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On the Mechanism of Indirubin Formation in the Baeyer-Emmerling Synthesis

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Abstract Indigo red, the co-product formed in the Baeyer-Emmerling indigo synthesis, has been synthesized by two different methods. One is from Baeyer and the other is due to Wahl. The last procedure has been improved afterwards. However, both methods employ reactants that are absent in the Baeyer-Emmerling synthesis. Thus, the way by which indirubin (indigo red) is formed in the reaction medium of the original synthesis is unknown. We have cleared this and also why this co-product is always present in this synthesis. Baeyer intended a regioselective synthesis of indigo blue, but many years later biological activity was found in the co-product and now this important compound is used in cancer treatment.

Keywords Indigo blue, Indigo red, Indirubin, Purpurin, Reaction mechanisms, Reactive intermediates

1. Introduction

The first synthesis of indigo was accomplished by Baeyer and Emmerling, and Baeyer improved it for ten years. It has been updated by us in a critical review [1].

The present communication is devoted to the co-product that is formed in this synthesis. An unexpected fact was the presence of another coloured product (indigo red) which first was termed indigo purpurin and years later Baeyer named it indirubin. This co-product troubled Baeyer since he desired obtain indigo blue as the sole product.

Natural indigo, isolated from different plants, also contains the red isomer. The beneficial properties of indigo were discovered by Chinese Medicine, and afterwards it was cleared that the red component was the actual responsible for the medicinal properties. Actually indirubin is used in cancer treatment [2, 3].

There are two syntheses of indirubin, one is from Baeyer and the other is from Wahl. However, in both syntheses are used alien reactants that are not present in the Baeyer-Emmerling procedure.

Now we present two routes showing how indirubin is formed in the Baeyer-Emmerling synthesis, and in the subsequent modifications of it. The provided reaction mechanisms not only explain the formation of indirubin, but also why indigo red is always produced as a companion of indigo blue in this synthesis, and in a minor proportion.

2. Antecedents

Baeyer and Emmerling achieved the first synthesis of indigo [4, 5]. This is remarkable because at that time the structure of indigo was unknown. They knew that treatment of natural indigo with nitric acid gave an oxidation product, isatin, whose chemical structure had been advanced by Kekulé just one year before and was not yet well established.

Baeyer attempted the reverse reaction, isatin reduction. However, other products than indigo were obtained, depending on the employed reagents and the raction conditions. So, Baeyer and Emmerling treated isatin with phosphorus trichloride with the purpose of increasing the reactivity of the resulting chloro derivative. The reaction is based on the lactam-lactim isomerism, the phosphorus halide acting on the imidol. We provide the reaction mechanism in Figure 1.

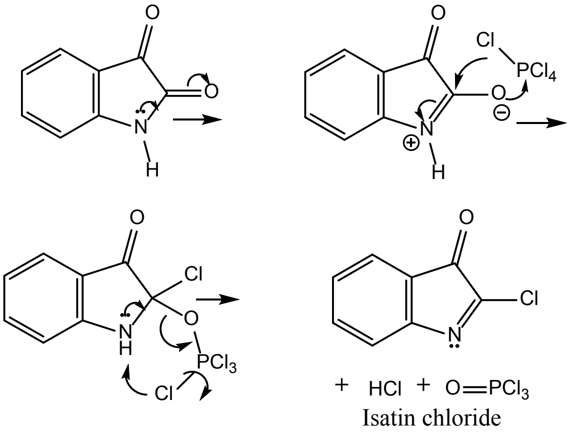

Figure 1. Preparation of isatin chloride from isatin and phosphorus pentachloride via the amphion

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The obtained imidoyl chloride was reduced in situ, in a one-pot experiment, with yellow phosphorus.

An unexpected fact was the concomitant presence of a red coloured compound besides indigo blue. They termed the co-product indigo purpurin (indigo red).

