



Studies on Spectral and Antifungal Activity of Some Complexes of Chromium, Nickel and Copper Metals with p-phenylenedibiguanide [C₁₀H₁₆N₁₀, Ph (BigH)₂] Molecule

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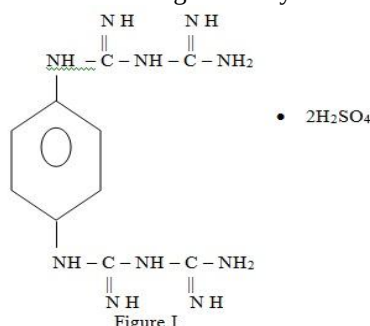
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Abstract: These complexes have been found to be effective in controlling the growth of *Aspergillus versicolor* in PDA (Potato Dextrose Agar) and SDA (Sabourand and Dextrose Agar) mediums. Some metal complexes of p-phenylenedibiguanide [Ph(BigH)₂, C₁₀H₁₆N₁₀] with metals: Chromium (Cr), nickel (Ni) and copper (Cu) have been synthesized and isolated in the pure state. These metal complexes have been found effective in inhibiting the growth of fungi named, *Aspergillus niger* and *Aspergillus versicolor*. The metal complexes of p-phenylenedibiguanide [Ph(BigH)₂, C₁₀H₁₆N₁₀] ligand were dissolved in DMSO and were tested with Dilution test method and growths were separated and examined under different concentrations of the afore said complexes in PDA (Potato Dextrose Agar) and SDA (Sabourand and Dextrose Agar) medium. The data of growth inhibition of the fungi so obtained were then compared with those of different metal complexes..

Keywords: *Aspergillus versicolor*, *Aspergillus niger*, p-phenylenedibiguanide, PDA, SDA

I. Introduction

p-Phenylene dibiguanide¹⁻² behaves as a quadridentate molecule has been found to give as usual 4- coordinated copper and nickel complexes and only 6-coordinated dichromium tris-p-phenylenedibiguanide base. The elemental elucidation of the coordination complexes of p-phenylene dibiguanide with bivalent metal ions have been done till now, but its biochemical importance is still lacking. We have therefore, work on its antifungal property and compared this activity in two different fungus (*Aspergillus niger*³⁻¹⁴ & *Aspergillus versicolor*¹⁵) under two different conditions while applying its different concentration To investigate this antifungal activities of quadridentate biguanide ligand p-phenylene dibiguanide ligand complexes, we have prepared the ligand and complexed with Ni and Cu metal and then done antifungal activity on it.



II. Materials and Methods

The ligand and its complexes were prepared by the reported method as described below:

A. p-Phenylene dibiguanide sulphate²⁵ [C₁₀H₁₆N₁₀].2H₂SO₄

For its preparation p-phenylene diamine (10g) C₆H₄(NH₂)₂, dicyandiamide (17g) C₂H₄N₄, 22 c.c. of 32% HCl and 25c.c. of H₂O were heated under reflux for about 2 hours. The refluxing mixture was cooled and to this mixture 100 c.c. of alcohol C₂H₅OH was added. The solution was then boiled with 5g charcoal for a few minutes and filtered. The cold filtrate was then treated with 30 c.c. of dilute H₂SO₄ (1:2 by volume) and kept for 2 or 3 days which deposits crystals of p-phenylenedibiguanidesulphate C₁₀H₁₆N₁₀.2H₂SO₄. The crystals of the sulphate were filtered, washed first with cold H₂O and then with alcohol & dried in air.

Found

$$\begin{aligned} \text{N} &= 37.60\% \\ \text{SO}_4^{2-} &= 25.71\% \end{aligned}$$

$\text{C}_{10}\text{N}_{10}\text{H}_{16} \cdot 2\text{H}_2\text{SO}_4$ requires

$$\begin{aligned} \text{N} &= 37.43\% \\ \text{SO}_4^{2-} &= 25\% \end{aligned}$$

B. *Tris(p-phenylenedibiguanide)chromium (III)* $[\text{Cr}_2(\text{C}_{10}\text{N}_{10}\text{H}_{14})_3] \cdot 5\text{H}_2\text{O}$

The complex chromium base $[\text{Cr}_2(\text{C}_{10}\text{N}_{10}\text{H}_{14})_3] \cdot 5\text{H}_2\text{O}$ was obtained as rose red precipitate by adding a solution of chrome alum to that of p- phenylene dibiguanide sulphate in caustic soda. The product was washed with ice cold water till free from sulphate then with alcohol and afterwards as dried over solid potassium hydroxide, KOH in a dessicator. The substance is moderately soluble water and reacts alkaline to litmus. It liberated ammonia from ammonium salt solutions.

