

(RESEARCH ARTICLE)



The chemistry of Heller's test for urine Indican detection

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Abstract

The Heller's color test for indican in urine is interesting because besides detecting this important biomarker it reveals the dual function of nitric acid since it can accomplish the synthesis of indigo blue and indigo red, as well as it can degrade them to isatin, depending upon the reaction conditions. Moreover, the Heller's test inspired other tests for indican detection, using other oxidizing reagents. Actually, indican in urine is detected by a colour chart with different tones of blue indicating the concentration of indican via indigo blue formation. In this paper we provide the series of reactions that occur during the test, there are nitrations followed by redox reactions. The organic intermediates are oxidized and the inorganic reagent is reduced to nitrous acid. However, indigo blue and indigo red can be degraded by nitric acid on heating. The electron flow is given in each step.

Keywords: 2; 3-Dioxindoline; Indigo Blue; Indigo Red; Indigoids Degradation; Indoxyl Sulphate; Ipso Attack

1. Introduction

A test for indicanuria is important since high indican level can be due to hypochlorhydria, inhibited peristaltic movement or poor production of bile. Urine indican increases with high protein diets or inefficient protein digestion, [1]. Other studies on indican are related to diabetes mellitus, [2]. There is an interesting article on the value of estimating urinary indican, [3]. Heller gave the name uroxanthin to the yellow compound which later was identified as indoxyl sulphate, physiological indican, Figure 1.

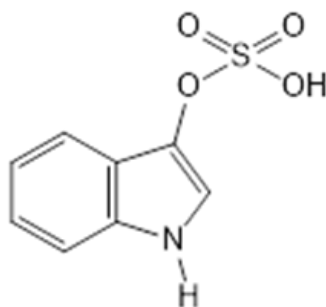


Figure 1 Urinary indican

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Treatment with nitric acid produced two new pigments: one blue and red the other, that is, the indigoids derived from plants. In this communication we describe the reactions leading to these compounds from urine indican. This is a follow up of our studies on reaction mechanism, [4-8].

2. Antecedents

The test under study is due to Johann Florian Heller (1813-1871), Czech physician and chemist working in Vienna, [9]. He edited the Archive for physiological and pathological chemistry, known as 'Heller's Archiv', [10]. He is considered as one of the founders of modern clinical chemistry.

Heller gave the name of uroxanthin (indican) to a yellow substance found in small quantity in healthy urine but found in large quantities in diseased urine. The action of acids produces two new pigments: uroglaucin (indigo blue) and urrhodin (indigo red). These pigments are occasionally found in the sediment of abnormal urine. According to Heller they are products of the oxidation of uroxanthin, [11].

The test is as follows: 5 ml of hydrochloric acid (d. 1.19) are mixed with 2 ml of urine then add 5 drops of strong nitric acid and heat to boiling. In the presence of indican a beautiful violet colour is produced, which at first plays into a blue, but afterwards move into a red, [12].

This colour change is interesting since indigo red is formed through more steps, as we will see in the 'Discussion'.

To separate the two oxidation products, the sediment thrown down by nitric acid is collected. Extraction with ether takes up urrhodin (indigo red), and evaporation leaves rosy-red granules, which can be crystallized by very slow evaporation of an alcoholic solution. The crystals are nearly black, but thin layers are carmine-red.

The residue from the extraction is boiled with alcohol from which uroglaucin (indigo blue) is obtained as blue powder. Under the microscope, fine pointed needles are observed, in star-like form. It may be sublimed.

Carter used a variant of Heller's test for indican he employed nitric acid and sulphuric acid, [13].

Fordos named these coloured compounds as urocyanose and uro-erythric acid, [14. 15]. The latter term is interesting because the acidic property of the compound is due to the lactim form present in indirubin (indigo red).

