$$C_2H_5OCH_2COOC_2H_5 + Zn + BrCH(CH_3)COOC_2H_5 \longrightarrow$$

$$C_2H_5OCH_2C(OZnBr)(OC_2H_5)\cdot CH(CH_3)COOC_2H_5 \xrightarrow{H_2O} C_2H_5OCH_2C(OH)(OC_2H_5)\cdot CH(CH_3)COOC_2H_5 \longrightarrow$$

$$C_2H_5OCH_2COCH(CH_3)COOC_2H_5.$$

IX.

We have used so far in our work ethyl ethoxyacetate, ethyl α-ethoxypropionate, ethyl bromoacetate and ethyl α-bromopropionate and have synthesized all four of the theoretically possible β-ketone esters by application of this reaction. The boiling points of these new ketone esters are recorded below. The description of their syntheses and chemical behavior will be discussed in future papers.

Ethyl γ-Ethoxyacetoacetate, $C_2H_5OCH_2COCH_2COOC_2H_5$.—Boiling points: 120–125° at 30 mm.; 130–136° at 45 mm.; 116–120° at 26–27 mm.; 110° at 20–21 mm.; 132° at 52 mm.; 135° at 55 mm.

Ethyl α-Methyl-γ-ethoxyacetoacetate, $C_2H_5OCH_2COCH(CH_3)COOC_2H_5$.—Boiling points: 116° at 24 mm.; 113–116° at 18–20 mm.

Ethyl γ-Methyl-γ-ethoxyacetoacetate, $C_2H_5OCH(CH_3)COCH_2COOC_2H_5$.—Boiling points: 110–115° at 19 mm.

Ethyl α-Methyl-γ-methyl-γ-ethoxyacetoacetate, $C_2H_5OCH(CH_3)COCH(CH_3)COOC_2H_5$.—Boiling points: 108–115° at 16 mm.

The synthesis of ethyl γ-ethoxyacetoacetate is incorporated in the following paper by L. H. Chernoff and the writer.

NEW HAVEN, CONN.

[CONTRIBUTIONS FROM THE SHEFFIELD LABORATORY OF YALE UNIVERSITY.]

RESEARCHES ON PYRIMIDINES. LXII. THE SYNTHESIS OF PYRIMIDINES RELATED STRUCTURALLY TO PYRIMIDINE-NUCLEOSIDES.

BY TREAT B. JOHNSON AND LEWIS H. CHERNOFF.

Received March 26, 1913.

Through the investigations of Levene and his co-workers[1] it seems to have been definitly established that the highly complex nucleic acids are composed of characteristic complexes designated by the term *nucleotides*. The latter are compounds consisting of phosphoric acid conjugated with a complex composed of a carbohydrate and a purine or pyrimidine. In other words, substances like yeast and thymus nucleic acids may be viewed as *polynucleotides*, corresponding to the polysaccharides in the sugar series, and consist of combinations of the molecules of *mononucleotides*, with loss of the elements of water. The latter correspond to the monosaccharides of the sugar series, and are represented in nature by the

[1] *Ber.*, **41**, 1905; **42**, 2703, 2744; **43**, 3150, 3164; **44**, 1027; **45**, 608. THIS JOURNAL, **12**, 411, 421; **11**, 85.

simple nucleic acids—guanylic and inosinic acids.[1] This nucleotide structure is apparently common to all nucleic acids.

$$\left[\begin{array}{l}\text{Carbohydrates} \longrightarrow \text{polysaccharides and monosaccharides.}\\ \text{Nucleic acids} \longrightarrow \text{polynucleotides and mononucleotides.}\end{array}\right]$$

By the hydrolysis of mononucleotides, under proper conditions, it is possible to detach the phosphoric acid from these substances and obtain simpler complexes of a carbohydrate and a purine or pyrimidine. These compounds are called *nucleosides*,[2] and direct proof of their presence has been presented by the isolation of the pentose nucleosides—guanosine II, adenosine I, cytidine III, and uridine IV, from yeast nucleic acid,[2] and of guanine-hexoside from thymus nucleic acid.[3] On further hydrolysis these nucleosides undergo decomposition with cleavage of the carbohydrate and formation of the purines—guanine and adenine (VI and V) and the pyrimidines-cytosine and uracil (VII and VIII), respectively. The relationship between yeast nucleic acid, for example, and its various decomposition products may be represented, in chemical terms, by the following formulas:

