Open Access Research Journal of
Chemistry and Pharmacy
Journals home page: https://oarjpublication/journals/oarjcp/
ISSN: 2783-0276 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

The chemistry of Klett's test for urinary indican

Francisco Sánchez-Viesca* and Reina Gómez

Department of Organic Chemistry, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City (CDMX), Mexico.

Open Access Research Journal of Chemistry and Pharmacy, 2022, 02(01), 010–013

Publication history: Received on 22 April 2022; revised on 18 June 2022; accepted on 20 June 2022

Article DOI: https://doi.org/10.53022/oarjcp.2022.2.1.0021

Abstract

Urinary indican is a biomarker. This analyte can be detected by a series of oxido reduction reactions after acidolysis of indoxyl sulfate. Then the key intermediates condense to afford indigo blue and indigo red, the latter in less proportion. In his test for indican Klett used ammonium persulfate in acidic medium, the reactive species being the powerful oxidant peroxydisulfuric acid. Since the reaction series occurring in this test has not been advanced we provide it as well as the reaction mechanism with the electron flow. This test has both pharmacological and chemical interest. Its mechanism is sui generis, for instance there is desulfation at one position and then sulfation in other site, and a symmetrical reagent is reacting as a rather polarized one.

Keywords: Acidolysis; Indigo Blue; Indigo Red; Indoxyl Sulfate; Peroxydisulfuric Acid; Redox Reactions

1. Introduction

Biological indican is absorbed into the blood from the intestines and eventually appears in the urine. If the stomach acid is not adequate, if there is fail to digest protein, or if the diet does not supply sufficient fiber, the resultant overgrowth of unfavorable bacteria can release toxic products, such as indican, that the body must remove, [1].

Thus the tests for indicanuria are very important. Besides the detection of this biomarker, the Klett test is interesting because the use of ammonium persulfate in acidic medium follows a sui generis reaction route due to the peroxo group. The structure of indican is in Figure 1.

This communication is a follow up of our studies on reaction mechanism, [2-6].

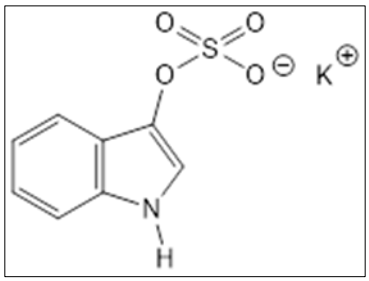

Figure 1 Graphic formula of indoxyl sulfate potassium salt

*Corresponding author: Francisco Sánchez-Viesca
Organic Chemistry Department, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City (CDMX), Mexico.

2. Antecedents

The test under study is due to A. Klett, Czech chemist working in Imperial Austria. He published his test in the Chemiker Zeitung [7] and it was registered in England [8], Germany [9], the United States [10] and Italy [11].

The test is as follows**:** To 10 ml urine add 5 ml 25% hydrochloric acid and a crystal of ammonium persulfate and shake with a little chloroform. The latter is colored blue if any indican is present.

There is a series of papers related to indican in urine, [12].

Peroxydisulfuric acid, $H_2S_2O_8$, is a colorless solid and one of the most powerful peroxyacid oxidant available. It can be generated from potassium or ammonium persulfate in acidic solution, as in Klett test. It is an anhydride of sulfuric acid and persulfuric acid (Caro's acid, H_2SO_5). Peroxydisulfuric acid may be prepared by the reaction between cold concentrated sulfuric acid and concentrated hydrogen peroxide, [13].

The first reaction with urinary indican is the neutralization of the anion in indoxyl sulfate potassium salt followed by an acidolysis. There is a paper on the mechanism of the acid hydrolysis of sodium aryl sulfates [14] but there is no acid in the key step of an acidolysis. A more recent paper refers to uncatalyzed hydrolysis of aryl sulfate [15]. Other communication is related to choline sulfatase, [16]. In the next section we provide a coherent mechanism for the acid hydrolysis of indoxyl sulfate, the urinary indican.

