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The Mechanism of Frabot Test for Uric Acid

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Abstract

Uric acid is an Analyte. Excess uric acid ─ hyperuricemia ─ leads to gout and renal stones. Frabot noted that uric acid added to an alkaline tungstate solution developed an intense blue colour. A colorimetric method widely utilized to quantify uric acid depends on the reduction of the chromogen sodium tungstate by uric acid to produce a measurable colour change. The reaction route from the initial products to the coloured compounds and the oxido-degradation of the organic substance has not been advanced. In this communication the electron flow is given in each step of the series of reactions that take place. These are isomerization to imidol, Michael addition to enone, oxirane formation in a redox step, hydrolysis of imido group, oxirane ring opening, ring contraction to five-member ring, ring opening via alkoxide, and finally assisted decarboxylation to the end product, 5-ureido hydantoin (allantoin).

1. Introduction

Uric acid, 2,6,8-trihydroxypurine (lactim form), crystallizes in the lactam form, this tautomer being the more stable. However, a mixed structure involving only one lactim form is pertinent to explain the chemical deportment of the compound, as it will be seen in the 'Discussion'.

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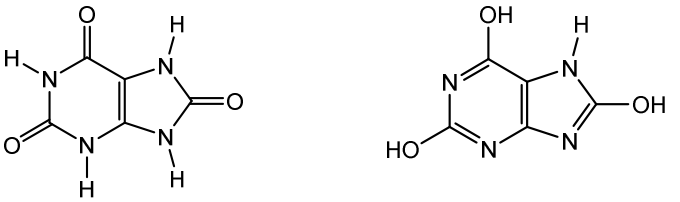

Figure 1. Tautomeric structures of uric acid.

The purine structure comprises two fused rings: a pyrimidine and an imidazole. In the Frabot test reaction starts at the pyrimidine ring and epoxidation of the C═C double bond occurs. The following reactions will be disclosed afterwards, including a ring contraction.

Uric acid is a biomarker and thus an Analyte. In this communication the reaction route of the oxido-degradation of uric acid by means of the chromogen sodium tungstate is described. This paper is a follow up of our studies on reaction mechanism, [1-5].

2. Antecedents

Frabot noted in 1904 that uric acid added to an alkaline tungstate solution developed an intense blue colour. He published his test in France [6], and was abstracted in England, [7]. The test has been also registered in the United States, [8, 9].

In organic chemistry sodium tungstate is used as a catalyst for the epoxidation of alkenes and oxidations of alcohols into aldehydes or ketones, [10]. It is also known for its antidiabetic effects, [11].

Sodium tungstate dihydrate, $Na_2WO_4.2H_2O$, combines with hydrogen peroxide for the oxidation of secondary amines to nitrones, [12]. Disodium dioxido(dioxo)tungsten (IUPAC name), is used as precipitant for alkaloids and proteins, and to catalyze oxidation reactions.

Alkali metal tungstates, M_2WO_4, are prepared by dissolving the trioxide in the appropriate alkali: $WO_3 + 2OH^- \longrightarrow WO_4^{-2} + H_2O$.

The acidification of boiling tungstate solution causes the precipitation of yellow tungstic acid, H_2WO_4. Proton magnetic resonance studies indicate that the hydrogen is present as a water molecule, and this compound is in reality hydrated oxide, [13].

Some notes about the substrate. Under physiologic conditions uric acid exists mainly as a monosodium salt. At pH less than 5.75, as may occur in the urine, the predominant form is nonionized uric acid. The solubility of monosodium urate is about 18 times

greater than uric acid in aqueous solutions. This solubility differential provides the therapeutic rationale for alkalinization of the urine pH to greater than 6.0 in patients forming uric acid stones, [14].

The normal range of serum uric acid level is between 3.5 and 7.2 mg/dL, [15]. Levels beyond 7 mg/dL result in supersaturated solutions that are prone to crystal formation.

Normal values range for uric acid in urine is 250 to 750 mg/24 hours.

3. Discussion

The structure of sodium urate has been proposed with the negative charge at N-1, in accordance with the acidic properties of the imido group, [16], Figure 2, **a**. However, this anion can be in resonance with two imidate structures, **b**, **c**. The more stable cross conjugated structure is preferred and has been taken into account for uric acid monosodium salt, [17]. This structure lefts an enone capable of 1,4-addition reaction.

Therefore, sodium tungstate reacts with the α,β-unsaturated ketone, **d**. Cf. [18]. An epoxide is formed by electron return to the oxygen atom of the wolframate and elimination of tungstenous salt (oxido-reduction step), **e**.

The next step is hydrolysis of the re-stablished imide, **f**, **g**. This reaction occurs via the gem-alkoxide, **h**; a carboxylate is formed, and the resulting negative charged nitrogen atom opens the oxirane ring, **i**, giving an alkoxide at C-4, **j**. This ion is the driving force for ring opening in this symmetric structure, **k**.

The last step is ready elimination of the β-oxo-carboxylate, **l**. The end product is 5-ureido-hydantoin (allantoin).

The blue products formed in the test are W_2O_5 [19], and W_3O_8 [20]. Now let us clear up the formation mode of these derivatives. Tungsten pentoxide, W_2O_5, comes from condensation of WO_3 and WO_2. These compounds are formed by reaction of a disodium salt with a water molecule giving NaOH and the monosodium salt, followed by formation of WO_3 or WO_2 via WO double bond formation and hydroxyl elimination (reverse reaction of salt obtention). The condensation occurs via polarized WO_3 and one-electron transfer from the unshared electron pair at WO_2. That is, W(VI) plus W(IV) gives 2 W(V).