	Nucleotides.		*Nucleosides.*		*Base.*
Yeast nucleic acid	$O:P(OH)_2.C_5H_8O_4.C_5H_4N_5$	$\longrightarrow$	$C_5H_9O_4.C_5H_4N_5$ Adenosine. I.	$\longrightarrow$	$C_5H_5N_5$ Adenine. V.
	$O:P(OH)_2.C_5H_8O_4.C_5H_4ON_4$	$\longrightarrow$	$C_5H_9O_4.C_5H_4ON_4$ Guanosine. II.	$\longrightarrow$	$C_5H_5ON_4$ Guanine. VI.
	$O:P(OH)_2.C_5H_8O_4.C_4H_4ON_3$	$\longrightarrow$	$C_5H_9O_4.C_4H_4ON_3$ Cytidine. III.	$\longrightarrow$	$C_4H_5ON_3$ Cytosine. VII.
	$O:P(OH)_2.C_5H_8O_4.C_4H_3O_2N_2$	$\longrightarrow$	$C_5H_9O_4.C_4H_3O_2N_2$ Uridine. IV.	$\longrightarrow$	$C_4H_4O_2N_2$ Uracil. VIII.

While it is known that these nucleosides are combinations of a pentose sugar (ribose[2]) with adenine, guanine, cytosine and uracil, on the other hand, we have practically no knowledge regarding the nature of the sugar linkings, nor do we know which positions are substituted, by the carbohydrate, in the purine and pyrimidine rings. Consequently we are unable to express structurally the exact constitution of these interesting substances. We do know, however, that the purine-nucleosides differ from pyrimidine-nucleosides in their behavior towards acid hydrolytic agents. The former are easily hydrolyzed by the action of dilute acids, while, on the other hand, the pyrimidine-nucleosides are very stable, and in order to effect a cleavage of the carbohydrate it is necessary to heat them with more concentrated acids and at a higher temperature

[1] Levene and Jacobs, *Ber.*, **42**, 2469; **41**, 2703; **43**, 335.

[2] Levene and co-workers, *Loc. cit.*

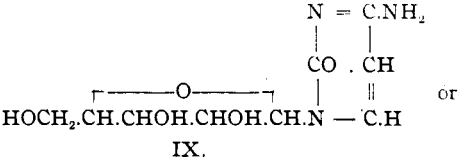
[3] Levene and Jacobs, *J. Biol. Chem.*, 12, 377.

(120–130°). This remarkable difference in behavior is of special interest from a chemical standpoint, because it indicates that the bases are not linked, in these two types of nucleosides, to the carbohydrate in a similar manner.

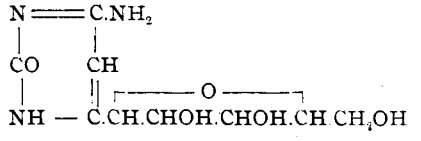
The primary object of the work described in this paper was to obtain new data by the application of synthetical methods, which would aid in establishing the constitution of these compounds. This paper is our second contribution on this subject[1] and includes a description of the properties and synthesis of a simple pyrimidine-nucleoside.

The two pyrimidine-nucleosides, uridine and cytidine, are combinations of the pentose sugar ribose with the pyrimidines uracil (VIII) and cytosine (VII), respectively. It has also been shown that these pyrimidines are linked, in these substances, to the sugar in a similar manner. This was established by the fact that cytidine is transformed into uridine by the action of nitrous acid.[2] Levene and La Forge[3] have since obtained evidence, which has enabled them to draw still further conclusions regarding the constitution of these compounds. They have found, for example, that these nucleosides react smoothly with nitric acid and bromine. They easily undergo substitution with introduction of a nitro group and a bromine atom in the 5-position of the pyrimidine ring and without detachment of the carbohydrate. These results, therefore, indicate that the sugar is not joined at position 5, as might be assumed, and led these investigators to the conclusion that the sugar may be joined to the pyrimidine in one of two other positions *viz.*, the 3- or 4-position of the ring. The possibility that the carbohydrate may be linked at both the 4- and 5-position of the ring was not considered. If the assumptions of these investigators be correct then the constitution of cytidine and uridine may be expressed by the following structural formulas.

Cytidine.



IX.



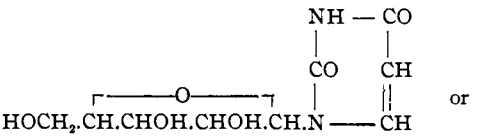
X.

