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On the mechanism of the oxido-degradation of uric acid by ferric chloride

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Abstract

The reaction of ferric chloride with uric acid giving ferrous ions, urea and oxalic acid, the Neubauer Reaction, has been developed in two recent spectroscopic techniques for uric acid determination. However, the reaction mechanism with uric acid has not been advanced. We provide the electron flow, step by step, from uric acid to the end products. Each step is commented, as well as other theoretical initial reaction sites. A substituted aziridinone is an interesting intermediate, and the formation of parabanic acid as precursor of the end products is in accord with experimental data.

Keywords: aziridinone, Neubauer reaction, parabanic acid, reactive intermediates

1. Introduction

Uric acid is a terminal metabolite of purines in primates including humans. Other mammals have the enzyme urate oxidase and excrete the more soluble allantoin oas the end product. Uric acid is formed primarily in the liver and excreted by the kidney into the urine [1], and is widely distributed in human tissues and extracellular spaces. Uric acid is also a water-soluble antioxidant that can scavenge free radicals. Urinary or serum uric acid concentration is an indicator of kidney condition. An increase of uric acid concentration may indicate renal dysfunction [2, 3].

The Neubauer Reaction and the modern spectroscopic methods derived from it will be treated in the Antecedents section.

The reaction mechanism of the original reaction is provided for the first time and it is fully commented in the 'Discussion'. It is a follow up of our studies on organic reaction mechanisms [4, 5, 6, 7, 8].

2. Antecedents

The reaction under study, the oxidation of uric acid by means of ferric chloride, yielding ferrous ions, urea and oxalic acid, is due to Carl Neubauer (1830-1879), [9, 10, 11]. He also studied creatine and creatinine [12], and this study was continued afterwards in the United States [13].

Uric acid has been oxidized by other methods, for instance, early by potassium permanganate in alkaline medium [14, 15]. More recently, uric acid has been oxidized electrolytically in dilute acetic acid at large graphite electrodes. However, this method affords several products [16].

The Neubauer reaction was developed in order to determine uric acid in biological fluids by a colorimetric method in the visible range and that is specific for uric acid. It uses 1, 10-phenanthroline as indicator of the ferrous ions formed in the original reaction [17]. That is, the red ferrous complex formed by six ligands from three 1, 10-phenanthroline molecules with a ferrous ion.

A very recent diagnostic technique to detect uric acid in urine is also based in the Neubauer reaction. It uses paper impregnated with potassium ferricyanide solution, urine sample, and ferric chloride solution. Prussian blue is formed with the ferrous ions from the uric acid oxidation. The coloration is compared with known ferrous concentrations and related to that of uric acid [18].

3. Discussion

The most suitable site in uric acid for reaction with a Fe+++ ion is the transient, negatively charged nitrogen atom that is formed during tautomerization from a lactim form to the lactam structure. A neutral radical results at the nitrogen, $FeCl_2$ is produced and the eliminated proton forms HCl. Figure 1, formulas a, b, c, d.

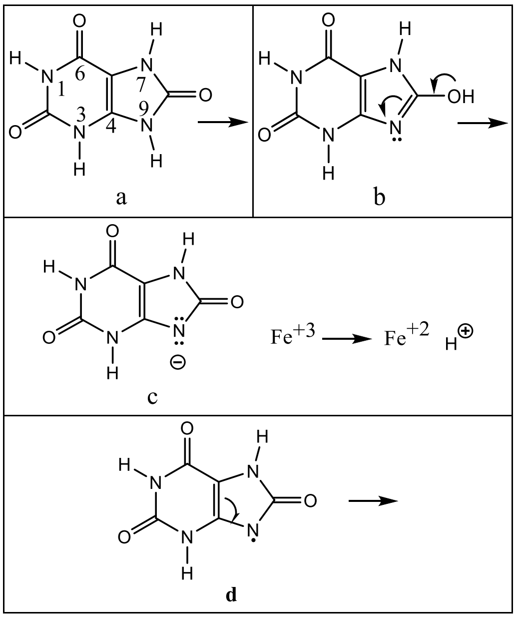

Fig 1: Formation of the initial free-radical.

The nitrogen radical reacts with the double bond**:** an imino group is generated, Figure 2, e, and the new radical at carbon couples with a nitrogen electron in the pyrimidine ring. There is ring contraction with concomitant formation of an aziridinone ring, f. This is unstable because, in addition to the strain in the three member ring, there is a free radical in its nitrogen. Hence, this ring is broken down and carbon monoxide is expelled, which acquires a triple bond structure, like in the iso-electronic N_2 molecule [19].

