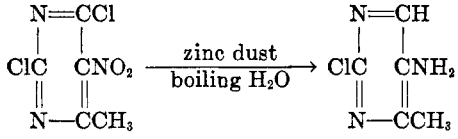
I II

A similar difference in the behavior of 2,6-dichloro-4,5-dimethylpyrimidine has been observed under the action of the same

reagents (173c). It is interesting to note that 4,5-dimethylpyrimidine, which is formed on complete reduction, yields 5-methylpyrimidine when oxidized with permanganate and then heated to eliminate carbon dioxide from the carboxylic acid formed by oxidation (173e).



The fact that in the reduction of halogen derivatives of the pyrimidines the character of the reduction product depends not only upon the reagent employed but also upon the nature of the other groups present in the molecule is shown by the following reaction (56h),

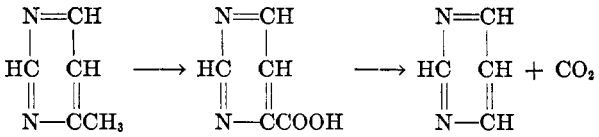


where it is to be noted that the chlorine atom in the 2-position remains unaffected, although it is usually very susceptible to the action of a number of different reducing agents. A similar illustration is to be found in the fact that 2-amino-6-chloropyrimidine is readily reduced, while 2-chloro-6-aminopyrimidine appears to be completely inert, when a mixture of these two isomeric compounds is subjected to the action of zinc dust and boiling water (196b).

In the preparation of pyrimidine hydrocarbons by condensing urea with diketones the primary product consists of a 2-oxypyrimidine which readily lends itself to reduction. Thus, for example, urea and acetylacetone in alkaline solution condense to give 2-oxy-4,6-dimethylpyrimidine (35, 62). This reacts with phosphorus oxychloride in the usual way and the product on treatment with zinc dust and boiling water yields 4,6-dimethylpyrimidine (55d, 181c).

Pyrimidine, the parent substance of this series, may be synthesized in either of the following ways:

(I) Urea condensed with ethyl acetoacetate gives 2,6-dioxy-4-methylpyrimidine, which, as has been stated, may be reduced to 4-methylpyrimidine (55i). The latter on oxidation with potassium permanganate and subsequent heating to eliminate carbon dioxide yields pyrimidine (55l).



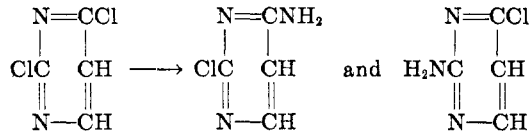
(II) This method differs from any thus far described in that barbituric acid (60, 61, 150, 193, 59, 180b, 22) is made the starting point in the preparation. This pyrimidine is digested with phosphorus oxychloride at 130–140°C. and the reaction mixture then distilled in a vacuum, when 2,4,6-trichloropyrimidine is obtained in yields approximately 80 per cent of theory (51a, 59, 29a). The latter pyrimidine, on reduction with zinc dust and boiling water, gives a 25 per cent yield of pyrimidine (51a).

Methods of preparation of amino derivatives of pyrimidine and its homologues

With very few exceptions amino, diamino, and other more highly aminized derivatives of pyrimidine have been prepared either from uracil and its homologues or from barbituric acid by first replacing the oxygen present in these compounds with chlorine, under the action of phosphorus pentachloride or oxychloride, and then treating the product with alcoholic ammonia. In some cases the oxychloride has been found to possess decided advantages over the pentachloride, reacting more readily and giving better yields of the reaction product. In order to obtain a clearer conception of the special methods by which substances belonging to this general class have been synthesized, it seems desirable by way of an introduction to review briefly the principal groups of chloro- and chloroaminopyrimidines which have up to the present time been prepared and studied.

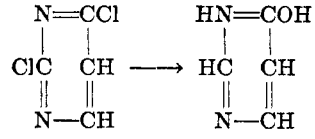
Uracil reacts with phosphorus oxychloride to give 2,6-dichloropyrimidine (III). Care should be taken in handling this

substance, since it has a very corrosive action and causes painful blisters (54). The same product may be obtained from 2-thiouracil (110c, 54) under the action of phosphorus pentachloride (205c). The dichloropyrimidine then reacts with alcoholic ammonia to give a mixture of 2-chloro-6-aminopyrimidine (IV) and 2-amino-6-chloropyrimidine (V) (205c, 196b, 54).

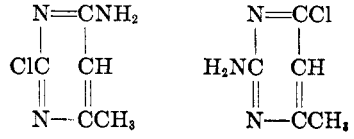


III IV V

On boiling this mixture with water and zinc dust the 2-amino-6-chloropyrimidine (V) is reduced to 2-aminopyrimidine, which is very soluble and can, therefore, be readily separated from the 2-chloro-6-aminopyrimidine which remains unchanged (196b). It may also be noted in this connection that 2,6-dichloropyrimidine behaves quite differently in the presence of hydrogen iodide and red phosphorus. In this case the chlorine in the 2-position is reduced while that in the 6-position is hydrolyzed (196d, 104a):



4-Methyluracil (14a) dissolves readily in phosphorus oxychloride on warming to give 2,6-dichloro-4-methylpyrimidine, and the latter when treated with alcoholic ammonia at 100°C. gives the following mixture (VI and VII):

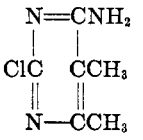


m.p. 218° m.p. 181–2°

VI VII

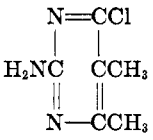
The second of these two isomers (VII) is identical with a substance obtained by treating Jaeger's 2-amino-6-oxy-4-methylpyrimidine (67a, 15b) with phosphorus oxychloride, and in this way its constitution was established (55f, m). The separation of the two isomers (VI and VII) depends upon the fact that 2-amino-6-chloro-4-methylpyrimidine is soluble in benzene and may therefore be removed by extraction (56a).

4,5-Dimethyluracil (10a, 173a) reacts with phosphorus oxychloride to give 4,5-dimethyl-2,6-dichloropyrimidine, and this on treatment with alcoholic ammonia at 100°C. gives (173j) a mixture of VIII and IX.



m.p. 215°

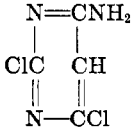
VIII



m.p. 182°

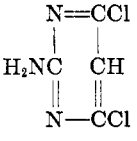
IX

Barbituric acid[6] is insoluble in phosphorus oxychloride but when heated in a sealed tube at 130–140°C. it reacts to give 2,4,6-trichloropyrimidine (51b, 52b, 53, 29c). In the presence of alcoholic ammonia this substance exhibits a marked difference in behavior at different temperatures, one halogen atom only being replaced at 100°C., two at 160°C., and all three at temperatures above 300°C. The product obtained at ordinary temperatures (i.e., up to 100°C.) consists of a mixture of X and XI.



m.p. 271°

X

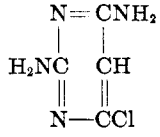


m.p. 221°

XI

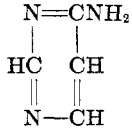
[6] See reference 59 for improved method yielding 80 per cent of theory, and for a complete bibliography of previous methods.

The second isomer is soluble in benzene and may therefore be removed by extraction. Its structure is known from the fact that it may be prepared by treating malonylguanidine with phosphorus oxychloride (29d). On further treatment with alcoholic ammonia both isomers yield the same 2,6-diamino-4-chloropyrimidine and this fact serves as additional evidence in confirming the structural formulas given above. 2,6-Diamino-4-chloropyrimidine

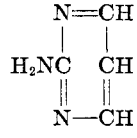


on reduction with zinc dust and boiling water yields 2,6-diaminopyrimidine. 2,4-Chloro-6-aminopyrimidine yields 6-aminopyrimidine with the same reagents, and under the action of other reagents a variety of different products may be obtained. The other chloroaminopyrimidines mentioned previously react in the same way, so that these different classes of compounds may be used as starting points for the preparation of a relatively large number of substances.

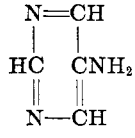
Since aminopyrimidines existing in two isomeric modifications (XII and XIII) are available by the above methods of synthesis, it has seemed desirable to consider in two separate classes all substances which may be regarded as derived from one or the other of these two forms. It will not be possible to mention the salts, picrates, etc., in the case of all of these substances, but literature references will supply these omissions. A third class derived from 5-aminopyrimidine (XIV) as the mother substance may also be noted in this connection, since it will be considered in some detail later in the text.



XII

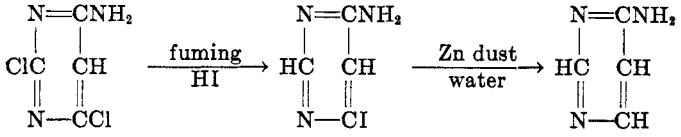


XIII

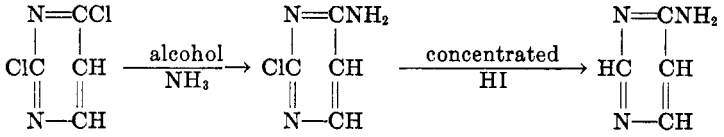


XIV

6-Aminopyrimidine (XII) has already been referred to as resulting from the intensive hydrolysis of the purine adenine. It may be synthesized in the following way (29h),



or as follows (205c, 196c, 104a):



6-Amino- and 6-anilino-4-methylpyrimidine may be prepared (a) by reducing 2-chloro-4-methyl-6-aminopyrimidine and 2-chloro-4-methyl-6-anilinopyrimidine, respectively, with zinc dust and water (56a), or (b) by treating 6-chloro-4-methylpyrimidine with alcoholic ammonia and with aniline, respectively (55v). 2-Amino-4-methylpyrimidine may also be obtained by reducing 2-chloro-4-methyl-6-aminopyrimidine with zinc dust and water (55s). A nitroamino derivative of this pyrimidine is formed as a result of nitration (56d).

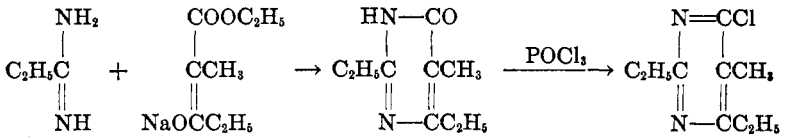
6-Amino- and 6-anilino-2-phenyl-4-methylpyrimidine result from treating the corresponding 6-chloro derivatives in the above way (163, 162b).

6-Amino- and 6-anilino-2-p-tolyl-4-methylpyrimidine have been prepared from the corresponding 6-chloro derivatives by heating with alcoholic ammonia at 180–190°C. and with aniline in benzene, respectively (116d).

6-Amino- and 6-anilino-2,4-dimethylpyrimidine result from treating the corresponding 6-chloropyrimidines with alcoholic ammonia (175c, 178, 106a) and with aniline, respectively (175d).

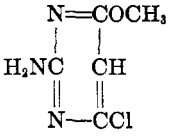
6-Amino- and 6-anilino-4,5-dimethylpyrimidine may be prepared respectively from 2-chloro-4,5-dimethyl-6-aminopyrimidine and the corresponding 6-anilinopyrimidine by reduction with zinc dust and water (173l).

6-Amino- and 6-anilino-2,4-diethyl-5-methylpyrimidine may be synthesized in the following way:

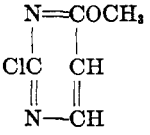


The chlorination product is then treated with alcoholic ammonia and aniline, respectively (148).

2-Aminopyrimidine (XIII), like its isomer 6-aminopyrimidine, may be obtained in either of two ways: (a) from barbituric acid by passing to 2,4,6-trichloropyrimidine, and then to 2-amino-4,-6-dichloropyrimidine, the latter being reduced under the action of zinc dust and boiling water (29d). It should be noted in this connection that attempts to reduce 2-amino-4,6-dichloropyrimidine with sodium in methyl alcohol resulted in the formation of the oxygen ether,



This on subsequent treatment with zinc dust and boiling water yields 2-aminopyrimidine as the final product (52a). (b) From uracil by passing to 2,6-dichloropyrimidine and then to 2-amino-6-chloropyrimidine, the latter being reduced with zinc dust and boiling water (104a). A modification of this procedure consists in treating 2,6-dichloropyrimidine with sodium methylate. The product consists of the methyl ether,



which may be transformed into 2-aminopyrimidine by treating it first with alcoholic ammonia and then reducing the methoxy group under the action of zinc dust and hydrogen chloride in absolute alcohol (58c).

A 2-anilinopyrimidine corresponding to the above may be prepared by the synthesis of 2-phenylanilino-6-oxypyrimidine and its subsequent treatment first with phosphorus oxychloride and then with zinc dust and water (98a).

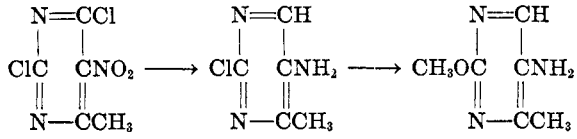
2-Amino-4-methylpyrimidine may be prepared from 4-methyluracil by passing to the corresponding 2,6-dichloro derivative and then to 2-amino-6-chloro-4-methylpyrimidine, reduction being effected in the usual way (55p). It may also be prepared from Jaeger's 2-amino-4-methyl-6-oxypyrimidine (67a, 15a) by treating with phosphorus oxychloride in the usual way and then reducing with zinc dust (55p).

2-Amino-4,5-dimethylpyrimidine may be obtained from 4,5-dimethyluracil (10a) by passing first to the corresponding 2,6-dichloro derivative and then to 2-amino-6-chloro-4,5-dimethylpyrimidine (173j). The latter is identical with the compound obtained by condensing guanidine with ethyl acetopropionate and treating the condensation product with phosphorus oxychloride (173f). The 2-amino-6-chloro-4,5-dimethylpyrimidine which is formed in either of these two ways is reduced smoothly by zinc dust and water (173i).

2-Amino-4,6-dimethylpyrimidine may be obtained from 2-oxy-4,6-dimethylpyrimidine (35) by treatment with phosphorus oxychloride and then with alcoholic ammonia in the usual way (181e). When aniline is used in place of ammonia, the corresponding 2-anilino derivative is formed.

Only one member of the class of monoaminopyrimidines in which the amino group occupies the 5-position has as yet been described in the literature. This is *4-methyl-5-aminopyrimidine* and is prepared by the application of an entirely different method of procedure in that it is formed by the direct reduction of a nitro group in the 5-position of the pyrimidine ring. For example, 2,6-dichloro-4-methylpyrimidine yields on nitration a corresponding 5-nitro derivative (56e). This on reduction with

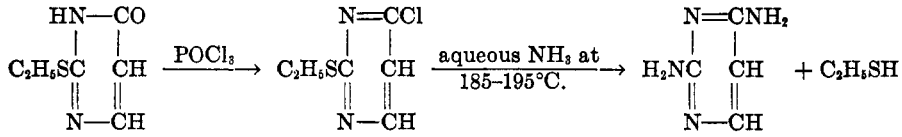
zinc dust and subsequent treatment with sodium methylate undergoes the following transformations,



and the product when treated with fuming hydrogen iodide and phosphonium iodide yields 4-methyl-5-aminopyrimidine.

Relatively few diaminopyrimidines have been prepared and studied. The substances investigated have been synthesized by methods closely analogous to those developed in the preparation of the monoaminopyrimidines,—having in most instances been obtained as transition products either from the 2,6-dihalogen derivatives of uracil and 4,5-dimethyluracil, or from the 2,4,6-trihalogen derivative of barbituric acid. Other general methods for the preparation of 2,6-diaminopyrimidines involve (a) the synthesis of 2-amino-6-oxypyrimidines by condensing guanidine with β-ketonic esters, followed by the conversion of these 6-oxy condensation products into their respective 6-chloro and then into 6-amino derivatives by application of the usual methods; (b) the synthesis of 2-mercapto-6-oxypyrimidines by condensing pseudothioureas with β-ketonic esters, followed by the conversion of the condensation products into the corresponding 2-amino or 2-anilino derivatives with subsequent replacement of oxygen in the 6-position by one or the other of these or similar groups.

Diaminopyrimidines which contain amino groups in the 5-position of the ring are prepared by special methods which involve at some point in the procedure the reduction of a nitro or a nitroso group in this position. *2,6-Diaminopyrimidine* may be obtained from 2,6-diamino-4-chloropyrimidine (29a) by treating it first with hydrogen iodide and red phosphorus and then reducing the resulting 2,6-diamino-4-iodo derivative with zinc dust and water (29i). The same substance may also be prepared by starting with 2-ethylmercapto-6-oxypyrimidine as represented below (203c, 100a).



2,6-Dianilidopyrimidine was obtained from 2-ethylmercapto-6-chloropyrimidine by heating with two molecular proportions of aniline at 150°C. (100c).

