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The Chemistry of the Folin Test for Uric Acid

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Abstract In the interaction of uric acid with sodium tungstate in phosphoric acid, followed by alkalinisation in order to develop a blue colour (Folin Test for uric acid) there are two reaction series, organic and inorganic. These are described and a mechanism has been provided for each reaction along with sustaining references to similar chemical deportment. The process is an oxidative degradation that involves addition to double bond, organometallic ester, oxido-reduction step, epoxide formation, pyrimidine ring breakdown, hydration, decarboxylation, and oxirane isomerization. The final product, 2,4-dioxo-5-ureido-imidazolidine, has been obtained before by other methods differing in oxidant and reaction medium, with no mechanism advanced. The chemistry related to the involved inorganic compounds is also treated in detail.

Keywords Allantoin, Allophanic acid, Isocyanate, Oxidative degradation, Phosphotungstic reagent, Reaction mechanism, Reactive intermediates, Uric acid

1. Introduction

Continuing our studies on organic reaction mechanisms [1-4], we turned our attention to the Folin Test for uric acid.

Besides the laboratory results, many times the colour tests induce the theorist's interest in order to disentangle the reactions that are occurring.

The clinical significance of uric acid is well known. At high uric levels (hyperuricemia) uric acid crystallizes and the crystals deposit in joints, tendons and surrounding tissues resulting in an attack of gout.

In this paper we study the reaction mechanism of the Folin test, the reaction of uric acid with sodium tungstate in phosphoric acid that yields an intense blue colour [5]. We describe the mechanism of the steps of the uric acid oxidative degradation to allantoin and the coloured tungsten derivatives that are formed.

There is a memorial article dedicated to Otto Folin [6]; and the reagent, though not complicated, is also commercially available (Supplier: VWR Chemicals).

The discussion has been enhanced with logical connectors, similar reactivities, and 3D molecular structures.

2. Antecedents

As said before, Folin's reagent employs sodium tungstate in phosphoric acid. Closely related inorganic reagents that also give blue coloured products are the Buckingham reagent for alkaloids [7, 8] and the Froehde reagent [9, 10]. The first uses ammonium molybdate in concentrated sulphuric acid. The second utilizes sodium molybdate in the same acid, and Froehde employed it in a test for morphine. Years later the Irish physician E. W. Davy published a new test for carbolic acid (phenol) employing the sulphomolybdic reagent [11-13]. This discloses that the phenol group in morphine is responsible for the positive reaction of this compound.

Molybdene and tungsten are transition elements that are vicinal in the d-block, group VI (the chromium group). Molybdates and tungstates are mild oxidants.

Tungsten (Z=74) is in the third series and its electron structure is: Xe core, $4f^{14}$, $5d^4$, $6s^2$ [14]. Electron transfer from the 6s to the 5d orbitals gives d^6 orbitals with additional stability. The hexavalent tungstates are example of this.

Acidified tungstates when reduced give blue pigments and colorations [15]. When the acidified tungstate solution is reduced with hydrazine, hydrogen sulphide, or other reagents, an amorphous intense blue product is obtained [16].

The blue products are W_2O_5 [17], and W_3O_8 [18], and both come from WO_2, tungsten(IV) oxide (tungsten suboxide).

This pertinent information will sustain the reaction mechanism proposed in the next section.

3. Discussion

Uric acid is tautomeric and presents the lactam form (2,6,8-trioxopurine) and the lactim form (2,6,8-trihydroxypurine), Figure 1.

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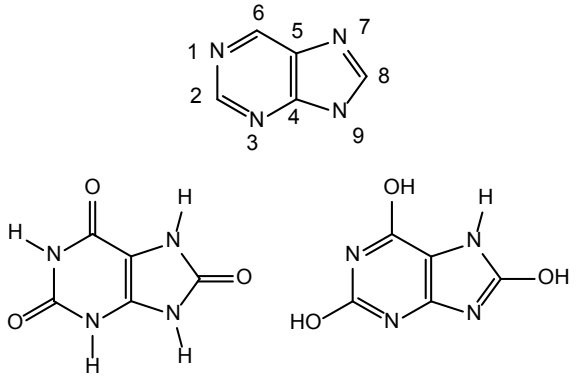

Figure 1. Purine frame, and uric acid lactam and lactim forms

Uric acid crystallizes in the lactam form [19], computational chemistry also indicating that tautomer to be the most stable [20]. However, with phosphoryl chloride uric acid reacts in the lactim form, yielding a trichloro derivative with imidoyl chloride groups [21].

After this previous information, the mechanism of the reaction of uric acid with the phosphotungstic reagent was disclosed as follows.

The ureido groups are rather stable due to the resonance effect with the nitrogen atom vicinal to the carbonyl group. Thus, reaction begins by tungstic acid addition to the double bond since the compound is not aromatic, similarly to nitric acid addition to indigo double bond [22].

