[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Researches on Pyrimidines. CLV. The Synthesis of Thyminylamine and its Conversion into Uracil[1]

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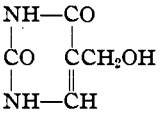
It has previously been shown that the substitution of a carbinol radical —CH_2OH in the 5-position of a 2,6-dioxypyrimidine is productive of an alcohol which easily undergoes a carbon–carbon cleavage, when subjected to hydrolysis, with formation of formaldehyde and the original pyrimidine. Such an instability was observed, for example, by Kircher[3] in 1911, who showed that the 5-carbinol derivative of 4-methyluracil II is quantitatively decomposed by digesting in boiling water with regeneration of 4-methyluracil and the liberation of formaldehyde.



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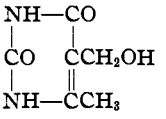

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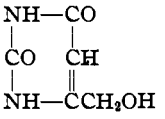

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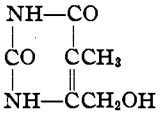
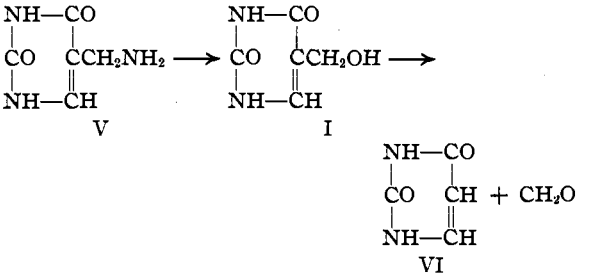


IV

A corresponding instability of a pyrimidine–carbinol linkage of this type was later found not to be the case when the carbinol radical is substituted in the 4-position of the pyrimidine cycle. Johnson and Chernoff,[4] in an investigation of synthetic pyrimidine nucleosides, prepared the thymine derivative IV which is isomeric with Kircher's pyrimidine II, and found that this compound can be heated with 10% sulfuric acid at 125° without alteration. There was no evidence of the formation of formaldehyde. Later these same investigators synthesized the corresponding carbinol derivative of uracil represented by formula III, and again made the discovery that the carbinol group could not be split off by hydrolysis,[5] with formation of uracil and formaldehyde.

These results revealed for the first time a fundamental difference in the stability of 4- and 5-carbinol substitutions in pyrimidines of the uracil type. It was, therefore, important to determine whether the fourth representative of the above series, thyminyl alcohol I, would conform to our prediction, and show the same behavior on hydrolysis as its higher homolog II.

As all attempts to produce thyminyl alcohol I by the action of formaldehyde on uracil have thus far proved unsuccessful, the authors decided to synthesize the corresponding and unknown primary base, *thyminyl-amine* V, and then convert this into the desired thyminyl alcohol I by the action of nitrous acid. A description of the preliminary reactions which have made possible the final synthesis of this interesting amine has already been given in the preceding paper of this series.[6] A complete description of the different procedures that the authors have finally applied successfully for the preparation of this interesting amine V is given in detail in the Experimental Part of this paper.



V I VI

We now find that thyminylamine V, as well as thyminyl alcohol I, are both characterized by their instability when subjected to hydrolytic conditions. Both pyrimidines are decomposed by boiling with water with cleavage between two carbon atoms and regeneration of uracil VI. The primary aliphatic amine radical —CH_2NH_2 in the pyrimidine V is destroyed by hydrolysis with formation of uracil, ammonia and formaldehyde. The chemical evidence favors first the formation of thyminyl alcohol I as an inter-

(1) For preliminary paper see Researches on Pyrimidines, CXLVI, THIS JOURNAL, **57**, 1139 (1935).

(2) This paper was constructed from a thesis presented by Dr. Anne Litzinger in June, 1936, to the Graduate Faculty of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy. This research was partially supported by a special grant from the Research Committee of the American Medical Association.

(3) Kircher, *Ann.*, **388**, 293 (1911).