2,6-Diamino-5-carbethoxypyrimidine was prepared from the corresponding 2-ethylmercapto-5-carbethoxy-6-chloropyrimidine in a similar way (200b).

2-o-Toluidino-6-amino- and 2-p-toluidino-6-anilido-pyrimidine have been obtained by starting with 2-ethylmercapto-6-oxypyrimidine and treating it with *o*- and *p*-toluidine, respectively. The products obtained in this way were treated first with phosphorus oxychloride and then with alcoholic ammonia at 140–150°C. or with aniline in benzene solution, respectively. The corresponding 2-*o*-toluidino-6-anilidopyrimidine was obtained by warming the corresponding 6-chloro derivative with aniline in benzene solution (116c).

2,6-Diamino-4-methylpyrimidine was prepared from the corresponding 2,6-dichloro-4-methylpyrimidine (56j) and also from 2-amino-4-methyl-6-oxypyrimidine by the usual methods (55o, 56j).

2,6-Diamino-4-methyl-5-nitropyrimidine was prepared by first nitrating 2,6-dichloro-4-methylpyrimidine and then replacing the chlorine in the usual way (56e).

2-Amino-6-anilino- and 2-anilino-6-amino-4-methylpyrimidine were obtained from 2-amino-6-chloro- and 2-anilino-6-chloro-4-methylpyrimidine by treatment with aniline and alcoholic ammonia respectively (55q).

2,6-Diamino-4,5-dimethylpyrimidine and 2-amino-6-anilino-4,5-dimethylpyrimidine were prepared from 2-amino-6-oxy-4,5-dimethylpyrimidine in the usual way (173g).

2-Anilino-6-amino- and 2,6-dianilino-4,5-dimethylpyrimidine were obtained from the corresponding 2-chloro-6-amino (173k) and 2,6-dichloro (173m) derivatives.

4,6-Diaminopyrimidine was prepared from 4-iodo-6-aminopyrimidine by treatment with alcoholic ammonia (29f).

5,6-Diamino-4-methylpyrimidine was obtained from 2-chloro-4-methyl-5,6-diaminopyrimidine by reduction with hydrogen iodide and red phosphorus (56g), and *2,5-diamino-4-methylpyrimidine* from 2-chloro-4-methyl-5-aminopyrimidine by treatment with alcoholic ammonia (56i).

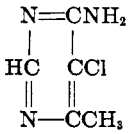
A few more highly aminized pyrimidines have also been prepared and studied. These are: *2,4,6-triaminopyrimidine,* prepared from the corresponding trichloro derivative of barbituric acid (52a) and also from 2-methylmercapto-4,6-dichloropyrimidine (199b). This substance on nitration yielded a 5-nitro derivative which reduced to give *2,4,5,6-tetraaminopyrimidine* (52c). *2,4-Dianilino-6-aminopyrimidine,* prepared from 2-methylmercapto-4-chloro-6-aminopyrimidine by heating with two molecular proportions of aniline at 140–150°C. for ten hours (100e). *2,5,6-Triamino-4-methylpyrimidine,* prepared by nitrating 2,6-diamino-4-methylpyrimidine and then reducing the product with tin and hydrochloric acid (56l).

II. THE PROPERTIES OF PYRIMIDINES AND AMINOPYRIMIDINES

Halogen derivatives

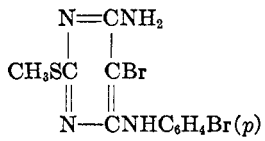
In considering the preparation of pyrimidines and aminopyrimidines from the corresponding halogen derivatives by processes of reduction and aminization, variations in the behavior of halogen in the 2-, 4-, and 6-positions were noted and nothing further needs to be added in this connection.

It remains to be noted, however, that both chlorine and bromine derivatives have been reported in the literature in which the halogen occupies the 5-position in the pyrimidine molecule. Thus for example, 4-methyl-6-aminopyrimidine reacts readily with chlorine in hydrochloric acid solution to give the corresponding 5-chloropyrimidine.



A similar reaction occurs when a molecular quantity of bromine is added to 4-methyl-6-aminopyrimidine (56c) and also to 4-methyl-2,6-diaminopyrimidine (57a) under similar conditions. The halogen in these cases appears to be very firmly seated and to resist replacement by amino groups under the action of alcoholic ammonia (56c).

While mercapto- and thio-pyrimidines have been reserved for special consideration a little later, it may be said in this connection that substances which contain halogen in the 5-position have been reported in the case of 2-mercapto-6-aminopyrimidines and their derivatives. These have been prepared by both direct and indirect methods. For example, *2-methylmercapto-4-chloro-5-bromo-6-aminopyrimidine* and also *2-methylmercapto-4-p-bromoanilido-5-bromo-6-aminopyrimidine*,



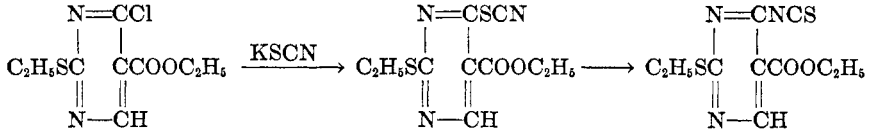
were prepared by dissolving the corresponding unbrominated pyrimidines in acetic acid and adding a molecular quantity of bromine (100d). Under these conditions the product separates in the form of a salt of hydrogen bromide, and the free base is obtained by neutralizing with 10 per cent sodium hydroxide solution. The free base of the second of these compounds was also obtained by the indirect method of heating 2-methylmercapto-4-chloro-5-bromo-6-aminopyrimidine with *p*-bromoaniline. In a similar manner *2-methylmercapto-5-bromo-4,6-diaminopyrimidine* may be prepared by treating the corresponding 4-chloro derivative with aqueous ammonia at 150–160°C. 2-Ethylmercapto-5-bromo-6-aminopyrimidine was obtained by the same indirect method.

Iodine cannot be introduced into the pyrimidine nucleus successfully by direct iodination. It has however been observed to replace chlorine in the 6-position, and also in the 4-position (55u, 34, 29b, 29g). In both positions it is readily reduced by zinc dust and water and readily aminized by alcoholic ammonia at high temperatures.

Amino derivatives

The 2-, 4-, and 6-amino- and anilino-pyrimidines which have been described as being formed from the corresponding halogen derivatives under the action of ammonia or aniline, require specific conditions of temperature and pressure if adequate yields are sought. In the case of 2,4,6-trichloropyrimidine, for example, only one halogen atom is replaced at ordinary temperatures or even up to 100°C., the product consisting of a mixture of 2,4,6- and 4,2,6-aminodichloropyrimidine. At 160°C. a second amino group enters the molecule and 2,4-diamino-6-chloropyrimidine is formed. Finally at temperatures above 200°C. all three chlorine atoms are replaced (52a, 29a). Variations in the behavior of chlorine in different positions toward aqueous and alcoholic ammonia have been frequently reported in the literature (55w, 56b, 58c, 173b, 175a, 181b, 82).

2-Mercapto-6-aminopyrimidines may be prepared indirectly by treating the corresponding 6-chloro derivatives with potassium thiocyanate in toluene and other solvents. For example:



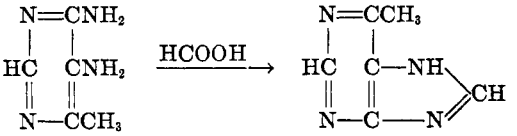
The resulting isothiocyanate when dissolved in absolute alcohol and heated for two hours at 150°C. gave the thionurethane, $\overline{\overline{N \cdot C(SC_2H_5)=N \cdot CH=C(COOC_2H_5)C}} \cdot NHCSOC_2H_5$, and when heated for six hours gave the corresponding 6-amino derivative (88). The structure of the latter substance was confirmed by comparison with a specimen of 2-ethylmercapto-5-carbethoxy-6-aminopyrimidine previously synthesized by Wheeler and Johns (200a).

The corresponding 5-bromo-6-chloropyrimidine also gives an isothiocyanate under the action of potassium thiocyanate and this again may be transformed into the corresponding 6-amino derivative (198d, 108a, 116a).

5-Aminopyrimidines cannot be prepared from the corresponding

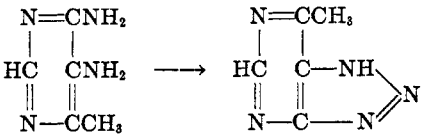
halides, since halogen in this position is not acted upon by aqueous or alcoholic ammonia. They may however be obtained from the corresponding 5-nitro and 5-nitroso compounds by the reducing action of tin and hydrochloric acid (56f, 58e). Substances which contain an amino group in this position can be detected by means of characteristic color reactions. The test is applied by treating the pyrimidine in solution in ammonia under very particular conditions with phosphotungstic acid, when a brilliant blue color is developed (101d, 49, 48, 144).

5,6-Diaminopyrimidine, and also a triaminopyrimidine which possesses this grouping, can be identified by the fact that they are readily transformed into the corresponding purines under the action of different reagents such as formic acid, urea, thiourea, and nitrous acid. For example (56g),



4-Methyl-5,6-diaminopyrimidine 6-Methylpurine

The same diaminopyrimidine, on treatment with urea and thiourea respectively, yields 6-methyl-8-oxypurine and 6-methyl-8-thiopurine, and when its hydrochloride is treated with sodium nitrite in aqueous solution it is transformed into the cyclic diazo derivative or the triazole, azimido-6-methylpyrimidine.

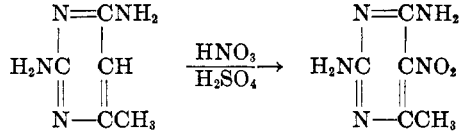


The corresponding 2-chloro-4-methyl-5,6-diaminopyrimidine yields chloromethylpurine when treated with formic acid, 2-amino-4-methyl-5,6-diaminopyrimidine yields 2-amino-6-methylpurine, and 2-thio-4,5,6-triaminopyrimidine when treated in the same way and then desulfurized yields adenine (188b).

A pentachloropyrimidine, $C_5Cl_5N_2$, has been reported which reacts with alcoholic ammonia to give a monoamino derivative $C_5HCl_4N_2 \cdot NH_2$ (57a). All classes of amino compounds yield

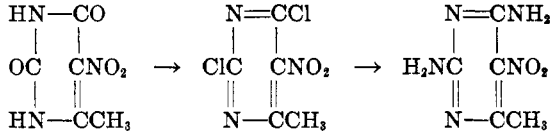
the corresponding oxypyrimidine derivatives on treatment with nitrous acid.

5-Nitropyrimidines and aminopyrimidines may be obtained by direct nitration (56k, 52a, 163).

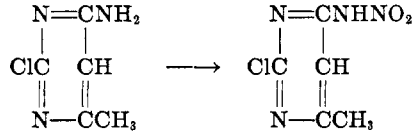


These substances are all reduced under the action of tin and hydrochloric acid to the corresponding amino derivatives.

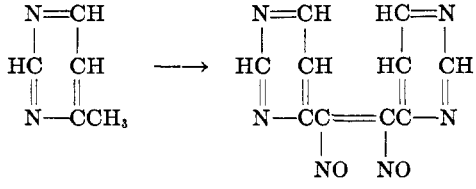
It should be noted, however, that the behavior of pyrimidine and aminopyrimidines under the action of nitric acid varies considerably. This is especially true in the case of aminopyrimidines. Thus, for example, 4-methyl-6-aminopyrimidine and the corresponding 2-chloro derivative yield nitroamines when treated with this reagent (56d).



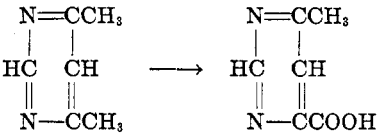
Or, nitro derivatives may be obtained indirectly from the corresponding nitrouracils by the usual method of replacing the oxygen first by chlorine and then by an amino group (56e).



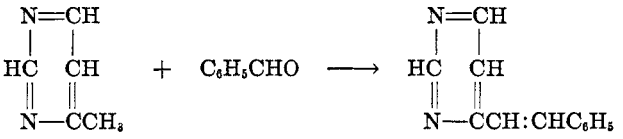
4-Methyl- and 4,6-dimethyl-pyrimidine react still differently, yielding what have been represented structurally as glyoxime superoxides. Very little attention has been paid to the chemistry of these types of nitroso compounds (57b).



4-Methyl- and 4,5-dimethyl-pyrimidine show certain peculiarities of behavior which call for comment, the hydrogen atoms of the methyl group in the 4-position displaying certain of the properties of so-called "reactive hydrogen." Thus, for example, 4-methylpyrimidine is readily oxidized by potassium permanganate to give the corresponding carboxyl derivative (55k). 4,5-Dimethylpyrimidine behaves in a similar manner (173e, 181a).



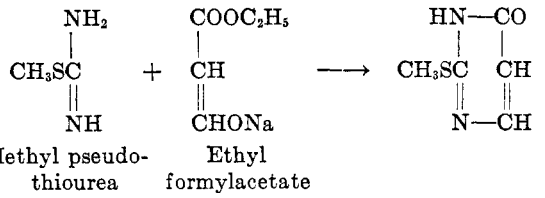
The fact that methyl groups in this position are readily oxidized led to the assumption that they might also be reactive in condensations and this proved to be correct, since 4-methylpyrimidine was found to condense with benzaldehyde in the presence of zinc chloride (58e).



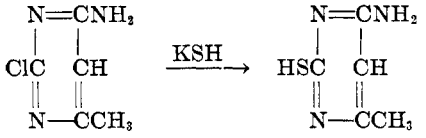
2,4-Dimethylpyrimidine has also been observed to react in the same way (175b). The activity of this hydrogen is probably also responsible for the curious behavior of these substances in the presence of nitric acid. This has already been referred to (57b).

Mercaptopyrimidines

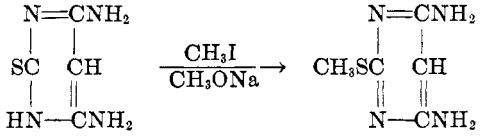
Mercaptoaminopyrimidines have been prepared in which the mercapto group occupies the 2-, 4-, and 6- positions, mono-, di-, and tri-substitution products having been described by different investigators in this field. 2-Mercaptopyrimidines may be obtained synthetically by condensing pseudothioureas with β-ketonic esters and β-diketones (207a).



In this case the corresponding 6-aminopyrimidines can be obtained by means of the usual transformations. 2-Mercaptopyrimidines may also be prepared by the interaction of the corresponding chloropyrimidines with potassium hydrogen sulfide (55t, 181e),



or by alkylating the corresponding thiopyrimidines (188a).



6-Mercaptopyrimidines are prepared exclusively by the second of these three methods (55r, 173h). 2,6-Dimercaptopyrimidines and 2,4,6-trimercaptopyrimidines have been obtained by means of the same general methods (55n, 173n, 29j).

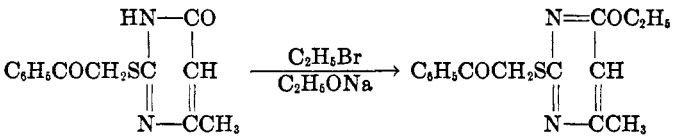
The mercapto groups present in these compounds can usually be hydrolyzed under the action of hydrobromic or hydrochloric acid to give the corresponding oxy derivatives with evolution of mercaptan. They are also replaceable in the 2-position by amino and substituted amino groups (106d, 100b, 98b, 198h, 116b).

The importance attached to the preparation of 2-mercaptopyrimidines by synthetic methods lies in the fact that the presence of this group limits the action of phosphorus oxychloride to the 6-position of the pyrimidine cycle, and this makes possible the preparation of a variety of different derivatives of known structure.

Oxygen ethers

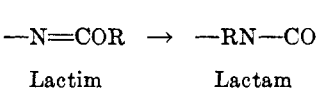
Oxygen ethers in which methoxy and ethoxy groups occupy one or more of the 2-, 4-, and 6-positions in the pyrimidine ring have been prepared from the corresponding halogen derivatives under the action of sodium methoxide and ethoxide, respectively (162b, 55m, 56h, 58b, 173o, 181d, 203d). It should be noted that Buttner in studying the action of sodium methoxide on trichloropyrimidine made the discovery that the formation of mono-, di-, or tri-methoxy derivatives, respectively, was subject to temperature control, the trimethoxy derivative being obtained at 100°C. under pressure (29k).

Ethers of this class have also been obtained by direct alkylation of 2-mercapto-6-oxypyrimidines under the action of alkyl halides in the presence of sodium ethoxide (112).



The same substance gave the corresponding benzyl ether under the action of benzyl chloride. A 2-ethylmercapto-5-ethoxypyrimidine and the corresponding 2,5-diethoxypyrimidine are representatives of a different type which have also been prepared (104b).

