

On the Formation Mechanism of Indigo Blue and Indigo Red from Vegetable Source

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Abstract: In this communication a series of ionic reactions is advanced in order to explain not only indigo blue formation but also the continuous presence of indigo red as companion, and why this compound is always found in minority. Indican is the glycoside found in *Indigofera tinctoria* from which the mixture of indigo blue and indigo red is obtained by means of air oxidation in alkaline medium. A reaction mechanism for the hydrolysis of indican, which was advanced decades ago, is criticized in this paper. Fifty years ago a radical mechanism was suggested for indigo formation. However, the supposed reactive species, indoxyl radical, was not detected by electron spin resonance. Besides, there is no free radical promoter, and indirubin (indigo red) was not considered. A series of ionic reactions explain the formation of both indigoids, and why indigo red occurs in minor proportion. Indoxyl, 3-hydroxyindole, is the aglycone from indican. Reaction of indoxyl with oxygen is catalyzed by calcium hydroxide, indoxyl hydroperoxide being formed. Internal reaction in this intermediate produces 3-oxo-indolenine, which reacts with indoxyl carbanion to give leucoindigo. Further steps lead to indigo blue. Indirubin is formed from indoxyl hydroperoxide by dehydration to isatin, a keto lactam. Condensation of isatin salt with 3-indolinone affords indigo red. This step is retarded due to electric hindrance.

Keywords: Indigo Blue, Indirubin, Indican, Indoxyl, Organic Hydroperoxide, 3-Oxoindolenine, PeroxyHemiaminal

1. Introduction

Since the early days of indigo studies, the effort was directed to the synthesis of the dye. Baeyer obtained indigo before knowing its structure. This fact reveals the enormous economic interest existing. In this article an ionic route is provided. This explains the formation mode, from vegetal source, of both indigo blue and indigo red (indirubin). The electron flow is given in each reaction. The work is supported by the chemical department of both reagents and substrate. This way the reaction sequence leading to these compounds has been cleared up.

This paper is a follow-up of other studies on reaction mechanism [1-5].

2. Literature Review

Indican is the glycoside, found in *Indigofera tinctoria*, from which the mixture indigo/indirubin is obtained. The

hydrolysis of indican (indoxyl- β -D-glucoside) yields glucose and indoxyl, the aglycone.

According to Ballou [6], the alkaline hydrolysis of the phenolic glycosides consists in the ionization of the 2-hydroxy group of the sugar in the alkaline medium and this reacts with the glycosidic center, causing hydrolysis with the formation of a 1,2-epoxy derivative. Ring opening of the epoxide restores the hydroxyl group at C-2.

This ephemeral epoxide involved in a forth and back mechanism, is very improbable. If the hydroxyl ion can form an alkoxide as the reactive species, as proposed, direct attack of the base to the anomeric center, with elimination of an ambident enolate from the heteroside, is preferred to the invoked alkoxide formation and the multistep proposal.

Fifty years ago a radical mechanism was suggested for indigo formation. In this proposal, two indoxyl radicals can couple to form leucoindigo, but indoxyl radicals could not be

detected by electron spin resonance, [7]. Besides, indirubin was not considered, much less why the minority of this constant secondary product. This theory is presented in a journal of scientific education [8], notwithstanding there is no free radical promoter.

In recent years the mechanism of the Baeyer-Drewsen synthesis of indigo was provided [9], as well as the mechanism of the Baeyer-Emmerling first indigo synthesis, [10, 11].

Indirubin and derivatives have attracted attention due to their healing properties. Topical indigo naturalis is clinically proved to be an effective therapy for psoriasis, indirubin being the active substance, [12]. These compounds have been used in cancer treatment, for instance in glioblastoma [13] and glioma [14]. Indirubin has been discovered to be the active compound of a formula used in traditional Chinese medicine to treat the symptoms of leukemia, [15].