Baeyer was interested in the obtention of indigo blue, so the presence of another product, always as a companion of the desired compound was annoying. He devoted several years to improve the synthesis, and the yields were increased but the purpurin was always present in smaller or larger quantities [6-9], even though other reagents were used in the reduction step, such as zinc-acetic acid or hydrogen iodide.

Indirubin has been synthesized by two methods. Baeyer [10, 11] obtained it from indoxyl (3-hydroxyindole) and isatin in alkaline medium (sodium carbonate). The reaction is given in Figure 2.

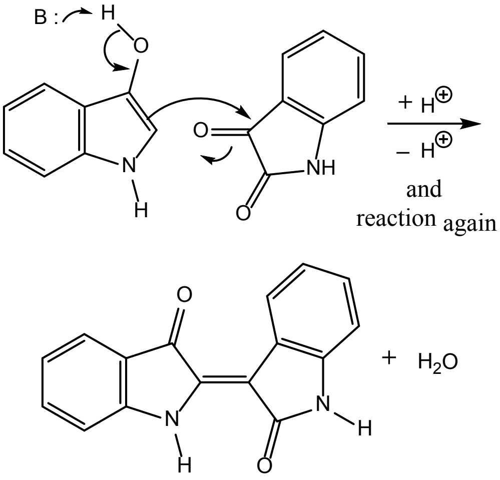


Figure 2. Baeyer's indirubin (indigo purpurin) synthesis

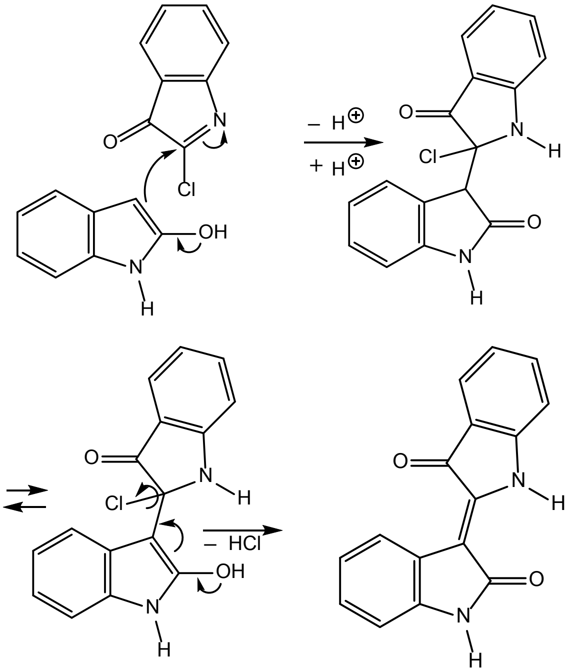


Figure 3. Wahl's indirubin synthesis from oxindole as 2-hydroxyindole

Years later in France, Wahl prepared indirubin by reaction of oxindole (indolin-2-one) with isatin chloride (2-chloroindolenin-3-one) in boiling benzene [12, 13].

There are experimental improvements to this synthesis [14]. The reaction mechanism is in Figure 3.

However, neither indoxyl nor oxindole are present in the Baeyer-Emmerling indigo synthesis.

3. Discussion

Now let's see if indoxyl or oxindol used in the previous syntheses can be formed in the Baeyer-Emmerling reaction medium.

Indoxyl (3-hydroxyindole or indolin-3-one) is discarded since it would come from partial reduction of isatin, the starting reactant. However the more reactive group in isatin is the ketone, not the lactam. For instance, Baeyer reduced isatin with zinc and dilute hydrochloric acid and obtained dioxindole (3-hydroxyoxindole), which he named hydroisatin and gave it the formula of enediol [15, 16], Figure 4.

