extractives and nicotine, while smoking tobacco has a high solubility in petroleum ether, ethyl ether and alcohol.

Plants having a high calcium content had most of their nicotine so combined in stable form as to be quite insoluble in either petroleum ether or ethyl ether.

Black Leaf 40, as well as powdered tobacco when mixed with different calcium compounds, had the nicotine so fixed as to make it difficult to extract it completely even by the official method.

The vegetable waxes, volatile oils and loosely combined nicotine contained in the petroleum ether extracts are responsible for much of the irritating effect of the tobaccos grown; tobaccos could be greatly improved by extraction with this solvent.

The ratio of leaf to stalk was much greater in Switzerland County (Miami clay loam) than in Tippecanoe County, Indiana (Sioux silt loam). The ash content averaged about 3% lower for tobacco grown in the latter county than for that from the clay loam soil.

Plants which had been treated with various amounts of acid phosphate fertilizer had a small petroleum ether extract and possessed the most agreeable aroma of any plants grown.

Although the composition of tobacco may be greatly modified and improved by different fertilizer treatments, the tobacco which commands the best price of any examined was grown on relatively poor sandy or clay soil, unsuited to corn, but producing a tobacco characterized by a low percentage of extractives, proteins and nicotine and suitable for making a cigar of such pleasing taste and aroma as to command a good market price.

West La Fayette, Indiana

[Contribution from the Department of Chemistry, Yale University]

# STUDIES ON CATALYSIS. IV. THE BEHAVIOR OF THE AMINO GROUP WHEN CYTOSINE AND NITRO-URACIL ARE REDUCED IN THE PRESENCE OF COLLOIDAL PLATINUM

By Elmer B. Brown and Treat B. Johnson

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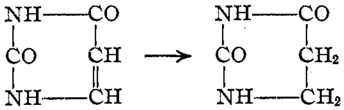
In the study of the application of catalytic reduction by means of colloidal platinum or palladium and molecular hydrogen to the pyrimidine series, the writers have previously reported the quantitative formation of hydro-uracil II by reduction of uracil I.[1] By the application of this method



I II

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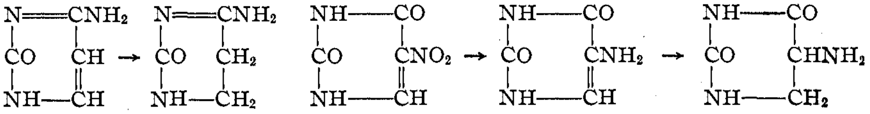
[1] This Journal, **45**, 2702 (1923).

of reduction to the pyrimidines, cytosine III and 5-nitro-uracil V, it was expected that the corresponding reduction products, hydrocytosine IV and hydro-amino-uracil VII, would be obtained on the addition of one and four mols of hydrogen, respectively. It was found that both cytosine and



III IV V VI VII

nitro-uracil were readily reduced under the conditions applied in the case of uracil,[1] but the products formed proved to be so unstable that neither of the compounds IV and VII was obtained as the final product of the reactions. In the reduction of cytosine, hydro-uracil II is formed smoothly as the only product of reduction and hydrolysis. The amino group in Position 6 is removed quantitatively during the reduction by the hydrolytic action of the acid solution produced by reduction of the chloroplatinic acid added to supply colloidal platinum.

When 5-nitro-uracil is reduced, hydrolysis also accompanies the reduction-reaction and ammonia is formed. When the reaction is stopped after the addition of 3 molecular equivalents of hydrogen to destroy the nitro group, the main product formed is 5-amino-uracil VI. Even at this stage of the reduction process partial hydrolysis takes place with formation of 5-hydroxy-uracil (isobarbituric acid). When the reduction is continued until 4 equivalents of hydrogen are added the hydrolysis of the amino group is practically complete and a new product is formed whose constitution has not been definitely established. The properties of this new compound are quite different from those of 5-hydroxy-uracil and hydro-uracil. Distillation of the solution after reduction in the presence of magnesium oxide liberates an amount of ammonia approaching closely the quantity theoretically possible by loss of the amino group in Position 5. The fact that the reduction product does not possess basic properties, and dissolves with difficulty in dil. sodium hydroxide solution indicates that we are not dealing here with a simple cyclic acid amide structure.