3. Discussion

The first step in Heller's test for indican is the acid hydrolysis of indoxyl sulphate. The liberated 3-hydroxyindole is nitrated at C-2, giving 2-nitro-3-indolinone, Figure 2, a, b. Protonation of the zwitter ion at the nitro group connects the electrodotic [14] property of N-1 with a good leaving group, c. Thus, 3-oxo-indolenine and nitrous acid are formed, d. The first is the key intermediate for leucoindigo.

Other reaction path is aromatization before nitrous acid detachment, this permits a second nitration either at C-2, ipso attack, Cf. [15], or preferably at C-3, e, due to the electron donor effect of the enamine and less steric hindrance compared to the presence of the nitro group at C-2. However there can be four nitro groups in a single carbon atom as in tetranitromethane. Protonation of the nitro group at C-3 favours elimination of nitrous acid with concomitant ketone formation, f, g.

Acid hydration of 2-nitro-3-oxo-indolenine, g, produces isatin by a five member concerted mechanism, h, i.

The 2,2-dinitro intermediate, j, can also yield 2-nitro-3-oxo-indolenine, g, by nitrous acid loosening, j, and continue to isatin as before, g, h, i

Reaction of 3-oxo-indolenine with indoxyl gives leucoindigo, Figure 3. Nitration of one unit of this bis-indole compound, is followed by nitrous acid loss yielding indigo blue.

Indigo red results by dehydration of isatin and indoxyl via reaction between this and the keto group at isatin.