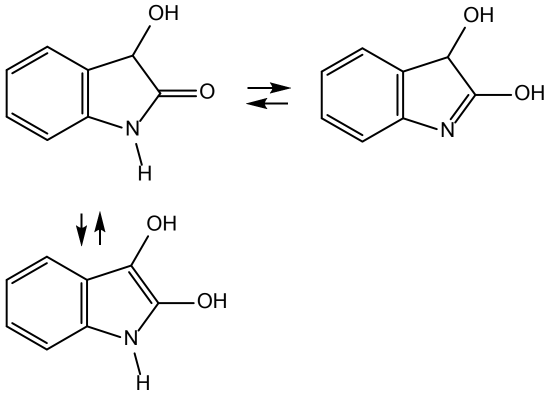


Figure 4. Isomeric structures of dioxindole

The second indirubin synthesis, that of Wahl, requires oxindole, also absent in the Baeyer-Emmerling synthesis. Its presence would imply carbonyl deoxygenation of isatin and it cannot be accomplished by zinc-acetic acid. Baeyer obtained oxindole by isatin reduction with tin and hydrochloric acid or with sodium amalgam [17, 18].

Besides, in the two steps procedures realized after the initial one-pot experiment we cannot take isatin into account since the second step was the reduction of isatin chloride obtained from isatin. If the chloride contained some unreacted isatin, this residue cannot be sufficient to form the important co-product.

Thus indirubin must be formed from isatin chloride and the reaction intermediates resulting from the reduction reaction.

Baeyer reported that isatin chloride reduction with zinc and acetic acid afforded indigo blue and indirubin (indigo red), the blue in higher yield.

We have found that these products are formed via four reaction series or routes. The required indole moieties are the carbanion arose from the reduction steps of isatin chloride, the remaining isatin chloride, and the chloro ketone derived from the first reduction of isatin chloride, Figure 5.

The nucleophile is the electrodotic anion [19], and the above mentioned neutral compounds are the two electrophiles.

Since both last substrates have two reactive sites this originates the four reaction series. In isatin chloride the imidoyl chloride is more reactive than the ketone; in the α-chloroketone, chlorine substitution is favourable instead of carbonyl reaction. These reactivities explain the preponderance of indigo blue since it derives from reactions at the most reactive sites, whereas indirubin is formed in the other way.

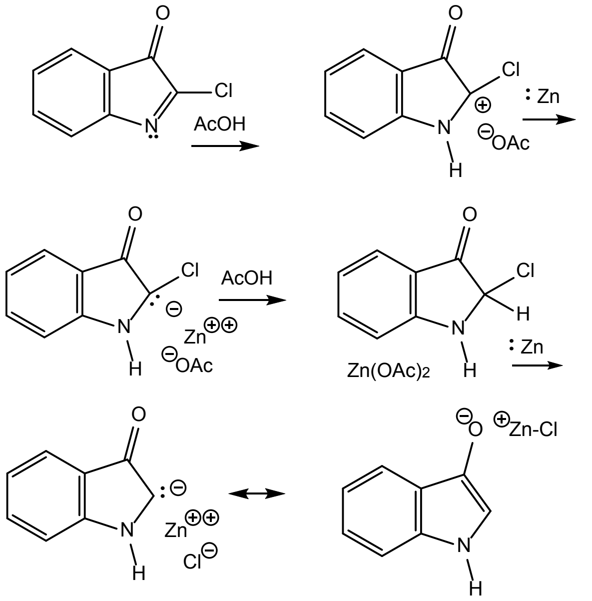


Figure 5. Three-reactant reaction medium from which the products derive, showing chloro zinc enolate with C- or O- metallation

