dine and the 2,4-diketo-tetrahydrothiazole gave the 2,4-diketo-5-anilido-methylene-tetrahydrothiazole, HN———CO

OC—S—C = CHNHC$_6$H$_5$

This is very difficultly soluble in hot alcohol from which it crystallized in almost colorless plates melting at 233°.

Calc. for $C_{10}H_8O_2N_2S$: N, 12.73%. Found: 12.96%, 13.03%.

As a matter of interest it may be noted here that experiments show that the true thiohydantoins containing a methylene group condense with the formamidines giving substitued amino methylene derivatives of the type,

RN — CO
SC |
HN — C = CHNHC$_6$H$_5$

isomeric with the thiazole derivatives.

These investigations are being continued in this laboratory.

LAWRENCE, KANSAS.

[CONTRIBUTIONS FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY.]

RESEARCHES ON PYRIMIDINES. LXXXI. SECONDARY-PYRIMIDINE-NUCLEOSIDES AND THEIR UNIQUE BEHAVIOR ON HYDROLYSIS.[1]

BY TREAT B. JOHNSON AND SIDNEY E. HADLEY.

Received June 28, 1916.

This is the fourth paper from this laboratory on pyrimidine nucleosides and, like those preceding it, deals with the chemistry of uracil and thymine combinations containing alcohol groupings in position 4 of the pyrimidine ring. The classification adopted namely, primary, secondary and tertiary

NH — CO
| |
CO CH
| ||
NH — CH
(Uracil)
(I).

NH — CO
| |
CO CCH$_3$
| ||
NH — CH
(Thymine)
(II).

NH — CO
| |
CO CCH$_3$
| ||
NH — C.CH$_2$OH
(III).

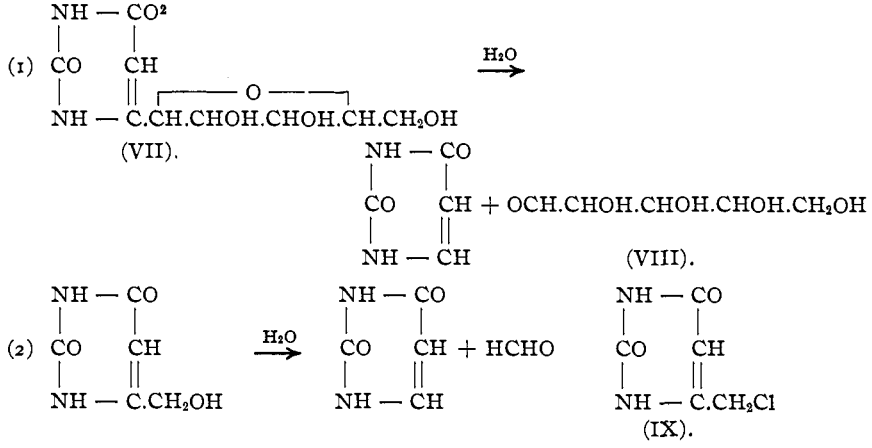
NH — CO
| |
CO CH
| ||
NH — C.CH$_2$OH
(IV).

NH — CO
| |
CO CH
| ||
NH — CCH(CH$_3$)OH
(V).

NH — CO
| |
CO CH
| ||
NH — CC(CH$_3$)$_2$OH
(VI).

[1] The observations recorded in this paper have developed from an investigation on nucleosides which was originally started in this laboratory by Dr. Lewis H. Chernoff in 1913. The paper has been constructed from a dissertation presented by Mr. Sidney Edward Hadley to the Faculty of the Graduate School of Yale University, 1916, in candidacy for the Degree of Doctor of Philosophy.

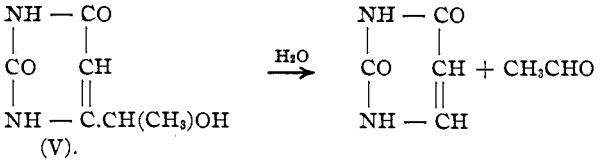
nucleosides, applies to pyrimidine combinations containing the typical alcohol groups represented in Formulas IV, V and VI, respectively. The two primary nucleosides of uracil and thymine (IV and III) have already been synthesized and their chemical behavior investigated.[1] Both compounds are characterized by their stability, and they exhibit the normal behavior of primary alcohols. Neither combination is broken down by heating with acids with rupture of the carbon union in Position 4 of the pyrimidine ring giving uracil and thymine, respectively. Instead of reacting according to Equation 2 the uracil nucleoside (IV) and also the thymine nucleoside (III) interact with acids giving their corresponding alkyl salts, of which the halide (IX) is a typical representative. In other words, pyrimidine alcohols of this type react as might be predicted and do not conform in behavior to that of natural pyrimidine nucleosides like *uridine* (VII). This combination undergoes hydrolysis with acids giving uracil and a sugar.



