

The chemistry of the Kossel's test for purine bases

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Abstract

Albrecht Kossel discovered the purine adenine and the pyrimidine thymine. He extended the murexide test for uric acid to adenine, guanine, hypoxanthine and xanthine. Since the structural differences in these compounds alter the pathways in these tests, we disclosed the reaction course in these assays. We provide the reaction sequence from the bi-annular base to the final product, the colored sodium purpurate.

Keywords: Alloxan; Degradation; Oxidation; Purpuric acid; Reactive intermediates; Uramil

1. Introduction

In this communication we present the chemistry involved in the tests that Kossel carried out with purine bases. The importance of these two-ringed, nitrogen-rich molecules is due to the role that they have in biochemistry as components of the double helix, that is, in living beings.

Corresponds to the theorist disentangle what is happening in a spot test. We describe the series of hydrations, eliminations, ring opening, hydrolysis, degradation, isomerizations, oxidations and couplings that occur during these assays. The reaction course is given in accordance with the reactivity of the substrate, and we provide the reactions of the different functional groups present in these molecules.

This paper is a follow up of our studies on reaction mechanisms [1-5].

2. Antecedents

The German scientist Albrecht Kossel (1853-1927) specialized in the chemistry of the cell and its nucleus. He discovered the purine adenine and the pyrimidine thymine [6, 7] He carried out colour reactions with adenine, guanine, hypoxanthine and xanthine, adding nitric acid and bromine water to the solution and evaporating. On addition of a drop of sodium hydroxide a red colour is obtained (Kossel's test), [8].

In fact, this is a combination of the murexide test [9] for uric acid (nitric acid) and a variant of the Treumann test [10] for theobromine (chlorine water). What is new is the use of the above mentioned purine bases, and sodium hydroxide instead of ammonium hydroxide as in the murexide test.

Adenine, 6-aminopurine, is a purine nucleobase. It has a role as a metabolite in humans, *Saccharomyces cerevisiae*, *Escherichia coli*, and mouse. It is vitamin B 4.

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Guanine is a 2-aminopurine carrying a 6-oxo substituent. It is obtained abundantly from guano, the excreta of sea birds.

In this communication we disclose the degradation and oxidation steps that occur in the tests with adenine and guanine. We provide and discuss the sequence of steps that occur from the alkaloid to the final product, the colored sodium purpurate.

3. Oxido-degradation of adenine to uramil

In adenine, Figure 1, **a**, the aromaticity indices are: pyrimidine, 67, and imidazole, 47, [11].

Thus, reaction begins at the imidazole ring. Acid catalyzed hydration of the imino group yields an intermediate with a carbinoldiamino group, **b**. Protonation of the less hindered nitrogen atom in this system leads to N-C fission and ring opening, yielding 4,6-diamino-5-formamido-pyrimidine, **c**. Acid hydrolysis of the formylamine via the gem diol affords formic acid and 4,5,6-triaminopyrimidine, **d**.

The amino group at C-6 is part of a guanyl group. Protonation at N-3 induces tautomerization to a non-aromatic cross conjugated structure, **e**. Hydration of the conjugated imino groups to the hemiaminals occurs, **f**, and there is ammonia elimination from C-6, a lactam being formed, **g**.

Then reacts the more stable C=C double bond. The vicinal lactam has no appreciable influence due to its resonance. Protonation gives rise to a carbonium ion far from the carbonyl group, and it is neutralized by water. A carbinolamine results at C-4, **g**, and releases ammonia by protonation at nitrogen, forming a lactam, **h**.