Substances belonging to this general class are of considerable interest because many of them tend to undergo rearrangements into the lactam modification. Such transformations belong to

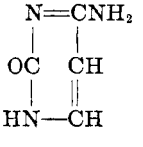


the type of imido ester rearrangements (117, 118, 202, 195, 95) and in the case of 2,6-dimethoxypyrimidine have led to the discovery of a synthetic method for preparing pyrimidine nucleosides (66c, 81).

III. AMINOÖXYPYRIMIDINES WHICH YIELD URACIL OR ITS DERIVATIVES ON HYDROLYSIS

Monoaminomonoöxypyrimidines

Following the general method of classification outlined in the first chapter of this paper, the amino derivatives of pyrimidine which contain one or more oxygen atoms in union with the carbon atoms of the pyrimidine nucleus, must now be considered. Such substances fall naturally into two groups, (a) those which yield uracil or derivatives of uracil (thymine, etc.) on hydrolysis, and (b) those which yield barbituric acid and its derivatives. The simplest compounds which may be regarded as belonging to the first group are those containing one oxygen and one amino group. Substances having this composition may be accounted for structurally in seven different ways if tautomeric variations in form are disregarded (lactim–lactam modifications) (203b).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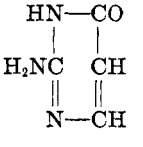
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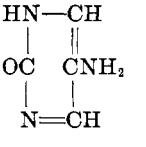
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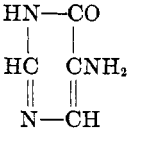
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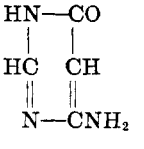
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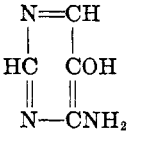
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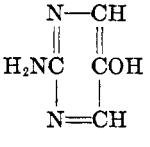

VII

Only the first two of these seven possible molecular configurations could be expected to yield uracil on hydrolysis. These have now been identified as belonging respectively to the two well-known substances, *cytosine* (I) and *isocytosine* (II). Cytosine has been observed to result from the hydrolysis of nucleic acids or other naturally occurring products, but isocytosine has not as yet been found among the degradation products of nucleic acid. Both pyrimidines have been synthesized by methods which admit of no doubt as to their structure. Numerous alkyl

and aryl derivatives of each of these two types of substances have also been synthesized, and in every case where the substituting group is not in union with the amino nitrogen atom and is, therefore, not eliminated with it during the process of hydrolysis, such compounds yield corresponding uracil derivatives under the action of hydrolyzing agents. Since cytosine and its derivatives differ from isocytosine and its derivatives both as to their respective methods of synthesis and also as to their properties, the two types of substances will be considered separately.

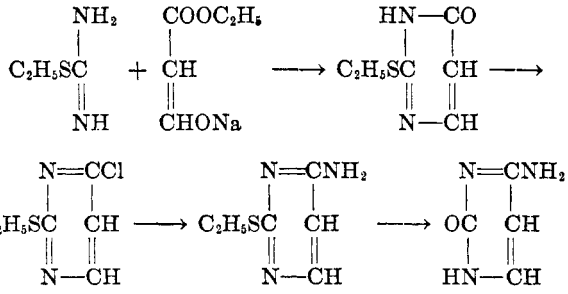
1. Cytosine

The only *general* method of synthesis which has been found applicable to cytosine and to such of its many possible derivatives as have up until now been investigated, consists in condensing a pseudothiourea with a β-ketonic ester. The product, which contains a mercapto group in the 2-position and an oxygen atom in the 6-position, is treated with a phosphorus halide—phosphorus pentachloride or oxychloride—and then with ammonia in the usual way, and finally desulfurized by warming with mineral acids. In certain cases this procedure has been modified slightly by using thiourea in the initial condensation and then transforming the resulting 2-thio-6-oxypyrimidine into the corresponding 2-mercapto derivative by treatment with metallic sodium dissolved in absolute alcohol. Further transformations of the product obtained in this way follow the general course outlined above. Direct desulfurization, CS $\rightarrow$ CO, can also be accomplished by digesting a 2-thiopyrimidine with chloroacetic acid. This is a very practical method of operating when the alkylation method is not necessary for the further steps in synthesis.

Cytosine was first discovered as a cleavage product of thymus nucleic acid (121) and is very widely distributed in nature, having been obtained from the testicles of sturgeon and herring (123) and from various other animal organs (130, 132a). It has also been found among the hydrolytic products of wheat embryo (158b, 203g) and of yeast cells (132b, 127). The free base has been isolated in the form of its chloroplatinate (123) and of its picrate which was then converted into the sulfate (131). In 1903

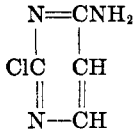
the base was transformed into uracil by deaminization with nitrous acid (124).

The structure of cytosine was tentatively suggested by Kossel and Steudel (124), but the first actual synthesis of the substance was accomplished by Wheeler and Johnson (203a). This consisted in condensing ethyl pseudothiourea with ethyl formylacetate in alkaline solution and treating the product first with phosphorus oxychloride, then with alcoholic ammonia, and finally desulfurizing by warming with hydrochloric acid.



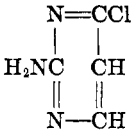
While the technique of this method is difficult for an inexperienced worker, it served for many years as the only practical method for preparing cytosine in quantity. The greatest obstacle in applying this process is to be found in completely eliminating the mercaptan which is evolved during the final hydrolysis without conversion of the resulting cytosine into uracil. Many workers also object to the production of mercaptan odors in laboratory practice.

It is now possible to avoid the use of sulfur compounds in the synthesis of cytosine itself by applying a second special method of procedure which possesses the added advantage of simplifying the operating technique. This consists in starting with 2,6-dichloropyrimidine. The latter is readily prepared from uracil (32) by treatment with phosphorus oxychloride (54). By allowing 2,6-dichloropyrimidine to react with ammonia in alcohol solution at 100°C., Gabriel obtained a mixture of the isomeric chloroaminopyrimidines,



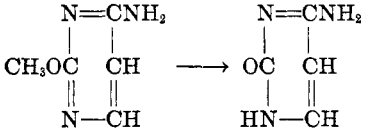
2-Chloro-6-aminopyrimidine

and



2-Amino-6-chloropyrimidine

in the proportions of 40 per cent and 60 per cent, respectively. This mixture may be treated in either of two ways: (a) It may be distilled with steam, when 2-amino-6-chloropyrimidine is removed while its isomer remains behind. The latter can then be hydrolyzed to give cytosine by heating with water at 140°C. The yield is, however, not good because of the fact that some of the 2-chloro-6-aminopyrimidine is transformed into uracil during this process. (b) It may be treated with sodium methylate. In this case a mixture of 2-methoxy-6-aminopyrimidine and 2-amino-6-methoxypyrimidine is obtained without loss of material. The two isomeric ethers may then be separated by recrystallization from hot water in which 2-amino-6-methoxypyrimidine is extremely soluble. In this way yields approximating 40 per cent of 2-methoxy-6-aminopyrimidine may be obtained from 2,6-dichloropyrimidine (66a). When this ether is warmed with dilute hydrochloric acid it passes quantitatively into cytosine without danger of hydrolysis of the latter to uracil.



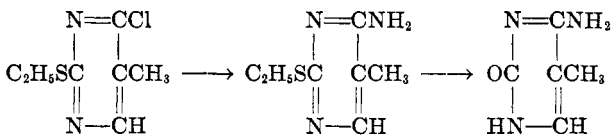
Cytosine may be readily identified in a number of different ways. It separates from its aqueous solutions with one molecule of water of crystallization, and therefore differs from 5-methyl- and 5-ethyl-cytosine in this respect and also in its solubility in water.[7] Its salts with hydrochloric and sulfuric acids and also its acetyl derivative, picrate, and picrolonate show characteristic properties (196f). Since cytosine is readily hydrolyzed to form

[7] A comparative table showing the relative solubilities of these substances has been compiled (109a).

uracil it gives a characteristic color test when treated successively with bromine water and barium hydroxide (205a). When oxidized by shaking with air in mixtures of sodium bicarbonate and ferrous sulfate, it gives a characteristic reaction (7). It also gives a red color when treated with diazobenzenesulfonic acid (90g).

2. Derivatives of cytosine

Comparatively few of the many possible derivatives of cytosine have as yet been reported in the literature. Of these *5-methylcytosine* is unquestionably the most important. This substance was synthesized by Wheeler and Johnson (204a) as early as 1904, but it was not until 1925 that its presence among the hydrolytic products of tuberculinic acid was discovered by Johnson and Coghill (91). The identity of these two substances was established by means of crystallographic comparison of their respective picrates. Synthetic 5-methylcytosine may be prepared by methods closely analogous to those which have been applied in the synthesis of cytosine. The process consists in preparing 2-ethylmercapto-5-methyl-6-chloropyrimidine by condensing ethylpseudothiourea with the sodium salt of ethyl formylpropionate in alkaline solution and then treating the condensation product with phosphorus oxychloride. The resulting pyrimidine is first purified by distillation under reduced pressure and then heated with alcoholic ammonia for three hours at 127°C. The product is then desulfurized by warming with concentrated hydrochloric acid (204a):



5-Methylcytosine is five times more soluble in water than cytosine and ten to twelve times more soluble than uracil or thymine. It is readily transformed into thymine by heating with 20 per cent sulfuric acid at 150–160°C., or in general by the prolonged action of hot mineral acids. It forms basic salts with halogen acids as, for example, $(C_5H_7ON_2)_2 \cdot HBr \cdot H_2O$. Its

picrate has the same solubility in water as that of cytosine; its chloroplatinate is very soluble. It is precipitated by phosphotungstic acid (204a). It gives a negative color test when treated successively with bromine water and barium hydroxide (205a), but yields acetol (65b) when treated successively with bromine water and barium hydroxide under specific conditions. Since it undergoes hydrolysis to give thymine it may be expected to yield acetol, pyruvic acid, and urea when shaken with air in the presence of sodium bicarbonate and ferrous sulfate or other similar oxidation media under well-defined conditions (84). The latter test can be applied in the presence of uracil, cytosine, and also of sugar (33a). 5-Methylcytosine gives an intense red color when treated with diazobenzenesulfonic acid (90h).

5-Ethylcytosine has been synthesized by condensing ethyl pseudothiourea with ethyl formylbutyrate and then treating the condensation product in the usual way (110a). Unlike cytosine and 5-methylcytosine, it separates from its aqueous solutions in an anhydrous condition. It is also distinguished from both of these substances by its solubility in water and by the fact that it is not hydrolyzed by 20 per cent sulfuric acid, the amino group being apparently very firmly seated in the pyrimidine molecule.[8]

Cytosine-5-carboxylic acid and the corresponding *acid amide* have been synthesized by the application of the same general procedure (201, cf. 200a). Other derivatives of cytosine in which the substituents occupy the 5-position are *cytosine-5-acetic acid* (79) and *5-oxycytosine* (109a).

Derivatives of cytosine in which the substituents occupy the 4-position in the pyrimidine molecule have been synthesized in an analogous manner. For example, *4-methylcytosine* is obtained by condensing ethyl acetoacetate with ethyl pseudothiourea in alkaline solution. The product when boiled with phosphorus oxychloride yields 2-ethylmercapto-4-methyl-6-chloropyrimidine, and this when heated in a sealed tube with alcoholic ammonia at 140–150°C. is transformed into the corresponding 6-aminopyrimidine. The latter is readily converted into the hydrochloride of 4-methylcytosine by digesting with concentrated hydrochloric

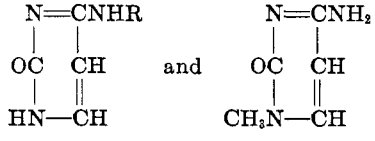
[8] The comparative solubilities of these substances and their respective salts have been determined (110b).

acid. The base is precipitated from the hydrochloride by neutralization of the hydrochloric acid with alkali.

4-Methylcytosine can be readily distinguished from 5-methylcytosine because of the fact that it is far less soluble in water and does not melt at 310°C., while the latter substance effervesces at 270°C. It gives a very characteristic basic hydrochloride containing three molecules of pyrimidine to one molecule of hydrogen chloride. When crystallized from concentrated solutions of the common acids it yields a series of normal salts. Its importance is to be found in the fact that it has been used in the synthesis of certain purine derivatives which will be described later (68).

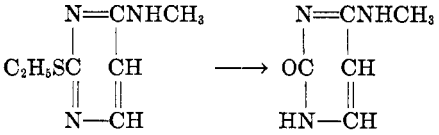
Other interesting derivatives of cytosine which contain substituents in the 4-position are *cytosine-4-aldehyde* (111) and *4-phenylcytosine* (94). In the case of the latter substance the method of preparation was modified, and thiourea was substituted for ethyl pseudothiourea in the condensation with ethyl benzoylacetate. The resulting 2-thio-4-phenyl-6-oxypyrimidine was then transformed into the corresponding 2-ethylmercapto derivative and this into the desired product in the usual way. 4-Phenylcytosine is characterized by the fact that it separates from its solutions in two different crystalline modifications (cf. 99b). *4-Methyl-5-ethylcytosine* has also been prepared by using thiourea in the initial condensation and then proceeding in the manner described above (83c).

Alkylation products of cytosine, 4-methylcytosine, and 5-methylcytosine have been prepared in relatively large numbers, by the application of both direct and indirect methods. Such substances fall naturally into two groups:—those in which the substituent replaces one or more hydrogen atoms of the amino group in the 6-position, and those in which replacement has taken place on the nitrogen atoms occupying the *N*-3-positions in the pyrimidine nucleus, as for example,



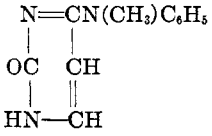
(Group I) (Group II)

Substances belonging to the first group are usually obtained by treating the corresponding 6-chloropyrimidine with an alkyl or aryl amine but may be prepared by alkylation of the amino nitrogen under the action of an alkyl halide in alkaline solution (198e, 103a, 90a, 72a, 73). Methylated derivatives of cytosine belonging to this group may also be obtained from the corresponding methylated 2-mercaptopyrimidines under the hydrolyzing action of hydrochloric acid:

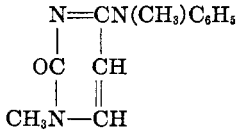


Several examples of this method of preparation have been reported (71, 72b, 74, 76).

Compounds belonging to the second group have been prepared by direct alkylation of cytosine or derivatives of cytosine in alkaline solutions under the action of methyl iodide (90b, 72a, 73). Alkylation products have also been prepared under the action of dimethyl sulfate (75, 77). The presence of substituents in the *N*-3-position of the pyrimidine ring may be detected by the fact that substituting groups in this position inhibits the action of diazobenzenesulfonic acid and negative color tests result. The formation of a red color with this reagent is not, however, dependent upon the presence of a free amino radical in the 6-position in the case of either cytosine or 5-methylcytosine. Thus, for example,

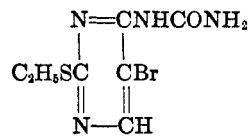


gives a red color with diazobenzenesulfonic acid, while



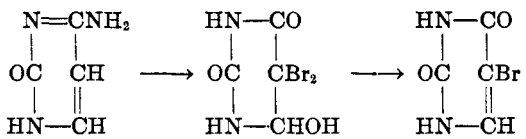
shows no change under the same conditions (90f). This reaction may be of possible significance in allocating the sugar linkage on the pyrimidine molecule in nucleic acid combinations. It is also interesting to note in this connection that the introduction of methyl groups into the cytosine molecule increases the solubility and lowers the melting point of the product (90c).

The number of halogen derivatives of cytosine which have been investigated up to the present time is extremely limited. *5-Bromocytosine* has been prepared by treating 2-ethylmercapto-6-oxypyrimidine dissolved in acetic acid with a molecular quantity of bromine. The resulting product was converted first into 2-ethylmercapto-5-bromo-6-chloropyrimidine, the chlorine replaced by an amino group and the mercapto radical then removed by boiling with concentrated hydrochloric acid (204c). The same substance was also obtained from 2-ethylmercapto-5-bromo-6-ureapyrimidine,



by boiling with concentrated hydrochloric acid (198f).

In studying the behavior of cytosine under the direct action of bromine water, Wheeler and Johnson (203d, 205a) found that the primary product consisted of oxydibromohydrouracil. This on digestion with alcohol was converted quantitatively into 5-bromouracil:



3-Methylcytosine has since been observed to behave in the same way under the action of bromine water, yielding 3-methyl-5-bromouracil (90d). 3,5-Dimethylcytosine yields 3-methylthymine under the same conditions.