A ferric ion takes the remaining interannular-electron; a rather stable carbocation vicinal to two nitrogen atoms is formed, and then neutralized with water. The imino group is hydrated and a symmetric intermediate results. Formulas g, h, i.

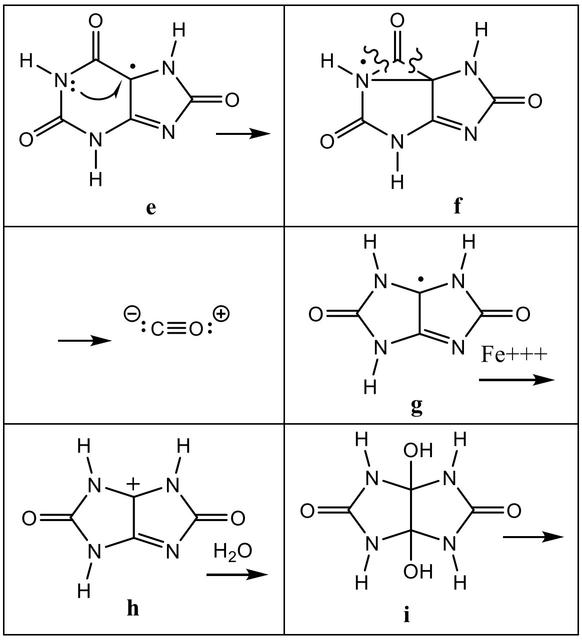

Fig 2: Symmetric intermediate after ring contraction and CO elimination.

A tautomeric ring opening of hemiaminal type occurs, giving an ureido chain, Figure 3, j. Another similar fission in the remaining pseudo-carbinolamine yields urea and parabanic acid, trioxoimidazolidine (oxalyl urea), k.

Hydrolysis of parabanic acid forms at first instance oxaluric acid, l, whose hydrolysis affords the final products: urea and oxalic acid. M.

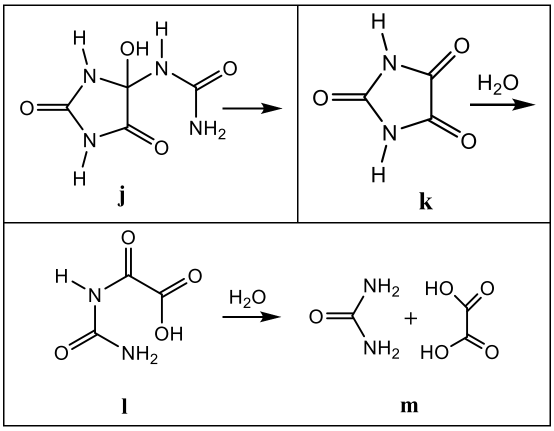

Fig 3: Last steps of uric acid degradation.

Oxaluric acid has the property to yield urea and oxalic acid by autocatalyzed hydrolysis in aqueous solution [20], but in the Neubauer reaction some hydrochloric acid has been formed already.

Aziridinones, α-lactams, have been synthesized rather recently [21], and have been found as intermediates in some reactions [22]. Aziridinone is a key compound in the formation of the first proteinogenic amino acids and polypeptides [23].

Other possible initial reaction sites have been tried since there are other lactim forms to be considered. The negatively charged N-3 can be easily stabilized by resonance occurring in the six member ring, and thus is less prone to react with a ferric ion than that from N-9, as was indicated in the reaction sequence.

The lactim from N-1 is rather stable since it has cross conjugation. In support of this statement, the reported structure of sodium urate derives from this lactim [24].

Finally, a reaction starting with a free radical at N-7 would yield alloxan, 2, 4, 5, 6-tetraoxohexahydropyrimidine (5-oxobarbituric acid), which is not obtained. This oxidation product is formed under strongly acid conditions. Parabanic acid can be prepared from alloxan and fuming nitric acid [25].

4. Conclusion

The reaction of uric acid with ferric chloride to give ferrous ions, urea and oxalic acid dates from an early communication by C. Neubauer. This reaction has been used recently in order to develop spectroscopic techniques for uric acid determination. However, the detailed course of this oxidative degradation occurring in this analyte has not been studied. In this communication we provided the electron flow, step by step, of this important multi-reaction process. The sequence is based on the reactivities of the intervening functional groups, as well as in experimental data.

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