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### Research Paper

### Oxidative degradation of indole using mixed ligand Co(II) complex and hydrogen peroxide : Selective formation of indole dimers.

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**Abstract:** Mixed ligand Cobalt (II) complex is a good catalyst for decomposition of  $H_2O_2$  consequently this reagent was used for the oxidation of indole to give three major products namely indirubin, indigo and isatin in good yield. Indirubin being the major product.

### Introduction

Oxidation of the indole has been extensively studied because of the formation of a variety of products which are of biological as well as commercial importance. Some of the important products of oxidation of indole are oxindole, indoxyl, isatin and the dimers, indirubin and indigo. It has been reported that different oxidizing agents have different effect on the indole ring and the mode of oxidation is diverse with respect to the site of initial attack and the extent of oxidation. The multiplicity of products obtained during oxidation is possibly due to oxidative dimerization of indole. Biologically, catabolism by gut bacteria of tryptophan gives several oxidation products of which indoxyl and indirubin are physiologically important. The physiological importance of indole has

generated considerable interest in the biological oxidative transformation of indole and its derivatives. The study assumes significant importance because of the observation that the indole derivative namely typtamine may be associated with the occurrence of some mental disorders<sup>1</sup>. Further, it has been reported that the products of biological degradation of indole namely, indirubin and indigo are potent aryl hydrocarbon (AhR) receptor ligands<sup>2</sup>. The toxic potentials of xenobiotic AhR is currently a major concern for the regulatory agencies that are responsible for public and environmental health.

Among the usual chemical oxidizing agents used for the oxidation of indole and its derivatives is  $CrO_3$  in acetic acid which preferably cleaves the  $C_2.C_3$  bond particularly when electron withdrawing groups are present in the indole ring. In addition to the usual products of ring

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cleavage, dimers have also been isolated<sup>3,4</sup>. Oxidative cleavage of a similar type also occurs when oxidation is carried out with  $\text{KMnO}_4$ <sup>5</sup>. Some selective oxidizing agents used are  $\text{OsO}_4$ <sup>6,7</sup>,  $\text{MnO}_2$ <sup>8</sup> periodic acid and periodates<sup>9,10</sup> and potassium nitrosodisulfonate<sup>11</sup>. Among the important reports on the oxidation of indole and indole derivatives are oxidation of indole with Indium (III) chloride /2-iodoxybenzoic acid (IBX) which yields the isatin as the major product<sup>12</sup>. The formation of an unusual dimer in the oxidation of indole by  $\text{Pb}(\text{OAc})_4$  is also reported by Takayama et al<sup>13</sup>. Further, a one pot synthesis of indigo by the oxidation of indole with a molybdenum complex and cumene-hydroperoxide is also reported<sup>14</sup>. As a general observation, inorganic oxidizing agents do not usually give the dimers<sup>15</sup>. In the various methods of oxidations reported, the products were analyzed for the purpose of establishing a possible mechanism of oxidation as also for a possible pathway of dimerization. The most elegant study involves the mode of action of biological oxidants on indole and indole derivatives. The endogenous production of indirubin and indigo in the human body was explored by Gillen et al<sup>16</sup>. They have also studied the oxidative transformations of indole with cytochrome P 450 and reported the formation of dimers namely indirubin and indigo in major quantity<sup>17,18</sup>.

## Results and Discussion

The oxidation of indole and indole derivatives with hydrogen peroxide have been carried out in the presence of acetic acid and ammonium molybdate<sup>19,20</sup>. It has been observed that  $\text{H}_2\text{O}_2$  alone is not a good oxidizing agent for indole further the dimerized products have not been isolated in substantial quantities. However,  $\text{H}_2\text{O}_2$  oxidation was utilized for structure determination of indole derivative and for establishing the mechanism of oxidative degradation rather than for the isolation of