[1] Johnson and Chernoff, *J. Biol. Chem.*, **14**, 307.

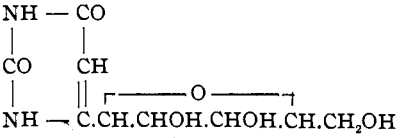
[2] Levene and Jacobs, *Ber.*, **44**, 1027.

[3] *Ber.*, **45**, 608.

Uridine.

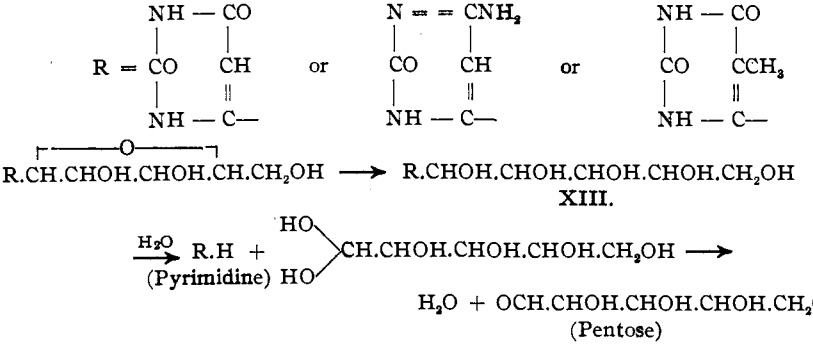


XI.



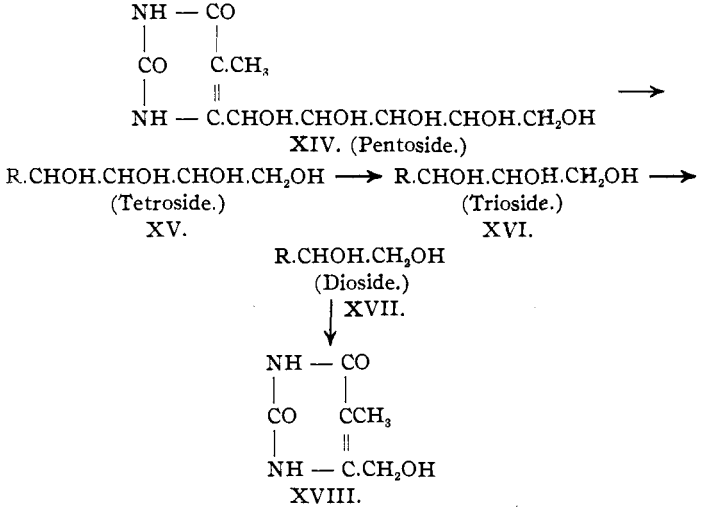
XII.

The synthetical work now in progress has developed from the assumption that these pyrimidines, in their corresponding nucleosides, are linked to the carbohydrate at the 4-position and that this linkage is between two carbon atoms as represented by formulas (X) and (XII). This constitution is the most probable of the two, because of the stability of these nucleosides towards hydrolytic agents. The results, which we have obtained (unpublished), indicate that a combination like that represented in formulas (IX) and (XI) would be extremely unstable in the presence of acids. A pyrimidine-nucleoside may be considered, therefore, as an addition-product of a pyrimidine and a sugar. The formation of ribose from such a complex, by hydrolysis, would then involve, theoretically, two distinct changes, *viz.*, a rupture of the furane ring forming the glucoside (XIII) and finally a cleavage of the carbohydrate from the pyrimidine. These various changes may be expressed as follows:



XIII.

type of the series or the simplest nucleoside of this type. It would still retain the same glucosidic linking. The four pyrimidine-nucleosides, which would be formed from *thymine-nucleoside* (XIV) by such a process, are represented by the following formulas:



Since the pyrimidine-nucleosides undergo hydrolysis with formation of a pentose, the simpler forms represented by formulas (XV), (XVI), (XVII) and (XVIII) would likewise be expected to undergo hydrolysis with formation of erythrose $CH_2OH.(CHOH)_2CHO$, glycerose $CH_2OH.CHOH.CHO$, glycolylaldehyde $CH_2OH.CHO$ and formaldehyde, respectively.