5-Iodocytosine is prepared by dissolving synthetic cytosine in

dilute aqueous potassium hydroxide and adding finely powdered iodine (102b). *4-Chlorocytosine* is prepared by treating 2-ethylmercapto-4,6-dichloropyrimidine with alcoholic ammonia and then desulfurizing by boiling with concentrated hydrochloric acid (199b). *4-Chloro-5-bromocytosine* was obtained in the form of its hydrobromic acid salt by suspending 4-chlorocytosine in glacial acetic acid and adding a molecular quantity of bromine. The free base was separated by first warming the salt with dilute, aqueous sodium hydroxide and then neutralizing the solution with dilute acetic acid (100f).

It is to be noted that chlorine in the 4-position of certain pyrimidines and also both bromine and iodine in the 5-position are extremely inert and cannot be replaced by an amino group on treatment with aqueous or alcoholic ammonia. Chlorine in the 4-position was not reduced by tin and hydrochloric acid, nor by hydrogen iodide and red phosphorus (199c). Iodine in the 5-position of cytosine behaves in a remarkable way when treated with alcoholic ammonia, being reduced practically quantitatively to cytosine (102a).

A number of different derivatives of cytosine have been prepared which contain a nitro group in the 5-position. Substances of this type command considerable interest because of the fact that they yield 2-oxy-4,5-diaminopyrimidines on reduction, since the latter have been used extensively in the synthesis of the purines.

5-Nitrocytosine was first obtained by treating cytosine with a mixture of nitric and sulfuric acids (204d). Its structure at that time was assumed to be that of a nitramine. Later it was prepared by dissolving 2-ethylmercapto-6-aminopyrimidine in a mixture of nitric and sulfuric acids, when hydrolysis of the mercapto group took place simultaneously with nitration. The product, which consisted of 5-nitro-6-aminopyrimidine, was found to be identical with the substance previously obtained by nitrating cytosine. Its structure was finally definitely established by heating it under pressure with 20 per cent sulfuric acid, when it was converted into 5-nitrouracil (103b). The product may be readily methylated in the 3-position by treating the potassium salt with methyl iodide (72a).

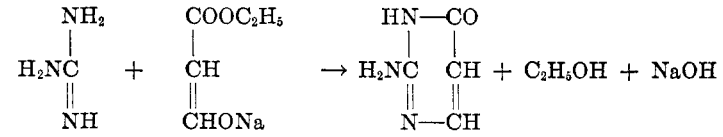
4-Methyl-5-nitrocytosine was obtained in almost quantitative yields when 4-methylcytosine was dissolved in sulfuric acid and then treated with nitric acid (sp. gr. 1.5). The structure of the product was definitely established by heating in a sealed tube with 30 per cent sulfuric acid for two hours at 130–140°C., when 4-methyl-5-nitrouracil was obtained (cf. 13a). The product may be readily methylated in the 3-position by treating with dimethyl sulfate (75).

2-Oxy-5-nitro-6-methylaminopyrimidine was obtained from 7-methylcytosine by dissolving the latter in concentrated sulfuric acid and adding fuming nitric acid to the solution (71). The product may be readily methylated in the 3-position of the ring by treating its potassium salt with methyl iodide (73). *2-Oxy-4-methyl-5-nitro-6-methylaminopyrimidine* was obtained from 2-oxy-4-methyl-6-methylaminopyrimidine in the same way (72c). The corresponding 5-nitro derivatives of 2-oxy-6-ethylaminopyrimidine (76) and of 2-oxy-4-methyl-6-ethylaminopyrimidine (74), were obtained by the use of the same general method.

Cytosine is unacted upon by most reducing agents but under the action of hydrogen in the presence of colloidal palladium the 4,6-ethylene double bond is saturated and it is hydrolyzed smoothly to give hydrouracil as the only product (24b). This behavior is remarkable because it emphasizes the relative instability of the amino group in a reduced pyrimidine nucleus as compared with its very great stability in both the 6- and the 5-positions when ethylene carbon atoms are present in the ring.

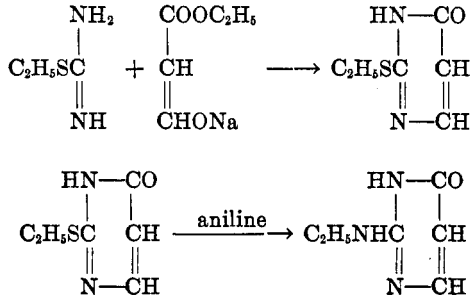
3. Isocytosine

Three general methods have been used successfully for the synthesis of isocytosine and its alkylated derivatives. The first consists in condensing guanidine (203d) with β-ketonic esters in alkaline solutions, as, for example:

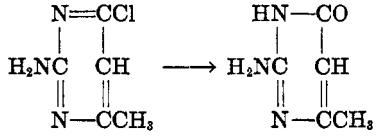


Guanidine — Sodium salt of ethyl formylacetate — Isocytosine

The second method consists in condensing pseudothioureas with β-ketonic esters and then digesting the product with ammonia, an amine,[9] aniline, etc. (102c).



The third general method consists in the hydrolysis of a halogen atom present in the 6-position of 2-aminopyrimidines (55q):



The above reaction was brought about by the action of hydrogen iodide and red phosphorus, but hydrolysis may be induced by prolonged refluxing with water (66b). In certain cases it has been found convenient to treat the substance with sodium methoxide and then warm the resulting 2-amino-6-methoxypyrimidine with hydrochloric acid (66b).

Isocytosine itself has been prepared by use of the first and third of these general methods (203d, 66b). It has also been prepared independently by the hydrolysis of 2-amino-6-methoxypyrimidine (58c).

4. Derivatives of isocytosine

Derivatives of isocytosine have been described which contain alkyl and aryl groups on the carbon atoms in the 4- and 5-positions in the ring. Such substances have been synthesized by condensing either guanidine carbonate or ethyl pseudothiourea

[9] For the use of methylamine in this type of transformation see reference 106e.

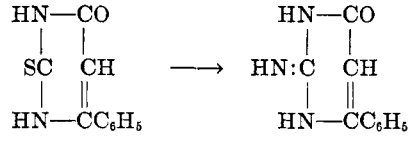
with β-ketonic esters. Another class of derivatives in which the hydrogen of the amino group in the 2-position has been replaced by hydrocarbon residues is also represented by a fairly large number of substances. These have been prepared for the most part by the application of the second general method. Only one derivative containing a substituent in the *N*-1-position has as yet been described.

4-Methylisocytosine was prepared by condensing guanidine carbonate with ethyl acetoacetate (67a, cf. 15b), and also by treating 2-amino-4-methyl-6-chloropyrimidine with hydrogen iodide and red phosphorus (55o).

5-Methylisocytosine was obtained by condensing guanidine carbonate with ethyl formylpropionate in the presence of barium hydroxide (89a). This substance is characterized by the fact that in the presence of dilute aqueous sodium hydroxide, the pyrimidine ring opens to give a mixture of *cis* and *trans* α-methyl-β-guanidineacrylic acids. Attempts to hydrolyze 5-methylisocytosine to thymine by heating in a sealed tube with concentrated sulfuric acid for five hours were unsuccessful.

4,5-Dimethylisocytosine was prepared by condensing guanidine carbonate with ethyl methylacetoacetate (67c, cf. 173c). This substance hydrolyzes readily in acid solution to give 4,5-dimethyluracil (173c).

4-Phenylisocytosine has been prepared by condensing guanidine carbonate with ethyl benzoylacetate (67d), and also by treating 2-thio-4-phenyl-6-oxypyrimidine with ammonia (191).



The fact that the substance appears to exist in isomeric modifications led to a repetition of Jaeger's experiments by Johnson and Hill (99a).

4-Methyl-5-ethylisocytosine was prepared from 2-ethylmercapto-4-methyl-5-ethyl-6-oxypyrimidine by heating with alcoholic ammonia for three hours at 150–160°C. (83b).

Isocytosine-5-acetamide was prepared by heating the corresponding 2-ethylmercaptopyrimidine with alcoholic ammonia at 170–180°C. for two hours (115).

2-Amino-4-methyl-6-oxypyrimidine-5-acetic acid was prepared by heating the corresponding 2-ethylmercaptopyrimidine with alcoholic ammonia at 170–180°C. for four hours (98f).

5-Ethoxyisocytosine was obtained by condensing guanidine carbonate with the sodium salt of ethyl α-ethoxy-β-oxyacrylate in the presence of barium hydroxide (109b).

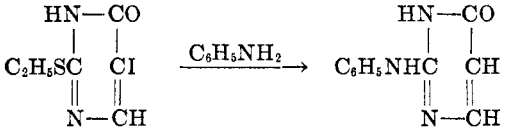
The following substances represent derivatives of isocytosine in which the hydrogen of the amino group in the 2-position has been replaced by alkyl or aryl radicals.

2-Methylamino-5-methyl-6-oxypyrimidine was obtained from the corresponding 2-methylmercaptopyrimidine by treatment with methylamine at 140–150°C. for two hours (106c).

2-Methylamino-4-methyl-6-oxypyrimidine was prepared (a) from the corresponding 2-methylmercaptopyrimidine by treatment with methylamine at 140–150°C. (106e); (b) from 4-methylisocytosine by treatment with methyl iodide in the presence of sodium methylate (67b); and (c) by condensing methylguanidine carbonate with ethyl acetoacetate (146).

2-Methylamino-4-carboxyl-5-methyl-6-oxypyrimidine was obtained from the corresponding 2-ethylmercaptopyrimidine by heating with aniline in alcohol solution (83a).

2-Anilino-6-oxypyrimidine was obtained from 2-ethylmercapto-6-oxypyrimidine by warming with aniline in alcohol solution (102c). It was also prepared by heating 2-ethylmercapto-5-iodo-6-oxypyrimidine with aniline on a steam bath for several hours (102c).



The corresponding 5-bromo derivative was obtained by heating 2-ethylmercapto-5-bromo-6-oxypyrimidine with excess of aniline on a steam bath for several hours. In this case there was no

evidence that any reduction of the bromine in the 5-position had taken place during the process (198c).

N-1-Methyl-2-anilino-6-oxypyrimidine[10] was prepared by treating 2-anilino-6-oxypyrimidine with methyl iodide in the presence of sodium methylate (98c). When formed in this way the substance separates from its solutions with one molecule of water of crystallization. An anhydrous modification of the same substance was obtained by heating *N*-1-methyl-2-ethylmercapto-6-oxypyrimidine with aniline at 100°C. for several hours (98c).

2-Benzalamino-6-oxypyrimidine was prepared by heating isocytosine with benzaldehyde at 160–180°C. for three hours (96).

2-Methylanilino-6-oxypyrimidine was obtained from the corresponding 2-ethylmercaptopyrimidine by heating with methylaniline at 100°C. for fourteen hours (98d).

2-Anilino-5-ethoxy-6-oxypyrimidine was prepared from the corresponding ethylmercaptopyrimidine by heating with aniline on a steam bath for several hours (98e).

None of the alkylated derivatives of isocytosine has been investigated with systematic thoroughness, although isocytosine itself has been studied in some detail. It melts at 276°C. and is precipitated by potassium bismuth iodide, but when dissolved in sulfuric acid it yields a brick-red precipitate with this reagent. It gives the murexide reaction. Its salts with hydrochloric and sulfuric acids are very soluble in water but its picrate and double salts with gold and platinum are not very soluble (203e). When boiled with acetic anhydride it yields 2-acetamino-6-oxypyrimidine (196g).

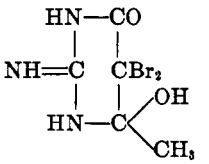
5-Bromoisocytosine has been prepared by dissolving isocytosine in glacial acetic acid and adding a molecular quantity of bromine (203f). The substance separates in the form of its hydrobromic acid salt under these conditions, but aqueous solutions of this salt yield the free base on treatment with ammonia. In this connection it is interesting to note that although 5-bromoisocytosine possesses both acid and basic properties, and therefore is soluble in aqueous ammonia, it nevertheless separates unaltered

[10] This is the only derivative of isocytosine which has as yet been described that shows replacement of hydrogen in the *N*-1-position.

from its ammoniacal solutions on boiling off the ammonia. 5-Bromoisocytosine, heated with ammonia at 190–215°C. for six hours, reacts to give 2,5-diamino-6-oxypyrimidine (100i).

When isocytosine is treated with bromine in aqueous solution, on the other hand, it behaves quite differently and yields a bromine derivative which, while not identical with the dibromoöxydihydrouracil that is formed when cytosine is treated in this way, gives an intense blue color when barium hydroxide is added to its aqueous solutions. This color is quite readily distinguished from the purple or violet-blue color produced by cytosine or uracil under the same conditions and is further characterized by the fact that it completely disappears when an excess of barium hydroxide has been added. This reaction therefore serves as a delicate test for isocytosine (205b).

5-Bromo-4-methylisocytosine is formed as a secondary product when 4-methylisocytosine is treated with bromine water. The primary product which is formed under these conditions consists of a substance closely analogous to dibromoöxydihydrouracil, viz.:



This when warmed with alcohol yields 2-amino-4-methyl-5-bromo-6-oxypyrimidine. The latter does not react smoothly when treated with ammonia, the product consisting of an oil mixed with small quantities of a crystalline compound. That 4-methyl-5-aminoisocytosine was formed as a result of this action seems probable, however, since the crystalline compound when separated appeared on analysis to have this composition (67a).

5-Nitroisocytosine was prepared by treating isocytosine with a mixture of concentrated sulfuric and nitric acids under special conditions of temperature and concentration (100h). The structure of the product was determined by its conversion into

5-nitrouracil. 5-Nitroisocytosine is readily reduced in ammoniacal solution under the action of aluminum amalgam. Nitrous acid does not react with isocytosine in solution in glacial acetic acid, an acetic acid salt of isocytosine being the only product which was obtained as a result of this treatment (100h).

Isocytosine and methylisocytosine in the form of their silver salts react with acetobromoglucose to give the corresponding tetraacetyl-*d*-glucosides. The latter on deacetylation with ammonia yield the corresponding *d*-glucosides. Both compounds are split by emulsin and takadiastase but not by maltase (63, 64).

That the amino group in the 2-position is firmly seated would appear from the fact that isocytosine and 4-methylisocytosine both react with phosphorus oxychloride to yield the corresponding 6-chloro derivatives without decomposition. Subsequent replacement of the halogen under the action of different reagents can be effected without involving the amino group (196b, 55m, 173a). The amino group in 2-amino-4,6-dichloropyrimidine seems to be equally stable (29a).

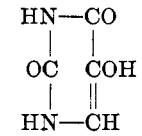
Monoaminodioxypyrimidines

The only monoaminodioxypyrimidines which can be included among the derivatives of uracil contain an amino group in the 5-position, since amino groups in the 2-, 4-, or 6-positions in dioxypyrimidines hydrolyze to give barbituric acid or its derivatives. Very few members of this class of pyrimidines have been investigated up to the present time, the most important being 5-aminouracil and 4-methyl-5-aminouracil. General methods for the preparation of such substances consist (a) in heating the corresponding 5-bromo- or 5-chloro-pyrimidine with ammonia and (b) in reducing the corresponding 5-nitropyrimidine.

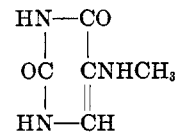
5-Aminouracil has been obtained by heating 5-bromouracil (13e, 207b, 204b) with aqueous ammonia. The product was identified by means of its picrate (204b, 16c, 198a). 5-Aminouracil has also been prepared by reducing 5-nitrouracil (119b, 198a, 107b). It should be noted that 5-nitrouracil may be prepared in a number of different ways, viz., by nitrating uracil, 2-ethylmercaptouracil, or 2-thiouracil (198b, 78a, 107a), and

also by heating 5-nitrouracil-4-carbonic acid (13b, 16b). The reduction of 5-nitrouracil has been made the subject of extended investigation and can be accomplished more or less successfully under the action of a number of different reducing agents, i.e., with tin and hydrochloric acid (12, 10c), with zinc and hydrochloric acid (13c), with zinc and ammonia (16a), with ferrous sulfate in alkaline solution (209), and with ferrous sulfate and ammonia (107b). A method which appears to afford better yields (70 to 86 per cent) than any of those reported above consists in using aluminum amalgam in the presence of slightly alkaline aqueous ammonia (16b). In all these methods of reduction there is always some formation of isobarbituric acid owing to hydrolysis.

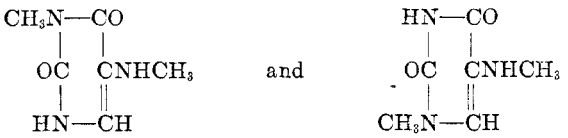
Attempts to bring about reduction catalytically in the presence of colloidal palladium also resulted in a partial hydrolysis of the amino group with the formation of isobarbituric acid (24a).