The most significant and interesting aspect of our work is this observation of the ease with which the amino group is removed from the pyrimidine molecule when the ring becomes saturated. The stability of this group in cytosine is such that the latter pyrimidine may be digested with concd. hydrochloric acid or 33% sulfuric acid for hours without appreciable hydrolysis of the amino group. The same stability is met also in nucleic acids containing cytosine which can be heated with sulfuric acid for hours at 125° without production of uracil.

The fact that hydrocytosine is not obtained by catalytic reduction of cytosine, but is quantitatively transformed into hydro-uracil and ammonia in dilute acid solution at a temperature of 75°, is a result not to be expected from the work of Levene and La Forge on the pyrimidine nucleosides, uridine and cytidine.[2] These investigators report that both of these nucleosides are reduced in the presence of palladium with formation of the corresponding dihydro combinations, and that the latter compounds undergo hydrolysis by digestion with dil. sulfuric acid, yielding *d*-ribose and the two reduced pyrimidines, hydro-uracil II and hydrocytosine IV, respectively. In their paper, however, they give no description of hydrocytosine and, so far as the writers are aware, the compound has never been described in the literature.

Our results have revealed the remarkable influence which the double bond in the 4,5 position of the pyrimidine ring exerts on the stability of the amino group in Positions 5 and 6. Whether the linkage between sugar and cytosine in cytidine actually stabilizes the union of the amino group in the 6 position protecting it from hydrolysis during reduction remains to be established. The result reported by Levene and La Forge would indicate that such is the actual fact which, in the light of our work, is an observation of considerable biochemical interest. It will be of interest to determine whether the amino group in isocytosine is similarly affected by the saturation of the pyrimidine ring in the 4,5 position. It is very probable that the further study of these influences will give data which will aid in the unraveling of the complex constitution of nucleic acids.

## Experimental Part

**The Formation of Hydro-uracil by Reduction of Cytosine.**—All the cytosine used in this research was prepared by synthesis according to the method of Wheeler and Johnson.[3] The base was carefully purified by repeated crystallizations from water and hydrochloric acid to remove all traces of sulfur impurities. A description of a single experiment will reveal the general procedure adopted for catalytic reduction.

Three g. of cytosine hydrochloride, 0.5 g. of gum arabic, 100 cc. of distilled water and 10 cc. of 10% chloroplatinic acid are shaken for seven hours with molecular hydrogen under two atmospheres' pressure and at a temperature of 75°, as described in our previous paper on the reduction of uracil.[1] During this time an absorption of 450 cc. of hydrogen was obtained, or the calculated amount necessary for the complete reduction of the double bond.

The colloid was then precipitated by pouring the aqueous solution into an equal volume of acetone, and the filtered solution finally concentrated to a small volume on the water-bath. As the solution cooled, a granular precipitate separated which weighed 1.95 g. This product exhibited the crystalline structure of hydro-uracil and melted without further purification at 272–274°. A nitrogen determination confirmed our conclusion that we had in hand hydro-uracil and not hydrocytosine.

*Analysis.*. Calc. for $C_4H_6O_2N_2$: N (Kjeldahl), 24.56. Found: 24.40.

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

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

That the reduction and hydrolysis proceeds in the above manner is shown by the results of the following experiment in which the amount of the reduction is shown to be quantitative by the hydrogen absorption, and the hydrolysis also by the estimation of the ammonium chloride produced by distilling the reduction solution with magnesium oxide and converting the ammonia into ammonium chloride.

