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(RESEARCH ARTICLE)

An insight on the mechanism of the Hoppe-Seyler test for xanthine

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Abstract

Being xanthine an important biomolecule, it has attracted the attention of scholars from different points of view. In the analytical field some tests for identification have been developed. Hoppe-Seyler detected xanthine using calcium chlorohypochlorite and sodium hydroxide since a green ring, changing to brown, is formed. It is a challenge to the theoretical chemist disentangle what is happening at molecular level during the assay. In this communication emphasis is placed in a reactive tri-polar intermediate that arises in a reaction sequence. That is, a carboxylate and a newly formed zwitterion. Reaction with water can eliminate the last dipole (a negatively charged nitrogen and a carbonium ion). However, there is other pair of ions that are connected: the carboxylate and the carbonium ion at β-position. This situation produces immediate decarboxylation, eliminating two electrical charges in a single internal way, and forming a double bond. Neutralization of the negatively charged nitrogen atom gives an ureido.

This reaction sequence produces the principal reaction product, imidazo-imidazolone. The observed colours are due to halochromism.

Keywords: Hofmann reaction; Nitrene; Reaction intermediates; Redox reaction; Tripolar intermediate

1. Introduction

Xanthine is a purine base formed in most human body tissues and fluids, as well in other organisms, [1]. Several stimulants are derived from xanthine, like caffeine (1,3,7-trimethyl xanthine) and theobromine (3,7-dimethylxanthine), found in coffee and in cacao plant, respectively.

A xanthine isomer is alloxanthine (oxypurinol), a pyrazolopyrimidine, [2]. Figure 1.

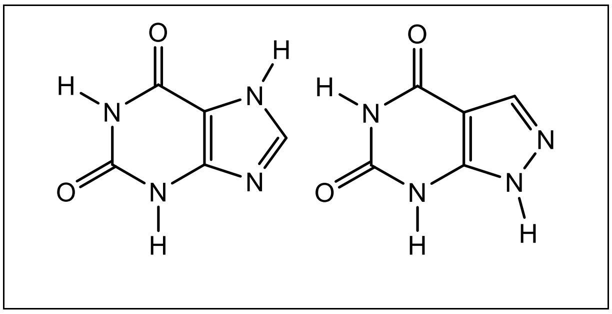

Figure 1 Xanthine and alloxanthine structures

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These biological active compounds have attracted attention from the analytical point of view, and several identification reactions have been developed. However, it is interesting to know what is happening in the test tube at molecular level, but it's not a simple question since we are dealing with two-ringed polyfunctional compounds with several options for reaction.

The present communication is an insight on the mechanism of the Hoppe-Seyler test for xanthine. This paper is a follow up of our studies on reaction mechanism, [3-7].

2. Antecedents

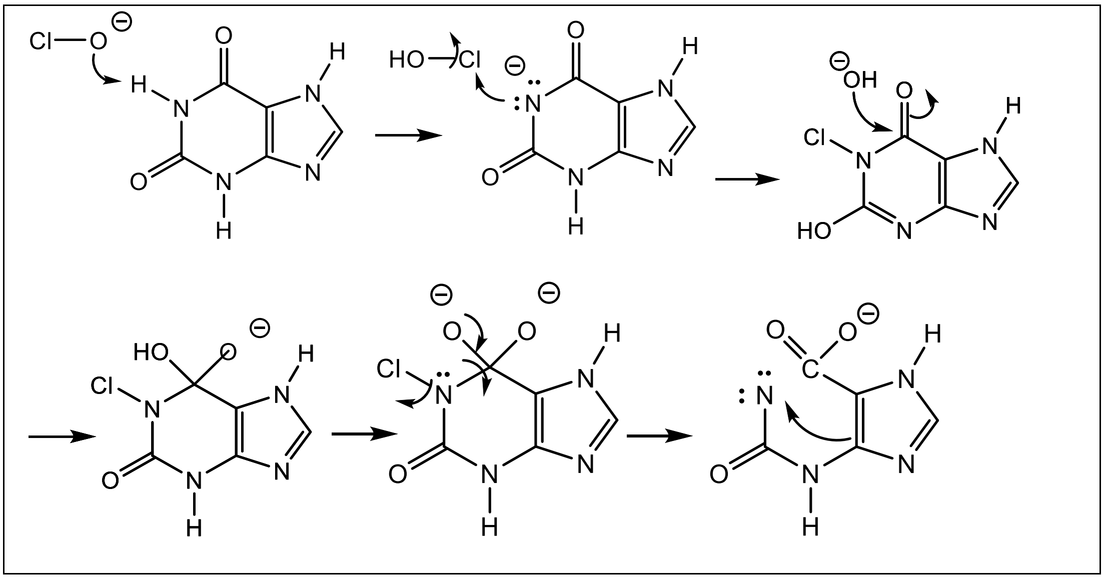
The test under study is due to Felix Hoppe-Seyler (1825-1895), German physiologist and chemist. In 1871 he discovered that the yeast extract contains invertase, the enzyme that converts sucrose to glucose and fructose, [8] He studied chlorophyl and hemoglobine which he obtained in crystalline form. He was founding editor (1877) of Zeitschrift für Physiologische Chemie, the first biochemical journal, [9]. He detected xanthine by reaction with calcium chlorohypochlorite in alkaline medium, giving green colour, [10-12]. 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