A number of methylated derivatives of 5-aminouracil have been reported in the literature. *1,3-Dimethyl-5-aminouracil* was prepared by treating the potassium salt of 5-aminouracil suspended in methyl alcohol with methyl iodide (107c). The product separated in the form of the salt of hydrogen iodide and the free base was isolated as a secondary product on treatment with silver oxide. *5-Methylaminouracil,*

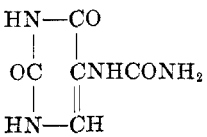


was obtained by treating 5-bromouracil with methylamine in aqueous solution (207b, 107e). *5-Dimethylaminouracil* was prepared from 5-bromouracil under the action of dimethylamine (199e). *N-1-* and *N-3-methyl-5-methylaminouracil*



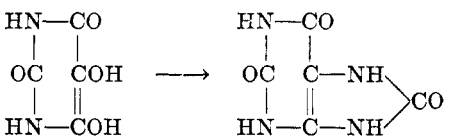
were obtained from the corresponding *N*-1- and *N*-3-methyl derivatives of 5-bromouracil by treatment with methylamine (107d, 97c, 90d).

Various salts of 5-aminouracil have been prepared and described, also its acetyl and benzoyl derivatives (16d). With potassium cyanate it gives pseudouric acid (10d),



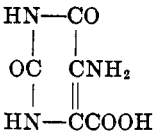
which does not lose water to give a purine but which, like xanthine, is readily oxidized to alloxan (10e; cf. 37).

5-Aminouracil may be transformed into uric acid by a process of simultaneous hydrolysis and oxidation with bromine water and then condensing the product, which consists of isodialuric acid,

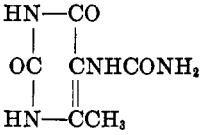


with urea in the presence of sulfuric acid to give a purine by incorporation of an amidazole ring (17).

4-Methyl-5-aminouracil has been prepared from 4-methyl-5-bromouracil (11a) by heating with concentrated aqueous ammonia for several hours. 4-Methyl-5-nitrouracil is easily oxidized to the corresponding nitrouracilcarboxylic acid (13b). The potassium salt of this pyrimidine acid is reduced in aqueous solution by zinc chloride to give *5-aminouracil-4-carbonic acid* (13d, 119a), which is apparently the only pyrimidine aminocarboxylic acid thus far described in the literature.



4-Methyl-5-aminouracil yields an acetyl derivative under the action of acetyl chloride (11d) and reacts with potassium cyanate to give a pseudouric acid (11c).

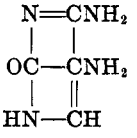


Like the corresponding pseudouric acid obtained from 5-aminouracil, this does not condense to give a purine compound under the action of dehydrating agents.

The chemistry of the alkyl derivatives of 4-methyl-5-aminopyrimidine is very incomplete and only one derivative belonging to this class of substances has been reported in the literature. *4-Methyl-5-methylaminouracil* has been prepared by treating 4-methyl-5-bromouracil (11b, 199e) with an excess of aqueous methylamine at 150°C.

Monoöxydiaminopyrimidines

There are five possible monoöxydiaminopyrimidines in which the oxygen atoms occupy the 2- or 6-positions of the pyrimidine ring and in which the amino groups are attached to carbon:



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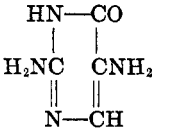

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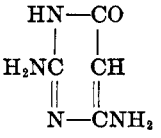
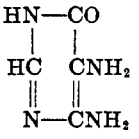
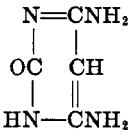


III



IV



V

Of these only the first two can yield uracil or a derivative of this pyrimidine (isobarbituric acid). Both have been synthesized by nitrating cytosine and isocytosine respectively and then transforming the nitro group into an amino group under the action of suitable reducing agents. As has been stated previously in another connection, considerable study has been given to the conditions under which the reduction of a nitro group may be most conveniently effected. In the case of the substances about to be described, aluminum amalgam in the presence of aqueous ammonia or water is used in a number of cases, but in the most recent work this has been replaced by freshly precipitated ferrous hydroxide in aqueous solution, which is recommended as possessing certain distinct advantages.

2-Oxy-5,6-diaminopyrimidine (5-aminocytosine) was obtained by suspending 5-nitrocytosine (204d, 103c) in cold water in contact with aluminum amalgam. The temperature was not allowed to rise above 35°C. during the reduction and the mixture was thoroughly agitated during the 30 minutes required for the reaction. Since the base is characterized by the ease with which it undergoes decomposition in aqueous solution with the evolution of ammonia, the aluminum hydroxide was filtered as rapidly as possible and the clear straw-colored solution evaporated to small volume in an atmosphere of hydrogen under diminished pressure. Under these conditions a crystalline substance mixed with considerable quantities of amorphous flocculent material was deposited. The base crystallizes from its aqueous solution with one molecule of water of crystallization and is quite stable when dry. When dissolved in water it decomposes slowly with the precipitation of amorphous material. Its aqueous solutions are alkaline to turmeric and react with hydrochloric, nitric, and sulfuric acids to form soluble salts. It is an excellent reducing agent, giving a silver mirror when added to an ammoniacal solution of silver nitrate and precipitating the metals from aqueous solutions of gold and platinum chloride. It reduces Fehling's solution instantly. It is precipitated from its aqueous solutions by phosphotungstic acid and by potassio-bismuth iodide (103e).

The reduction of 5-nitrocytosine has also been accomplished

under the action of freshly precipitated ferrous hydroxide (70). Because of the marked instability of this substance in the presence of hydrolytic agents and even in solution in water, the possibility of its being identical with "Kutscher's base" is obviously excluded.

2-Oxy-3-methyl-5,6-diaminopyrimidine has been prepared by treating the potassium salt of 5-nitrocytosine in aqueous solution with methyl iodide and then reducing the product with freshly precipitated ferrous hydroxide (72a).

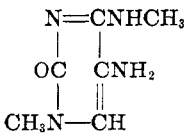
2-Oxy-4-methyl-5,6-diaminopyrimidine was prepared from 4-methyl-5-nitrocytosine (69), by suspending it in water and then reducing in the presence of aluminum amalgam under vigorous shaking, care being taken to keep the temperature below 45°C.

2-Oxy-3,4-dimethyl-5,6-diaminopyrimidine was obtained by alkylating 4-methyl-5-nitrocytosine with dimethyl sulfate and then reducing the nitro group with ferrous hydroxide (75).

Derivatives of 5-aminocytosine and 4-methyl-5-aminocytosine have been described in which the hydrogen of the amino group has been replaced by alkyl residues. In certain cases *N*-3-methyl derivatives of these substances have also been prepared.

2-Oxy-5-amino-6-methylaminopyrimidine was obtained by treating 2-ethylmercapto-6-chloropyrimidine with methylamine and then desulfurizing the product by boiling with hydrochloric acid. The resulting 2-oxy-6-methylaminopyrimidine was then nitrated by dissolving it in concentrated sulfuric acid and adding fuming nitric acid. The nitro group in the 5-position was finally reduced with ferrous hydroxide (71).

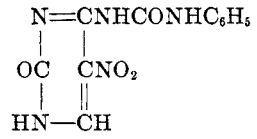
2-Oxy-3-methyl-5-amino-6-methylaminopyrimidine



was obtained by methylating the potassium salt of 5-nitromethylcytosine with methyl iodide and then reducing the resulting product (73).

2-Oxy-5-amino-6-ethylaminopyrimidine was synthesized from 2-ethylmercapto-6-chloropyrimidine by using the same procedure as has been described in the case of the corresponding methylaminopyrimidine (76).

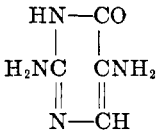
2-Oxy-5-nitro-6-phenylureapyrimidine,



has been prepared (103d) but has not been reduced to the corresponding amine.

2-Oxy-4-methyl-5-amino-6-methylaminopyrimidine was synthesized by starting with 2-ethylmercapto-4-methyl-6-chloropyrimidine and proceeding in a manner analogous to that described in the case of the corresponding 2-oxy-5-amino-6-methylaminopyrimidine (72b). *2-Oxy-4-methyl-5-amino-6-ethylaminopyrimidine* was synthesized in a similar way (74).

The only 5-amino derivative of isocytosine which has been described up to the present time is 2,5-diamino-6-oxypyrimidine.



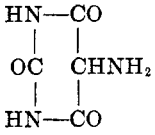
This was prepared in three different ways: (a) From isocytosine (203d) by nitrating under special conditions (100h). The structure of the resulting 5-nitro derivative was determined by converting it first into nitrouracil and then transforming the latter into the picrate of 5-aminouracil which has a definite melting point. 2,5-Diamino-6-oxypyrimidine was also readily obtained from 5-nitroisocytosine by reduction in ammoniacal solution with aluminum amalgam (100h). (b) From 5-bromoisocytosine (203f) by heating it with concentrated aqueous ammonia (100h). (c) From 2-ethylmercapto-5-amino-6-oxypyrimidine by heating it with alcoholic ammonia (100h). Of these three methods the first is the only one having practical value.

2,5-Diamino-6-oxypyrimidine is extremely soluble in water and separates from its aqueous solution with one molecule of water of crystallization. When boiled with water the solution assumes a red color and the base is apparently oxidized with the separation of a flocculent amorphous precipitate. It is a strongly diacid base and forms soluble salts with hydrochloric and nitric acids. Its sulfate and its picrate are only slightly soluble in water. It reduces the chlorides of gold and platinum to the corresponding metals and is precipitated from its aqueous solutions by phosphotungstic acid, mercuric chloride, and potassiobismuth iodide. The latter forms a brick-red precipitate. The base when heated with 20 per cent sulfuric acid at 130–140°C. in a sealed tube for three hours was partially hydrolyzed to 2-amino-5,6-dioxypyrimidine, but about 50 per cent of the original substance was recovered unchanged. The fact that this substance is relatively stable in the presence of sulfuric acid admits of the possibility of its being identical with "Kutscher's base." It is the only dioxydiaminopyrimidine which corresponds in its general properties to the base described by Kutscher as having this composition and as occurring among the hydrolytic products of yeast nucleic acid.

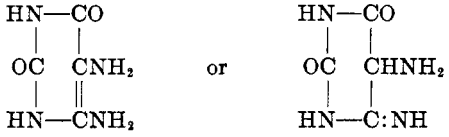
It should be noted that all 2-oxypyrimidines which contain amino groups in the 5,6-positions undergo condensation with formic acid, acetic anhydride, and urea to give purine derivatives. This type of condensation is not inhibited by the replacement of the hydrogen of the amino group nor of the hydrogen in the *N*-3-position in the ring by an alkyl hydrocarbon residue. A more detailed discussion of the various methods by which pyrimidines may be transformed into purines is reserved for later consideration.

IV. OXYAMINOPYRIMIDINES RELATED TO BARBITURIC ACID

In considering this class of substances it is necessary to distinguish between (a) direct substitution products of barbituric acid which result from the replacement of hydrogen by amino or other groups, such as for example, uramil,



its homologues and other derivatives; and (b) substances like 2,6-dioxy-4,5-diaminopyrimidine (or its imido form),

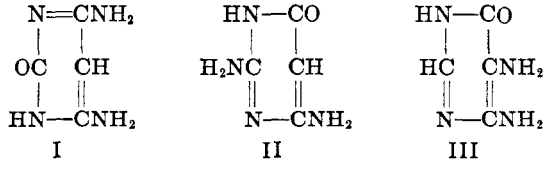


which while appearing to be substitution products of uracil actually yield barbituric acid or its derivatives on hydrolysis. It may be noted in passing that all 2,6-dioxypyrimidines which contain an amino group in the 4-position of the ring belong to the latter class. Since compounds of this type are not very numerous they will be considered first, and a discussion of uramil and products related to it will be reserved until later.

Aminoöxypyrimidines which hydrolyze to give barbituric acid or its derivatives may be separated into different subgroups arranged according to the number of oxygen atoms and amino groups present in the molecule. Any classification as arbitrary as this is open to certain objections, but these seem to be overbalanced by the advantages afforded in dealing with a somewhat confused mass of material.

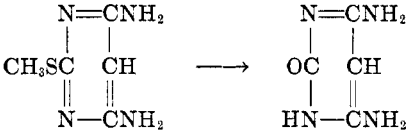
Monoöxydiaminopyrimidines

The statement has already been made that oxydiaminopyrimidines are capable of existing in five different structural modifications, only two of which yield uracil on hydrolysis. Both of these have been considered in some detail. Of the remaining three isomeric possibilities only the first two have



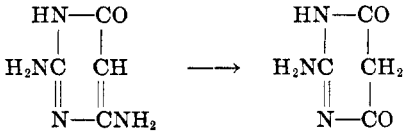
been synthesized up to the present time.

2-Oxy-4,6-diaminopyrimidine (I) was first prepared by Wheeler and Jamieson (199c) because it seemed possible that a substance having this composition might prove to be identical with a base which had been described by Kutscher (126) as resulting from the hydrolysis of yeast nucleic acid. While this did not prove to be the case, the substance which was actually obtained is nevertheless of definite theoretical importance. 2-Thio-4,6-diaminopyrimidine (188a) was made the starting point in this synthesis and was converted first into the corresponding 2-methylmercapto derivative under the action of methyl iodide. This product was then hydrolyzed by warming with hydrochloric acid:

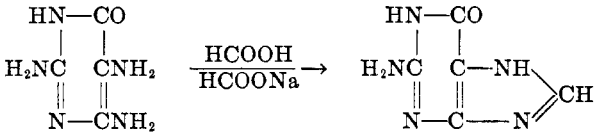


Great care must be exercised in the hydrolysis, since continued warming with hydrochloric acid results in the complete conversion of the product into barbituric acid with loss of ammonia. Under the conditions of the experiment the base separated in the form of the monohydrochloride, but when this salt is dissolved in water and ammonia added, the free base may be obtained in anhydrous condition. The latter is only slightly soluble in water and forms a characteristic picrate.

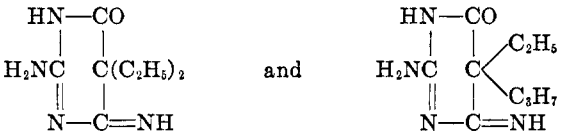
6-Oxy-2,4-diaminopyrimidine (II) was first synthesized by Traube (190d) by condensing guanidine with ethyl cyanoacetate in the presence of absolute alcohol and sodium ethoxide. This product when heated with 20–30 per cent sulfuric acid at 130–140°C. is completely decomposed, this fact eliminating at once the possibility of its being identical with "Kutscher's base." When warmed with dilute sulfuric acid, on the other hand, it hydrolyzes readily to give malonylguanidine (31b; cf. 151b, 189b).



6-Oxy-2,4-diaminopyrimidine reacts with hydrochloric and sulfuric acids to form salts. When the sulfate is dissolved in water and treated with sodium nitrite it gives a 5-nitroso derivative and this on reduction in aqueous solution with ammonium sulfide yields 2,4,5-triamino-6-oxypyrimidine. The latter, under the action of a mixture of formic acid and sodium formate, condenses to give guanine (189b).



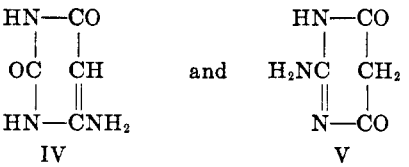
Derivatives of 6-oxy-2,4-diaminopyrimidine have been obtained in which the hydrogen atoms in the 5-position are replaced by alkyl groups (31e), viz.,



These substances are prepared by treating guanidine with the corresponding dialkylcyanoacetic esters in the presence of sodium ethoxide. Like the parent substance they hydrolyze on prolonged boiling with mineral acids, yielding the corresponding dialkylbarbituric acids.

Dioxymonoaminopyrimidines

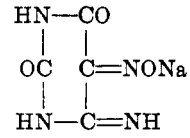
Only two of the four structural possibilities for compounds having this composition yield barbituric acid or its derivatives on hydrolysis, viz.,



Both types of compounds have been synthesized and various derivatives of each prepared.

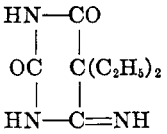
2,6-Dioxy-4-aminopyrimidine (4-aminouracil) was prepared by condensing ethyl cyanoacetate with urea in absolute alcohol solution in the presence of sodium ethoxide (31b; cf. 190a). The same substance may be obtained from cyanoacetylurea, NH_2-$CONH \cdot COCH_2CN$ (190b, 31a) by treatment with sodium hydroxide.