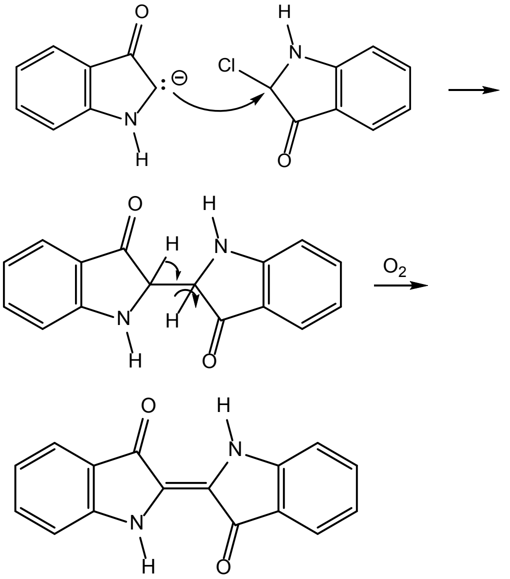


Figure 6. Obtention of indigo blue from the intermediate α-chloro ketone

Route 1 is in Figure 6. The carbanion, the more unstable form of the chloro zinc enolate reacts with the α-chloroketone. The C-2, C-2’ condensation yields indigo white from which the blue dye is formed by aerial oxidation (oxygen as hydride scavenger).

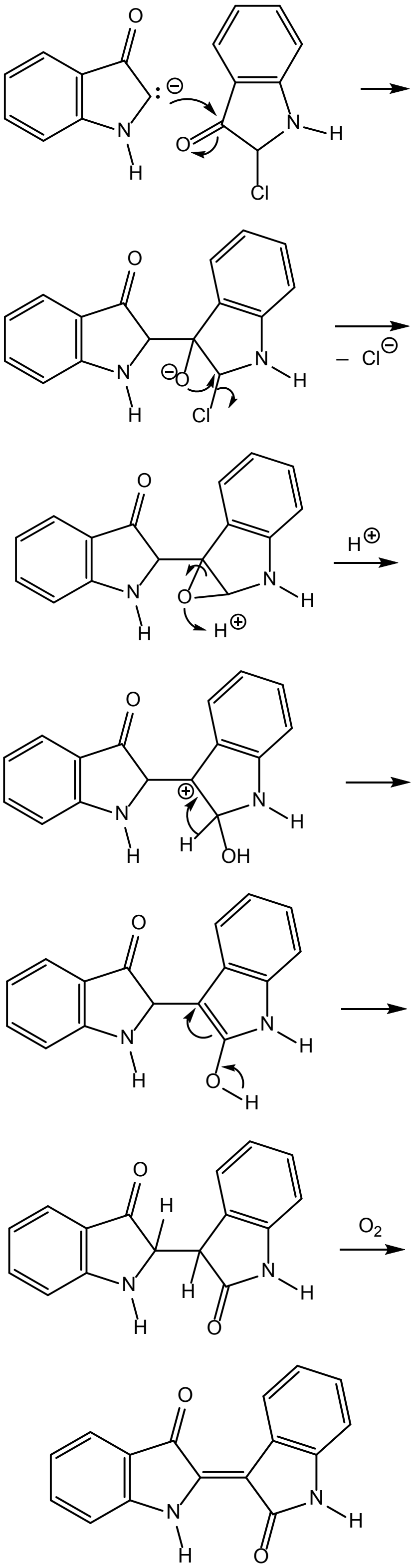


Figure 7. Synthesis of indirubin from the intermediate α-chloro ketone

Route 2 is in Figure 7. Reaction of the mesomeric carbanion with the α-chloroketone carbonyl group, second option to chlorine substitution, originates a halohydrin alkoxide. An intramolecular reaction gives an epoxide. Ring opening by intermolecular reaction with acetic acid produces an enol that rearranges to carbonyl (prototropic processes), and a lactam is formed. The last step in this route is aerial oxidation to indirubin.

Route 3 is in Figure 8. Now let's consider the reaction of the nucleophile with isatin chloride. The main condensation reaction takes place by nucleophilic addition to the C-N double bond. Then hydrogen chloride is easily eliminated due to the presence of an acidic hydrogen alpha to carbonyl, and indigo blue is formed.