The indicated protonation gives a rather stable carbocation between two nitrogen atoms. The alternative ionization is discarded since it would yield a carbonium ion vicinal to a carbonyl group. Then a tungstic ester is formed, Figure 2. The 3D structure shows the proximity of the ester oxygen at C-4 to the C-5 position.

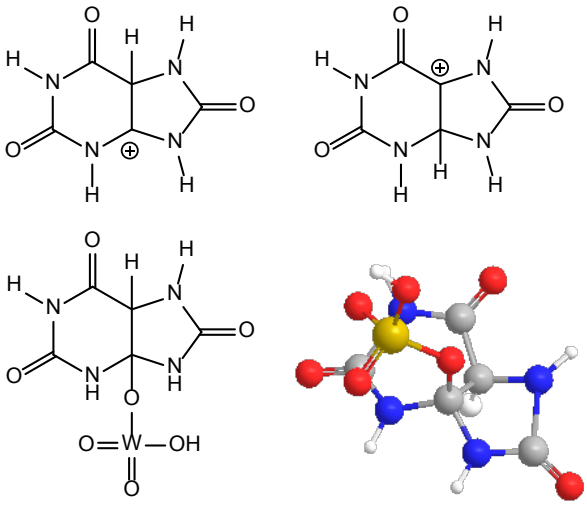

Figure 2. Uric acid preferred and discarded protonations, and tungstic ester (2D- and 3D structures)

The oxido-reduction step occurs by protolysis of the organometallic ester, resulting an oxonium ion and the hydrate of tungsten(IV) oxide, Figure 3.

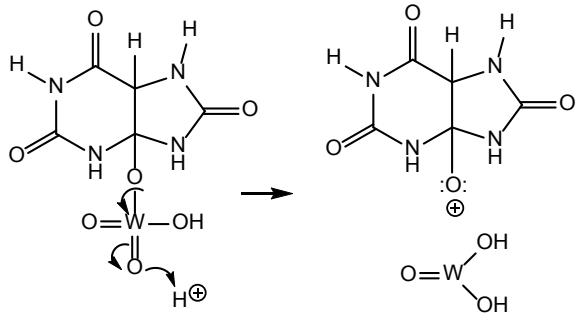

Figure 3. Ester protolysis, oxonium ion intermediate and tungsten oxide hydrate

The formed oxonium ion, a powerful electrophilic species breaks the pyrimidine ring, yielding an epoxide, as in other oxidations [23], and an isocyanate group in the resulting chain, Figure 4. Now the molecule is steric hindrance free as it can be seen in the 3D structure.

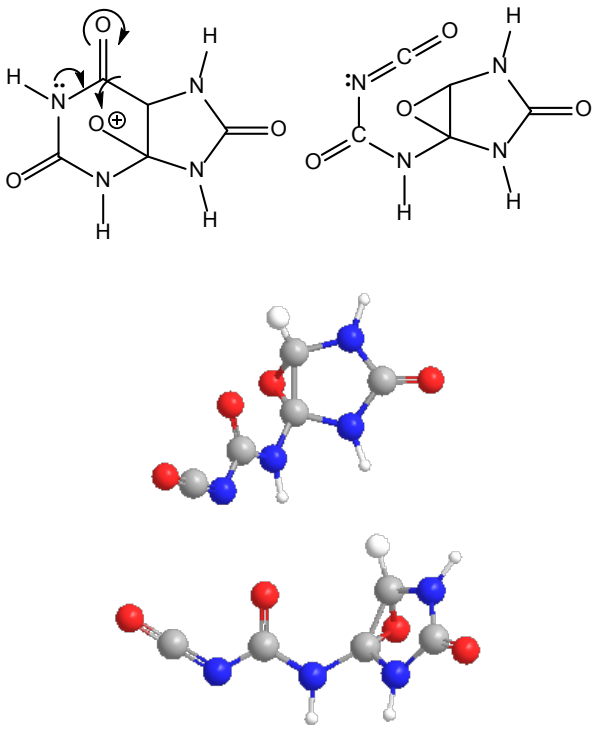

Figure 4. Epoxide formation and pyrimidine ring opening, with two 3D-structures of the resulting molecule

The breakdown is facilitated by the concerted electron contribution of the near nitrogen atom of the imido group in the pyrmidine ring. This group is not present in the fused 2-imidazolidinone ring and thus there is no electrodotic effect [24] from this heterocycle. A carbon-carbon breakdown promoted by an oxonium ion occurs in the oxidation of indigo to isatin [22].

The isocyanate is prone to hydration [25] and the unstable allophanic acid [26] undergoes decarboxylation, yielding an ureido radical at C-5 in the 2-imidazolidinone ring, Figure 5.