2,6-Dioxy-4-aminopyrimidine is both acidic and basic, dissolving in alkali and ammonia and also in concentrated mineral acids to form salts. Its sodium salt when treated with sodium nitrite in aqueous solutions yields a salt of the corresponding violuric acid:



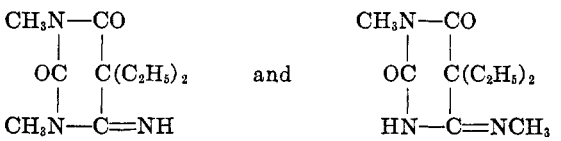
N-Alkyl derivatives of 2,6-dioxy-4-aminopyrimidine may be obtained by condensing alkyl ureas with ethyl cyanoacetate in the presence of sodium ethoxide,—*N*-3-methyl-4-amino-2,6-dioxypyrimidine being obtained from methylurea (190b, 31c, 97b) and an *N*-1-*N*-3-dimethyl derivative from dimethylurea (190i; cf. 156). Both have been transformed into the corresponding 5-nitroso and 5-amino compounds by application of the same general procedure as has already been described in the case of 2,6-dioxy-4-aminopyrimidine. The resulting methylated 4,5-diaminopyrimidines yield 3-methylxanthine (190h; cf. 43) and 1,3-dimethylxanthine, respectively, under the action of a mixture of formic acid and sodium formate. 1,3,7-Trimethylxanthine and 1,3-dimethyluric acid may also be obtained from 1,3-dimethyl-2,6-dioxy-4,5-diaminopyrimidine on treatment with methyl formate and with ethyl chloroformate and ammonia (190h), respectively.

Derivatives of the tautomeric modification of 2,6-dioxy-4-aminopyrimidine in which two hydrogens in the 5-position have been replaced by alkyl groups have also been prepared. For example, *C*-diethyl-4-iminobarbituric acid,

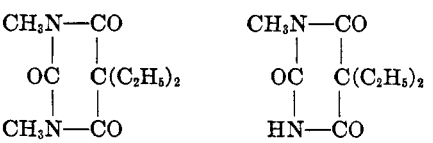


is obtained by condensing ethyl diethylcyanoacetate with urea in the presence of sodium ethoxide. Other representatives which have been prepared by substituting the corresponding alkyl cyanoacetic esters for ethyl cyanoacetate in this type of reaction are, for example, *C*-propyl, *C*-ethylpropyl, *C*-dipropyl-, and *C*-dibenzyl-4-aminobarbituric acids. These substances all hydrolyze on prolonged boiling with mineral acids to give the corresponding alkylated barbituric acids (31d).

Alkyl derivatives combining both of the two preceding types have also been reported. For example, 5-diethyl-4-iminobarbituric acid reacts with methyl iodide in the presence of sodium methoxide to give the corresponding *N*-1-methyl-, *N*-3-methyl-, and *N*-1-*N*-3-dimethyl derivatives (31d). *N*-1-Methyl-5-diethyl -4-iminobarbituric acid also reacts with methyl iodide in the presence of sodium methoxide to give a dimethyl derivative which is not identical with the *N*-1-*N*-3-diethyl-4-iminobarbituric acid referred to above. It is an interesting fact that these two isomeric products



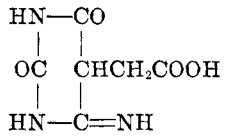
hydrolyze under the prolonged action of mineral acids to give diethylmalonyldimethylurea and diethylmalonylmethylurea respectively.



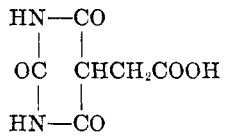
In this connection it may be noted that *N*-alkyl derivatives of the above types hydrolyze to give open chain compounds and in

this respect differ from the preceding class of substances which contain hydrogen atoms in both the *N*-1 and *N*-3 positions and which hydrolyze under the same conditions to yield ring compounds (alkylbarbituric acids).

4-Iminobarbituryl-5-acetic acid has been prepared by a modification of the general method of procedure which has just been discussed, and may be obtained by digesting ethyl cyanosuccinate with urea. During the process the ester group was saponified and the imino group was also hydrolyzed to some extent. This was shown by the fact that while a brown precipitate consisting of



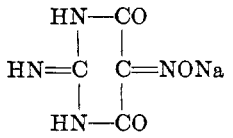
was obtained as the immediate product of the reaction, the filtrate from this when concentrated deposited crystals of barbituryl-5-acetic acid (105).



4,6-Dioxy-2-aminopyrimidine (V), malonylguanidine, was first synthesized by Michael (150, 151a, 149) as a result of condensing guanidine rhodanide with the sodium salt of diethyl malonate in absolute alcohol. It has also been prepared by adding two molecular quantities of free guanidine to one molecular quantity of diethyl malonate in the absence of any solvent. The product in this case forms a solid crystalline mass, which consists of the guanidine salt of malonylguanidine. This dissolves readily in water to give a strongly alkaline solution from which, on the addition of dilute acetic acid, free malonylguanidine is precipitated as a crystalline solid (189a).

Malonylguanidine when suspended in water and treated with bromine gives a dibromo derivative having the composition $C_4H_3O_2N_3Br_2$ (189a). Treated with fuming nitric acid, it yields a

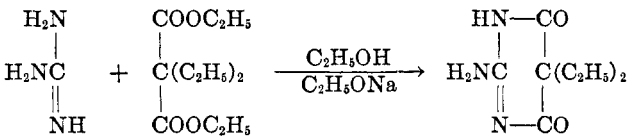
5-nitro derivative and with sodium nitrite, the sodium salt of the corresponding isonitroso derivative, viz.,



2-Iminovioluric acid
(sodium salt)

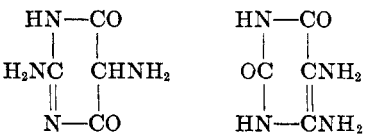
The corresponding ammonium salt of 2-iminovioluric acid may be obtained by dissolving malonylguanidine in aqueous ammonia and adding an equivalent amount of sodium or potassium nitrite. Dilute hydrochloric acid is then gradually added until the solution is strongly acid, when it is diluted and treated with an excess of ammonia. If sufficiently dilute the clear solution develops a dark violet coloration on boiling and when cooled deposits violet colored needles which consist of the ammonium salt of 5-isonitrosomalonylguanidine. This salt may be used in the preparation of the corresponding 2,5-diamino derivative of 4,6-dioxypyrimidine.

C-Dialkyl malonylguanidines have been prepared by condensing alkylmalonic esters with guanidine in absolute alcohol under the action of sodium ethoxide as the condensing agent (45a). This reaction is of general application.



Dioxydiaminopyrimidines

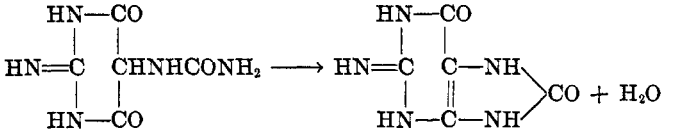
Pyrimidines which contain two oxygen atoms and two amino groups and which on hydrolysis yield barbituric acid or its derivatives may exist in two different structural modifications (VI and VII):



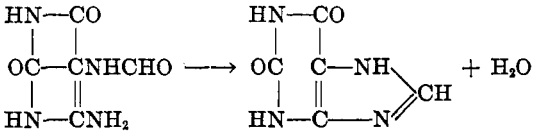
VI VII

Both types of substances have been synthesized but very little is known regarding their derivatives. *4,6-Dioxy-2,5-diaminopyrimidine* (VI) was prepared by Traube (189c) by dissolving the ammonium salt of 5-isonitrosomalonylguanidine in dilute hydrochloric acid and saturating the solution with hydrogen sulfide. It is interesting to note that reductions of this type with hydrogen sulfide in acid solution are very unusual, since the observation has frequently been made that nitro and nitroso groups are unacted upon by this gas except in the presence of alkali (113a; cf. 4c).

4,6-Dioxy-2,5-diaminopyrimidine dissolves in boiling aqueous potassium cyanate, and this solution when cooled and treated with hydrochloric acid yields the corresponding urea (189d, 38d). According to Fischer the latter may be condensed to the corresponding purine if it is warmed with 20 per cent hydrochloric acid.



2,6-Dioxy-4,5-diaminopyrimidine (VII) has also been prepared by Traube (190f). Like its isomer it was obtained from the corresponding 5-isonitroso derivative under the reducing action of hydrogen sulfide. The product behaves like all pyrimidines which contain amino groups in the 4,5-positions and is readily transformed into the corresponding purine under the action of formic acid and sodium formate. It is interesting to note that in this case, however, the reaction takes place in two stages,—the primary product of the reaction consisting of a formyl derivative which must be isolated and heated at 220°C. in order to effect a closure of the imidazole ring (190g):



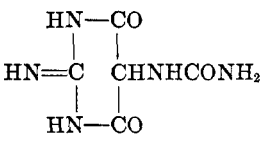
2,6-Dioxy-4,5-aminopyrimidine also condenses readily with urea to give quantitative yields of uric acid (101c).

The syntheses of 4,6-dioxy-2,5-diaminopyrimidine and 2,6-dioxy-4,5-diaminopyrimidine have played a very important rôle in solving what was for many years a most perplexing problem in the field of biochemistry, since without a knowledge of the configuration of these two substances the structure of divicine and vicine could not have been elucidated. As is generally known, divicine, vicine, and convicine have been frequently reported as resulting from the hydrolysis of various plant tissues. All three were isolated from vetch seeds and also from beans by Ritthausen and his coworkers (170, 168, 169, 171, 167). Vicine has also been isolated from beet juice by Lippmann (145), from vetch seeds and peas by Schulze (176), and also from vetch by Winterstein (210). Both vicine and convicine are well-crystallized substances slightly soluble in water, vicine being distinguished from convicine by the fact that it forms a soluble sulfate. Both are nucleosides since they have been found to yield a sugar and a base on hydrolysis. In the case of vicine the base is divicine (168), and the sugar is dextrose (40, 128, 143).

The elucidation of the structure of divicine was accomplished in the following stages. Schulze and Trier (177a) were the first to suggest that a structural relationship might exist between it and pyrimidines. This idea was developed in greater detail by Johnson (80) and by Johnson and Johns (101a) who called attention to the similarity in composition between divicine and the isomeric dioxydiaminopyrimidines described by Traube. Both of these substances had been isolated by Traube in the form of their sulfates. The latter were again prepared by Johnson and Johns according to Traube's method and the free bases separated by treatment with the exact amount of sodium hydroxide. In comparing these two free bases with divicine a series of precipitation and color tests were applied which directly paralleled similar tests described by Ritthausen (169). The results as tabulated (101b) indicated so close an agreement in chemical behavior between 2,6-dioxy-4,5-diaminopyrimidine and divicine as to lead to the conclusion that the two substances were identical.

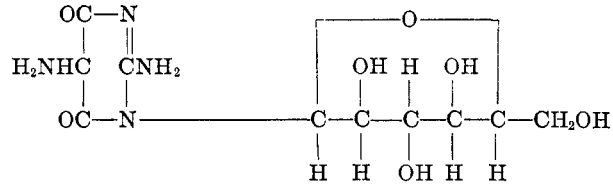
The further discovery that 2,6-dioxy-4,5-diaminopyrimidine condenses readily with urea to give uric acid while 4,6-dioxy-2,5-diaminopyrimidine does not, led to the statement that conclusive evidence in support of the former configuration might be obtained by fusing divicine with urea. In applying this reaction to natural divicine, Fischer (40) obtained negative results, but the data furnished by this experiment was of such a character as not to disprove completely the configuration for divicine suggested by Johnson and Johns. In fact, Fischer was led to conclude that divicine and 2,6-dioxy-4,5-diaminopyrimidine were stereoisomers, the latter representing a maleinoic modification.

This conclusion was not accepted by Levene who, on the other hand, as a result of independent investigations during the same year was able to call attention to the fact that the properties of divicine coincide very closely with those of *4,6-dioxy-2,5-diaminopyrimidine*. Both form anhydrous sulfates, both give off 50 per cent of their nitrogen as nitrogen gas when treated with nitrous acid, and neither condenses with urea to give uric acid (128). This configuration for divicine was finally established by Levene as a result of further investigations undertaken in coöperation with Senior (143) by means of which he was able to demonstrate that divicine yields (a) guanidine with a cleavage of the pyrimidine ring under the oxidizing action of potassium chlorate and hydrochloric acid, and (b) 2-iminopseudouric acid,



on treatment with potassium cyanate and hydrochloric acid.

The following configuration for vicine was developed by Levene on the basis of this evidence taken in conjunction with the fact (a) that both vicine and divicine yield 50 per cent of their nitrogen in the form of nitrogen gas when treated with nitrous acid, thus pointing conclusively to the presence of two free primary amino groups in each of these substances, and (b) that vicine had been shown to give divicine and dextrose on hydrolysis (143):



The ease with which vicine is hydrolyzed is, moreover, in accord with the assumption that a dihydropyrimidine nucleus, as represented above, is present in its molecule. Nucleosides which contain cytosine and other unsaturated pyrimidine ring structures are characterized by their relatively great resistance to the action of hydrolyzing agents.

The configuration of *convicine*, which occurs along with vicine in vetch and broad beans, has not as yet been conclusively established. Ritthausen concluded that convicine, like vicine, was glucosidic in character. When hydrolyzed with 20 per cent sulfuric acid, it yields alloxantin in amounts corresponding to 36 to 37 per cent of its weight (169). Prior to this time Schulze and Trier (177b) had suggested the following equation to account for the hydrolysis of convicine,

$$C_{20}H_{28}N_6O_{16} \cdot 2H_2O + 4H_2O \rightarrow C_8H_6O_4N_8 \cdot 2H_2O + 2NH_3 + 2C_6H_{12}O_6$$

and at the same time advanced the hypothesis that convicine might be related structurally to the pyrimidine nucleosides.

In speculating upon these relationships, Johnson in 1914 pointed out that convicine agrees exactly in composition with either an aminoglucoside of dialuric acid or a glucoside of uramil. At the present time this hypothesis as to the possible configuration of convicine has been somewhat modified as a result of investigations carried on by T. B. Johnson in collaboration with H. J. Fisher (93). In preparing convicine from broad beans by a modification of the methods described by Ritthausen and Levene in isolating vicine, Johnson and Fisher obtained a product which corresponded to the later formula, $C_{10}H_{15}O_3H_8 \cdot H_2O$, proposed by Ritthausen. Hydrolysis of this substance yielded alloxantin in crystalline form and glucose, which was obtained from the filtrate in the form of glucosazone. Since no glucosamine hydrochloride could be detected when hydrochloric acid was used

for the hydrolysis, there is no evidence for the assumption that glucosamine is linked to the base in convicine.

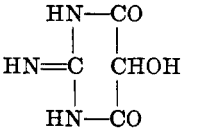
Supposing that $C_{10}H_1O_3H_8 \cdot H_2O$ correctly represents the composition of convicine it is obvious that alloxantin itself cannot be present in the molecule in the form of a glucoside. A base of the composition $C_4H_5N_3O_3$ which might be expected to result after the splitting-off of the sugar must be of such a configuration that (a) it will lose ammonia readily during the process of hydrolysis and (b) it will possess some well-defined relationship to alloxantin.

In considering this second prerequisite it may be said that while the actual molecular configuration of alloxantin has not as yet been conclusively determined, it has nevertheless been definitely demonstrated that an equilibrium is established between alloxan, dialuric acid, and alloxantin when any one of these substances is dissolved in water (18, 19, 20).

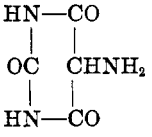
Alloxan + dialuric acid $\rightleftarrows$ alloxantin

It follows therefore that if either alloxan or dialuric acid were formed as a result of the hydrolysis of convicine, the presence of alloxantin among the hydrolytic products could be readily accounted for, since it is much less soluble than either of the other two substances and would, therefore, tend to separate in the form of a precipitate.

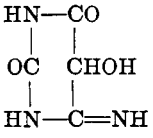
In brief, a base of the composition $C_4H_5N_3O_3$ and of such configuration as to lose ammonia readily to give either alloxan or dialuric acid, would satisfy the structural requirements demanded by convicine. Since it is obvious that an amino derivative of alloxan would contain too many atoms to meet these conditions, the problem is limited to a consideration of the imino derivatives of dialuric acid. Three structural isomers are possible, namely, 2-iminodialuric acid (VIII), uramil (IX), and 4-iminodialuric acid (X):



VIII



IX



X

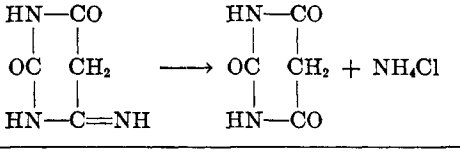
These three substances may now be considered separately in a further development of this analysis of the problem.[11]

2-Iminodialuric acid (VIII) would be expected to give guanidine in any cleavage of the pyrimidine molecule resulting from the oxidizing action of potassium chlorate and hydrochloric acid.[12] Actually convicine has been observed to yield urea (which was isolated as the xanthyl derivative) when oxidized under these conditions. Moreover there is no instance recorded in the literature where an imino group in the 2-position in a uracil or barbituric acid derivative is hydrolyzed under the action of dilute acids with the evolution of ammonia.[13]

Uramil (IX) would be expected to give the corresponding pseudouric acid under the action of potassium cyanate,[14] while actually convicine is recovered unchanged when treated with this reagent. Moreover there is again no instance recorded in the literature of an amino group in the 5-position being easily split off from a pyrimidine by hydrolysis.

