



The Mechanism of Schiff Test for Uric Acid in Urine

Francisco Sánchez-Viesca^{1*} and Reina Gómez²

¹ Organic Chemistry Department, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City (CDMX), Mexico; e-mail: sanchezviescafrancisco@gmail.com

² Organic Chemistry Department, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City (CDMX), Mexico; e-mail: reinagomez.quim@gmail.com

* Corresponding author

Abstract

Uric acid in urine can be detected by reaction with silver nitrate in alkaline medium. A redox reaction occurs and black elemental silver is observed in the filter paper used in this test. However, the mechanism of the series of steps occurring in the uric acid molecule, including a second redox reaction, has not been advanced. A reaction route is provided, each step is fully commented giving also the electron flow. The mechanism involves free radicals and ionic intermediates as well. There is ring contraction with concomitant oxo-aziridine formation, imide hydrolysis, imine hydration to carbinolamine, isomerization to ketone with concurrently ring opening, and assisted decarboxylation.

1. Introduction

Uric acid is an analyte. The body produces uric acid by purine catabolism. Purines are in the body tissues and in many foods and drinks like liver, anchovies, sardines, and beer. Uric acid usually dissolves in the blood, passes through the kidneys, and is excreted in the urine (ca. 600 mg/day). Gout usually happens when extra uric acid forms crystals that get stuck in the joints. Symptoms of gout include pain, mainly in the big toe, ankle or knee; red, shiny skin around the joints; joints that feel warm to the touch, [1].

The liver and the intestinal mucosa produce most of the uric acid. The kidneys

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eliminate two thirds of the uric acid, with the gastrointestinal tract excreting the other one third. Uric acid is a weak acid.

The elimination of milk or other dairy products might increase the risk for hyperuricemia and gout. Early-onset hyperuricemia have strong genetic components. Normal values for uric acid in blood range between 3.5 to 7.2 mg/dl, [2].

An increase of uric acid concentration may indicate renal dysfunction, [3, 4].

In this communication we provide the reaction mechanism of Schiff test for uric acid, it is a follow up of our studies on reaction mechanism, [5-9].

2. Antecedents

The test under study is due to Hugo Schiff (1834-1915), [10, 11]. The test is as follows: a small amount of urine is treated with 2% sodium carbonate. Moisten a filter paper piece with silver nitrate solution, and add one or two drops of mixed urine sample. The colour of the filter paper turns to dark brown or black due to elemental silver formation.

Uric acid has been oxidized by other methods, for instance, by potassium permanganate in alkaline medium [12, 13], with lead dioxide [14-16], and with alkaline hydrogen peroxide [17, 18].

Uric acid is 2,6,8-trioxopurine, Figure 1.

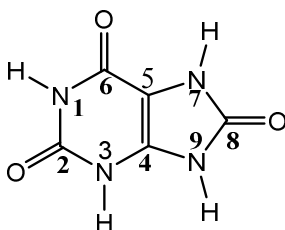


Figure 1. Uric acid structure.

3. Discussion

In Schiff's test for uric acid the reactive site is the imido group in the dioxypyrimidine ring. Figure 2.