In the light of these interesting results it was of special interest to extend our investigation of these new alcohol combinations and synthesize representatives of secondary nucleosides. Such combinations approach in constitution still closer to that assigned to natural nucleosides (uridine), and consequently it was important to determine whether they are less resistant to the action of acids than the primary compounds. The secondary nucleoside (V) was the first representative to be studied, and the primary object of this preliminary paper is to record a characteristic and unique transformation which this combination undergoes on hydrolysis. *It does not interact with acids, under the conditions favorable for the hydrolysis of uridine, with production of uracil and acetaldehyde.*

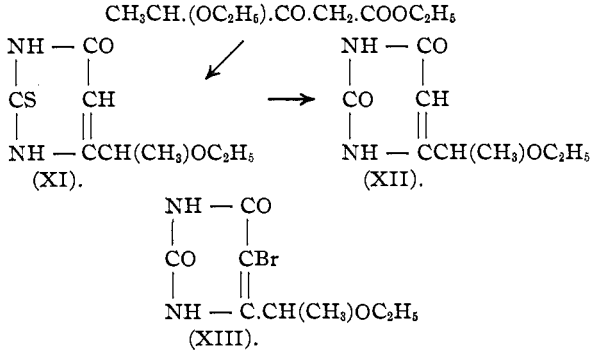
[1] Johnson and Chernoff, *J. Biol. Chem.*, **14**, 307 (1913); THIS JOURNAL, **35**, 585 (1913); **36**, 1742 (1914).

[2] Levene and La Forge, *Ber.*, **45**, 608 (1912).



(V).

In order to obtain the nucleoside (V) according to the method already applied successfully in our previous work,[1] it was necessary for us to synthesize first an ether of this nucleoside. The ethyl ether was the derivative selected and was prepared easily in the following manner: Ethyl γ-ethoxy-γ-methylacetoacetate[2] (X) was first synthesized in quantity by condensing ethyl α-ethoxypropionate with ethyl propionate in the presence of metallic sodium (Claisen condensation). This was then allowed to interact with thiourea when they combined smoothly, in the presence of sodium ethylate, giving the 2-thiopyrimidine represented by Formula XI. This pyrimidine was desulfurized by digestion with chloroacetic acid giving the desired nucleoside ether represented by Formula XII. This pyrimidine can be prepared easily in quantity by this method, and proved to be especially suitable for experimental study on account of its purity, crystalline character and insolubility in water. It interacted normally with bromine to give the bromopyrimidine (XIII).

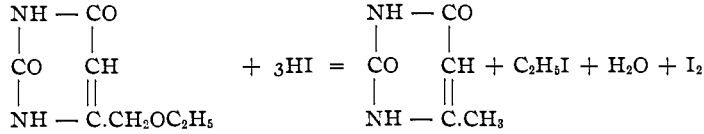


We have now made the most interesting observation that this nucleoside ether (XII) reacts in a characteristic manner when digested with hydrobromic and hydriodic acids. While the ethers of the primary nucleosides, so far examined, interact normally with hydrobromic acid, forming the corresponding alkyl halides and water, this pyrimidine reacts in an entirely different manner, giving a crystalline substance free from halogen and possessing weakly basic properties. Furthermore the pyrimidine (XII) interacts with hydriodic acid giving the same product as is obtained by hydrolysis with hydrobromic acid. This is a very

[1] *Loc. cit.*

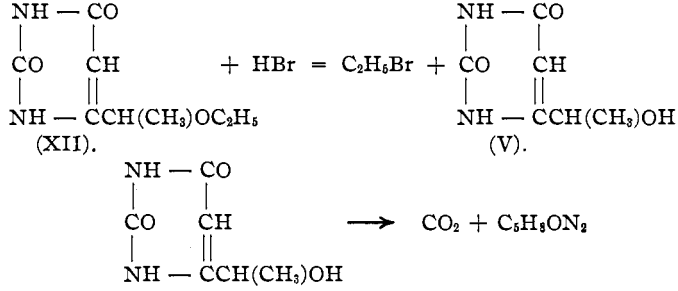
[2] Johnson, THIS JOURNAL, 35, 582 (1913); Chernoff, Dissertation, Yale University, 1914.