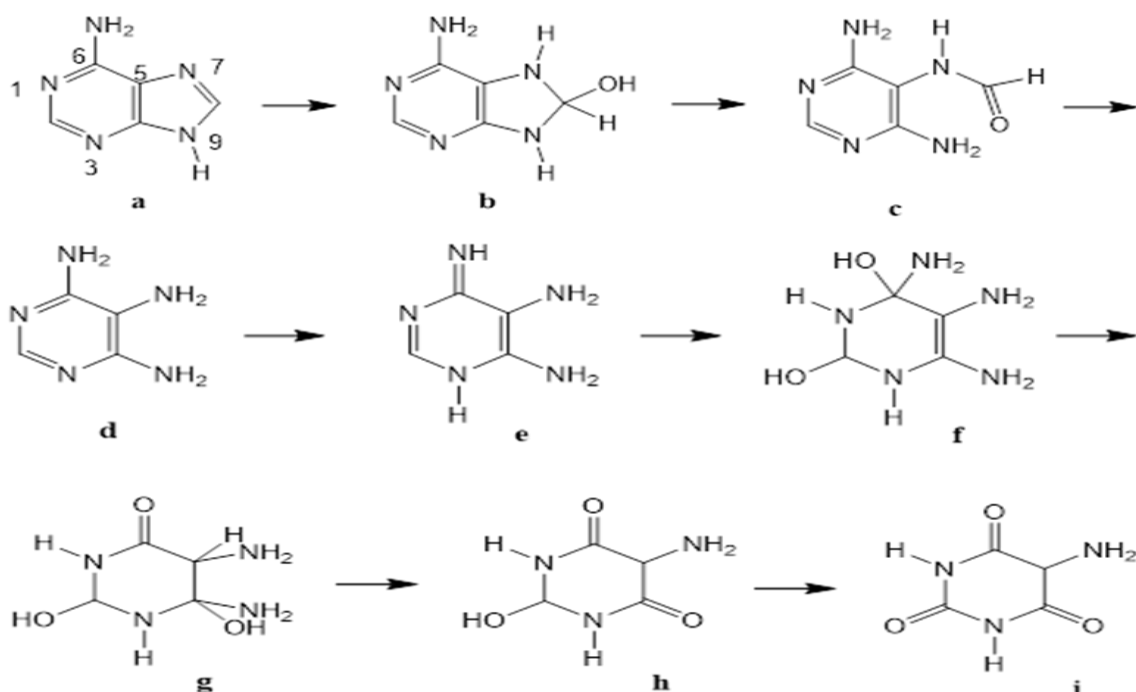


Figure 1 Degradation and oxidation of adenine to uramil

Adenine has been degraded to a one-ring molecule, it has lost two nitrogen atoms and added three oxygen atoms, that is, has undergone a radical transformation.

Oxidation of the hydroxy group at C-2 yields uramil, 5-aminobarbituric acid, **i**, a key intermediate in the synthesis of purpuric acid, the target molecule.

This oxidation can be achieved by nitric acid. The nitronium ion forms an organic nitrate. Protonation of its negative oxygen induces detachment of nitrous acid with concomitant C-H fission and carbonyl formation. This way an ureido group results. The carbonyl at C-2 is also part of an imide.

The other oxidizer present, bromine water, can also achieve this oxidation. Hydrolysis of bromine yields hypobromous acid and hydrobromic acid. Protonated hypobromous acid is the reactive species, a source of bromonium ion. An unstable organic hypobromite is formed. A carbonyl group results when hydrogen bromide is expelled via fission of the C-H bond. Cf. [12-14].

4. Degradation of guanine to uramil

A different reaction course takes place with guanine, Figure 2, **a**. The six member ring is not aromatic, thus reaction starts at this ring. Isomerization of the guanyl group creates an exocyclic imino group, **b**, and this can be hydrated faster than the endocyclic one. The reaction intermediate, **c**, gives an ureido group after ammonia removal, **d**.

Acid catalyzed hydration of the imino group in the aromatic imidazol ring affords a carbinoldiamino group, **e**. The nitrogen atom attached at β position to the α,β -unsaturated carbonyl group is an enamine that contributes electrons to this system. Thus, this nitrogen is less prone to protonation and a formamido group results now at C-4, **f**, not at C-5 as in the previous case. Hydrolysis of the formyl amine yields formic acid and the enediamine, **g**.

Finally, acid catalyzed hydration of the carbon-carbon double bond forms uramil, **i**, after ammonia loss from the resulting hemiaminal, **h**.