When 1.1872 g. of cytosine hydrochloride was reduced according to the conditions described above, 175 cc. of hydrogen was absorbed (calc., 170 cc.). In the reduction solution was suspended 2 g. of magnesium oxide in 200 cc. of water, which previously had been freed from all traces of ammonia by boiling for two hours, and the mixture then distilled. The liberated ammonia when completely absorbed neutralized 81.18 cc. of 0.1 *N* acid.

Calc. for $C_4H_7ON_3Cl$: (one nitrogen) 9.43. Found: 9.57.

Therefore, in the experiment described above the hydrocytosine was hydrolyzed by the weak acid solution produced from the reduction of 10 cc. of 10% chloroplatinic acid. The ease with which cytosine is converted into hydro-uracil in the reduction process described is quite remarkable in the light of the work of Levene on the catalytic reduction of cytidine, and it will be very interesting indeed if it should be established that the reduced pyrimidine from the nucleoside is more resistant to hydrolysis than the product obtained from the reduction of synthetic cytosine.

**Effect of Alkali on the Catalytic Reduction of Cytosine.**—Reduction experiments on cytosine in alkaline solution showed that this medium was not any better suited for the reduction of this pyrimidine than in the case of uracil, where the reduction was too slow to be of practical value. In order to establish definitely that the difficulty experienced in the reduction was due to the alkaline medium and not to impurities which it might contain, the sodium hydroxide solution was prepared from pure metallic sodium and distilled water and used in the following experiment:

A mixture of 2 g. of cytosine, 10 cc. of 2.3 *N* sodium hydroxide solution, 100 cc. of water, 2 g. of gum arabic and 6 cc. of chloroplatinic acid was shaken for 8 hours at 75° with molecular hydrogen, during which time 75 cc. of hydrogen was absorbed after complete reduction of the chloroplatinic acid. The colloid had not separated, and practically no further absorption of gas took place on standing. After the mixture had stood overnight 3 cc. of chloroplatinic and 7 cc. of acetic acid were added and the experiment was continued. The reduction then proceeded normally and within four hours 360 cc. of hydrogen was absorbed, bringing the total absorption to the amount theoretically required for the complete reduction of the double bond. The fact that the reduction proceeded normally after the addition of the acetic acid shows that the retardation was due to the alkaline medium and not to poisonous substances contained as impurities in the sodium hydroxide solution. After concentration of the solution 1.8 g. of hydro-uracil separated on cooling. That the reduction was complete was demonstrated by the fact that the solution failed to give the characteristic color reaction for cytosine when tested according to the directions of Wheeler and Johnson.[4]

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[4] Wheeler and Johnson, *J. Biol. Chem.*, **2**, 183 (1907).

The fact that both uracil and cytosine are reduced slowly in alkaline solution indicates that either these pyrimidines form salts of a different structure from the original substances which add hydrogen with greater difficulty, or that the alkaline solution affects greatly the progress of the reduction process.

**The Reduction of the Nitro Group in Nitro-uracil.**—Nitro-uracil was prepared by the action of fuming nitric acid on pure uracil as follows:

A mixture of 100 g. of sulfur-free uracil and 1 liter of fuming nitric acid (d., 1.5) was triturated until the uracil completely dissolved. The excess of nitric acid was then removed by heating in a water-bath at 60°, and the crystalline residue recrystallized twice from hot water. A calculated yield (140 g.) of pure nitro-uracil was thus obtained.