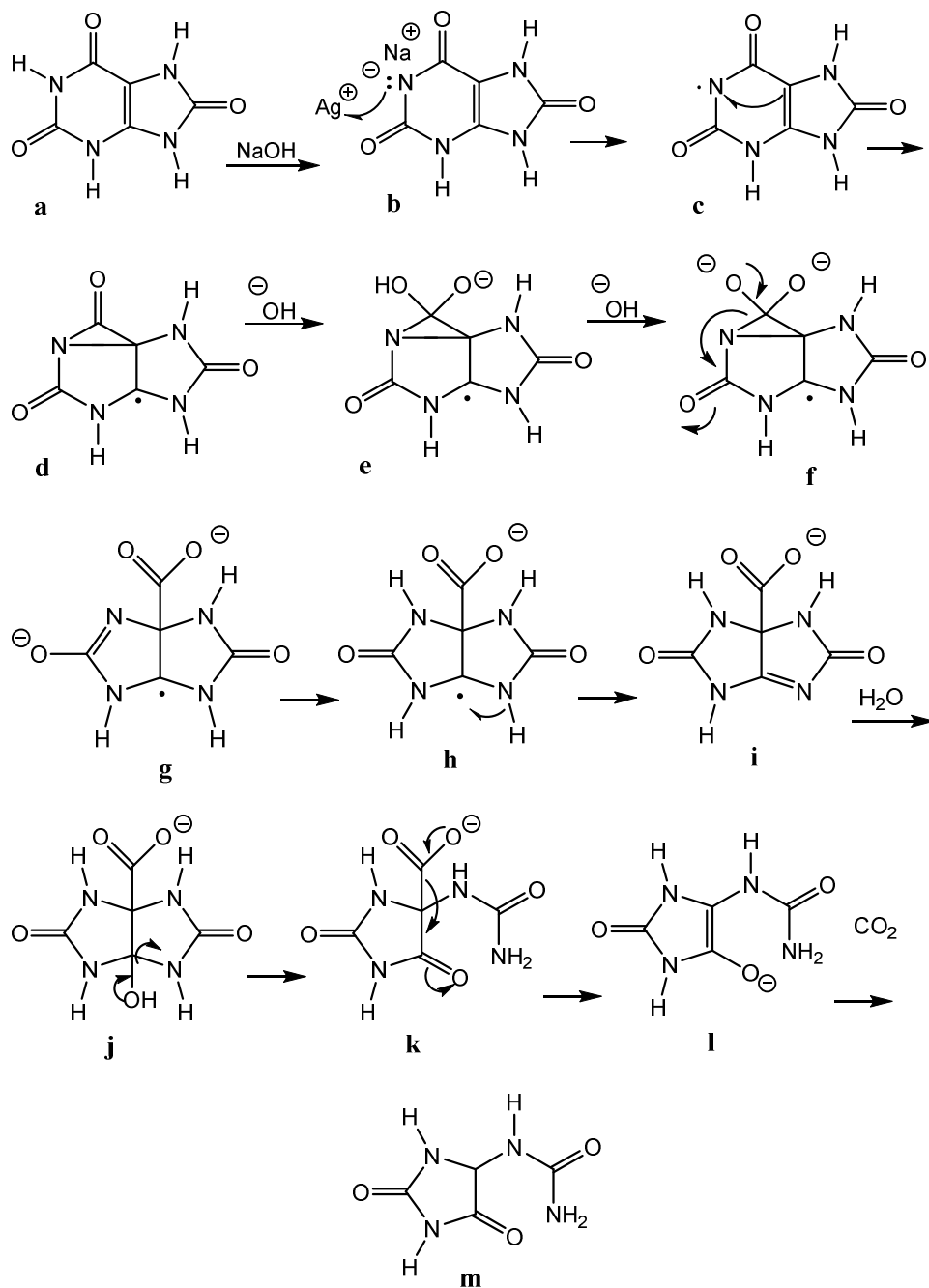


Figure 2. Oxido-degradation of uric acid by means of silver nitrate in alkaline medium.

The acidic hydrogen of this group reacts with sodium carbonate to yield the imidate, a, b. Silver ion abstracts one electron from the negative charged nitrogen atom, c. Elemental silver is formed and the free radical on nitrogen couples with one electron from the double bond. A five member ring results together with an oxoaziridine, d. Alkaline hydrolysis of this tensioned imide takes place via the double alkoxide, e, f.

A carboxilate and a cyclic ureido result, g. The interannular free electron in this symmetric structure forms an imino group by hydrogen atom elimination and coupling, h, i. The hydrogen free radical reacts with silver ion (second redox reaction), giving black elemental silver and a hydrion. The resulting nitric acid reacts with sodium carbonate. The imino group, conjugated with a carbonyl, is hydrated to a carbinolamine, j. This transient hemiaminal gives rise to a carbonyl group and an ureido chain by ring opening, k.

Finally, decarboxylation is enhanced by the presence of a β -carbonyl group, k, l. The end product is 5-ureido-2,4,dioxoimidazolidine (5-ureidohydantoin, allantoin), m. This compound results also in other uric acid oxidation reactions, [19].

A related reaction is Neubauer test for uric acid, [20-22]. It employs ferric chloride as reagent. The end products are urea and oxalic acid. Notwithstanding that reaction starts at the same nitrogen atom generating a free radical, the acidic and boiling medium gives rise to different end products, via a distinct reaction route and mechanism.

4. Conclusion

The reaction route of Schiff test for uric acid in urine has been provided. The imido group in uric acid (ring A) forms the imidate in basic medium. Silver ion abstracts an electron from this anion. The resulting free radical couples with an electron from the carbon-carbon double bond. A five member ring is formed together with an azaridinone. The tensioned carbonyl in the three member ring is part of an N-substituted imide which is hydrolyzed easily to carboxilate. The remaining interannular free radical produces hydrogen atom elimination forming an imino group. There is hydrolysis to carbinolamine and isomerization to carbonyl and ring opening. Finally decarboxylation occurs. The end products are 5-ureidohydantoin (allantoin) and elemental silver.

Conflicts of Interest

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

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