the dimers. It is reported that the mechanism of oxidation by Cytochrome P 450 resembles the chemical oxidation by hypervalent transition metal oxidants and that the reaction is initiated by an electrophilic attack on the indole ring. In the present study, the result of oxidation of indole with  $\text{H}_2\text{O}_2$  catalyzed by a mixed ligand Co(II) complex is reported. The study was carried out with the objective of identifying the pigments that may result and to optimize conditions under which the dimers may be obtained in good yield. It has been reported that Co(II) complexes decompose  $\text{H}_2\text{O}_2$ <sup>21</sup> and therefore this combination of mixed ligand Co(II) complex and  $\text{H}_2\text{O}_2$  expected to be an appropriate reagent for studying the oxidation of indole and indole derivatives especially for the isolation of indirubin and indigo in good yield. The mixed ligand Co(II) complex was prepared as per reported procedure using glycine and 8-hydroxyquinoline as the ligands<sup>21</sup>. In a typical procedure, a mixture of the Co(II) complex and indole was dissolved in methanol at 60°C in a water bath and to this solution, 30% v/v  $\text{H}_2\text{O}_2$  was added dropwise with constant stirring. The progress of the oxidation was monitored by TLC. After completion of the oxidation, as indicated by TLC, the solvent methanol was removed by reduced pressure distillation and the product mixture was separated by Column chromatography using silicagel column and a mixture of 20% ethyl acetate and petroleum ether as the eluent. It was observed that indirubin, and indigo were the major products of the oxidation with isatin being a minor byproduct. The products were separated by HPLC and their structures established on the basis of their UV-visible, <sup>1</sup>H-NMR spectra and mass spectra. A probable pathway for the oxidation is given in **Scheme 1**.

The mixed ligand Co(II) catalyst could be prepared from easily available and cheap starting material and could be recycled. A

many as four sets of reactions were carried out with the recovered catalyst without appreciable loss in activity.

### Materials and Methods

Starting materials and authentic samples were obtained from Aldrich Chemical Co. Indole was purified by precipitation from an aqueous ethanol solution. Melting points were determined in open capillaries and are not corrected, UV spectra were recorded in Perkin Elmer Lambda UV spectrometer, <sup>1</sup>H-NMR were recorded in Bruker 300 MHz spectrometer with TMS as internal standard in DMSO-d<sub>6</sub> solutions and Mass spectral data were obtained from SAIF, NEHU Shillong. HPLC analysis was performed in Shimadzu 6.2x80 mm octadecylsilane column (mode SPD-M10AVP) using gradients composed of solvent A (MeOH: H<sub>2</sub>O) and solvent B (MeOH: DCM). The column was eluted at flow rate of 1 mL/min. The Co(II) catalyst was prepared by a procedure reported<sup>21</sup> using glycine and 8-hydroxyquinoline as the ligands. The structure of the complex formed, have also been reported therein.

### Experimental Section

**Oxidation of indole with mixed ligand Co(II) complex and H<sub>2</sub>O<sub>2</sub> :** A stirred solution of indole (1.0 mmol) and the Co(II) complex (0.25mmol) in 5 mL of methanol was preheated in a water bath to 60°C for 10 mins till a clear solution was obtained and to the resulting solution, varying amounts of H<sub>2</sub>O<sub>2</sub> (30% v/v) (Table I) was added dropwise. The reaction mixture was kept at 60°C with stirring and the progress of the reaction was followed by TLC. It was observed that with 3 mL of H<sub>2</sub>O<sub>2</sub> solution the oxidation was complete in 5.5 hrs. Methanol was removed by reduced pressure distillation and 100 mL water was added to the solid product. The crude product was recovered by filtration and washed 4 times with ice cold water (

50mLx4) and dried. The crude product when subjected to separation by chromatography with silica gel (60-120 mesh) using 20% ethyl acetate/petroleum ether (60-80) afforded several fractions. The two major fractions and one minor fraction were eluted and recovered. These fractions were identified as indirubin, indigo and isatin respectively by comparison with authentic samples. A blank experiment was also carried out using H<sub>2</sub>O<sub>2</sub> without the use of the Co(II) catalyst. The results of the reaction with and without the catalyst is shown in **Table I** and **Table II** respectively. The results indicate that without the Co(II) catalyst the oxidation is sluggish and gives low yields of the desired products. HPLC experiment showed indirubin eluted at t<sub>R</sub> 21.1 min, isatin at t<sub>R</sub> 7.4 mins and indigo at t<sub>R</sub> 11.96 mins. All the products were recorded in a UV detector and compared with authentic samples. The mechanistic aspects of the conversion is being investigated.