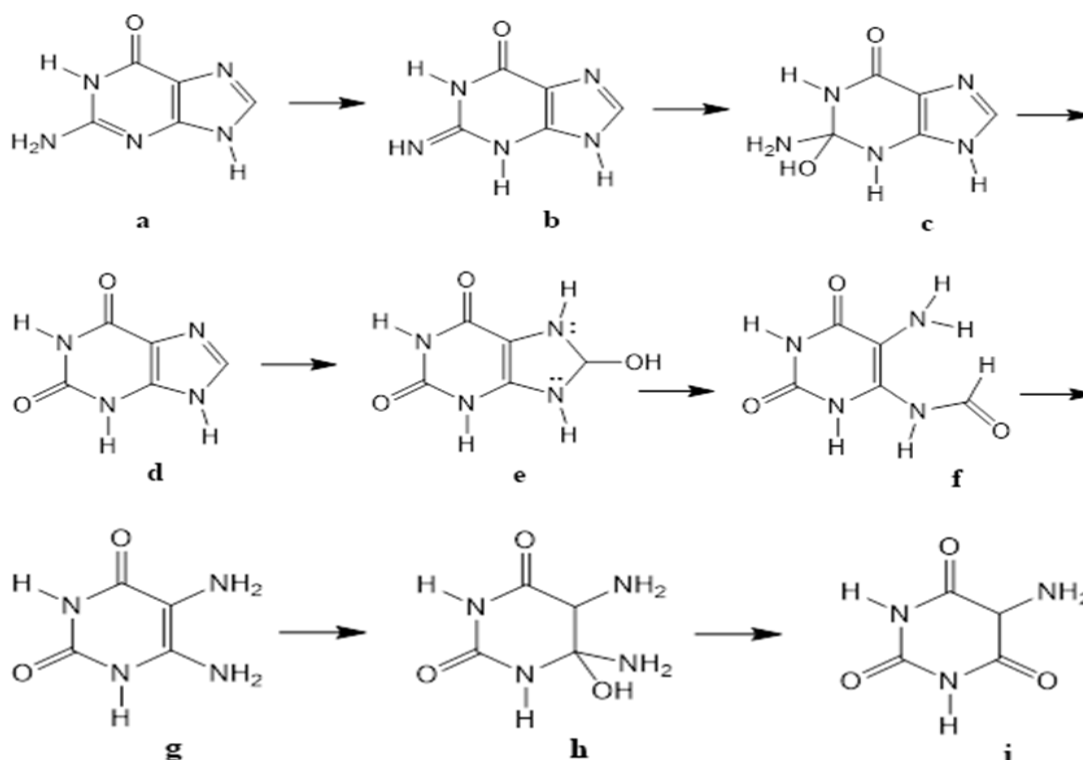


Figure 2 Degradation of guanine to uramil

5. Obtention of alloxan and purpuric acid.

Alloxan, 5-oxobarbituric acid, is the second key intermediate in the synthesis of purpuric acid. Oxidation of uramil to alloxan can be accomplished either with nitric acid or with bromine water, the reagents used in Kossel's test.

In the nitric acid oxidation of the amino group in uramil, Figure 3, **a**, a nitronium ion forms a nitramine, **b**, which is degraded to the imine via protolysis and nitrous acid elimination, **c**, (oxido-reduction step), [9]. Subsequent hydrolysis of the ketimine affords alloxan, 2,4,5,6-tetraoxoperhydropyrimidine.

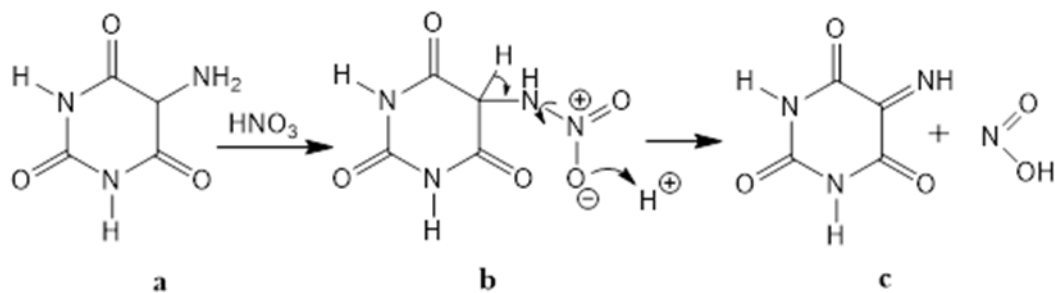


Figure 3 Nitric acid oxidation of uramil.