Found

$$\begin{aligned} \text{Cr} &= 10.15\% \\ \text{N} &= 40.96\%, \\ \text{H}_2\text{O} \text{ (by loss at } 105^\circ\text{C)} &= 6.33\% \end{aligned}$$

$[\text{Cr}_2(\text{C}_{10}\text{N}_{10}\text{H}_{14})_3] \cdot 5\text{H}_2\text{O}$ requires

$$\begin{aligned} \text{Cr} &= 10.17\% \\ \text{N} &= 41.09\% \\ \text{H}_2\text{O} &= 8.85\% \end{aligned}$$

C. *p-Phenylenedibiguanidiniumnickel (II) sulphate* $[\text{Ni}(\text{C}_{10}\text{N}_{10}\text{H}_{14})]\text{SO}_4 \cdot 2\text{H}_2\text{O}$

It was obtained as a brick red flocculent precipitate when an ammonium solution of p-phenylene dibiguanide sulphate $\text{C}_{10}\text{N}_{10}\text{H}_{16} \cdot 2\text{H}_2\text{SO}_4$ was treated with that of nickel sulphate, NiSO_4 . This was washed and dried as usual. The substance is very sparingly soluble in hot water but insoluble in alcohol.

Found

$$\begin{aligned} \text{Ni} &= 12.30\% \\ \text{N} &= 29.84\% \\ \text{SO}_4^{2-} &= 20.50\% \\ \text{H}_2\text{O} \text{ (by loss at } 105^\circ\text{C)} &= 5.68\% \end{aligned}$$

$[\text{Ni}(\text{C}_{10}\text{N}_{10}\text{H}_{14})]\text{SO}_4 \cdot 2\text{H}_2\text{O}$ requires

$$\begin{aligned} \text{Ni} &= 12.57\% \\ \text{N} &= 29.98\% \\ \text{SO}_4^{2-} &= 20.50\% \\ \text{H}_2\text{O} &= 7.70\% \end{aligned}$$

D. *p-Phenylenedibiguanidiniumcopper (II) sulphate* $[\text{Cu}(\text{C}_{10}\text{N}_{10}\text{H}_{16})]\text{SO}_4 \cdot 2.5 \text{H}_2\text{O}$

It was obtained as a violet coloured powder by adding an ammonical solution of p- phenylene dibiguanide sulphate to an ammonical solution of CuSO_4 . The product was filtered washed and dried as usual. It is very sparingly soluble in water.

Found

$$\begin{aligned} \text{Cu} &= 13.20\% \\ \text{N} &= 28.89\% \\ \text{SO}_4^{2-} &= 19.90\% \\ \text{H}_2\text{O} \text{ (by loss at } 105^\circ\text{C)} &= 9.37\% \end{aligned}$$

$[\text{Cu}(\text{C}_{10}\text{N}_{10}\text{H}_{16})]\text{SO}_4 \cdot 2.5 \text{H}_2\text{O}$ required

$$\begin{aligned} \text{Cu} &= 13.20\% \\ \text{N} &= 29.10\% \\ \text{SO}_4^{2-} &= 19.97\%, \\ \text{H}_2\text{O} &= 9.36\% \end{aligned}$$