4–Iminodialuric acid (X), on the other hand, which has recently been synthesized by Bogert and Davidson (isouramil (21)), appears to fulfill all requirements. It is known, for example, that pyrimidines which contain an imino group in the 4-position hydrolyze readily under the action of dilute mineral acids with the evolution of ammonia. In the case of the above substance this hydrolysis actually results in the formation of dialuric acid.

Two examples of this type of hydrolysis may be cited, out of the many to be found in the literature, as pertinent to the present discussion. 4-Aminouracil, which represents a reduced form of 4-iminodialuric acid, is transformed into barbituric acid and ammonia when treated with dilute hydrochloric acid:



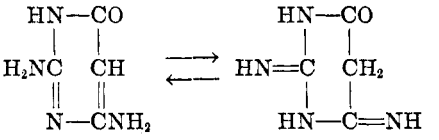
[11] Only the lactam CO.NH constructions are considered in this review.

[12] Compare the formation of guanidine from divicine (143).

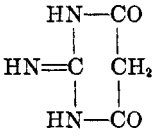
[13] When the imino group occupies the 2-position in certain tetrahydropyrimidines it is easily hydrolyzed with evolution of ammonia (unpublished results).

[14] Compare the formation of 2-iminopseudouric acid from divicine (143).

2,4-Diamino-6-oxypyrimidine, which can also be expressed as a diimino derivative of barbituric acid,

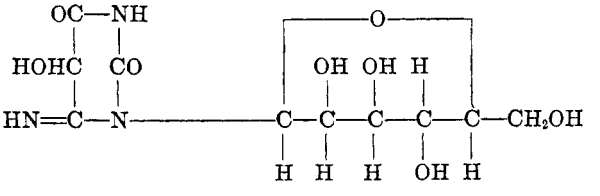


reacts in a similar way to give malonylguanidine,



and ammonia when warmed with dilute mineral acids. This second illustration is particularly significant because it serves to emphasize the difference in the stability of amino and imino groups in the 2- and 4-positions respectively.

It has been observed moreover that convicine contains one free amino group, since it yields a quantity of free nitrogen gas equivalent to such a configuration when treated with nitrous acid. From this it would seem to follow that the sugar linkage cannot be located on this group. All of the above relationships may therefore be embodied in the following tentative formula for convicine:

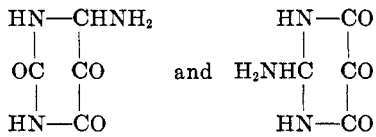


This formula also accounts for the relative ease with which convicine undergoes hydrolysis.[15]

[15] Compare the similar behavior of vicine, which is also accounted for by the presence of a dihydropyrimidine nucleus in the glucosidic combination, the latter having been observed to split as readily as in the case of the common glucosides.

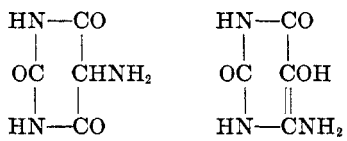
V. DERIVATIVES OF BARBITURIC ACID AND THEIR RELATION TO MEMBERS OF THE PURINE GROUP

Several different molecular configurations are theoretically possible in which three oxygen atoms and one amino group are associated in a reduced pyrimidine ring structure such as, for example, pyrimidines I and II,



I II

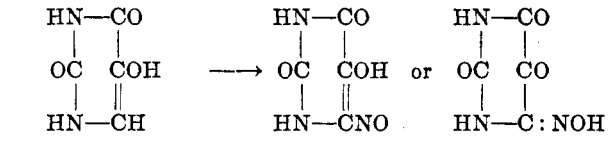
but no compound corresponding to either of these two formulas has as yet been described in the literature. The only pyrimidines possessing this general composition which have been studied are 5-aminobarbituric acid or, as it is more commonly known, uramil (III), and its isomer, isouramil (IV), recently synthesized by Bogert and Davidson (21).



III IV

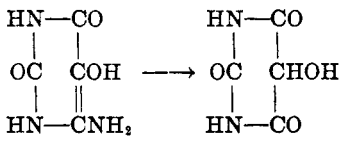
Isobarbituric acid (I) served as the starting point for the synthesis of isouramil. Davidson and Bogert have observed that this pyrimidine interacts with nitrous acid to give a nitroso compound which may be expressed by either of the two formulas, VI or VII. On reduction of this nitroso derivative with ammonium sulfide, the nitroso group is easily reduced and the pyrimidine is converted into isouramil (IV), whose structure is established by the fact that it undergoes hydrolysis in acid solution with evolution of ammonia and formation of dialuric acid (VIII). Isouramil does not melt below 290°C., and is insoluble in hot water, alcohol, or acetic acid. It dissolves immediately

in sodium hydroxide solution, ammonia, and dilute hydrochloric acid, and by the oxidation of nitric acid it is oxidized to alloxan.



Isobarbituric acid

V VI VII



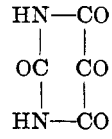
Isouramil Dialuric acid

IV VIII

The extremely interesting and important pyrimidine uramil (III) has held the attention of research workers from very early times and has been intimately associated with developments in the fields of both pyrimidine and purine chemistry. Uramil was first described by Liebig and Wöhler in connection with their investigations on uric acid, which have since become classic. Their discovery may be said to mark the beginning of a century of research during which time both pyrimidines and purines have been intensively studied. The result of this work was to establish the molecular configurations of the more important substances belonging to each of these two classes and in this way to elucidate the interrelationships existing not only between individuals of the same class, but also between the two general groups of compounds. In the field of purine chemistry these developments culminated in the classic investigations of Emil Fischer and his coworkers, which will be referred to again later. The fact that in the early stages of their development the chemistry of pyrimidines was so closely interwoven with that of purines makes it difficult to discuss one without reference to the other. This is particularly true in the case of uramil, so that a brief

preliminary survey of the early investigations in the field of uric acid seems necessary to any clear understanding of the chemistry of uramil and its derivatives.

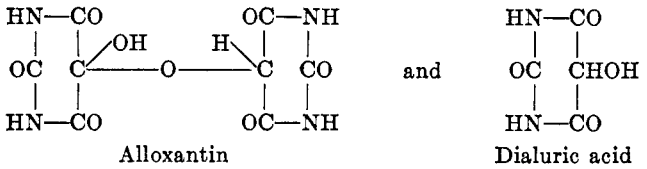
Uric acid was discovered by Scheele in 1776. Forty years later Brugnatelli (25, 26) oxidized it with nitric acid and chlorine water and isolated the pyrimidine *alloxan.*



In the following year the English chemist, Prout (166), repeated the work of Scheele and found that the product which was obtained by oxidizing uric acid with nitric acid reacted with ammonia to form an intense red color which was destroyed by acids. This led to the discovery of *murexide,* which has since been found to represent the ammonium salt of purpuric acid and which finds application at the present time as a color test for uric acid and related purines. In continuing this investigation Prout (165) was able to show that alloxan may also be obtained from murexide on oxidation.

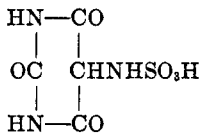
It was not until more than half a century after the discovery of uric acid by Scheele that a systematic study of its properties was undertaken by Wöhler and Liebig (211a). It was then prepared in pure condition, analyzed, and its transformations under the action of different oxidizing agents, at different conditions of temperature and concentration, investigated. The results obtained from these experiments demonstrated that the process of oxidation involved the formation of a number of substances in addition to those already described. It was found, for example, that while uric acid reacts with concentrated nitric acid to give alloxan, it also reacts with dilute nitric acid to give *alloxantin.* These investigations led moreover to the discovery of a definite relationship between alloxantin and alloxan, since they may be transformed one into the other under the action of oxidizing and reducing agents respectively. Further investigations of alloxan (which was obtained in pure condition and

correctly analyzed) revealed the fact that under the action of reducing agents it yielded not only alloxantin but also the pyrimidine *dialuric acid,*



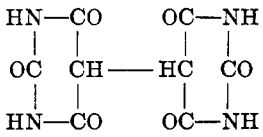
Alloxantin Dialuric acid

and under the action of sulfuric acid, another new and equally important pyrimidine, namely, *thionuric acid*:



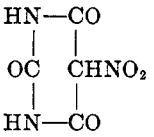
The isolation of the latter led directly to the discovery of *uramil,* which is readily formed from thionuric acid under the hydrolyzing action of dilute mineral acids. The structure of these substances as represented above was not of course established until a number of years later, because the theory of organic chemistry had not at that time progressed far enough to admit of such an interpretation of the molecular formulas which were obtained as a result of these experiments.

The investigations of Liebig and Wöhler were continued by two of their pupils, Schlieper and Gregory, who published important papers on alloxan, hydurilic acid, nitrohydurilic acid, allituric acid, and dilituric acid. It should be noted in this connection that Schlieper (174), in the course of preparing alloxan, incidentally succeeded in isolating *hydurilic acid.*



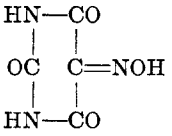
This discovery was important because of the fact that this substance was made the starting point for later investigations by

Baeyer. Schlieper also obtained *dilituric acid* (nitrobarbituric acid),

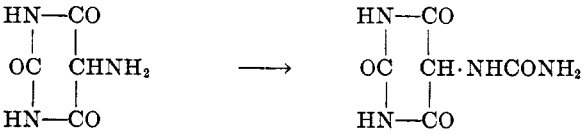


as one of the products formed in the oxidation of alloxantin by nitric acid.

Renewed impetus to the further study of the interesting relationships existing between pyrimidines and purines was supplied by Baeyer, a pupil of Schlieper, who in 1863 was able to apply the theories of structural organic chemistry, as they had by that time been developed by Kekulé, to a systematic elucidation of the molecular configurations of these various compounds. As has been said, the starting point of Baeyer's investigations was hydurilic acid. This he succeeded in transforming into violuric acid,



as a result of oxidation with nitric acid, demonstrating at the same time that violuric acid readily yields dilituric acid on further oxidation with the same reagent. From the evidence furnished by these and other experiments he was led to the important conception that a direct transition from barbituric to uric acid might be established through the intermediate formation of bromobarbituric acid, dibromobarbituric acid, dialuric acid, alloxan, violuric acid, dilituric acid, uramil, and pseudouric acid (5). The latter substance was obtained from uramil under the action of aqueous potassium cyanate (4a),



but its conversion into uric acid was not accomplished until many years later, when Fischer and Ach (42a) finally succeeded in condensing the urea side chain to an imidazole ring with the elimination of one molecule of water under the action of molten oxalic acid.

The period of activity initiated by Baeyer was also characterized by the following achievements: A correct structural formula was assigned to barbituric acid by Mülder (155) and its synthesis from urea and malonic acid under the action of phosphorus oxychloride was accomplished by Grimaux (61). The synthesis of nitroso-, nitro-, and amino-malonic acids was effected and attention directed to the relationship of these substances to the corresponding derivatives of barbituric acid (5). The conclusion of this period of great productivity was finally marked by the brilliant speculations of Medicus (147), who proposed structural formulas for uric acid, guanine, and other purines, which were not only illuminating at the time, but which have since been demonstrated to be correct.

In summary, it may again be said that this general historical review has been introduced at this point in order to call attention to the fact that uramil is related on the one hand to such complex combinations as uric acid, murexide, purpuric acid and alloxantin, and on the other hand, to the relatively simpler derivatives of barbituric acid. Because of this definitely established connection it seems desirable in considering the various methods by which uramil may be prepared, to arrange them in three groups representing:

I. The hydrolysis of complex structures, the exact configuration of which in certain instances has not yet been conclusively determined, as, for example, murexide.

II. Transitions (a) from barbituric acid and (b) from alloxan.

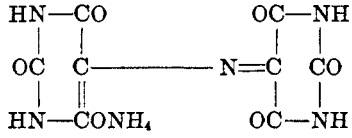
III. Direct synthesis from simpler units.

It is hoped that such an arrangement may serve not only to simplify a discussion of the methods for preparing uramil, but also aid in the subsequent treatment of its various alkylated derivatives.

I. In considering methods of preparation belonging to the

first group, nothing further need be said in regard to the oxidation and hydrolysis of uric acid. This process is one which while very complex, nevertheless involves the formation of murexide, or other salts of purpuric acid, and alloxantin as primary products. These may therefore be considered respectively as starting points in the preparation of uramil.

Murexide, as has already been said, is now regarded as the ammonium salt of purpuric acid. The latter is not itself capable of existing in the free state, although many of its derivatives are known. The formula for murexide which is most generally accepted as correct is one proposed by Möhlau (153, 154; cf. 160a, 180a), viz.,

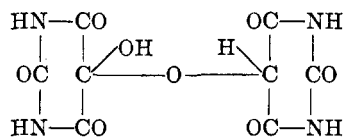


Purpuric acid, from which this salt is derived, might therefore be assumed to represent a condensation product of uramil with alloxan. It might be assumed further that the resolution of this complex on hydrolysis would serve to define this relation, but unfortunately the experimental evidence is confusing. For example, murexide has been observed under certain conditions to give uramil (46 per cent), alloxan (47 per cent), and ammonia (154), while under still other conditions it breaks down to give an ammonium salt of dialuric acid and alloxan. In the latter case uramil could not be assumed to represent a primary product from which murexide forms or into which it hydrolyzes (160a). In general it may be said that the character as well as the quantity of the decomposition products that have been obtained from murexide and also from potassium purpurate depend upon conditions of temperature and concentration even under the action of the same hydrolytic agent (160b). Murexide has been synthesized by condensing molecular quantities of uramil and alloxan in the presence of ammonium carbonate (154).

$$\underset{\text{Uramil}}{C_4H_5O_3N_3} + \underset{\text{Alloxan}}{C_4H_2O_4N_2} + (NH_4)_2CO_3 \rightarrow \underset{\text{Murexide}}{2C_8H_8O_6N_3} + 2H_2O + 2CO_2$$

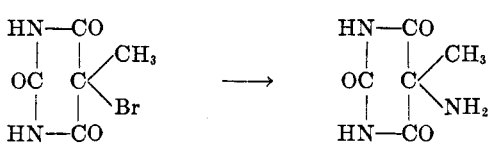
It has also been prepared from the ammonium salt of dialuric acid and alloxan under the action of ammonium acetate and ammonium carbonate (160b).

Alloxantin has been found to occur among the oxidation products of uric acid, the hydrolytic products of murexide, and the reduction products of alloxan. It has also been prepared by condensing alloxan with dialuric acid and is generally thought to possess the following structure:

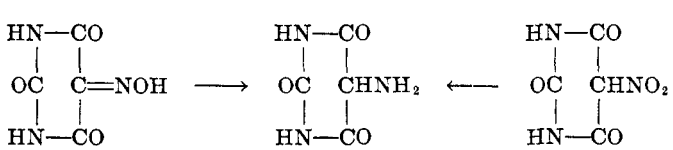


Treated with ammonia and amines it reacts to give uramil or alkylated derivatives of uramil, respectively (154, 160b, 180a). The salts of purpuric acid with ethylamine and methylamine have also been observed to yield 7-methyl- and 7-ethyl-uramil, respectively (154).

II (a) In preparing uramil from barbituric acid a number of different methods of procedure are possible. For example, 5-bromobarbituric acid when treated with ammonia yields uramil; the corresponding 5-alkyl-5-bromobarbituric acids behave similarly (45c).

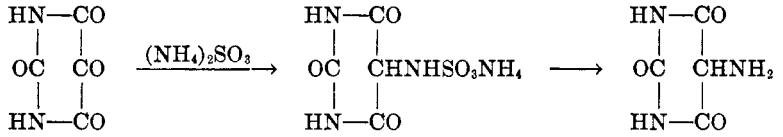


Uramil may also be prepared from violuric and dilituric acids on reduction. *N*-1- and *N*-3-alkyluramils can be prepared from the corresponding compounds in the same way (4b, 179, 208, 186).



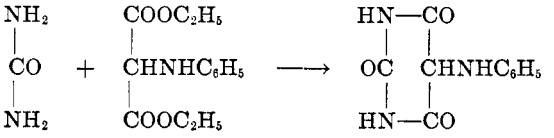
It may be obtained from thionuric acid (211a) and also from the ammonium salt of dialuric acid (160a) on hydrolysis.