Oxidation of uramil by bromine water, Figure 4, can also be achieved. Hydrolysis of bromine yields hypobromous acid and hydrobromic acid. Protonated hypobromous acid, a halogen containing complex, acts as a bromine cation, **a**. Thus a bromo amine is formed, **b**, and elimination of hydrogen bromide gives the imine, **c**, **d**. Finally, hydration of the imino group and ammonia loss after protonation at nitrogen, **e**, gives rise to alloxan, mesoxalylurea, **f**. It has a high affinity for water due to the high reactivity of the intermediate carbonyl in the mesoxalyl group, and therefore exists as the monohydrate, Cf. [15].

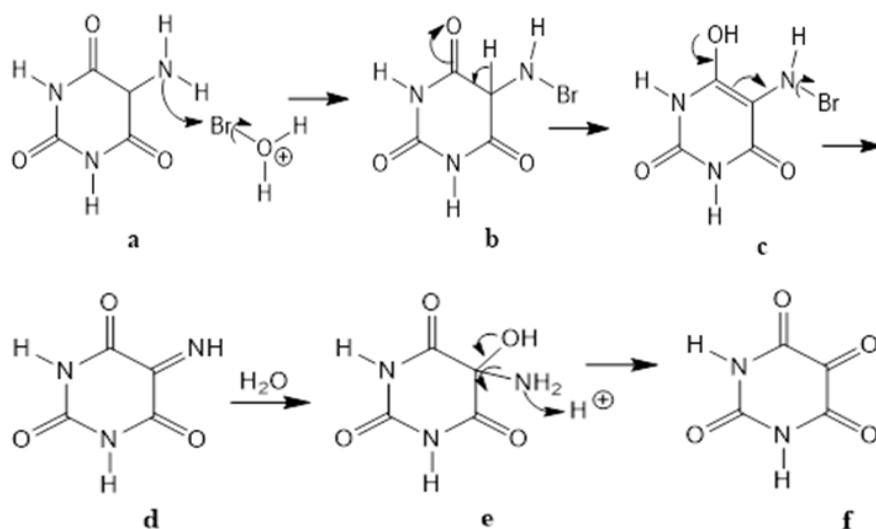


Figure 4 Bromine water oxidation of uramil to alloxan

Afterwards, condensation of uramil with alloxan yields purpuric acid, 5,5'-nitrido-dibarbituric acid. For this name see references [16, 17].

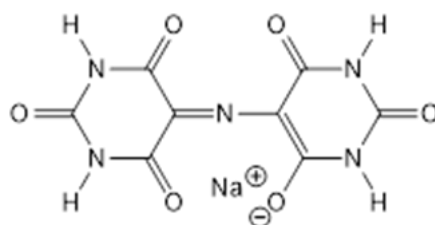


Figure 5 Sodium purpurate

In alkaline medium there is enolization in the uramil fragment, creating conjugation with the azomethine group. The salt of the enol is formed, that is, the colored purpurate, Figure 5.

In aqueous solution purpuric acid hydrolyzes slowly to uramil and alloxan, [18]. Additional information is found in [19, 20].

6. Conclusion

We provided the series of reactions that occur in the Kossel's test for adenine and guanine.

The reaction course from the purine base to the final product, the coloured sodium purpurate, includes hydration, ring opening, hydrolysis, elimination, tautomerization, degradation, hydroxyl oxidation, amine oxidation to imine, and condensation.

The reaction sequence was established in accordance to the reactivity derived from the structure of the substrate, and each step conforms to the chemical department, in acidic medium, of the involved functional group.

Thus, the synthesis of sodium purpurate from adenine, and also from guanine, has been studied, and commented step by step.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors or any other person.

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