



## Insight into the chemistry of Folin's test for uric acid

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World Journal of Chemical and Pharmaceutical Sciences, 2024, 05(01), 001–004

Publication history: Received on 28 July 2024; revised on 05 September 2024; accepted on 08 September 2024

Article DOI: <https://doi.org/10.53346/wjcps.2024.5.1.0025>

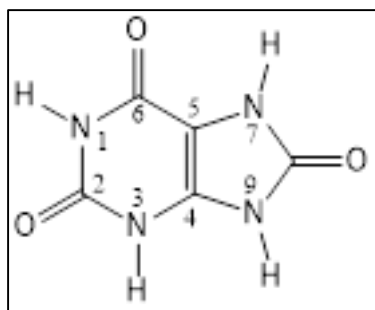
### Abstract

Folin developed an analytical technique for uric acid spectroscopic determination. It is based on the interaction of uric acid with sodium tungstate in phosphoric acid. This produces an intense blue colour after alkalization, whose concentration is measured. However, the structure of the organic product obtained was not mentioned. The present communication gives the references related to each reaction step, as well as information about the reaction products obtained in uric acid oxidations carried-out in acidic or in alkaline mediums. The reaction route is as follows: addition of tungstic acid to the C-C double bond, acidolysis of the organo-metallic ester, producing tungstic subacid and an epoxide. Oxirane opening to vicinal diol after reaction with water. Two carbinolamines result, which are broken by protonation at the nitrogen; carbonyl groups are formed at C-4 and C-5 with concomitant elimination of a urea molecule. This way alloxan monohydrate is formed. The blue products obtained are described.

**Keywords:** Acidolysis; Addition to double bond; Alloxan; Carbinolamine; Epoxide formation; Glycol; Tungsten blue

### 1 Introduction

Uric acid is an Analyte. Its clinical importance is well-known. So, its determination – quantitative analysis – is of utmost importance.



**Figure 1** Uric acid structure

Otto Folin developed a reagent that gives blue colour in the reaction of uric acid with sodium tungstate in phosphoric acid (chromogenic reagent). He emphasized his study in the concordance of the results with the known quantities of uric acid employed in the assays. The spectroscopical determination was based on the concentration of the pigment produced (tungsten blue). His study is centred exclusively in the analytical point of view, leaving apart the Organic

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Chemistry, that is, the chemical structure of the oxidized product. Not to mention any reaction route and less a reaction mechanism.

In this communication we present a new approach to this reaction, proposing a short, straight, reaction mechanism that is in accordance with the strong acidic medium. This study is a follow up of our studies on reaction mechanism, [1-5]

## 2 Antecedents

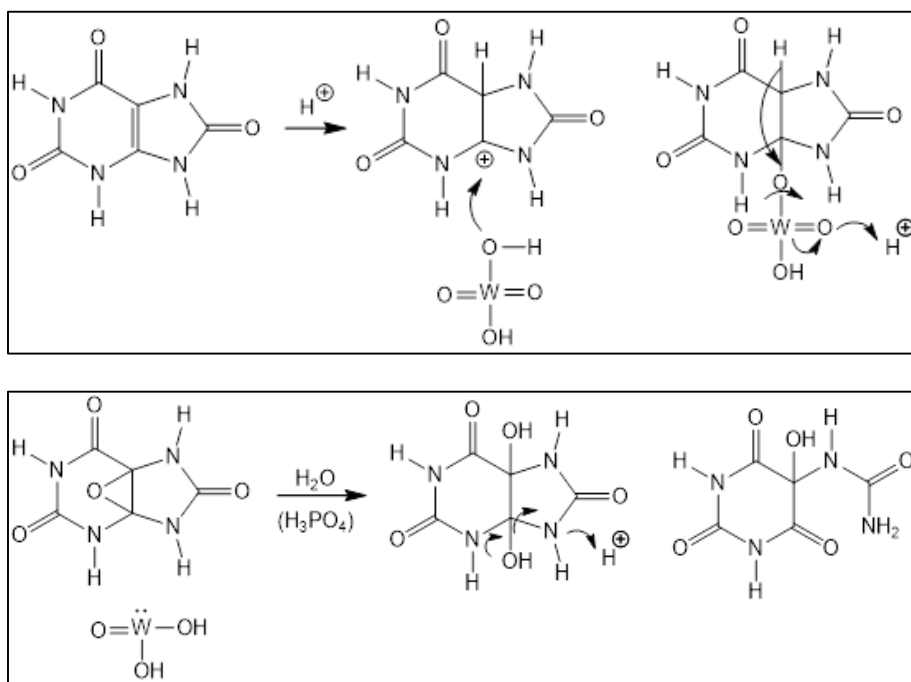
The test under study is due to Folin and Denis [6]. Folin was a German chemist working in the United States (Harvard University). As said before, the reagent for uric acid determination is sodium tungstate in phosphoric acid.