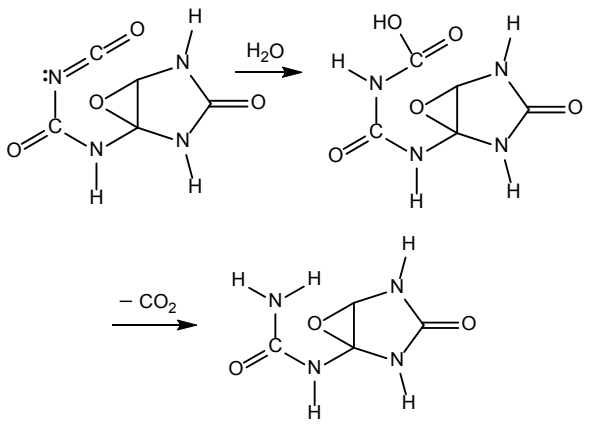

Figure 5. Hydration and decarboxylation of the intermediate isocyanate

Ring opening of the epoxide (protolysis) affords an alcohol and a carbocation. Neutralization forms an enol that rearranges to a carbonyl at C-4 in the heterocycle, affording an imido group, Figure 6.

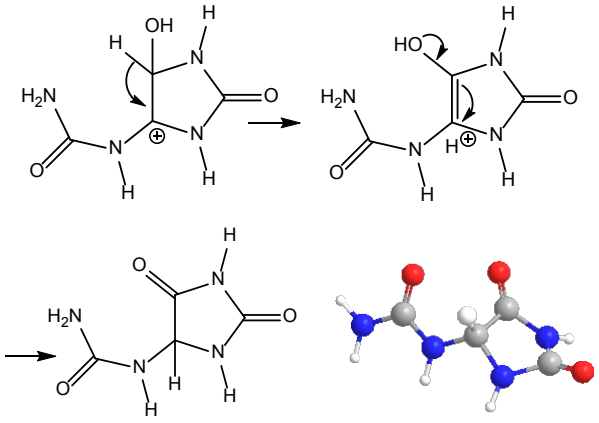

Figure 6. Steps after epoxide opening and end product (2D and 3D)

The indicated protonation yields a carbocation between two nitrogen atoms, whereas the alternative opening would form a carbonium ion vicinal only to one nitrogen atom.

The resulting product, 2,4-dioxo-5-ureido-imidazolidine, is commonly named allantoin or 5-ureidohydantoin, and has been obtained by very different methods, such as alkaline oxidation with potassium permanganate [27, 28] or by enzymatic procedures [29]. In these cases there is no reaction mechanism advanced.

Now let us take a look at the nature of the test blue colour. As we have seen above, the reduced tungsten derivative arising from the concomitant oxidation of the organic compound, is tungsten(IV) oxide hydrate, $WO(OH)_2$. Folin states that the reaction occurs in acidic medium but that the colour develops only after alkalinisation of the reaction medium. For this purpose, sodium carbonate is preferred in order to avoid precipitate formation, as occurs with potassium carbonate. Thus, the monovalent anion of $WO(OH)_2$ (tungsten subacid) splits off a hydroxyl group, giving WO_2, tungsten(IV) oxide (tungsten suboxide). This dioxide forms W_2O_5 and W_3O_8, products that have been described as tungsten blue, as mentioned in the previous section. The reactions are drawn in Figure 7.

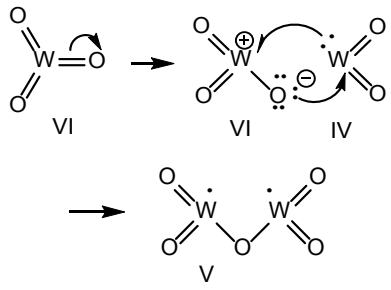

Figure 7. Formation of tungsten pentoxide from tungstic anhydride and tungsten dioxide

The other blue compound formed is W_3O_8 to which Latimer and Hildebrand give the structure $(WO_2)_2WO_4$. This is in agreement with the composition $(MoO_2)_2$ (MoO_4) given by Pesez and Poirier [30] for the blue Mo_3O_8.

The reactions are as follows:

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

Then transfer of one electron from a neutral molecule of tungsten dioxide (in Fig. 7) to the above oxycation gives two radicals WO_2^{+}.

Thus W_3O_8 is a salt, a tungstate anion and two oxycations with tungsten (V).

4. Conclusions

Although many colour tests are known since many years the reactions involved in them are frequently unknown. Sometimes there are hints, but no more. It is the theorist who unravels what is happening, step by step, in the reaction medium. This is the case with the Folin test for uric acid. What has been done is provide a complete reaction mechanism that involves both the organic chemistry of the tested substance and the inorganic chemistry related to the employed reagent, as well as the participation of the reaction medium.

The reaction series includes the following intermediates: an organometallic ester, an epoxide, an isocyanate, an allophanic acid, an alcohol and an imide.

The occurring reactions are: addition to double bond, mixed ester formation, protolysis, oxidative degradation involving epoxide formation and breakdown of the purinic pyrimidine ring, hydration, decarboxylation, a second protolysis, and isomerization.

As it can be seen, it is rather a complex process. However, the final product is in agreement with experimental results obtained with other oxidizing agents and different reaction mediums.

The provided mechanism was inferred from well known reactivities, i.e., from the chemical deportment of the involved functional groups (nomothetic explanation, by means of general laws or principles).

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