The first step in this sequence of reactions is interaction of the hypochlorite anion with the acidic hydrogen of the imido group in the pyrimidine ring, forming hypochlorous acid. Figure 2. This acid is a positive-chlorine donor [13, 14], that yields an N-chlorimide, which is hydrolysed by the alkaline medium via a double anion in order to avoid hydroxyl ion regression. A carboxylate is formed and the negative charged nitrogen atom separates a chloride ion (oxidation step). The nitrene thus formed is a variant of the Hofmann reaction [15-17]. This radicle attracts electrons from the carbon-carbon double bond, and a zwitter ion results. At first glance both ions can be neutralised by reaction with water [18], giving an ureido group and a pseudo-carbinolamine (external neutralisation). However, there are a carboxylate and a carbonium ion at β-position, this induces immediate decarboxylation (rapid internal neutralisation), with double bond formation, yielding an imidazo-imidazolone (dominant product) after neutralization of the nitrogen.

The ureido group in the imidazolone is not prone to alkaline hydrolysis as well as the aromatic imidazole ring, although both rings can be opened in more drastic conditions or more reaction time than that in a test-tube colour-reaction. [19-21].

The green and brown colours observed during the test are due to halochromism, [22, 23].



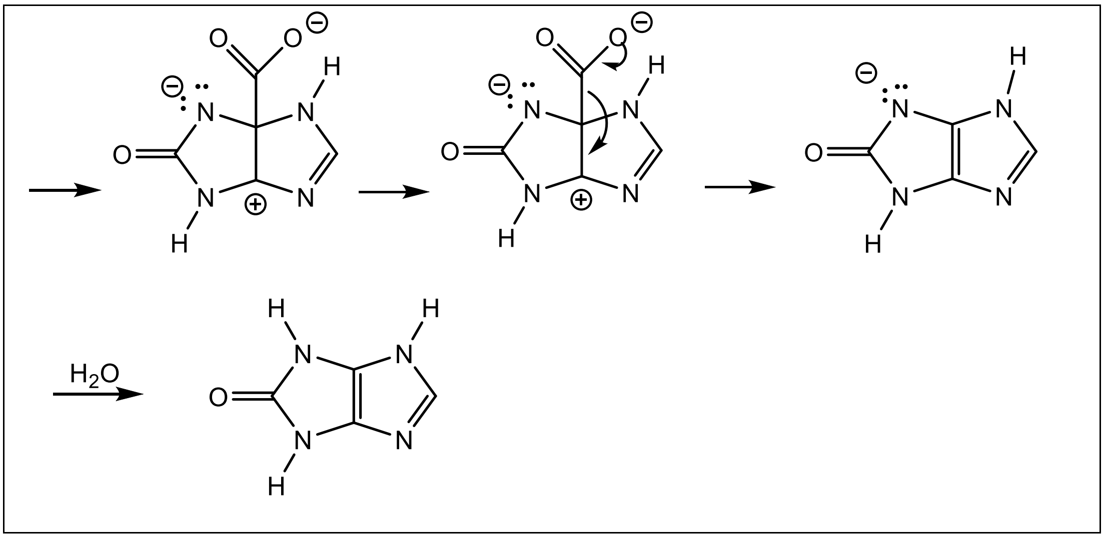


Figure 2 Formation of the principal product in Hoppe-Syler test for xanthine

4. Conclusion

In this communication, the dominant product in the Hoppe-Seyler test for xanthine has been established. This was deduced from the reactivity of a tri-polar intermediate: two ions can be neutralised by water in an external reaction: a negatively charged nitrogen atom can take a proton, and a carbonium ion can add a hydroxyl.

However, the carbocation is at beta-position to a carboxylate and this situation must enhance decarboxylation notably, with concomitant double bond formation and neutralization of both ions. This internal reaction is faster than the external one and must prevail. This is a direct variant of the easy decarboxylation of β-ketoacids, whose reaction mechanism is well known. Then occurs neutralisation of the negative nitrogen.

In this manner imidazo-imidazolone is formed as the principal reaction product in Hoppe-Seyler test for xanthine.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest to declare.

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