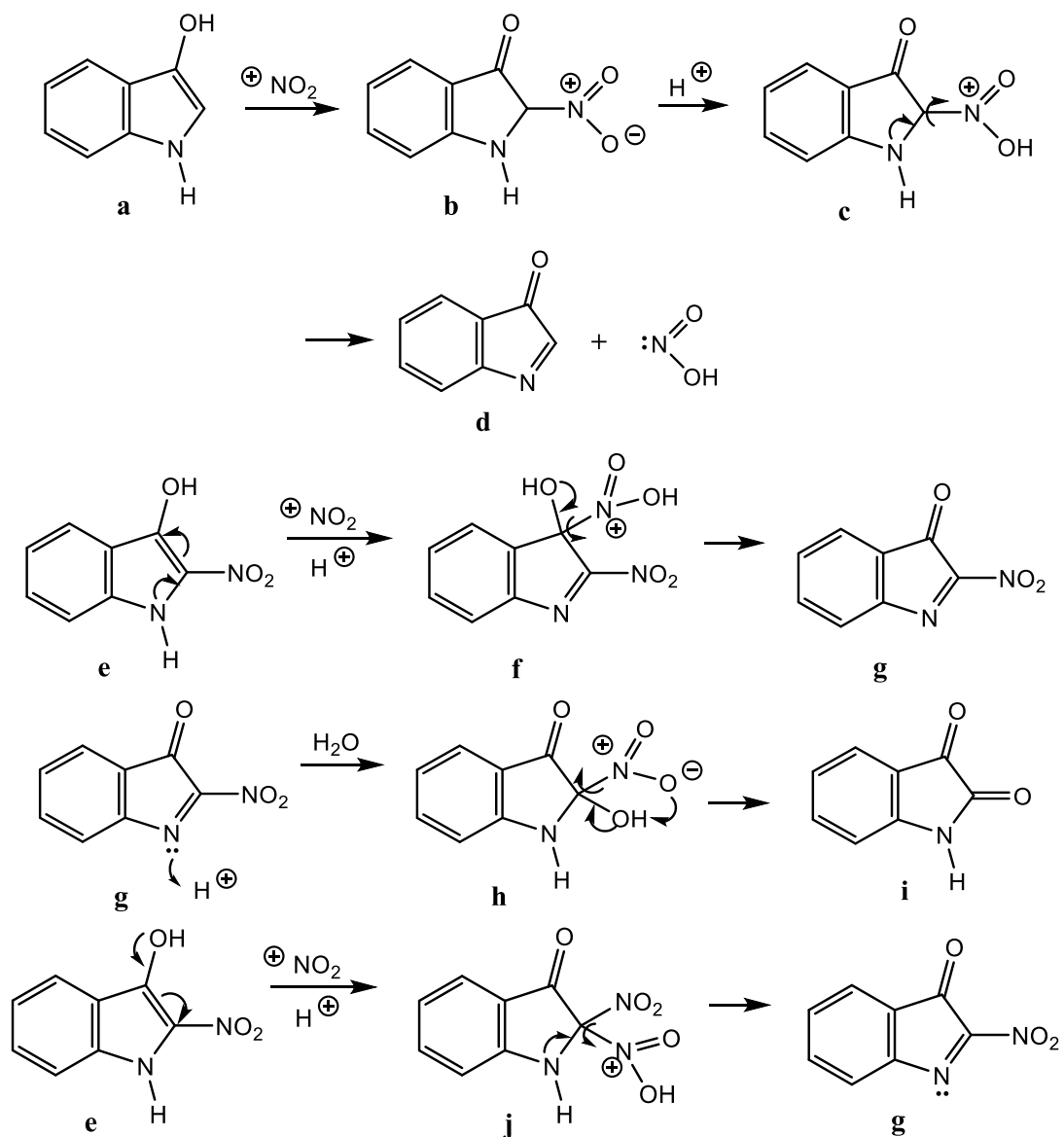
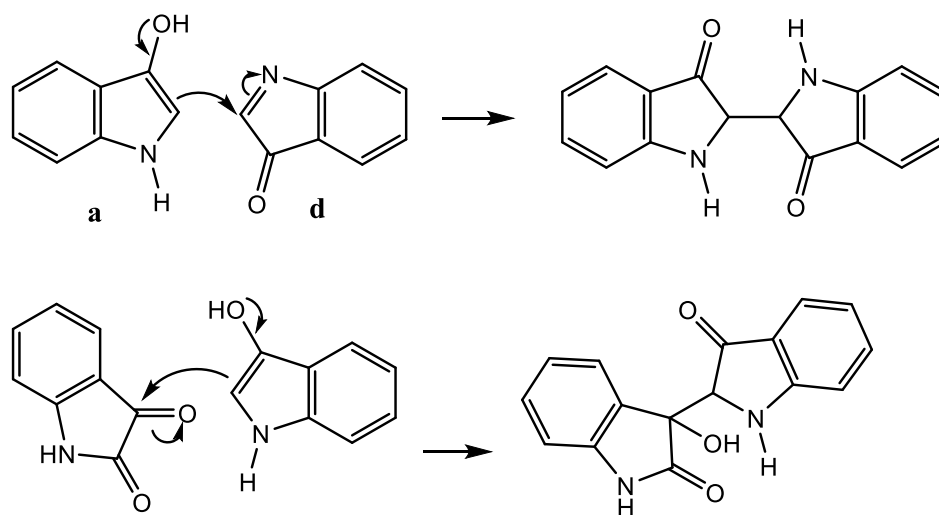


Figure 2 Route to the key intermediates in Heller's Test



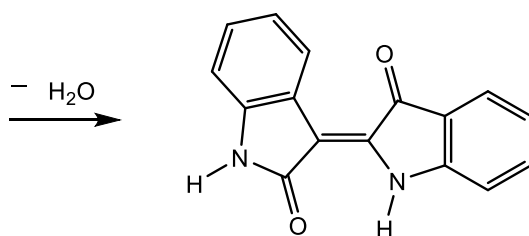


Figure 3 Formation of indigo blue and indigo red

Laurent in France obtained isatin by heating slurry of indigo with nitric acid [16, 17], Figure 4. The attack of nitronium ion to the double bond is due to the higher temperature, not to the concentration of the acid. So, it is a case of improving the impact for reaction. The degradation occurs by addition of nitro and hydroxyl groups to the double bond, initiated by a nitronium ion followed by reaction with water. Protonation of the nitro group causes epoxide formation with concomitant nitrous acid release. Protonation of the epoxide, ring opening and addition of a nitrate ion yields an alcohol and a nitrate. Protonation of the dipole originates nitrous acid separation, C–C fission and two independent keto groups are formed, that is two molecules of isatin result.