We have absolutely no knowledge of the chemical properties of even the simplest type of nucleosides as represented by formula (XVIII), and it was not until recently that the writer was able to undertake their investigation. We are now able to contribute important data regarding the nature of glucosidic linkings of this type. In the preceding paper[1] the writer has stated that β-ketone esters can be prepared by the application of Reformatsky's reaction. One of the first ketone ester that we prepared by this method was ethyl α-methyl-γ-ethoxyacetoacetate (XXI). It was obtained by condensation of ethyl α-bromopropionate (XX) with ethyl ethoxyacetate (XIX) in the presence of amalgamated zinc. The reaction may be expressed as follows:

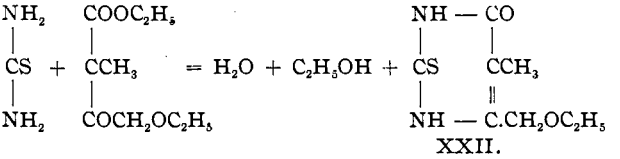
$$\underset{\text{XIX.}}{C_2H_5OCH_2COOC_2H_5} + \underset{\text{XX.}}{Br.CH(CH_3)COOC_2H_5} + Zn.Hg = \underset{\text{XXI.}}{C_2H_5OCH_2.COCH(CH_3)COOC_2H_5}.$$

Starting with this new ketone ester and thiourea we have now suc-

[1] Johnson, THIS JOURNAL.

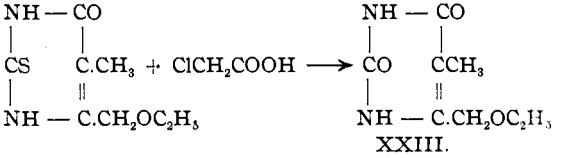
ceeded in synthesizing the simplest nucleoside of thymine *viz.*, 2,6-dioxy-4-hydroxymethyl-5-methyl pyrimidine (XVIII). The various steps involved in its synthesis are as follows:

(1) The ketone ester (XXI) was first digested in alcohol with the required proportions of thiourea and sodium ethylate when a pyrimidine condensation was effected and 2-thio-4-ethoxymethyl-5-methyl-6-oxy-pyrimidine (XXII) was formed. This new condensation is expressed by the following equation:



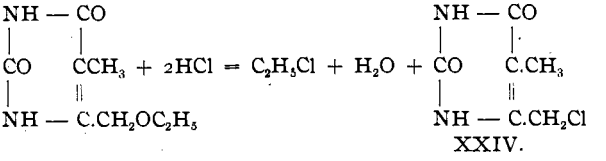
XXII.

(2) This thiopyrimidine (XXII) was then digested with chloroacetic acid, when it was desulfurized practically quantitatively and we obtained the corresponding oxypyrimidine (XXIII).



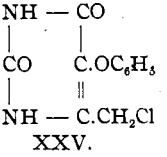
XXIII.

(3) This pyrimidine (XXIII) was then heated with concentrated hydrochloric acid. The ethyl group was detached, by the action of this reagent, in the form of ethyl chloride and the pyrimidine was transformed smoothly into the chlormethylpyrimidine (XXIV). We obtained no evidence here of the formation of thymine and formaldehyde. This interesting change is represented by the following equation:



XXIV.

(4) Pyrimidine halides of this type (XXIV) have not been investigated, only one, so far as the writer is aware, has been described, *viz.*, 2,6-dioxy-4-chlormethyl-5-phenoxypyrimidine (XXV).

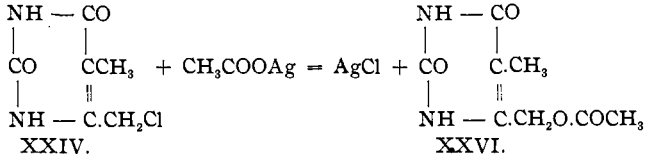


XXV.