3. Study Method and Process

This is a Theoretical Organic Chemistry study. It is based on the chemical department of reagents and substrate. All is in accordance with the reaction medium, the nature of the oxidizer and the catalyst employed. The several steps leading to indigo blue and indigo red are fully commented and the electron flow in each reaction is provided. In this communication a series of ionic reactions explain the formation of both dyes and why indigo red occurs in minor proportion. All this process is sustained by the pertinent references.

4. Discussion

Indoxyl, Figure 1, gives in alkaline medium, $\text{Ca}(\text{OH})_2$, the enolate. This is stabilized by resonance affording a carbanion at C-2, an ambident anion. The methylene group in the indoxyl keto form is acidic due to the acidifying properties of the carbonyl group. The carbanion at C-2 is also ambident.

Molecular oxygen is a high energy molecule and this kind of molecules has relatively weak bonds. The double bond energy in O_2 is higher (300 kJ/mol) than the double bond energy in CO_2 , [16]. This makes oxygen an electron acceptor, [17, 18].

In the secondary carbanion, out of four hybrid orbitals, three are involved in sigma bonds, whereas fourth hybrid orbital contains unshared electron pair.

The indoxyl carbanion adds to the oxygen double bond forming organic peroxide which is neutralized by water to a hydroperoxide, and a peroxy hemiaminal is obtained.

The electrodotic group [19] eliminates a hydroperoxide anion, a good leaving group, and 3-oxo-indolenine is formed (oxidation step). In this cross conjugated molecule there has been Umpolung, polarity inversion, at C-2. This position is completely free of steric hindrance and is preferred for reaction.

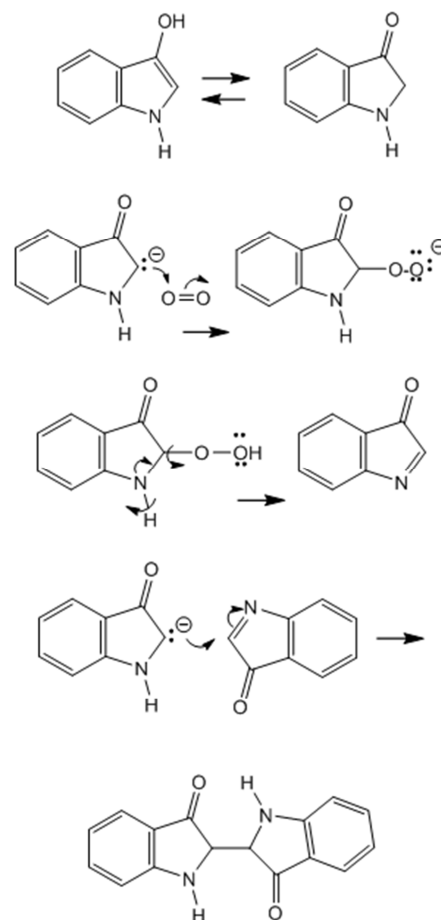


Figure 1. Formation of leucoindigo via indolenin-3-one.

Since air oxygen is passed gradually through the reaction mixture, the remaining indoxyl carbanions can react with the indolenine derivative, yielding leucoindigo. One unit of this bis-indole intermediate repeats carbanion formation and reaction with oxygen, Figure 2.

Elimination of hydroperoxide anion can be accomplished in the same unit as before, or by means of a carbanion generated in the other unit. This way a double bond is formed and indigo blue results.

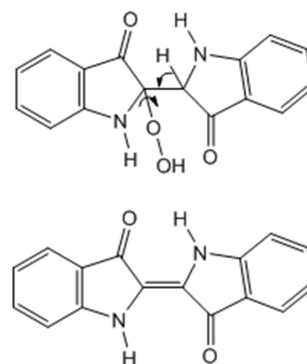


Figure 2. Oxidation of leucoindigo and indigo blue formation.

The eliminated hydroperoxide anions are neutralized by water yielding hydrogen peroxide, a byproduct in the process for obtaining natural indigoids.

Now let's see the formation of the secondary product, indirubin (indigo red).