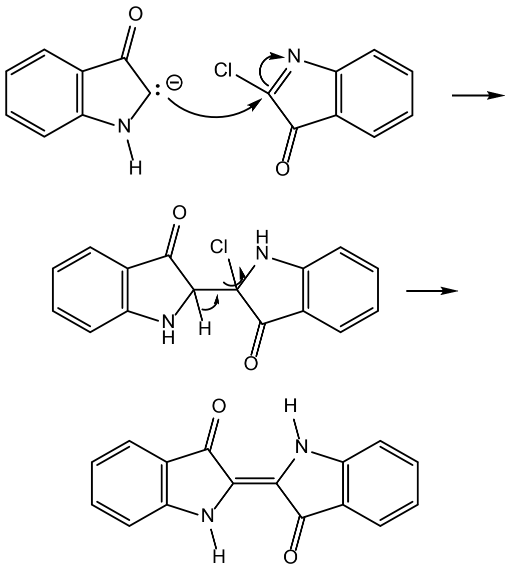
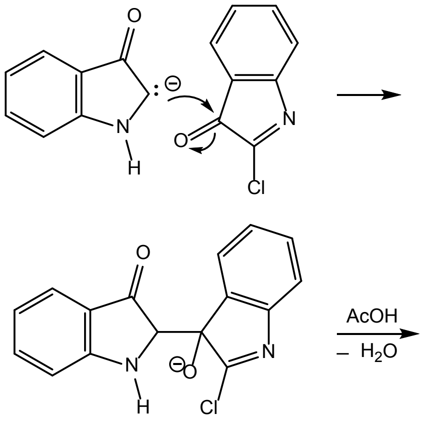


Figure 8. Indigo blue formation from isatin chloride

Route 4 is in Figure 9. The secondary condensation reaction between the electrodotic carbanion and isatin chloride is reaction with the keto group. Unlike epoxide formation as in route 2, now there is intermolecular reaction with acetic acid and dehydration, since now the chlorine atom is at sp^2 carbon atom, not at sp^3 one. Finally, water addition to the imidoyl chloride yields an unstable chloro alcohol that forms the lactam of indirubin.



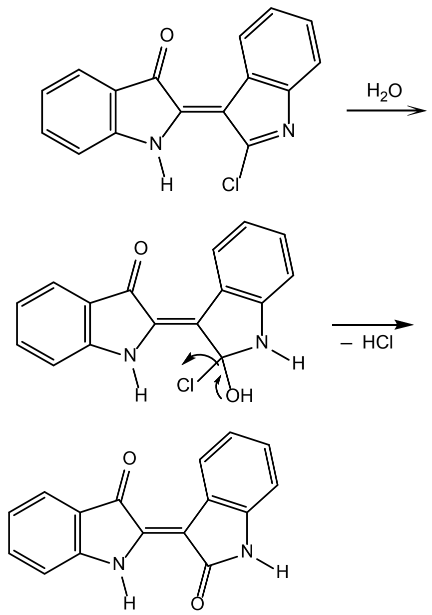


Figure 9. Indirubin synthesis from isatin chloride

4. Conclusions

It is a challenge for the theorist to unravel the course of a reaction, providing a sound reaction mechanism that is in accordance with the experimental facts and known chemical deportment.

We have found four routes to explain indirubin and indigo formation, two for each isomer.

There is a three-component reaction medium from which arise the obtained products. The nucleophile is a chloro zinc enolate and there are two electrophiles, isatin chloride (2-chloroindolenin-3-one), and 2-chloroindolin-3-one.

Each electrophile has two reactive sites. Indigo blue, obtained in higher yield, derives from reaction at the more reactive electrophilic sites, whereas indirubin (indigo red) comes from reaction at the less reactive site in each electrophile. By means of these intertwined reactions we have correlated yields with regiochemistry.

The presented reaction mechanisms not only explain how the products are formed but also why indirubin is always present as co-product in the Baeyer-Emmerling synthesis, and in a lesser proportion than indigo blue.

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