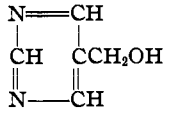
(4) Johnson and Chernoff, *J. Biol. Chem.*, **14**, 307 (1913).

(5) Johnson and Chernoff, THIS JOURNAL, **36**, 1742 (1914).

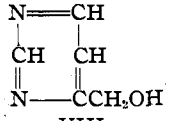
(6) Pyrimidine paper, CLIV, by Litzinger and Johnson, *ibid.*, **58**, 1936 (1936).

mediate, which being unstable then undergoes a normal hydrolysis like Kircher's pyrimidine-carbinol II, yielding uracil VI and formaldehyde. In fact, both transformations are practically quantitative. Because of the instability of thyminyl alcohol I in warm aqueous solutions, it has, thus far, been impossible for us to separate this pyrimidine from water solution after diazotization of the thyminylamine V without partial decomposition. Heretofore, we have discovered no pyrimidine constructions which reveal such characteristic differences in behavior between two carbon linkages attached to adjacent carbons in the pyrimidine ring.

It is the belief of the authors that these striking variations in chemical behavior will need to be considered carefully before corresponding constructions can be assigned to any pyrimidine compounds possessing the chemical properties of vitamin B_1.[7] Our next problem will be to determine whether the corresponding isomeric carbinol derivatives of a reduced pyrimidine substituted in positions 4 and 5, VII and VIII will show a corresponding difference in chemical stability. This study is now in progress in this Laboratory.



VII



VIII

Experimental Part

Thyminylamine or (Uracil-5-methylamine), V, $NHCONHCH{=}C(CH_2NH_2)CO$.—This amino derivative of thymine can be prepared according to the following procedures:[1] (1) by hydrolysis of ethyl 2-ethylmercapto-6-oxypyrimidine-5-methylurethan with either hydrochloric or sulfuric acid leading to the formation of the corresponding salts of thyminylamine; (2) by hydrolysis of ethyl thyminyl-urethan with hydrochloric or sulfuric acid; (3) by hydrolysis of ethyl-2-ethylmercapto-6-oxypyrimidine-5-methyl isocyanate by action of hydrochloric or sulfuric acid; (4) by hydrolysis of thyminyl isocyanate with acids.[6]

Thyminylamine Sulfate, $(C_5H_7O_2N_3)_2{\cdot}H_2SO_4{\cdot}H_2O$.—This salt is formed by the action of concentrated sulfuric acid[8] on ethyl 2-ethylmercapto-6-oxypyrimidine-5-methylurethan according to procedure (1), but care must be taken in applying the reaction. It was found by experimentation that the best results are obtained by applying the hydrolysis reaction for two hours at a temperature of 110°. By lowering this temperature (90–100°) or reducing the time of heating, the desired change was incomplete. On the other hand, if the temperature of the sulfuric acid solution reached 120° and the heating was more prolonged, considerable charring and decomposition occurred. Five grams of the mercapto pyrimidine is stirred into 10 ml. of concentrated sulfuric acid. On heating this mixture, evolution of carbon dioxide begins at about 80°, and within a few minutes the pyrimidine-urethan dissolves completely and with no apparent evolution of ethyl mercaptan. A pale yellow solution is obtained. This is then cooled and poured into 200 ml. of water and the solution heated on a steam-bath until the evolution of mercaptan ceases. The solution is exactly neutralized with barium hydroxide to remove all sulfuric acid, and then a quantity of 0.2 *M* sulfuric acid is added, or the amount necessary to form the sulfate of the dissolved amine. The barium sulfate is then separated by filtration and the combined filtrates and washings are concentrated *in vacuo* to a volume of 15 ml. The addition of alcohol (25 ml.) leads to an immediate precipitation of the sulfate of the desired amine (3 g.) melting at 240–245°. This salt is finally purified by dissolving it in the least quantity of hot water and diluting with alcohol until the hot solution becomes turbid. On cooling, the sulfate separates in the form of glistening plates melting at 245–246° with decomposition. While the salt is insoluble in alcohol, 1 g. dissolves in 1.5 ml. of hot and 4 ml. of cold water. Ethyl thyminyl urethan is converted directly into thyminylamine by the action of concentrated sulfuric acid at 110°. The purified salt did not give Wheeler and Johnson's test for uracil.[9] *Anal.* Calcd. for $(C_5H_7O_2N_3)_2{\cdot}H_2SO_4{\cdot}H_2O$: N, 21.11; H_2O, 4.50. Found: N, 21.17, 21.10; H_2O, 3.90.