significant result. The corresponding ethers of primary nucleosides react with this acid with formation of the corresponding alkyl pyrimidines. In other words, such nucleoside combinations are reduced by this reagent as is expressed by the following equation:



The final product of hydrolysis with these halogen acids is not the pyrimidine nucleoside represented by Formula V. Ethyl bromide and ethyl iodide are formed, respectively, during the transformation, as would be expected, but at the same time a secondary reaction sets in during hydrolysis, and a quantity of carbon dioxide is evolved which corresponds to practically one-sixth of the total carbon content of the nucleoside (V). Apparently the nucleoside (V) is the primary product of the reaction, but in this case is unstable in the presence of acids and breaks down further with destruction of the pyrimidine molecule.

This characteristic product of hydrolysis has been isolated, purified and analyzed and we find that it is not identical with uracil. It does not give Wheeler and Johnson's[1] test for this pyrimidine. The analytical values obtained for carbon, hydrogen and nitrogen agree with those required by the empirical formula $C_5H_8ON_2$. In other words, we are dealing here with an unique transformation which sharply differentiates secondary from primary nucleosides. The change is accomplished by hydrolysis and may be viewed as a dissociation of the nucleoside into carbon dioxide and the hydrolytic product $C_5H_8ON_2$ of unknown structure. The complete transformation of the nucleoside ether (XII) is expressed by the following equations:

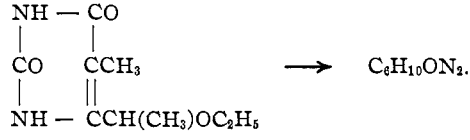


It is an interesting fact that the compound $C_5H_8ON_2$ and uracil ($C_4H_4O_2N_2$) both contain the same percentage of nitrogen—25%.

Regarding the structure of this interesting substance $C_5H_8ON_2$ and the mechanism of its formation from the nucleoside (V), we have not ob-

[1] *J. Biol. Chem.*, **3**, 183 (1907).

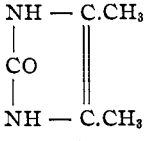
tained sufficient data to enable us to formulate definite conclusions. The reaction, however, is apparently a normal one and we already have obtained good evidence that the corresponding nucleoside ether of thymine (XIV) undergoes a similar transformation on hydrolysis, yielding a substance having the formula $C_6H_{10}ON_2$. In other words, these two



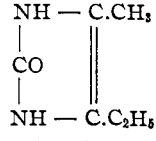
(XIV).

hydrolytic products are representatives of an homologous series differing in constitution by a CH_2 radical. A description of our work on secondary thymine nucleoside will be given in a later paper.

A careful search of the literature now reveals the interesting fact that of the 14 known compounds[1] having the formula $C_5H_8ON_2$ and the 18 conforming to the expression $C_6H_{10}ON_2$ two agree very closely in chemical and physical properties with our unknown hydrolytic products, namely: 2-oxy-4,5-dimethylimidazol (XV) and 2-oxy-4-ethyl-5-methylimidazol (XVI), respectively.



(XV).



(XVI).

The imidazol (XV) was first synthesized by Kunne[2] and it is stated by Biltz[3] to darken when heated at 290° and sublime without melting at 300°. Gabriel and Posner[4] assigned to the imidazol (XVI) a decomposition point of 270°. Our hydrolytic product obtained from the nucleoside (V) did not melt at 300° while we find that the corresponding derivative formed by hydrolysis of the pyrimidine (XIV) melts at 268° with decomposition. Owing to lack of time it has been impossible for us to prepare these known imidazols and compare them with our hydrolytic products. This work will be taken up just as soon as possible. Whatever constitution is to be assigned to our hydrolytic products it is apparent that we are dealing here with an unique transformation of the greatest biochemical interest. Our researches on synthetical pyrimidine nucleosides will be continued.

[1] Richter's "Lexikon der Kohlenstoffverbindungen."

[2] *Ber.*, **28**, 2040 (1895).

[3] *Ibid.*, **40**, 4801 (1907).

[4] *Ibid.*, **27**, 1038 (1894).

Summary.

The secondary nucleoside (V) undergoes hydrolysis, when digested with hydrobromic and hydriodic acids, giving carbon dioxide and a crystalline, basic product $C_5H_8ON_2$ of unknown constitution.

Experimental Part.

