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The chemistry of Obermayer test for indican in urine

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Abstract

The purpose of this article is to provide the reaction mechanism of the process occurring during the Obermayer colour test for indican in urine. This test differs from cognate tests because no chemical oxidant is used in order to oxidise 3-hydroxy-indol to indigo blue, but exclusively fuming hydrochloric acid is employed. From this fact it is deduced the intervention of aerial oxygen. The approach to the chemistry of this test is based on the chemical deportment of reagent and substrate, in union with cogent reasons (Theoretical Organic Chemistry). The proposed intermediates show a parallelism with those suggested in alkaline medium during the obtention of indigo blue from natural source. However, the formation of indigo red (indirubin) in acidic medium goes through different intermediates and the reaction mechanism is given here for the first time.

1. Introduction

There are two kinds of indican, one is the natural product isolated from *Indigofera tinctoria*, and it is the glucoside of indoxyl (3-hydroxyindole). The other type of indican is physiological indican, which is the sulpho derivative of indoxyl, and it is found in urine, [1]. Figure 1.

A test for indicanuria is important since high indican level can be due to hypochlorhydria, inhibited peristaltic movement, or poor production of bile, etc. [2].

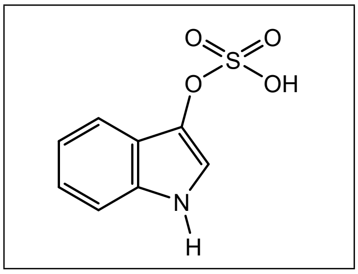

Figure 1. Physiological indican

Obermayer provided a colour test for urinary indican detection using fuming hydrochloric acid. A blue colour is observed in a chloroform extract due to formation of indigo, [3]. This test is very important because several theoretical points related to the chemistry and mechanism of indigo are undoubtedly cleared from it, as we will see in the 'Antecedents'.

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This communication is a follow up of our studies on reaction mechanism, [4-8].

2. Antecedents

Two important tests for urinary indican detection are due to two eminent chemists.

One test is due to Johann Heller. He used a mixture of hydrochloric acid, urine, and strong nitric acid. After heating to boil, a violet colour is produced in the presence of indican, [9]. In this test nitric acid is the oxidizer, the reactive species is nitronium ion, and the reduced compound is nitrous acid, [10]. So, there is no need of aerial oxygen as oxidant, as in the preparation of indigo from natural source, [11].

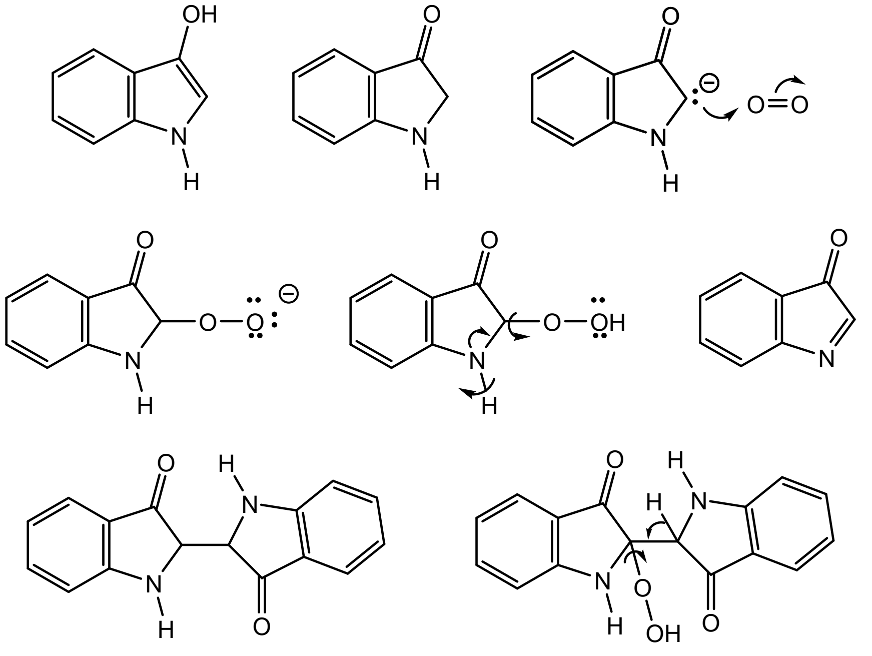
The other test for urinary indican is due to Olof Hammarsten. He employed chlorinated lime added to strong acidulated urine containing indican. A blue colour is developed, [12]. In this test hypochlorous acid generated in situ is the reactive species and oxidant. Almost all the intermediates leading to both indigo blue and indigo red (indirubin) are chlorinated compounds.

In the Obermayer test for indican in urine, no chemical oxidizer is employed, [13]. Since there is oxidation of indoxyl to 3-oxo-indolenine, and also to isatin (2,3-dioxo-indoline), the oxidant must be aerial oxygen. The ample availability of oxygen in the short time of a colour test, is surprising. The preparation of indigo from natural source requires mechanical aeration, although in big batches.

On the other hand, the use of fuming hydrochloric acid indicates that an ionic process is ongoing, instead of a free radical mechanism as proposed in the past [14] and still in use in a journal of chemical education, [15].

3. Discussion

After acid hydrolysis of the sulphate in indican, the phenol reacts with water [16] giving the phenolate whose electrodotic property [17] to C-2 makes it react with oxygen forming a peroxide which is neutralised by acid to the hydroperoxide. A kind of hemiaminal results. Figure 2.