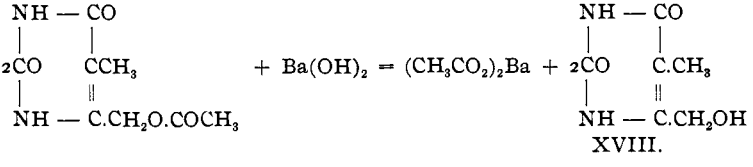
This was synthesized by Johnson and Hill.[1] Halides of this type are

[1] *Am. Chem. J.*, 48, 296.

very reactive and the halogen is easily removed by the action of alkali. We found, for example, that 2,6-dioxy-4-chlormethyl-5-methylpyrimidine (XXIV) was transformed almost quantitatively into the corresponding acetate (XXVI) when digested in aqueous solution with the required amount of silver acetate. This pyrimidine possessed characteristic properties and underwent hydrolysis, when digested with barium hydroxide solution, forming the corresponding alcohol or pyrimidine-nucleoside (XVIII). These final changes in our synthesis are represented by the following equations:

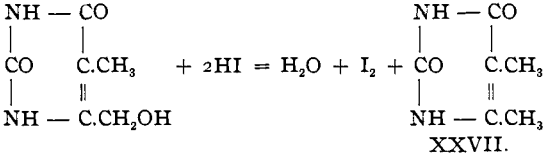


XXIV. XXVI.



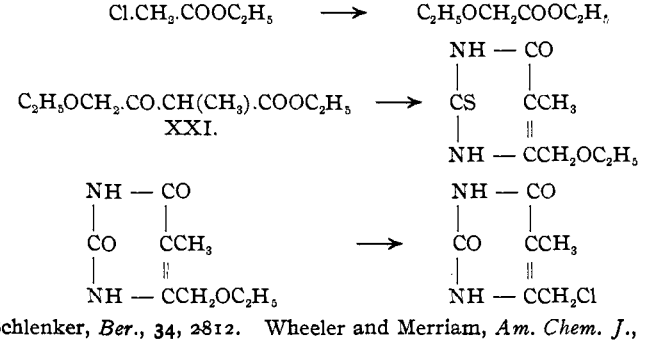
XVIII.

The constitution of this compound (XVIII) was established by the fact, that it was transformed quantitatively into 2,6-dioxy-4,5-dimethylpyrimidine[1] (XXVII) by reduction with hydriodic acid and red phosphorus.

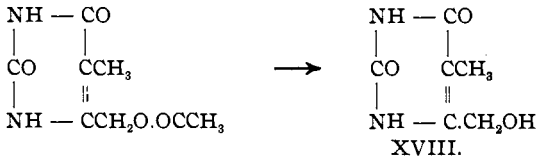


XXVII.

Starting with ethyl chloroacetate our synthesis of this simple nucleoside (XVIII), therefore, involves seven different operations, which may be expressed as follows:



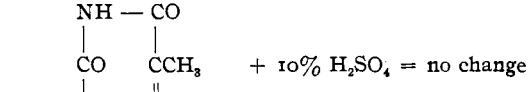
[1] Schlenker, *Ber.*, 34, 2812. Wheeler and Merriam, *Am. Chem. J.*, 29, 488.



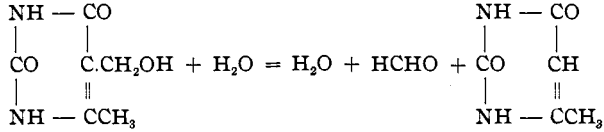
XVIII.

Furthermore the yields are excellent in every step except one, *viz.*, that involving the formation of the β-ketone ester (XXI). The reactions following are, however, so smooth that it is possible to accomplish the complete synthesis of the nucleoside (XVIII) by using only a small quantity of the ketone ester (5 grams).

We have now made the interesting observation that this simple nucleoside of thymine (XVIII) is extremely stable in the presence of sulfuric acid, and does not undergo hydrolysis, with formation of formaldehyde and thymine, when heated with this reagent. It has been shown by Levene and his co-workers[1] that the carbohydrate is cleaved from the pyrimidine-nucleosides by heating with 10% sulfuric acid at 125°. Our nucleoside was recovered unaltered after heating with sulfuric acid of this same strength for 3 hours at 125–130°. Whether formaldehyde will be detached by heating with sulfuric acid at a higher temperature will be determined by further investigation. The stability of this compound, in the presence of sulfuric acid, is all the more remarkable when compared with that of the isomeric pyrimidine (XXVIII), which has been prepared by Kircher.[2] This pyrimidine (XXVIII) is transformed quantitatively into 4-methyluracil (XXIX) and formaldehyde simply by heating in aqueous solution. From a chemical standpoint these results are very interesting and suggestive. Whether the linking between the carbohydrate complex and the pyrimidine at the 4-position will be more labil as we increase the length of the sugar chain must be decided by further investigations.



XVIII.



XXVIII. XXIX.

The investigation of pyrimidine nucleosides will be continued in this laboratory.