(b) Uramil and its monoalkyl derivatives may be prepared by starting with alloxan. In this case also it is possible to proceed in a number of different ways. Alloxan may be treated with ammonium sulfite or with the sulfites of primary amines, respectively. This method is capable of very wide application but cannot be used successfully with the sulfites of secondary amines (160d). The mechanism of the reaction is thought to consist in the formation of a salt of thionuric acid as a primary product and its subsequent hydrolysis in the presence of acids (211b, 186, 44, 38b).



Alloxan or its alkyl derivatives may be treated with hydroxylamine. This results in the formation of the corresponding oxime (violuric acid), which may then be reduced as described under II (a). Uramil may also be prepared by treating alloxan with phenylhydrazine and then reducing the hydrazone with tin and hydrochloric acid (125). The same hydrazone may also be obtained by starting with barbituric acid on treatment with diazobenzene hydrochloride. It will be noted in general that all of the above methods for preparing uramil from alloxan almost exactly parallel methods which have been described under II (a).

III. Finally 7-phenyluramil, for example, may be directly synthesized by condensing urea with diethyl anilinomalonate in the presence of sodium ethoxide (114c):



This method finds somewhat more general application in the synthesis of thiouramils by means of condensations with thiourea and will be referred to again later.

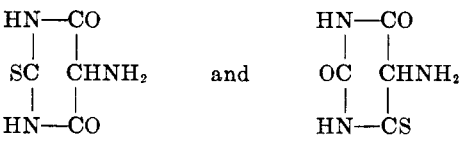
Uramil is almost completely soluble in hot water, from which it crystallizes on cooling in colorless needles which redden on exposure to the air. It reacts with alkalis to form salts but on prolonged boiling is resolved into urea and the salts of aminomalonic acid. Boiled with ammonia it is transformed into murexide and with nitric acid into alloxan. When dissolved in aqueous potassium cyanate it yields pseudouric acid and the latter, when heated with molten oxalic acid or when digested with hydrochloric acid, undergoes condensation to form uric acid (42a, 38c). Treated with ethyl cyanate or phenyl cyanate respectively, uramil is transformed into the corresponding substituted pseudouric acids, which may then be condensed to yield derivatives of uric acid which contain hydrocarbon residues in the 9-position (2, 39). As has been stated, a variety of different products may be obtained by condensing uramil with alloxan or with dialuric acid under different conditions (154, 160d). When treated with acetic anhydride and sodium acetate, uramil reacts to give a 7-acetyl derivative. The same product is formed under the action of acetyl chloride (160c).

Alkylated derivatives of uramil may be obtained under a variety of different conditions. The preparation of substances belonging to this class has already been referred to in connection with the different methods for preparing uramil. In summary, it may be said that *N*-1 and *N*-3 derivatives are commonly obtained by starting with the corresponding mono- or di-substituted barbituric acids or alloxans respectively and proceeding as described under II (a) and (b). They may also be prepared from alkylated alloxantins by treatment with ammonia and from alkylated derivatives of purpuric acid on hydrolysis (154, 160d). 7-Alkylated uramils may be obtained (1) by treating alloxan, or its *N*-1-*N*-3 derivatives, respectively, with alkyl sulfites and then hydrolyzing the product; (2) by treating alloxantin or purpuric acid with alkyl amines; (3) by synthesis from urea and amino compounds corresponding to diethyl anilinomalonate. All of the substances obtained in these various ways react with aqueous potassium cyanate to give the corresponding pseudouric acids, which in most cases condense to form alkylated uric acids

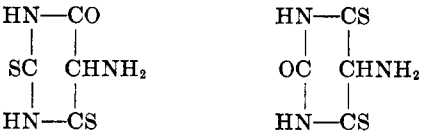
containing substituting groups in the 1-, 3-, and 7-positions, respectively or collectively. In general it is possible to distinguish pseudouric acids from the corresponding uric acids by the fact that the latter yield alloxantins while the former do not (38a; cf. 42b). In the case of pseudouric acids which have been obtained by treating uramil with alkyl cyanates the resulting uric acids contain substituents in the 9-position.

C-5-Alkyl derivatives of uramil may be prepared from 5-bromo-5-alkylbarbituric acids on treatment with ammonia (45b). Substances belonging to this class react with potassium cyanate to give pseudouric acids, but the latter do not undergo condensation with the formation of imidazole rings.

Since the term "uramil" is commonly used to include thiouramils, these must now be considered briefly. Two isomeric configurations are possible for monothiouramils, viz.,

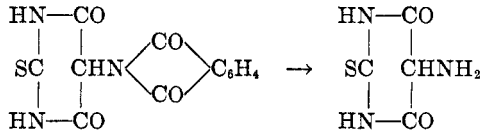


and also two for uramils in which two oxygen atoms have been replaced by sulfur (114a).



Both types of monothiouramils are known.

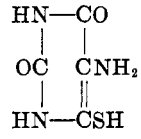
2-Thiouramil may be prepared by condensing thiourea with the hydrochloride of diethyl aminomalonate in the presence of sodium ethoxide. Under these conditions the product separates in the form of a sodium salt. The latter, when dissolved in water and treated with acetic acid, yields the free base (113b). The same substance may be obtained by condensing thiourea with phthalimidomalonic ester and then hydrolyzing the resulting product (114a).



In the latter case, however, the yield is not as good as in the first case. 2-Thiouramil is not desulfurized by warming with mineral acids and when warmed with sodium hydroxide it decomposes to give the sodium salt of aminomalonic acid. When dissolved in aqueous potassium cyanate it reacts to give 2-thiopseudouric acid (113b). Substituted 2-thiouramils have been obtained by condensing thiourea and allylthiourea, respectively, with diethyl anilinomalonate (114a).

2-Thiovioluric acid may be prepared by condensing thiourea with diethyl nitrosomalonate (113b). The corresponding 2-methylmercaptovioluric acid has also been synthesized (199d). This on reduction gives 2-methylmercaptouramil.

4-Thiouramil has been synthesized by Traube (188a) and has also been prepared by reducing potassium urate with ammonium sulfide at 155–160°C. (41a; cf. 157, 192). The product reacts with potassium cyanate to give the corresponding pseudouric acid. The latter cannot be condensed to form the corresponding uric acid (41b). 4-Thiouramil is a very strong monobasic acid. This is assumed to be due to the fact that in its tautomeric form



it contains a hydrogen atom in union with sulfur. The replacement of this hydrogen by metals results in the formation of salts which are very stable and which are not hydrolyzed under the action of acetic acid. 1,3-Dimethyl-4-thiouramil has also been prepared both by Fischer and Ach and by Traube (188a).

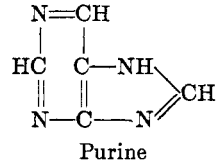
In general it may be said that monothiouramils are substances which have physiological interest but which do not react readily to give the corresponding pseudouric acids. No attempts to synthesize dithiouramils have been reported in the literature.

In connection with the use of thioureas and pseudothioureas in the synthesis of pyrimidines it may be noted that both types of substances have been employed extensively for condensation reactions leading to the formation of this cycle. When the condensation product consists of a 2-thiopyrimidine, it can frequently be transformed into the corresponding 2-oxypyrimidine under the action of chloroacetic acid, or into the corresponding 2-methylmercapto derivative under the action of methyl iodide in the presence of sodium methoxide. Substances belonging to the latter class usually hydrolyze readily to the corresponding 2-oxypyrimidines when warmed with mineral acids. The application of these two methods of synthesis has represented one of the most important contributions to pyrimidine chemistry during the present century, and has been particularly valuable in preparing pyrimidines of known structure, since the presence of a sulfur atom or a mercapto group in the 2-position can be utilized to limit the substitution of various other groups to the 2-position of the ring. It should be noted, however, that the presence of certain substituents in the ring tends to interfere with both of the above methods for the ultimate desulfurization of the products which may be prepared in this way. Notable illustrations of this have been observed in cases where position 5 is occupied by amino or substituted amino groups (89b, 78b, 114b), and also in cases where position 6 is similarly occupied (108b). It has been observed, however, that the influence of nitrogen in these same positions in the purine molecule is not so potent. Thus for example, 2-thio-4,5,6-triaminopyrimidine is not desulfurized under the action of acids like formic acid but undergoes condensation to give 2-thioadenine, the latter then being readily desulfurized to form adenine (188b). Other illustrations showing the ease with which 2-thiopurines may be hydrolyzed are recorded in the literature.

Since the chemistry of the uramils is so closely associated with the chemistry of the purines, it seems desirable at this point to refer briefly to some of the later developments in both fields. It was pointed out at the beginning of this chapter that certain

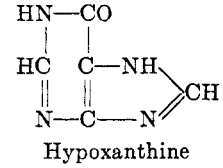
phases in the early development of pyrimidine chemistry could be attributed to the fact that uramil and related compounds had been observed to result from the oxidation and hydrolysis of uric acid. Since then other purines which occur more or less abundantly in nature (notably caffeine, theobromine, guanine, and adenine) have also been found to yield pyrimidines when treated in the same or a similar manner. In attempting to explain the relationship between these two classes of compounds the problem has been attacked in two ways, i.e, by studying the transformations and cleavage products of the purines and by synthesizing them from pyrimidines.

As a result of research in the field of purine chemistry, many new substances have been discovered, the configurations of their molecules have been correlated, transformations of one into the other have been effected, and finally they have in many instances been resolved into the corresponding pyrimidines. The achievements of Emil Fischer and his coworkers have been especially conspicuous in developments of this kind. Since this work has been very thoroughly reviewed and summarized by Levene (135) it needs no further comment. However, for purposes of clarity in the following discussion, it may be pointed out that purines, like pyrimidines, may for convenience be arranged into groups depending upon the number of oxygen atoms present in the molecule and that, therefore, all compounds belonging to this general class may be referred to the following types:



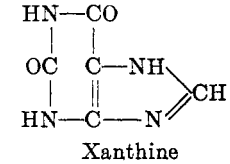
Purine

I



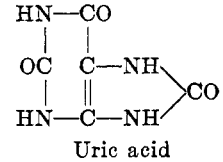
Hypoxanthine

II



Xanthine

III



Uric acid

IV

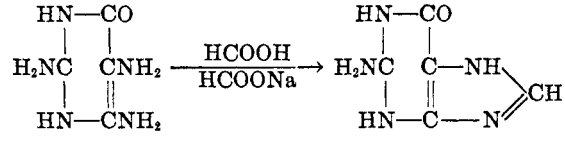
These types retain their individuality even when oxygen has been replaced by sulfur or by imino groups and when the hydrogen has been substituted by hydrocarbon residues. These relationships having been definitely established, it is now possible to summarize briefly the methods which are applicable to the synthesis of these substances from pyrimidines.

Such syntheses fall into two general classes if limited to methods which are capable of extended application: A, those involving the preparation of 5-aminopyrimidines (uramils, etc.), pseudouric acids, and the subsequent condensation of the latter; B, those involving the preparation of 4,5-diaminopyrimidines and their condensation under the action of different reagents. Of these two the latter is perhaps the more varied in its scope.

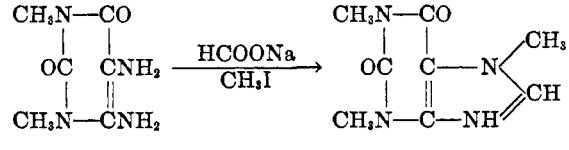
(A) Synthesis from 5-aminopyrimidines involves treatment of the substance with potassium cyanate with the formation of a substituted urea side chain in the 5-position. Such reactions usually take place readily. This is followed by the condensation of the urea side chain to form an imidazole ring. The ease with which the final phase in this process takes place varies greatly depending upon the character of the other substituents in union with the atoms of the pyrimidine ring. As has been noted in connection with the discussion of thiouramils, the presence of sulfur in the 2-position retards this reaction. Behrend has reported the same resistance to condensation in the case of the pseudouric acids obtained from 5-aminouracil and 4-methyl-5-aminouracil (10d, 11c, 13b, 14a). The unsuccessful attempts of Baeyer to synthesize uric acid from uramil have been referred to. In this case, however, the condensation of the urea side chain was ultimately effected by Fischer (38b) as a result of fusing pseudouric acid with molten oxalic acid. In studying condensations of this type Fischer later found that hydrochloric acid was very effective in bringing about this reaction. Even the pseudouric acid derivative of uramil when warmed with this reagent is transformed smoothly into uric acid. In the case of the corresponding methylated derivatives of uramil, the reaction is very much accelerated so that even dilute hydrochloric acid may be used to induce this change.

(B) Synthesis of purines from 4,5-diaminopyrimidines in-

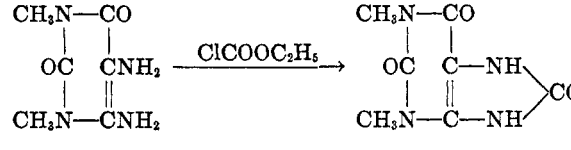
volves condensation with formic acid (in the presence of sodium formate) on the one hand, or with ethyl chloroformate or urea on the other. In cases where formic acid is used as the condensing agent an intermediate formyl derivative is frequently isolated, and the ease with which it suffers condensation to form an imidazole ring varies considerably with different compounds. The character of the imidazole ring depends upon the reagent chosen for the condensation, as is apparent from the following illustrations chosen at random (190 e, j, and k, respectively).



Guanine



Caffeine



1,3-Dimethyluric acid

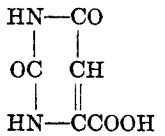
It may be added that by varying the character of the pyrimidine chosen the above methods of synthesis are applicable to the preparation of any purine belonging to the various groups classified under I, II, III, and IV (p. 286).

In conclusion, attention may again be directed to the fact that many important problems in biochemistry depend for their ultimate solution upon a knowledge of pyrimidine chemistry. This has already been pointed out in some detail in connection with a preliminary survey of the chemistry of the nucleic acids

and also in the more recent discussion of the chemistry of the purines. But there are innumerable other illustrations of the fact that pyrimidines play a conspicuous part in both animal and plant metabolism. Indeed the whole development of pyrimidine chemistry during the past century has been interlaced with fundamental biochemical problems. For example, the synthesis of cytosine and isocytosine was undertaken because of the fact that a substance having this composition had been isolated as a degradation product of both plant and animal tissues. The successful synthesis of cytosine confirmed the constitution of the major aminopyrimidine occurring in quantity in the nucleic acid molecule. The fact that a substance having the composition of a monoöxydiaminopyrimidine was isolated by Kutscher (126) from among the hydrolytic products of yeast nucleic acid led to the synthesis of 2-oxy-4,6-diaminopyrimidine (199a), 2-oxy-5,6-diaminopyrimidine (103a), 6-oxy-2,4-diaminopyrimidine (190a; cf. 100g), and 6-oxy-2,5-diaminopyrimidine (100g). These four substances represent all of the molecular configurations that are possible for a compound of this composition and of these the last is the only one which agrees in its general chemical properties with Kutscher's base (100g). The fact that identification of these two substances has not yet been established is due in part to the fact that the product isolated from natural sources has not been thoroughly investigated.

Another illustration of the way in which pyrimidine chemistry has been applied to the elucidation of biochemical problems is to be found in the investigation of some of the many possible isomeric aminodimethylpyrimidines having the composition of a base which has been isolated from among the hydrolytic products of Japanese *Schōyu* (Soja sauce) (185, 184). Such a substance might represent a derivative of 2-, 4-, or 6-aminopyrimidine. The isomeric modifications corresponding to 4-amino- and 6-amino-dimethylpyrimidines have not as yet been completely surveyed. While all dimethyl derivatives of 2-aminopyrimidine have been carefully examined, none of these corresponds in its chemical properties with the base described by the Japanese investigators (106a; cf. 90a). The final solution of this problem

is, therefore, one requiring further study. Another important development in the field of pyrimidine chemistry is the discovery that uracil-4-carboxylic acid synthesized by Wheeler (197) in 1907 has been shown to be identical with *orotic acid* found in milk (3).



Research developments in the field of pyrimidines have had of course their purely theoretical as well as their practical aspects. Gaps in the chemistry of different groups of compounds belonging to this general class are gradually being filled in and the importance of such work cannot be overestimated. Had it not been for the synthesis of the isomeric diaminodioxypyrimidines by Traube, the solution of the structural relations of divicine and vicine would have been very much retarded. 5-Methylcytosine was synthesized by Wheeler and Johnson many years before it made its appearance among the hydrolytic products of tuberculinic acid. Moreover, since some of the most important developments in biochemistry today are concerned with the so-called chemistry of the cell, it may be predicted with confidence that progress in the elucidation of the very complicated problems involved in this study will be associated in the future, as it has been in the past, with extended investigations in the field of pyrimidine chemistry.

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