Two g. of nitro-uracil, 0.5 g. of gum arabic, 90 cc. of water, and 100 cc. of 10% chloroplatinic acid were shaken together for $1^3/_4$ hour with molecular hydrogen at two atmospheres' pressure and at a temperature of 75°. The gas absorption proceeded rapidly at first; during the first forty minutes 500 cc. of hydrogen was absorbed and practically no further reduction took place. More chloroplatinic acid (3 cc.) was added and during the next twenty minutes an absorption of 250 cc. of hydrogen was obtained; on continuing the experiment for 45 minutes longer there was no further addition of hydrogen. The temperature of the reaction fluid at this time was 80°. The reaction mixture was allowed to stand overnight, during which time the colloid separated completely, leaving a clear solution. Since the hydrogen absorption was practically three molecular equivalents, the amount required for the reduction of the nitro group to the amine without the reduction of the double bond, further reduction was not attempted. The clear solution resulting from the filtration of the precipitated colloid was evaporated on a steam-bath to a small volume. As the mixture cooled, a crystalline product separated which was treated with water and the solution finally filtered to remove a small amount of flocky material which remained undissolved (see below). On neutralizing the acid solution with dil. aqueous ammonia a brown precipitate was obtained possessing all the properties of 5-amino-uracil. It was unstable in the air, slowly undergoing oxidation, and gave a positive murexide test when treated with nitric acid and ammonia. The picrate melted at 147°.

The difficultly-soluble flocky material obtained as a residue by dissolving the main portion of the reduction products as described above was further examined and identified as 5-hydroxy-uracil described by Behrend and Grünwald.[5] This material was readily dissolved by dil. aqueous alkali and was precipitated again by addition of acetic acid. It was difficultly soluble in both acid and cold water, but was readily dissolved by hot water from which it separated on cooling in microscopic crystals having the appearance of corpuscles under the microscope. The compound agreed in all its properties with the substance described by Behrend and Grünwald who always obtained it as a secondary product in the reduction of nitro-uracil to amino-uracil.

**Intensive Reduction of Nitro-uracil.**—In an experiment conducted with 1.06 g. of nitro-uracil, 0.7 g. of gum arabic, 90 cc. of water and 6 cc. of chloroplatinic acid, three molecular equivalents of hydrogen (450 cc.) were absorbed during the first fifteen minutes of agitation in the shaking machine. At the end of this period gas was still being absorbed and this continued until one additional equivalent was consumed. The reaction, however, was slow and the time required for the absorption of this additional equivalent of gas was $2^1/_4$ hours. During all this time there was no separation of the colloid.

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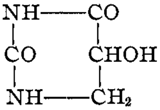
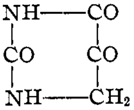
[5] Behrend and Grünwald, *Ann.*, **229**, 38 (1885).

After the reduction was apparently complete, 1 g. of magnesium oxide and 300 cc. of water were added and the solution was distilled to remove ammonia formed by hydrolysis. The amount obtained corresponded to 83.3% of the quantity theoretically available by complete loss of the amino group. In other words, the behavior here is in accord with that observed in the reduction of cytosine. The fact that we did not account for a theoretical production of ammonia is possibly due to the fact that we did not obtain complete reduction of the double bond in the pyrimidine ring. After removal of ammonia the solution was filtered and finally concentrated to a small volume and cooled. A brown substance separated which was difficultly soluble in 0.1 *N* aqueous alkali and insoluble in acids. In alkali of this strength 5-hydroxy-uracil dissolves at once. The substance separated from hot, dil. acetic acid in the form of characteristic diamond-shaped crystals which did not melt at 285°. Hydro-uracil melts at 272°. Whether we are dealing here with an alcohol or a diketone derivative must be decided by further research. It is possible that the carbonyl group in Position 6 is likewise partially reduced





in this case and that we did not have a pure substance.

**The Reduction of Yeast Nucleic Acid.**—An experiment was performed with the object of determining whether an appreciable quantity of ammonia is liberated by reduction of yeast nucleic acid in the presence of colloidal platinum.