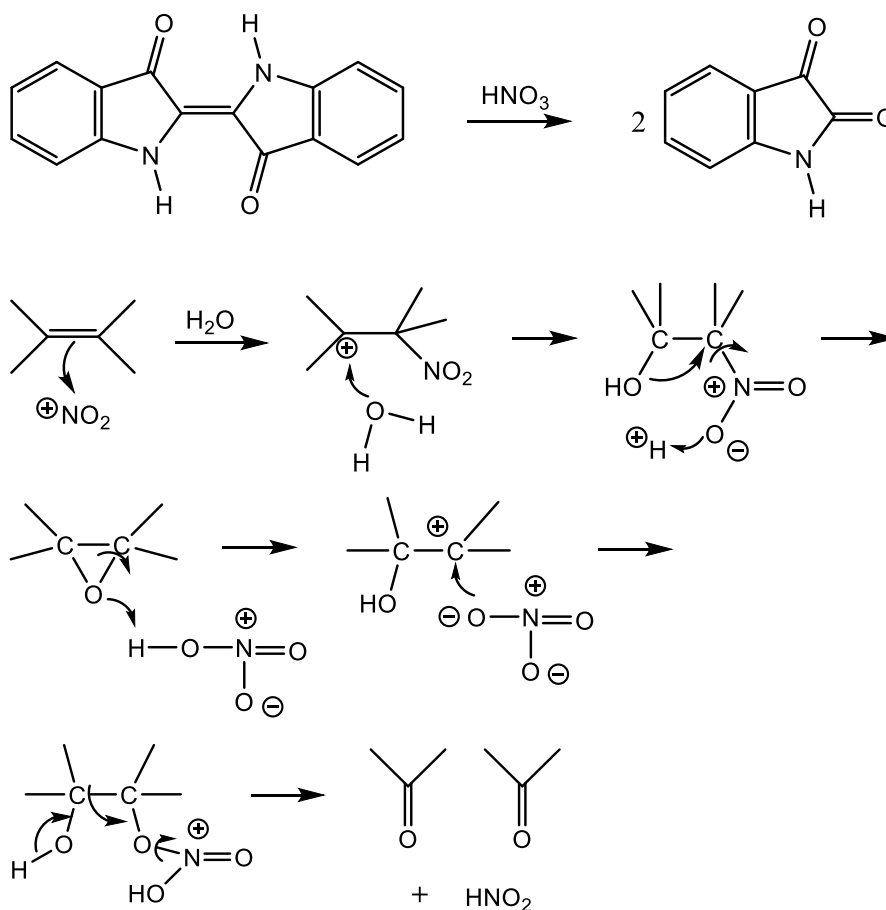


Figure 4 Degradation of indigo by means of nitric acid

4. Conclusion

In Antecedents Section we reviewed the pioneering medical-and chemical studies on the presence of indican in urine, as well as its relationship with medical disorders. The detection of indican was based on its chemical transformation into two colorful compounds to which Greek terms were assigned accordingly to their colour and its origin: uroglaucin for the blue and urrhodin for the red one. Other names are urocyanose and uroerythric acid. Yellow indican was designated uroxanthin. In this way indigo blue and indigo red were obtained, not from vegetal source but by swift

synthesis using nitric acid. There are nitrations, oxidation of the substrate, and reduction of the reagent, via acid catalysis and elimination reactions, as is detailed in the 'Discussion' section of this Theoretical Organic Chemistry Study.

We provided the reactions that occur during Heller's test, as well as the degradation steps of indigo in other reaction conditions. The electron flow was given in each step.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest among the authors or any other person.

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