3. Discussion

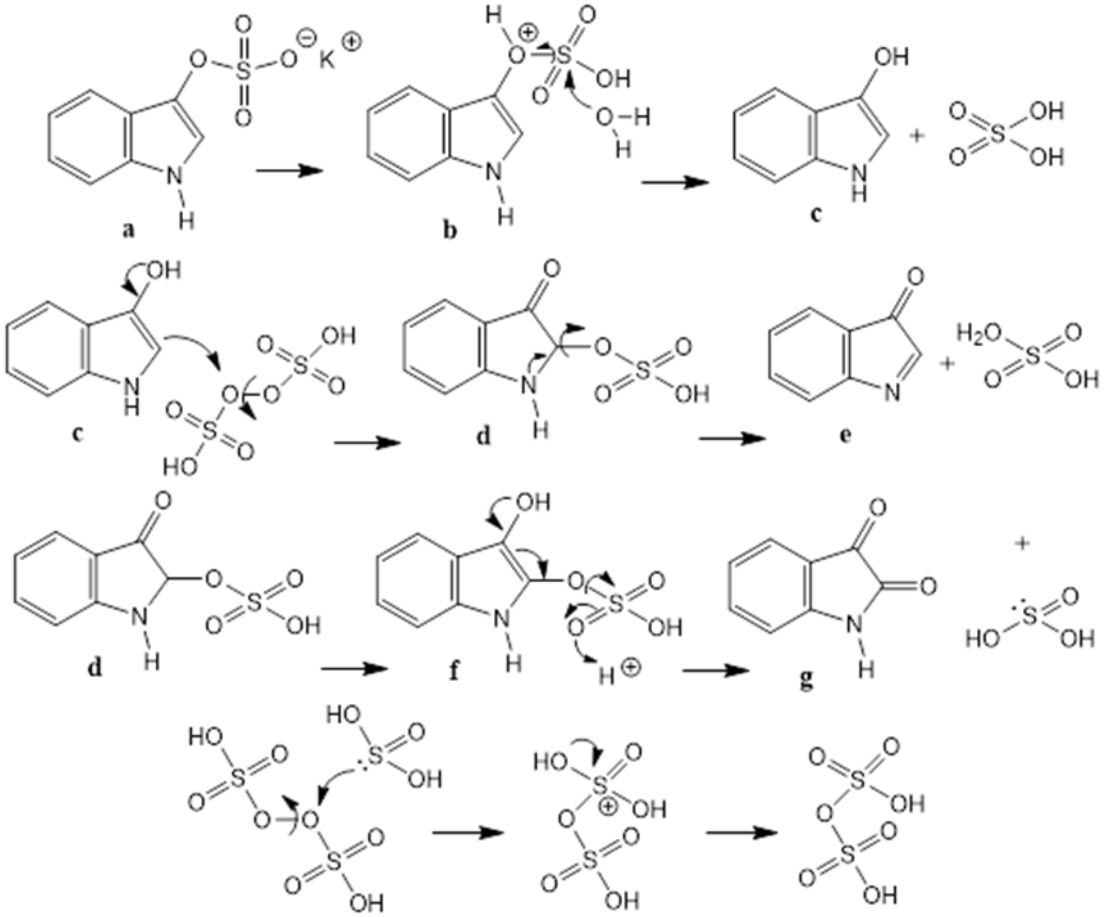

Figure 2 Formation of 3-oxo-indolenine and isatin intermediates for indigo blue and indigo red.

In the Klett test for indican in urine a series of reactions occurs. In acidic medium the anion of the potassium salt is neutralized. Then the acidolysis of the sulfate ester occurs by protonation of the oxygen linked to carbon. Figure 2, **a**, **b**. This produces an inductive effect towards the oxygen, increasing the δ+ at sulfur (proper of the sulfuril group). This fact

enhances a nucleophilic attack at sulfur by a water molecule, with concomitant S—O cleavage, yielding indoxyl and sulfuric acid, **c**.

