

On the Mechanism of the Hoppe-Seyler Test for Xanthine

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Abstract Xanthine, a dioxopurine, is a natural component of human urine. It was first isolated from a urinary calculus. This important biochemical substance can be detected by the colour test proposed by Hoppe-Seyler. He employed calcium chlorohypochlorite in alkaline medium, a green colour is observed with xanthine. Since the reaction pathway has not been advanced, the reaction mechanism of each step is provided. A redox reaction takes place after halogenation and alkaline hydrolysis via a variant of the Hofmann reaction. A nitrene is produced and reacts with the double bond in the uracil ring. A dipolar intermediate reacts with water, and the resulting imidzoline is hydrated by alkaline hydrolysis of the imino group in this cyclic amidine. A carbinolamide and a carbinolamine are formed whose isomerization gives rise to oxo derivatives and chain formation by ring opening. A second chlorination takes place, followed by dehydrohalogenation and isomerization. Assisted decarboxylation yields the final product, 5-ureidohydantoin.

Keywords Calcium chlorohypochlorite, Carbinolamine, Hofmann reaction, Imine alkaline hydrolysis, Nitrene, Redox reaction

1. Introduction

Xanthine is a two-oxygen derivative of adenine and guanine, the purine bases of DNA. Uric acid is a three-oxygen derivative of purine, and caffeine and theobromine are methyl xanthines.

Xanthine, 2,6-dihydropurine, was first isolated from a urinary stone. It has since isolated from muscle, liver, and tea leaves, and it is a natural component of human urine, [1].

A xanthine isomer is alloxanthine, a pyrazolopyrimidine, Figure 1.

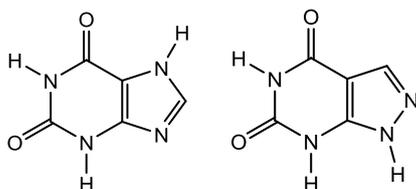


Figure 1. Xanthine and alloxanthine structures

In this communication we provide the pathway of xanthine oxidation by calcium chlorohypochlorite in alkaline medium.

This work is a follow up of our studies on reaction mechanism, [2-6].

2. Antecedents

Xanthine is a purine base. Like uric acid, it gives the murexide reaction [7], but by different route.

Traube prepared xanthine from 4,5-diamino-uracil by condensation with formic acid, [8,9].

Uracil is 2,6-dioxo-tetrahydropyrimidine, and it is a constituent of the vegetal nucleic acids, [10].

Felix Hoppe-Seyler (1825-1895), German physiologist and chemist, detected xanthine by reaction with calcium chlorohypochlorite in alkaline medium, giving green colour, [11-13]. He was the principal founder of biochemistry and molecular biology.

The test is as follows: add the substance to be tested to a mixture of chlorinated lime and sodium hydroxide in a porcelain dish. A dark green ring is formed at first, quickly changing to brown, and finally disappearing.

3. Discussion

Xanthine has two fused rings, a dioxypyrimidine (uracil), and an imidazole, also known as glyoxaline due to its preparation from glyoxal, ammonia, and formaldehyde, [14].

The acidic hydrogen of the imido group in xanthine reacts with hypochlorite anion giving hypochlorous acid. This acid generated in situ is the reactive species for N-chlorination, [15,16].

The hydrogen at N-3 is rather unavailable since a lactime (imidol) structure contributes to an $\alpha,\beta,\gamma,\delta$ -unsaturated carbonyl, Figure 2.

The imidazol ring being aromatic and basic is not proper for reaction with hypochlorite anion.

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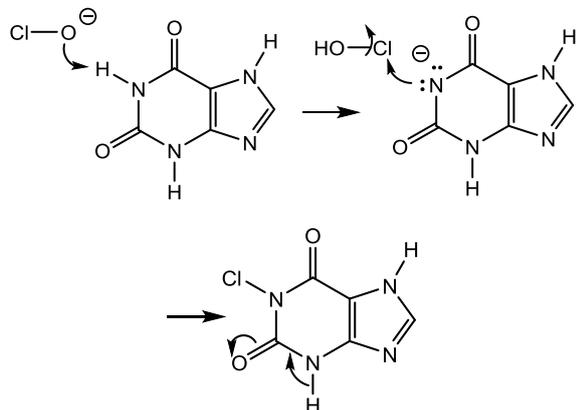


Figure 2. Xanthine halogenation and isomerization

The next step is basic hydrolysis of the chloroimide via the dialkoxide. A carboxylate and a nitrene result after elimination of a chloride ion. This step is a variant of the Hofmann reaction, that is, there is chlorine elimination in a $R\text{-CONX}^-$ salt to yield a nitrene, a transient radical. Cf. [17-20]. The nitrene attracts an electron pair from the double bond, Figure 3.