A mixture of 1.7 g. of carefully purified yeast nucleic acid, 100 cc. of water, 6 cc. of 10% chloroplatinic acid and 0.7 g. of gum arabic was shaken with molecular hydrogen under two atmospheres' pressure. After 6 hours the gage showed a hydrogen absorption of 75 cc. The reduction solution was allowed to stand for 10 to 12 hours, during which time the colloid did not separate. More chloroplatinic acid (3 cc.) was then added and the shaking continued for six hours when 40 cc. of hydrogen had been absorbed. The reduction was discontinued at this stage, and the solution diluted with water and finally digested with magnesium oxide to expel ammonia. The ammonia liberated neutralized 4.4 cc. of 0.1 *N* hydrochloric acid. When an equal amount of yeast nucleic acid, which had not been subjected to the reduction process, was distilled with magnesium oxide under similar conditions the ammonia generated neutralized 1.08 cc. of 0.1 *N* hydrochloric acid, showing that the nucleic acid was productive of more ammonia by digestion with magnesium oxide after it had been subjected to the hydrogenation treatment. A test for uracil and cytosine was made on the reduced nucleic acid solution, but no evidence of the presence of these pyrimidines was found, indicating that these combinations had been reduced in the nucleic acid molecule. We shall continue the study of the behavior of nucleic acids when reduced catalytically.

## Summary

1. Cytosine is reduced in the presence of colloidal platinum with formation of ammonia and hydro-uracil. The intermediate product, hydrocytosine, is apparently so unstable that it undergoes hydrolysis quantitatively with loss of the amino group in Position 6.

2. Nitro-uracil is reduced in stages with the formation, first, of 5-amino-uracil. This compound undergoes partial hydrolysis to form isobarbituric acid. Intensive reduction leads to a complete alteration of

the molecular structure with apparent reduction of the double bond in the 4,5 position. The natures of the final products of this change, however, have not been definitely established.

3. Yeast nucleic acid has been subjected to the action of molecular hydrogen in the presence of colloidal platinum and evidence obtained that ammonia is produced by such treatment.

New Haven, Connecticut

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[Contribution from the Chemical Laboratory of the University of California]

# THE EQUILIBRIUM BETWEEN BENZALDEHYDE AND BENZOIN

By Arthur Lachman

Received November 15, 1923

## Introduction

When benzaldehyde is acted upon by alkali cyanides, it undergoes a condensation reaction, yielding the substance called benzoin.

$$C_6H_5CHO + C_6H_5CHO = C_6H_5CH(OH)COC_6H_5 \qquad (1)$$

Many explanations have been offered of the mechanism of this reaction. The only careful quantitative study published so far was made in 1904 by Bredig and Stern,[1] who investigated the kinetics of the condensation. Their results may be summarized as follows.

Within wide limits, the rate of condensation proceeds as the square of the benzaldehyde concentration, for a given concentration of potassium cyanide. The rate is, furthermore, directly proportional to the concentration of the catalyst, which was found to be cyano ion, $CN^-$. No benzoin is formed in the absence of this ion. Hydrogen cyanide forms no benzoin; and the addition of acids stops the condensation. Hydroxyl

Table I

Reversal of Benzoin Condensation

Benzaldehyde concentration 0.28*M*; benzoin, 0.14 (initial values); potassium cyanide, 0.24 *M*. Temperature, 60°. Solvent, 66% ethyl alcohol.

| Original material | Time, hours | Benzoin present Mole | Percentage of possible benzoin |
|---|---|---|---|
| Benzaldehyde | 2.3 | 0.070 | 50 |
| | 3.2 | .094 | 67 |
| | 4 | .101 | 71 |
| | 5 | .113 | 79 |
| | 48 | .110 | 72 |
| | 72 | .089 | 62 |
| Benzoin | 48 | .073 | 51 |
| | 72 | .064 | 45 |

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[1] (a) Bredig and Stern, *Z. Elektrochem.*, **10**, 582 (1904); (b) Stern, *Z. physik. Chem.*, **50**, 513 (1904). The earlier literature has been summarized by Stern: further references are given by Lachman (c), This Journal, **45**, 1529 (1923).