Besides the hydroperoxide elimination by an intramolecular reaction, there is an alternative pathway. Secondary hydroperoxides may undergo dehydration to a ketone. This mode of decomposition is characteristic of liquid-phase. This β -elimination occurs by base catalysis: a nucleophilic attack to the β -hydrogen, [20, 21].

Thus, the remaining acidic proton at C-2 in the hydroperoxide intermediate can be taken by the hydroxyl ion.

The new carbanion forms a carbonyl group with concomitant hydroxyl ion elimination. This heterolytic fission of the peroxide linkage is involved also in the degradation of α -keto aldehydes (Friedmann reaction), [22, 23]. This way an α -keto lactam results, isatin, Figure 3.

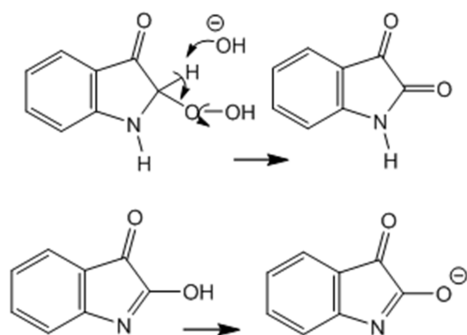


Figure 3. Isatin formation and its salt.

The preferred structure for this type of compounds is the lactim form, imidol, [24]. In alkaline solution the respective salt turns out. Compare [25].

Indirubin is formed by condensation of indoxyl, as 3-indolinone (ψ -indoxyl), and isatin, via the carbanion of the first and the keto group of the latter. Indirubin has been synthesized likewise, [26, 27], confirming our proposal, Figure 4.

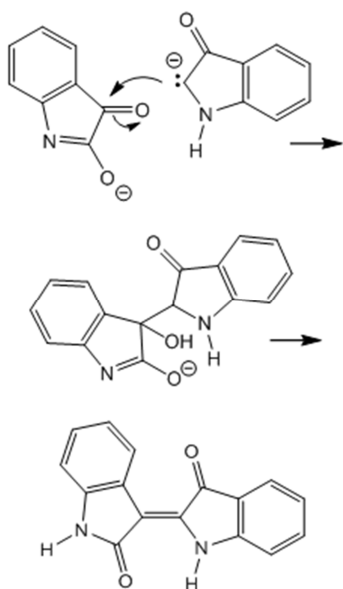


Figure 4. Formation of indirubin (indigo red).

Indirubin is always the secondary product, in more or less quantity. This intrigued Baeyer since he aimed to obtain only the blue compound. The minor proportion of indigo red can be explained since an additional reaction is needed for a second carbanion formation in the isatin synthesis, but the principal effect is electric hindrance, [28, 29], due to the negative charge in the salt of isatin, retarding reaction with the carbanion at ψ -indoxyl.

Corollary: we have proposed that in the natural process of indigo formation, indoxyl and its derivative 3-oxo-indolenine form the frame of the indigo molecule, that is, leucoindigo. Well, in the Baeyer-Drewsen indigo synthesis, after a long series of reactions, leucoindigo is formed by condensation of 1-hydroxyindoxyl and indolenin-3-one. The extra hydroxyl group is lost by dehydration, obviating one step, [30].

This is conclusive evidence that the two proposed reaction mechanisms are correct. The route corresponding to natural formation of indigo is shorter because the plant provides indoxyl, whereas the laboratory work starts from substituted benzene in order to obtain synthetic indigo.

5. Conclusion

The reaction series presented explains not only the formation of both indigo blue and indirubin (indigo red) but also why the latter occurs always as a secondary product, and the formation of hydrogen peroxide.

All is in accordance with the reaction medium, the oxidizer and the catalyst employed. Each step involves a known reaction, such as carbanion formation in alkaline medium, oxygen as electron acceptor, amino group as electron donor, hydroperoxide anion as good leaving group, hydroperoxide dehydration and condensation nucleophile-electrophile.

This ionic process is completely supported by the references.

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