[1] *Loc. cit.*

[2] *Ann.*, **385**, 293.

Experimental Part.

The ethyl ethoxyacetate $C_2H_5OCH_2COOC_2H_5$, which was used in this investigation, was prepared according to two different methods: (1) By the action of sodium ethylate on ethyl chloroacetate and (2) from chloromethylethyl ether $C_2H_5OCH_2Cl$ as follows: this was first converted into the nitrile $C_2H_5OCH_2CN$ and the latter changed to the ester by saponification and esterification in alcohol solution.

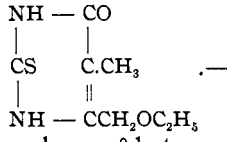
Ethyl α-Methyl-γ-ethoxyacetoacetate,[1] $C_2H_5OCH_2.CO.CH(CH_3).COOC_2H_5$. —This new β-ketone ester was prepared by condensing ethyl α-bromopropionate with ethyl ethoxyacetate by means of zinc-amalgam. The method of procedure was essentially as follows: Molecular proportions of the α-bromopropionate (84.4 grams) and the acetate (56.0 grams) were placed in a dry flask and 40.8 grams of dry, freshly amalgamated zinc suspended in the liquid. The flask was then connected with a return condenser and finally heated on the steam bath. At first there was no evidence of any reaction, but after warming a few minutes a violent reaction began and finally became so vigorous that it was necessary to plunge the flask into ice water at intervals to avoid too great heat. After the violent reaction was over the mixture was then heated on the steam bath for about 12 hours in order to thoroughly complete the reaction. We obtained a dark brown, syrupy fluid. This was then transferred to a separatory funnel and shaken with an excess of water when we obtained a heavy precipitate of a double zinc compound, which was dissolved immediately by the addition of cold, dilute hydrochloric acid. We obtained in this manner a transparent, red oil, which was separated from the acid solution and finally dissolved in ether. This ether solution was then thoroughly cooled and washed several times with a dilute solution of sodium hydroxide. The β-ketone ester was removed by this treatment and the alkaline solutions finally combined and acidified (cold) with cold, dilute hydrochloric acid. The ketone ester separated at once and was dissolved in ether. After thorough drying over anhydrous calcium chloride, the ether was removed and the ester purified by distillation under diminished pressure. It practically all distilled at 116° at 24 mm. The yield of purified ester was 7.5 grams. In a second experiment we used 100 grams of the bromopropionate, 73.0 grams of the acetate and 72.0 grams of amalgamated zinc and obtained 9.0 grams of the ketone ester boiling at 113–116° at 18–20 mm.

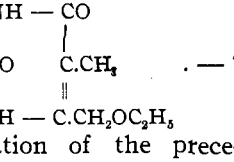
Molecular weight determination by the ebullioscopic method: 0.6731 gram substance in 15.56 grams of C_6H_6 gave $\Delta_0 = 0.619°$.

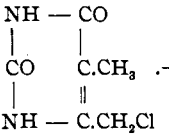
Calculated for $C_9H_{16}O_4$: M. W., 188.
Found: M. W., 186.

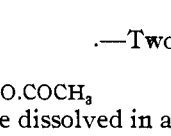
[1] The writer desires to call attention to the fact that we are using zinc-amalgam as a reagent in other investigations. We have already obtained important results of biochemical interest, which we hope to be able to present for publication in the near future.—(T. B. J.).

The other products of this reaction, which are insoluble in sodium hydroxide solution, will be examined later.

2-Thio-4-ethoxymethyl-5-methyl-6-oxypyrimidine,  .—This pyrimidine was formed by condensation of the above β-ketone ester with thiourea in the presence of sodium ethylate. In one experiment the following proportions were used: 7.4 grams of the ketone ester, 3.0 grams of thiourea and 1.8 grams of metallic sodium. The sodium was first dissolved in a small volume of absolute alcohol, the thiourea and ester then dissolved in the solution, and the mixture then digested on the steam bath for about 4 hours. The sodium salt of the thiopyrimidine began to form almost immediately, on heating, and deposited as a brown powder. After completion of the reaction the alcohol was then evaporated and the residue dissolved in a small volume of cold water and the solution filtered. On acidifying this solution (cold) with glacial acetic acid the pyrimidine separated at once in a crystallin condition. It was purified by crystallization from boiling 95% alcohol and separated on cooling, in hexagonal tables, which melted at 191–192° to a clear oil without decomposition. The yield of purified pyrimidine was 2.3 grams. This compound is very soluble in hot water and hot alcohol and difficultly soluble in cold. The experiment was repeated and from 9.0 grams of the ketone ester and the above proportions of thiourea and sodium ethylate we obtained 3.7 grams of the pyrimidine. Nitrogen determination (Kjeldahl): Calculated for $C_8H_{12}O_2N_2S$: N, 13.86. Found: N, 13.90.