Ethyl α-Ethoxypropionate and Ethyl α-Methoxypropionate.—These two esters, which were employed in this investigation, were prepared according to the directions of Schreiner[1] by the action of ethyl α-bromopropionate on sodium ethylate and sodium methylate, respectively. The α-bromo ester was prepared from propionic acid according to the directions given by Zelinsky.[2] The reactions in neither case were productive of quantitative yields of the alkoxyl esters. For example, 142 g. and 92 g., respectively, of the purified α-ethoxy and α-methoxy esters were obtained from 300 g. of the ethyl α-bromopropionate.

Ethyl α-Methyl-γ-methyl-γ-ethoxyacetoacetate, $CH_3CH(OC_2H_5).CO.CH(CH_3)COOC_2H_5$. **Preparation of the Ester by Application of Reformatsky's Reaction.**—This β-ketone ester can be prepared by application of Reformatsky's reaction, which has been modified by Johnson[3] and utilized for the preparation of β-ketone esters. Molecular proportions of ethyl α-ethoxypropionate and ethyl α-bromopropionate were condensed by warming in the presence of an excess of granulated zinc amalgam. There was an energetic reaction when such a mixture was warmed on a steam bath and, unless retarded, finally became so violent that it was necessary to keep at hand a cooling device to afford proper control of the reaction. After the violent reaction was over, heating on the steam bath was continued for 5–6 hours. To the syrupy liquid thus obtained ice and water were added, when a double zinc combination was precipitated. Keeping the mixture cold with ice, this was decomposed by addition of hydrochloric acid with formation of a clear oil. Ether was then added to dissolve the oil and finally sufficient sodium hydroxide was added to the ether solution to completely dissolve the zinc hydroxide and also the β-ketone ester present. The alkaline solution was saved and on acidifying this with hydrochloric acid, again keeping the solution cold with ice, the free β-ketone ester separated as a red oil. This was extracted with ether, washed with a little water and dried over calcium chloride. We obtained by this procedure 12 g. of the β-ketone ester (undistilled) from 70 g. of ethyl α-ethoxypropionate, which corresponds to only 12% of a theoretical yield.

Preparation of the Ester by Application of a Claisen Condensation.—Molecular proportions of ethyl α-ethoxypropionate and ethyl propionate

[1] *Ann.*, **197**, 13 (1879).

[2] *Ber.*, **20**, 2026 (1887).

[3] THIS JOURNAL, **35**, 582 (1913).

were mixed in a dry flask connected to a return condenser. This was then heated to 85° and a molecular proportion of sodium in wire form was added in small portions at a time. Heating was then continued for 3–4 hours, when the sodium completely dissolved and a red syrupy fluid was obtained. This was dissolved in ice water and the unaltered ester removed with ether. The aqueous solution was then acidified cold with hydrochloric acid and the free ketone ester extracted with ether. This was dried over calcium chloride and finally purified by distillation under diminished pressure. We obtained from 100 g. of ethyl α-ethoxypropionate 80 g. of crude ester which yielded on distillation 30 g. of the pure ketone ester boiling at 114° under a pressure of 14 mm.

Calc. for $C_{10}H_{18}O_4$: C, 59.36, H, 8.91. Found: C, 58.74; H, 8.79.

Ethyl α-Methyl-γ-methyl-γ-methoxyacetoacetate, $CH_3CH(OCH_3).CO.CH(CH_3).COOC_2H_5$.—This ester was prepared by condensation of ethyl propionate with ethyl α-methoxypropionate in the presence of metallic sodium. The procedure was similar to that described above. The yield of pure distilled ester in this case was 36% of the theoretical. It boiled at 105° at 14 mm. pressure.

Calc. for $C_9H_{16}O_4$: C, 57.44; H, 8.51. Found: C, 56.90; H, 8.40.

Ethyl γ-Ethoxy-γ-methylacetoacetate, $CH_3.CH(OC_2H_5).CO.CH_2.COOC_2H_5$.—This ester has already been described by Chernoff[1] who prepared it by application of Reformatsky's reaction with ethyl chloroacetate and ethyl α-ethoxypropionate. We find that the ester is more easily prepared and obtained in better yield by condensing the ethyl α-ethoxypropionate with ethyl acetate in the presence of sodium. The following proportions were taken: 100 g. of ethyl α-ethoxypropionate, 75 g. of ethylacetate and 17 g. of sodium. We obtained 65 g. of the β-ketone ester boiling at 107° at 16 mm. or a yield of ester corresponding to 58% of the theoretical. Chernoff assigned to his product the same boiling point but his yield was only 6.3% of theory. In another experiment we obtained, by condensation of 30 g. of ethyl α-ethoxypropionate with 30 g. of ethyl acetate, 13.5 g. of the β-ketone ester.