The other blue product, W_3O_8, results as follows. Two molecules of amphoteric tungstic acid react as base and acid, affording the salt W_2O_6:

$$O_2W(OH)_2 + H_2WO_4 \longrightarrow WO_2^{2+}\, WO_4^{2-} + 2\, H_2O$$

Transfer of one electron from a WO_2 molecule to the above oxycation gives 2 WO_2^+ ions and formation of the salt W_3O_8.

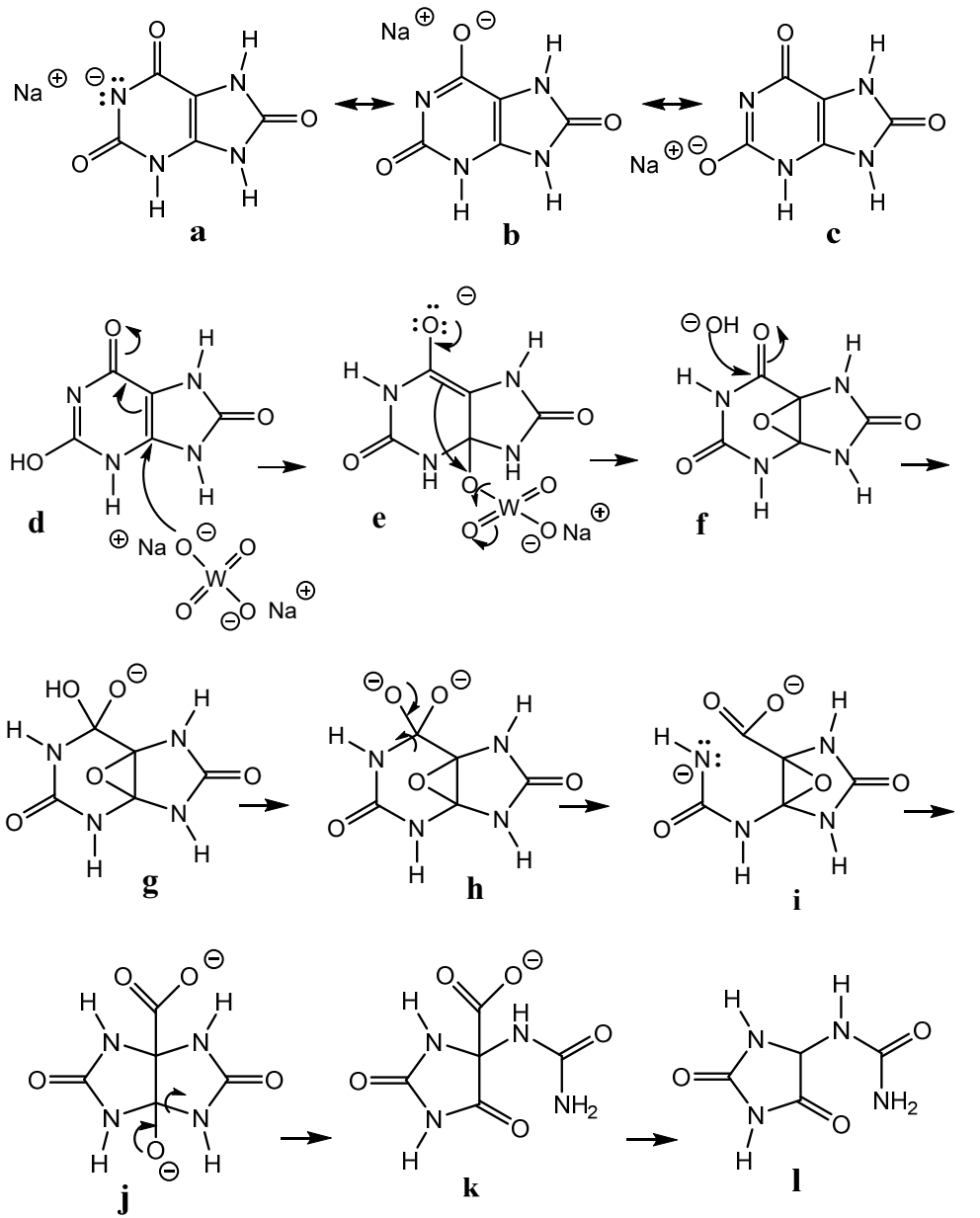

Figure 2. Reaction route of the oxido-degradation of uric acid by sodium tungstate.

4. Conclusion

Uric acid can be represented in a lactam structure or in a lactim one. We have found that these tautomeric forms must not be considered as building blocks, that is, this or that.

Uric acid can react as α,β-unsaturated ketone. However, neither of the two classic tautomeric structures exhibit the mentioned above type of ketone.

Nevertheless, a mixed structure with only one lactim form is adequate to explain uric acid reactivity. That is, the imidate proposed for monosodium urate, and the imidol derived from it, display a fairly stable cross conjugated structure with an α,β-unsaturated ketone.

A Michael addition of tungstate to this group affords an epoxide with concomitant elimination of tungstenous salt (redox step). Alkaline hydrolysis of the imide, restored by disappearance of the double bond and therefore cross conjugation, yields a carboxylate and a negative charged nitrogen that opens the epoxide (ring contraction step). The next reactions are commented in the 'Discussion'.

Conflicts of Interest

There are no conflicts to declare.

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