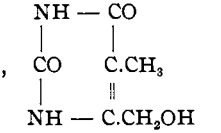
2,6-Dioxy-4-ethoxymethyl-5-methylpyrimidine,  . — This pyrimidine is easily obtained by desulfurization of the preceding thiopyrimidine. One and three-tenths grams of 2-thio-4-ethoxymethyl-5-methyl-6-oxypyrimidine and two molecular proportions of chloroacetic acid (1.1 grams) were dissolved in 30 cc. of water and the solution boiled for 5 hours. The solution was then allowed to cool when this pyrimidine separated in beautiful arborescent crystals. The compound was purified by crystallization from hot water and melted at 220° to a clear oil. It is soluble in hot alcohol. The yield was 1.1 grams. In a second experiment we obtained 3.4 grams of this pyrimidine from 3.7 grams of the thiopyrimidine. The yield therefore is practically quantitative. Nitrogen determination (Kjeldahl): Calculated for $C_8H_{12}O_3N_2$: N, 15.05. Found: N, 15.02.

2,6-Dioxy-4-chlormethyl-5-methylpyrimidine,  .—This chloropyrimidine can easily be prepared by heating the preceding ethoxypyrimidine with hydrochloric acid. For example, 1 gram of the ethoxypyrimidine and 20 cc. of concentrated acid were heated for 3 hours at 125–130°. When the bomb tube was opened, ethyl chloride was identified and a yellow solution was obtained. This was then concentrated on the steam bath and cooled, when the chloropyrimidine separated in the form of plates, which melted at 243° to a clear red oil. On evaporating the filtrate to dryness more of the same compound was obtained. The yield was excellent. We find that this chloride is also formed by heating the ethoxypyrimidine with hydrochloric acid at 100°, but it is necessary to heat for several hours before the transformation is complete. Three and four-tenths grams of the pyrimidine and 65 cc. of concentrated acid were first heated for 2 hours at 100°. On opening the tube ethyl chloride escaped and burned with a green flame. An examination of the reaction mixture showed, however, that the reaction was not complete. The heating was continued for 12 hours at 100° and the solution then concentrated and cooled, when we obtained the chloropyrimidine melting at 241–244°. The yield was 2.5 grams. It was purified by crystallization from water and melted at 243°. It gave a strong test for chlorine. The dust from this pyrimidine irritates the nose, causing violent sneezing and finally a severe headache. Nitrogen determination (Kjeldahl): Calculated for $C_6H_7O_2N_2Cl$: N, 16.04. Found: N, 15.81.

The Acetate of 2,6-Dioxy-4-hydroxymethyl-5-methylpyrimidine,  .—Two and four-tenths grams of pure silver acetate were dissolved in about 200 cc. of hot water and 2.086 grams of the above chloropyrimidine added to the hot solution. There was an immediate reaction, the pyrimidine dissolved and silver chloride deposited. This solution was finally boiled for 2 hours in order to complete the reaction. After filtering from silver chloride the solution was then evaporated to complete dryness and the residue dissolved again in hot water and the slight amount of silver in solution decomposed by treatment with hydrogen sulfide. After digesting with bone coal, the solution was then filtered and concentrated to a small volume. On cooling, the acetylpyrimidine separated in minute crystals. It is difficultly soluble in hot water and cold 95% alcohol. It was purified for analysis by crystallization from 95% alcohol and deposited in microscopic, corpuscular crystals,

which melted at 260–261° with effervescence. The yield of purified pyrimidine was about 1.5 grams. Nitrogen determination (Kjeldahl): Calculated for $C\ H_{10}O_4N_2$: N, 14.14. Found: N, 14.34.