2-Thio-4(α-ethoxyethyl)-6-oxypyrimidine (XI).—This new pyrimidine is easily obtained by condensation of ethyl γ-ethoxy-γ-methylacetoacetate with thiourea. The description of a single experiment will illustrate our general procedure for preparing the compound. Seventeen grams of sodium were dissolved in 300 cc. of absolute alcohol and 40 g. of thiourea then dissolved in the ethylate solution. Sixty-five grams of the above β-ketone ester were then added and the mixture heated on a steam bath for about 10 hours. The sodium salt of the thiopyrimidine separated. The excess of alcohol was removed by evaporation at 100° and the residue

[1] Dissertation, Yale University, 1914.

dissolved in water. On acidifying this aqueous solution with hydrochloric acid the above pyrimidine separated as a colorless solid. It was purified by crystallization from hot water and separated as stout prisms melting at 206–208°. The yield was 40 g.

Calc. for $C_8H_{12}O_2N_2S$: N, 14.00. Found: N, 14.20.

2,6-Dioxy-4(α-ethoxyethyl)pyrimidine (XII).—This pyrimidine was easily obtained by desulfurization of the corresponding sulfur compound with chloroacetic acid. Thirty-six grams of the thiopyrimidine were digested with 31 g. of chloroacetic acid in 600 cc. of water for 10 hours. The solution was then evaporated to dryness at 100°, several additions of alcohol being made near the end of the operation to aid the removal of the chloroacetic acid by esterification. The residue was triturated with cold water and the insoluble pyrimidine purified by crystallization from hot water. It separated on cooling in the form of colorless, elongated prisms which melted at 184–186°. The yield was 20 g.

Calc. for $C_8H_{12}O_3N_2$: N, 15.22. Found: N, 15.19.

2,6-Dioxy-4(α-ethoxyethyl)-5-bromopyrimidine (XIII).—This compound was prepared by allowing an excess of bromine to interact, at ordinary temperature, with 2,6-dioxy-4(α-ethoxyethyl)-pyrimidine in acetic acid. After evaporating the acid and triturating the reaction product with water this bromo compound separated in a crystalline condition. It crystallized from boiling water in flat prisms melting at 206° to a clear oil. The pyrimidine is very soluble in alcohol.

Calc. for $C_8H_{11}O_3N_2Br$: N, 10.65. Found: N, 10.74.

The Behavior of 2,6-Dioxy-4(α-ethoxyethyl)-pyrimidine on Hydrolysis with Acids: *The Action of Hydrobromic Acid.*—An important result that really directed our procedure in hydrolyzing this pyrimidine was our observation that carbon dioxide was given off when this pyrimidine was digested with acids. In consequence of this unexpected behavior we were obliged to hydrolyze under specific conditions in order to obtain consistent results. The method of operating, which was finally adopted as the most successful, was as follows: Five grams of the pyrimidine and 25 cc. of hydrobromic acid were heated under a return condenser at 130° in an oil bath. It dissolved almost immediately on warming, with evolution of a gas which was identified as carbon dioxide. This effervescence was continuous for about 2 hours. During this transformation ethyl bromide was also given off and condensed in globules in the condenser tube. After the evolution of carbon dioxide ceased, the heating was continued for about 3 hours longer. The resulting fluid was dark red in color and on evaporating, to remove hydrobromic acid, a semisolid residue was obtained. In order to remove the last trace of hydrobromic acid this was triturated with alcohol and the evaporation repeated. The residue was finally triturated with a small volume of cold water when

a colorless, granular substance deposited. This was separated and purified by crystallization from hot water. It deposited in the form of plates or distorted prisms which did not melt below 300°. More of the same compound was obtained when the original aqueous extract was neutralized with ammonia. In other words the substance was slightly basic, forming a hydrobromide which underwent dissociation on treatment with water. After final purification the product did not show a definite melting or decomposition point. It always began to turn brown when heated at 280° and remained in this condition at 300°. By repeated crystallization from hot water much material was lost as the compound appeared to undergo a change by such a treatment, being transformed into a more soluble product. What was formed here, we were unable to establish with the amount of material that was available for experimental purposes. The original compound was found to contain the same percentage of nitrogen as uracil. In fact uracil was the pyrimidine looked for here if the nucleoside underwent hydrolysis with formation of acetaldehyde and ethyl bromide. *The compound was not, however, uracil.* It was more soluble in water than uracil and did not respond to Wheeler and Johnson's test[1] for this combination. It also gave no test for bromine, proving that we were not dealing with a hydrobromic acid salt.