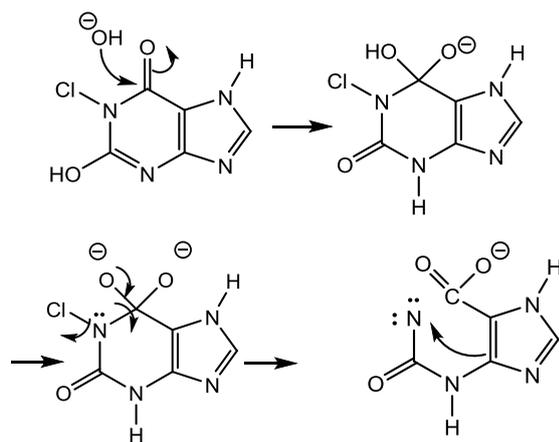


Figure 3. Chlorimide hydrolysis and nitrene formation

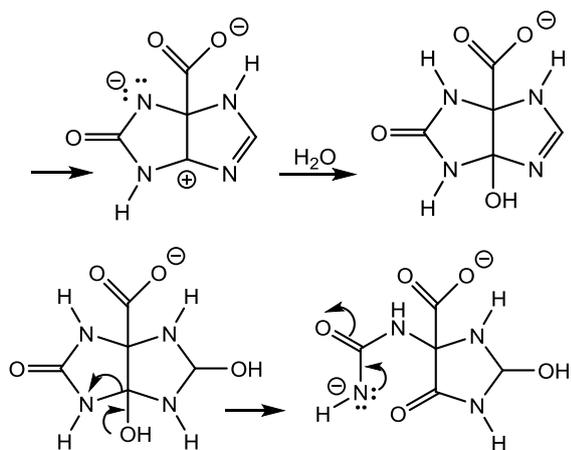


Figure 4. Cleavage of carbinolamide and anion stabilization

A negative charged nitrogen atom and a carbonium ion result, and both ions are neutralized by water.

The imino group in the new, not aromatic, imidazoline ring is hydrated by alkaline hydrolysis, Cf. [21].

Ring opening of the resulting carbinolamide (ring A) can form an isoureido chain by stabilization of the negative charged nitrogen atom, and an oxo group in ring B, Figure 4.

On the other hand, the carbinolamine (ring B) gives rise to an imide in ring A, and negative charged nitrogen that cannot be stabilized by resonance and thus can be chlorinated to the N-chloro intermediate. A dehydrohalogenation reaction produces an isoureido chain that isomerizes to the ureido form. In both of the above mentioned ring openings 5-ureido-hydantoin is formed, Figure 5.

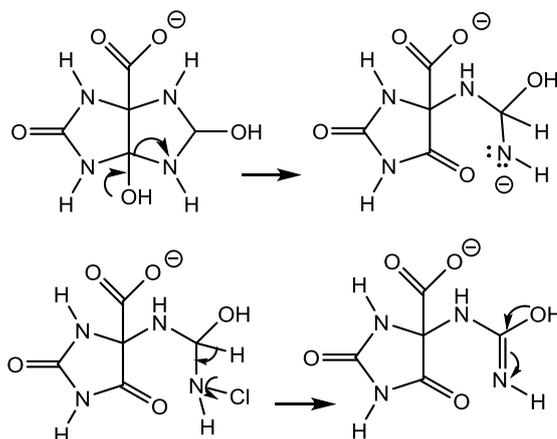


Figure 5. Ring fission from carbinolamine, chlorination, and dehydrohalogenation

Finally, assisted decarboxylation occurs due to the presence of a β -carbonyl group and delocalization of the original carbanion to oxygen, Figure 6.

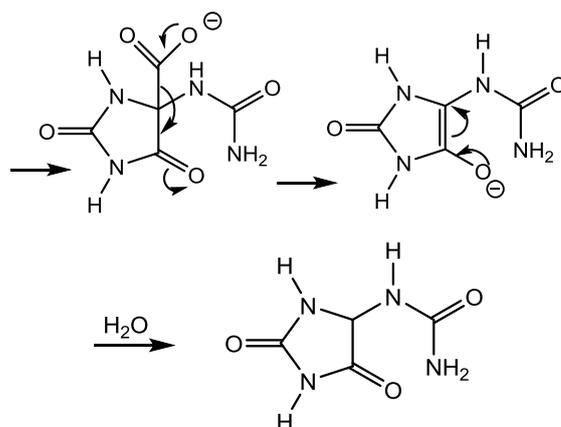


Figure 6. 5-Ureido-hydantoin by carbon dioxide release from β -carbonyl carboxylate

4. Conclusions

The mechanism of xanthine oxidation by means of calcium chlorohypochlorite in alkaline medium has been provided. This oxidation occurs via halogenation of the imido group, followed by hydrolysis of the haloimide. A nitrene results by ring opening and halide elimination, a

variant of the Hofmann reaction, with concomitant formation of carboxylate. The nitrene attracts an electron pair from the double bond. The dipolar intermediate is neutralized by water, and an imidazoline ring results. The imine of this cyclic amidine is hydrated by alkaline hydrolysis. A carbinolamide and a carbinolamine are formed. The first one gives a carbonyl and an ureido chain. The latter produces a carbonyl and an oxidable chain. In both cases the final product is 5-ureidohydantoin via assisted removal of the carboxylate.

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