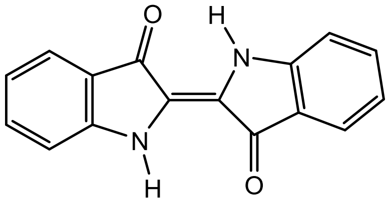


Figure 2. Synthesis of indigo blue in acidic medium

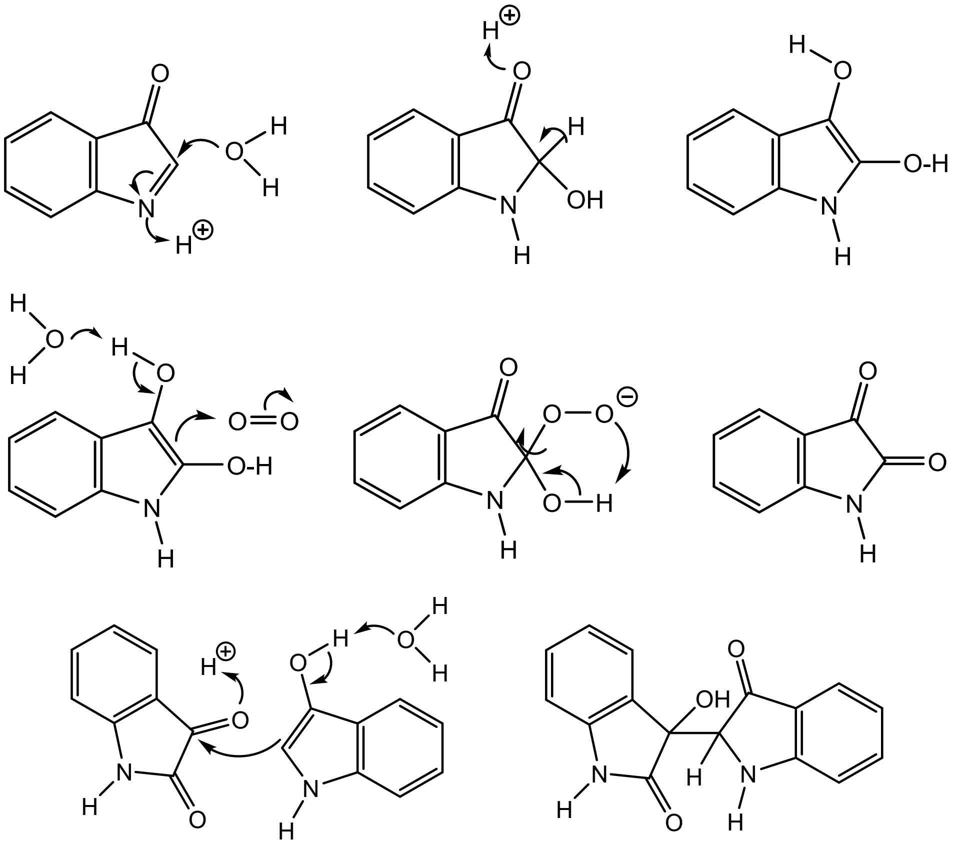
The nitrogen lone pair of electrons form a double bond with concomitant elimination of a hydroperoxide anion. This way 3-oxo-indolenine is obtained.

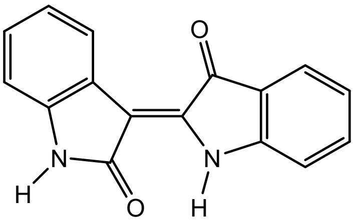
Before this elimination is complete, there is also isomerization to the phenolic structure. Thus, 2-hydroperoxy-indoxyl reacts with the imino group of 3-oxo indolenine via a push-pull mechanism, that is, the electron donor phenolate and the protonated imino group in the indolenine (acceptor moiety). This leuco-indigo derivative forms indigo blue by acidolysis, eliminating hydrogen peroxide.

Indigo red (indirubin) can also be formed by this method, and it would give a violet colouration. However, this colour is not observed because indigo red is a secondary product since its formation involves more steps and probably more indican is needed and also more reaction time than in a colour test.

The formation of indigo red involves acid hydration of the imino group in 3-oxo-indolenine, isomerization to phenol and reaction with oxygen at C-2, via double reaction at ipso carbon. Compare [18]. In the resulting five member intermediate there is proton caption followed by carbonyl formation with concomitant elimination of hydroperoxide anion (synchronous mechanism). This way isatin is formed, 2,3-dioxo-indoline, Figure 3.

The last steps to indigo red are reaction of indoxyl with the protonated keto group of isatin and acid dehydration of the intermediate alcohol. All these steps for indirubin synthesis are proper of acidic medium.





Figures 3. Synthesis of indigo red (indirubin).

The last steps to indigo red are reaction of indoxyl with the protonated keto group of isatin and acid dehydration of the intermediate alcohol, Figure 3. All these steps for indirubin synthesis are proper of acidic medium.

4. Conclusion

In the Obermayer test there are not chemical oxidants in order to oxidise indoxyl to indigo as in the Heller and Hammarsten tests for indican in urine. Fuming hydrochloric acid is employed alone. Thus, oxidation must occur through the participation of aerial oxygen. This is surprising since a colour test is brief and the oxidant is a gas and therefore it is dispersed. The preparation of indigo from natural source also employs aerial oxygen but there is removal of the reaction mixture for oxygen trapping. The alkaline medium is more proper for the electrodotic participation of a phenoxide. However, the strong acidic medium worked satisfactorily. There is parallelism in the key intermediates in both mediums leading to indigo blue, but it is not the case with indigo red.

5. Conflicts of Interest

There are no conflicts to declare.

6. Acknowledgement

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