Thyminylamine Hydrochloride (Uracil-5-methylamine), $C_5H_7O_2N_3{\cdot}HCl{\cdot}0.5H_2O$.—This salt is formed by hydrolysis of ethyl 2-ethylmercapto-6-oxypyrimidine-5-methylurethan, or ethyl uracil-5-methylurethan with concentrated hydrochloric acid in a bomb tube. The degradation is complete after heating for two hours at 103–107°. The acid solution is then concentrated to a small volume and the hydrochloride precipitated by dilution with alcohol. On recrystallizing from dilute alcohol the salt separated in the form of glistening plates melting at 242–243° with decomposition. This salt is more soluble in water and alcohol than the sulfate. When 2-ethylmercapto-6-oxypyrimidine-5-methyl isocyanate or thyminyl isocyanate is digested on a steam-bath with concentrated hydrochloric acid, thyminylamine hydrochloride is formed.[10] *Anal.* Calcd. for $C_5H_7O_2N_3{\cdot}HCl{\cdot}0.5H_2O$: H_2O, 4.88; N, 23.66 (anhyd. salt). Found: H_2O, 4.82; N, 23.86.

Thyminylamine, V, $C_5H_7O_2N_3$.—An absolutely pure specimen of this amine has not been prepared. The purest product so far obtained was prepared as follows. One gram of the purified sulfate is dissolved in 10 ml. of water and the sulfuric acid removed by precipitation as barium sulfate in a hot solution. After filtering and cooling, the free thyminylamine (0.5 g.) deposits as a colorless amorphous powder melting at 260–270° with decomposition. The amine is insoluble in the ordinary organic solvents and moderately soluble in water. Warm aqueous solutions of the base were invariably characterized by a

(7) Johnson and Litzinger, *Science*, **84**, 25 (1936).
(8) Jeffreys, *Ber.*, **30**, 900 (1897).
(9) Wheeler and Johnson, *J. Biol. Chem.*, **3**, 183 (1907).
(10) *Cf.* Naegeli and Lendorff, *Helv. Chim. Acta.*, **12**, 227 (1929).

distinct odor of ammonia resulting from a slow hydrolysis of the pyrimidine. The amine was purified for analysis by recrystallization from hot water, and separated on cooling as a colorless semi-crystalline powder melting at 265°. *Anal.* Calcd. for $C_5H_7O_2N_3$: N, 29.79. Found: N, 27.99, 27.93.

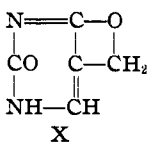
Behavior of Thyminylamine in Boiling Aqueous Solution.—An aqueous solution of 0.6 g. of thyminylamine in 40 ml. of water was boiled vigorously for twenty-five minutes, and the vapor condensed and conducted into 10 ml. of 4.5% boric acid solution. At the end of this time the borate solution was titrated with 0.02 *N* hydrochloric acid; 17.13 ml. of this standard acid was neutralized, being equivalent to 0.005824 g. of ammonia. Therefore, under these conditions, approximately 8.0% of the thyminylamine was decomposed. The acid distillate gave a positive aldehyde test with Schiff's reagent, indicating the presence of formaldehyde, and also a strong Wheeler and Johnson test for uracil.[9] It was our observation, as stated above, that thyminylamine could not be purified by crystallization from hot water without undergoing partial decomposition. The aqueous filtrates in every case responded to the characteristic color test for uracil and ammonia could be detected easily.