The ammonium persulfate in hydrochloric acid forms peroxydisulfuric acid, $H_2S_2O_8$, **c**. The peroxo group presents a chemical deportment similar to the observed in the also symmetrical chlorine molecule. In a nucleophilic reaction one atom reacts as chloronium ion and the other is released as chloride ion, [17, 18]. Thus the electron donor indoxyl reacts with an electrophilic oxygen atom at peroxydisulfuric acid. There is sulfation at 2-position with simultaneous sulfate ion detachment, **c**.

The electrodotic [19] property of N-1 eliminates a sulfate ion, oxidation step, and 3-oxo indolenine is formed, **d**, **e**. This is a key intermediate for leucoindigo.

An alternative reaction is enolization prior to sulfate elimination**:** protonation of the sulfate ester produces a redox reaction by a concerted six member mechanism, **f**, the reaction products are isatin and sulfurous acid, **g**, the latter reacts with peracid and water furnishing sulfuric acid and pyrosulfuric acid, $H_2S_2O_7$.

Reaction of 3-oxo-indolenine with indoxyl yields leucoindigo, Figure 3. Sulfation of one unit of this bis-indole intermediate, followed by H_2SO_4 elimination affords indigo blue: (2E)-Δ-2,2'-biindole-3,3'-dione.

Indirubin (indigo red) results by combination of isatin and indoxyl, that is, water elimination from the keto group of the first and the methylene group of ψ-indoxyl [20]. Indirubin is 2-(oxindol-3-ylidene)-indoline-3-one.

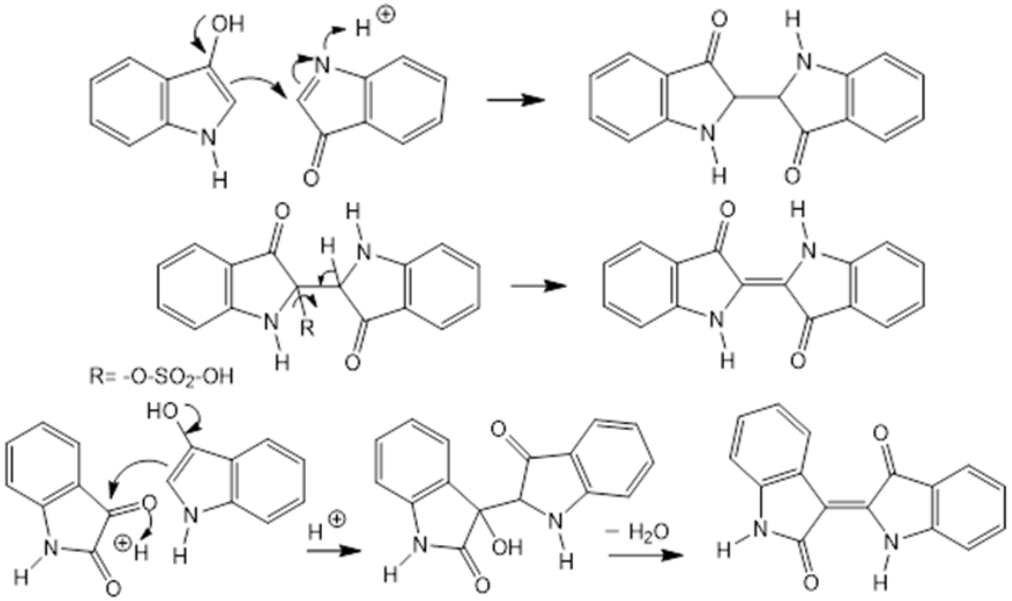

Figure 3 Condensations towards indigo blue and indigo red (indirubin)

4. Conclusion

A coherent mechanism for the acidolysis of indoxyl sulfate was provided as well as a description of the chemical deportment related to the series of reactions that takes place during Klett's test for indican in urine. The reactive species that intervene in the sulfation at C-2 in indoxyl is the peroxydisulfuric acid generated in situ from ammonium persulfate in the presence of hydrochloric acid. Then are oxido-reduction reactions that conduce to 3-oxo-indolenine and isatin which are intermediates for the obtention of indigo blue and indigo red. These compounds give the coloration observed in the test.

Compliance with ethical standards

Acknowledgments

Thanks are given to Martha Berros for support.

Disclosure of conflict of interest

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

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