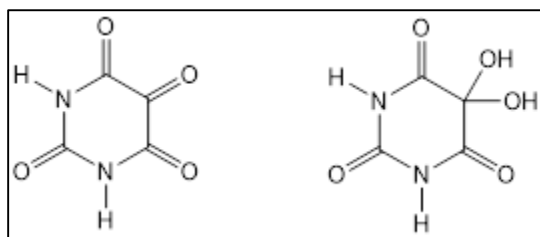
Sodium tungstate,  $\text{Na}_2\text{WO}_4$ , is a white, water-soluble solid, density 4.18. It is used as catalyst for epoxidation of alkenes, [7]. Its IUPAC name is disodium; dioxido(dioxo)tungsten. Sodium tungstate combines with hydrogen peroxide for the oxidation of secondary amines to nitrones, [8]. The commercial form is the dihydrate [9], and its aqueous solution is alkaline, pH 9-11. Commercial phosphoric acid contains about 80%  $\text{H}_3\text{PO}_4$ .

## 3 Discussion

The first steps in the interaction of uric acid with sodium tungstate in phosphoric acid, are formation of tungstic acid and protonation of uric acid double bond, Figure 2. This occurs at C-5, leaving a carbonium ion far from the carbonyl group at C-6. The carbocation is neutralised by a hydroxyl in the tungstic acid. Protonation of one double bond in the organometallic ester gives rise to a concerted reaction mechanism involving four electron shifts. Tungsten (VI) is reduced to tungsten (IV) oxide hydrate, and an epoxide is formed by loss of the hydrogen at C-5. See [7, 10]. This step discards the ring opening proposed previously, [11]. Then, the oxirane is opened by acid and the carbonium ion is neutralised by water (present in phosphoric acid and in tungstate dihydrate). A vicinal diol (glycol) is obtained. These hydroxyls are part of carbinolamines that are broken by acid (acidolysis), a urea molecule is eliminated and two carbonyls are formed (at C-4 and C-5) in the pyrimidine ring. That is, alloxan has been obtained (2,4,5,6-tetraoxo-hexahydropyrimidine). This oxido-degradation is in accordance with the fact that oxidation of uric acid in strong acidic medium yields alloxan or alloxan monohydrate [12, 13]. whereas alkaline oxidation yields allantoin (5-ureido-hydantoin), [14].

Now let's see the colouring matter. Acidified tungstates when reduced give blue pigments and colourations, [15]. When the acidified tungstate solution is reduced with hydrazine, hydrogen sulphide, or other reagents, an amorphous intense blue product is obtained, [16].





**Figure 2** Route of the interaction of uric acid with tungstate in phosphoric acid

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

As we have seen above, the reduced tungsten derivative obtained is tungsten (IV) oxide hydrate. Folin states that the reaction occurs in acidic medium but that the colour develops only after alkalization of the reaction medium with sodium carbonate. Thus, the monovalent anion of  $WO(OH)_2$  (tungsten sub acid) splits off a hydroxyl group, giving  $WO_2$ , tungsten ate. (IV) oxide (tungsten suboxide).

$W_2O_5$  is combination of  $WO_2$  (tungsten suboxide) with  $WO_3$  (tungstic anhydride) who is derived from mono-sodium tungstate. The structure of  $W_2O_5$  is  $O_2W-O-WO_2$  with a lone electron in each tungsten atom.

$W_3O_8$  comes from  $W_2O_5$  plus  $WO_3$ . It is a salt,  $2 WO_2^+ WO_4^-$ .

#### 4 Conclusion

The route of the interaction of uric acid with tungstate in phosphoric acid has been established. It comprises addition to C-C double bond, acidolysis of organometallic ester reduction of tungsten (VI) to tungsten (IV), epoxide formation, ring opening to glycol, and protolysis of carbinolamine. The final product is alloxan monohydrate, 2,4,5,6-tetra-oxo-hexahydropyrimidine monohydrate.

#### Compliance with ethical standards

##### Acknowledgments

Thanks are given to Luz Clarita for support.

##### Disclosure of conflict of interest

There is no conflict of interest to declare.

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