Behavior when Digested with Hydrochloric Acid.—0.35 g. of thyminylamine was refluxed with 15 ml. of strong hydrochloric acid for five hours, and the solution was then heated on a steam-bath for one and one-half hours longer or to complete dryness. We obtained a colorless solid residue of 0.25 g. which was recrystallized from hot water. It separated, on cooling, in the form of round, corpuscular crystals characteristic of the pyrimidine uracil and decomposed without melting at 308–312°. This solid gave a voluminous purple precipitate when it was tested according to the Wheeler and Johnson technique. It was apparent from the intensity of the color test that uracil represented the major proportion of the solid residue left after evaporation. However, the melting point is low for uracil (335°) and a nitrogen determination indicated some form of contamination. The question whether this is due to traces of thymine as a second product of hydrolysis has not been decided. In an equimolecular mixture of uracil and thymine the theoretical percentage of nitrogen would be 23.6. *Anal.* Calcd. for $C_4H_4O_2N_2$: N, 25.00. Found: N, 23.17, 23.16.

Action of Nitrous Acid on Thyminylamine

The Formation of Uracil-5-carbinol or Thyminyl Alcohol, $\overline{\text{NHCONHCH=C(CH}_2\text{OH)CO}}$.—Thyminylamine sulfate was dissolved in water and the sulfate ion removed by adding the required quantity of barium hydroxide to form barium sulfate. In the boiling solution were then dissolved two equivalents of barium nitrite, followed by the gradual addition of the required amount of 0.2 *M* sulfuric acid. The solution was then freed from barium sulfate by filtration and finally concentrated *in vacuo* to a volume of 1 cc. On adding absolute alcohol, a mixture of products was precipitated which melted from 240 to 300°. This gave a strong color test for uracil. By fractional crystallization from alcohol and ether we finally succeeded in separating a substance which melted from 190–200°, and which was probably an impure sample of the unknown thyminyl alcohol. All attempts to synthesize this alcohol by the action of formaldehyde on uracil have thus far proved unsuccessful. The carbinol radical is apparently not firmly bound at the 5-position of the uracil molecule. *Anal.* Calcd. for thyminylcarbinol, $C_5H_6O_3N_2$: N, 19.72. Found: N, 18.87, 19.01.

In a second experiment the oxides of nitrogen were conducted into an aqueous solution of thyminylamine for fifteen minutes. After concentrating the resulting solution by heating on a steam-bath, we obtained a product which decomposed at 270–295° and which also gave a good color test for uracil. Further purification finally led to a colorless substance melting at 195–200°. This material was very soluble in both cold and hot water, and was insoluble in alcohol. It was purified by crystallization from dilute alcohol. The structure of this substance was not established, but it is an interesting fact that a nitrogen determination agreed with the calculated for an inner anhydride derivative of thyminyl alcohol. *Anal.* Calcd. for $C_5H_4O_2N_2$: N, 22.57. Found: N, 22.69.



X

$\overline{\text{NHCO·NHCH=C(CH}_2\text{NHCSNH}_2\text{)CO}}$, **Thyminyl-thiourea,** was prepared by the action of ammonium thiocyanate on thyminylamine sulfate in hot aqueous solution. It was purified by crystallization from dilute alcohol and separated in the form of glistening plates melting at 204–205° to an oil. The yield was quantitative. *Anal.* Calcd. for $C_6H_8O_2N_4S$: N, 28.00. Found: N, 27.96.

Summary

1. Methods of synthesizing the pyrimidine—*thyminylamine*—have been described in this paper.

2. A study of this amine has revealed the fact that it is very susceptible to hydrolysis and is easily converted into uracil, formaldehyde and ammonia.

3. By diazotization of thyminylamine, the corresponding thyminyl alcohol is formed. This carbinol is likewise unstable and undergoes hydrolysis with formation of uracil and formaldehyde.

4. This discovery that the substitution of hydrogen in the methyl group of thymine by an hydroxyl or amino radical weakens the carbon linkage of this side chain, is a result of biochemical significance.

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