In another experiment 4 g. of the pyrimidine were digested with 20 cc. of hydrobromic acid for 7 hours and the excess of hydrobromic acid removed by heating on the steambath. The residue was then dissolved in 130 cc. of water, decolorized by digestion with bone coal and the solution finally cooled. One and five-tenths grams of the hydrolytic product separated. This did not melt at 300° and did not respond to the characteristic test for uracil. The aqueous solution was concentrated and cooled, when a thick syrup was obtained which gave a strong test for hydrobromic acid. This dissolved easily in cold water and on adding dilute sodium hydroxide solution a crystalline substance separated which was identified as the hydrolytic product described above. A trace of ammonia was also evolved when sodium hydroxide was added in excess. The weight obtained here was 1.3 g. This was purified by crystallization from water and showed no signs of melting at 300°. The average yield of the purified hydrolytic product was equivalent to about one-fifth of the weight of the nucleoside taken.

Calc. for $C_5H_8ON_2$: C, 53.56; H, 7.14; N, 25.00.

Found: C, 54.5, 53.56, 54.0; H, 6,85, 6.66, 7.17, 7.3, 7.00; N, 24.87, 24.86.

The reaction is expressed by the following equation:

$$C_8H_{12}O_3N_2 + H_2O + HBr = C_2H_5Br + CO_2 + H_2O + C_5H_8ON_2$$

The Action of Hydriodic Acid.—A preliminary experiment dealing with the behavior of 2,6-dioxy-4-(α-ethoxyethyl)-pyrimidine towards hy-

[1] *Loc. cit.*

driodic acid and phosphorus[1] was productive of results which indicated that this pyrimidine does not interact normally with this acid. Chernoff states that he did not obtain 4-ethyluracil, but a crystalline substance possessing entirely different properties. No analysis was made, but he states that the compound was purified by crystallization from water and did not melt at 280°. We have now investigated this reaction and find that the compound formed is identical with that produced by hydrolysis with hydrobromic acid. One gram of the ethoxypyrimidine was digested with 10 cc. of hydriodic acid at 140° for 3 hours. There was an evolution of carbon dioxide and ethyl iodide distilled from the solution. After completion of the reaction a little red phosphorus was introduced and the excess of acid removed by heating at 100°. A viscous residue was obtained which was dissolved in hot water, filtered and the solution allowed to cool slowly. Distorted prisms finally deposited. They turned brown when heated at 280° but did not melt at 300°. When this substance was mixed with that obtained by hydrolysis with hydrobromic acid and heated in a capillary tube there was no lowering of the melting point.

Calc. for $C_8H_8ON_2$: N, 25.00. Found: N, 25.1.

Quantitative Determination of the Carbon Dioxide Evolved by Hydrolysis of 2,6-Dioxy-4(α-ethoxyethyl)-pyrimidine with Hydrobromic and Hydriodic Acids: *Hydrolysis with Hydrobromic Acid.*—In order to determine quantitatively the amount of carbon dioxide given off during hydrolysis the operation was carried on in a specially constructed apparatus. Our procedure was to conduct a current of carbon dioxide-free air through the digestion flask (connected to a condenser) and lead it through a train of washing bulbs containing 1% hydrochloric acid, anhydrous calcium chloride and sulfuric acid. After this thorough washing, the gas mixture was then conducted through a weighed potassium hydroxide bulb where the carbon dioxide was absorbed and determined quantitatively. The analytical results obtained agreed very closely with that required by the equation given above, and were surprisingly constant when one takes into consideration that we are dealing here with a reaction which at best cannot be accurately controlled. They are in accord with the analytical values for carbon obtained by complete combustion.

2.1114, 1.3929 and 1.3962 g. pyrimidine gave 0.4809, 0.3372 and 0.2970 g. CO_2.

Calc. for one mol. of CO_2 from $C_8H_{12}O_3N_2$: CO_2, 23.9. Found: CO_2, 22.8, 24.4 and 21.0.

Hydrolysis with Hydriodic Acid.—Carbon dioxide determination:

(4) 0.9936 g. pyrimidine gave 0.1970 g. CO_2.

Calc. for one mol. CO_2 from $C_8H_{12}O_3N_2$: CO_2, 23.9. Found: CO_2, 20.0.

NEW HAVEN, CONN.

[1] Chernoff, Dissertation, Yale, 1914.