### Characterization of Pigments

**Indirubin** Colour deep pink, R<sub>f</sub> value: 0.76 in eluent [20% EtOAc: PE (60-80)], UV (MeOH) λ<sub>max</sub> 605 nm. Mass (MH<sup>+</sup>: m/z 263(262.07), <sup>1</sup>NMR spectra(400 MHz, DMSO-d<sub>6</sub>): δ8.61(d, J=8-4 Hz, H<sub>4</sub>), 8.82 (d, J=6, 4 Hz, H<sub>4'</sub>) 8.04(m, H<sub>6</sub>), 7.89(d, J=7.2 Hz, H-7') 7.81(m, H-6) 7.56 (m, H-5), 7.06 (m, H-5') 6.94 (d, J=8Hz, H-7)

**Indigo** : Colour blue, R<sub>f</sub> value: 0.72 in eluent 20% EtOAc: PE (60-80), UV (MeOH) λ<sub>max</sub> 550 nm Mass (MH<sup>+</sup> m/z 263(262.07) <sup>1</sup>NMR spectra(400 MHz, DMSO-d<sub>6</sub>): 10.52(s, NH), 7.62(d, 1H), 7.52(t, 1H), 7.33(d, 1H), 6.94(t, 1H)

**Isatin** : Colour pink R<sub>f</sub> value: 0.65 in eluent 20% EtOAc: PE (60-80), UV (MeOH) λ<sub>max</sub> 310 nm. Mass (MH<sup>+</sup>) m/z 148.

### Conclusion

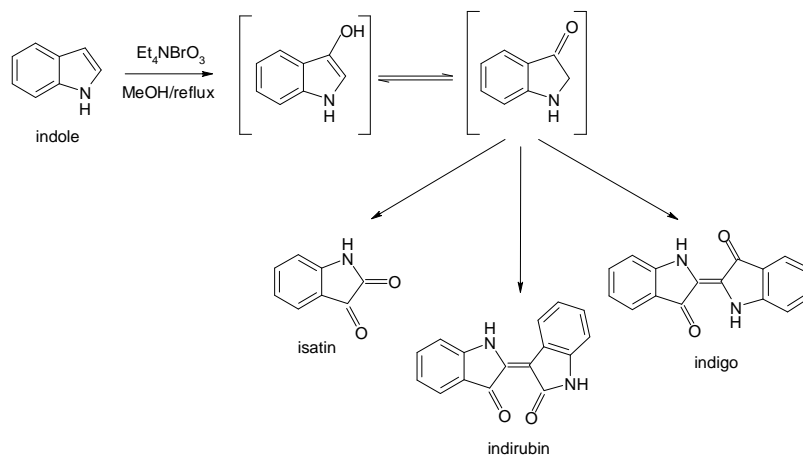
In conclusion, we report here the oxidation of indole with  $\text{H}_2\text{O}_2$  catalyzed by the mixed ligand Co(II) complex and the results indicate that the dimerized products are obtained in high yields. The usual monomeric products are obtained in minor quantities. Among the dimers, Indirubin is isolated as the major product. The reaction conditions are simple and product recovery is easy.

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### Scheme I

Oxidation of indole with methanolic solution of mixed ligand Co(II) complex



**Table I** The % conversion as a factor of time and volume of  $\text{H}_2\text{O}_2$  and Co(II) catalyst

Sl.No	Volume of $\text{H}_2\text{O}_2$ ( mL)	Reaction time ( hrs)	Percentage completion
1	1	0.5	40%
2	2	1.0	80%
3	3	5.5	100%

**Table 1I** The % conversion as a factor of time and volume of H<sub>2</sub>O<sub>2</sub> without catalyst

Sl.No	Volume of H <sub>2</sub> O <sub>2</sub> ( mL)	Reaction time ( hrs)	Percentage completion
1	2	1	25%
2	4	2	30%
3	6	4	40%

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