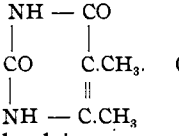
2,6-Dioxy-4-hydroxymethyl-5-methylpyrimidine,



This interesting pyrimidine was obtained by hydrolysis of its acetate. Five grams of crystallized barium hydroxide and 1 gram of the acetate were dissolved in the least possible amount of hot water and the solution boiled for 1 hour. The solution was then saturated with carbon dioxide gas in order to precipitate the barium as carbonate, and the solution finally filtered. The solution was then evaporated to dryness when the crude hydroxypyrimidine was obtained as an amber colored crystallin residue. This was purified by crystallization from hot water and separated on cooling, in distorted needles, which melted at 224–225° with decomposition. Nitrogen determination (Kjeldahl): Calculated for $C_6H_8O_3N_2$: N, 17.94. Found: N, 17.82.

An attempt to convert this simple nucleoside into thymine and formaldehyde by hydrolysis with 10% sulfuric acid was unsuccessful. One-half of a gram of the nucleoside was suspended in 25 cc. of 10% sulfuric acid and then heated in a bomb tube for 3 hours at 120–130°. When the tube was opened there was no pressure and no evidence that the pyrimidine had undergone any change. The sulfuric acid was precipitated by addition of the required amount of barium hydroxide and the solution then evaporated to dryness. We obtained a crystallin substance which was purified by crystallization from hot water. It separated in the form of distorted prisms, which melted at 223–224° with effervescence. The substance did not resemble thymine in any of its properties and was identified as the unaltered hydroxypyrimidine (nucleoside). We recovered all of the pyrimidine and used part of it in the following experiment:

The Conversion of 2,6-Dioxy-4-hydroxymethyl-5-methylpyrimidine into 2,6-Dioxy-4,5-dimethylpyrimidine,



One-tenth of a gram of the above nucleoside was dissolved in 5 cc. of hydriodic acid (sp. gr. 1.7) and the solution, after the addition of a few milligrams of red phosphorus, then boiled for 4 hours. After cooling, the acid solution was then diluted with water, filtered and an excess of dry silver carbonate stirred into the solution in order to remove the iodine, hydriodic and phosphoric acids. After filtering, the excess of silver was then precipitated as sulfide and the

solution concentrated on the steam bath and finally allowed to cool. Dimethyluracil separated in the form of prismatic crystals, which melted at 296° when heated rapidly. If the acid bath was heated slowly the substance melted at 292–294° with decomposition. A mixture of this compound with 4,5-dimethyluracil[1] melted at exactly the same temperature.

NEW HAVEN, CONN.

A PRELIMINARY STUDY OF THE BIOCHEMICAL ACTIVITY OF BACILLUS LACTIS ERYTHROGENES.

BY MARY LOUISE FOSTER.

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Bacillus lactis erythrogenes belongs to the group of pigment-forming bacteria which diffuse coloring matter through the culture medium. This organism was first described by Grotenfelt and later by Hueppe as a "non-motile rod, 0.3–0.5 × 1–1.4. Sporeless. Colonies round, gray-yellow to pure yellow, slowly liquefying with rosy coloration the surrounding gelatin..... Milk is coagulated by lab. ferment and peptonized. Nauseating odor. The red pigment is insoluble."[2] This organism, found occasionally in dairies, is non-pathogenic to man. The object of this study was to determin the compositions of the fluid from time to time and to follow in this way the course of the catabolism; to find out the specific cause of this change; and to study the nature of the pigment.

A flask of steril milk, inoculated with this organism, shows, after a few days, a faint blush which gradually intensifies in hue until the entire liquid is a deep blood red. At the same time the milk coagulates. Later it liquefies, lumps of solid material appear on the surface while at the bottom of the flask is a thick viscous mass. This gradually diminishes in bulk and a granular precipitate settles to the bottom with a clear red supernatant liquid. There is a strong disagreeable odor of glue. These phenomena take place at room temperature—about 20°—and require from two to six months for completion.

The flask, before any chemical analysis was made, was examined bacterially and was found to contain a pure culture of the organism. The liquid was alkalin to litmus and gave a negative result for *lactic acid* by Uffelmann's test; a faint trace of *formic acid* with mercuric oxide; a strongly positive Molisch test indicating the presence of *carbohydrate*, which was identified as lactose by the phenylhydrazine test. At another time in another flask, some glucose was found to be present. While this second flask contained the *bacillus lactis erythrogenes*, it was a different

[1] Schlenker, Wheeler and Merriam, *Loc. cit.*

[2] Flügge, Vol. II, page 305.