E. *Preparation of PDA(Potato Dextrose Agar)*²⁶

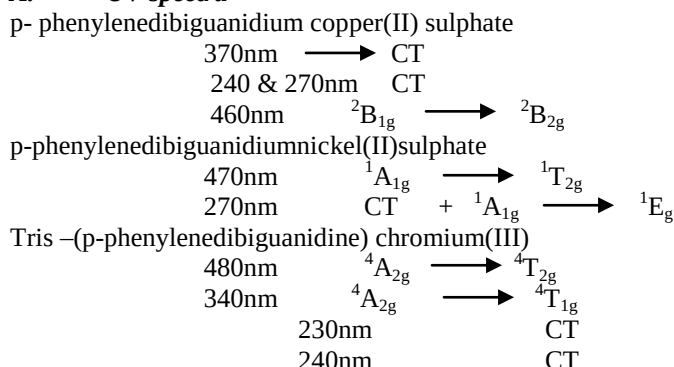
Potato tubers were taken peeled off and weighed 200g. It was chopped into small pieces and transferred to a beaker containing about 100ml of distilled water and boiled for 20minutes and filtered with muslin cloth. 20g Dextrose, 15g agar and 2g peptone were added into the extract and gently heated. The filtrate so obtained was made to 1 litre. The pH of the solution was maintained at 5.6 by using 1N HCl or NaOH and kept in Erlenmeyer flask. This solution so obtained was PDA medium and autoclaved at 121°C for 20minutes before using.

F. *Preparation of SDA (Sabourand and Dextrose Agar Medium)*²⁶

It was prepared by combining the ingredients water, dextrose, agar, peptone & antibiotics separately, in many different variations. In the case of using premix, the proper amount (around 70gms) was mixed with one litre of water and heated to dissolve the agar. pH of the medium was adjusted with one molar solution of hydrochloric acid to lower pH. The pH was maintained at 5.5. The medium was then autoclaved and stored at room temperature. The medium can be used to inoculate with fungal spores and mycelium inhibition growth was counted by usual method. The observation of the study is represented in the Table A.

III. Result and discussion

A. UV spectra



B. IR spectra

The IR band position of p-phenylenedibiguanide ligand and its complexes with copper, nickel and its complexes with Cu, Ni and Cr are recorded and probable assignments of IR bands have also been included in the table along with IR spectra report. This ligand contains ten nitrogen atoms capable of co-ordinate bond formation. The ligand is stable as sulphate in solid state and solution. The presence of $>NH$, is shown by peak at 3211.5, the $>NH$ stretching in $>N^+H$ shows by peak at 2374.1, 2339.5 & 2277.8 cm^{-1} . The $>NH$ deflection is shown at 1570.0 cm^{-1} and $-NH_2$ bending at 1351.8 cm^{-1} NH_2 rocking is confirmed by peak at 971.4 cm^{-1} . The phenyl ring skeleton is confirmed by stretching at 1432.7 due to C=C str in aromatic ring. The presence of peak at 823.8 assumes the p - substituted benzene ring.

The sulphate (SO_4^{2-}) shows stretching bands at 607.4 cm^{-1} (ν_4) and (ν_3). It is reported that ν_1 stretch is absent in ionic sulphate and ν_3 and ν_4 as strong band^[13]. Further non-conjugated C = N stretching is shown by peak at 1570.0. The terminal C - N stretching is shown at 1052.8 cm^{-1} . The IR spectrum of complex hydroxide of Cu & Ni and halide of Ni shows the absence of IR vibrations associated with SO_4^{2-} group. The complex sulphate Cu p-phenylenedibiguanidium sulphate, Ni p-phenylenedibiguanidium sulphate and Cr -p-phenylenedibiguanidium sulphate shows the characteristics of ionic sulphate molecule (sharp broad band at 1112.8 cm^{-1} & 618.3 cm^{-1} for Cu, 1113.2 cm^{-1} & 618.5 for Ni and 1115 cm^{-1} & 618 cm^{-1} for Cr). The complex base shows a strong band at 1058.5 cm^{-1} in Cu p-phenylenedibiguanidium hydroxide which suggests the presence of $\nu - OH$ of uncoordinated (OH) molecule. In fingerprint region the IR spectra of complex.

The biochemical properties¹⁶⁻²⁴ e.g. antibacterial activities of biguanide and substituted biguanide complexes have been studied for a long time. But no work is done regarding the antifungal activities of biguanides and substituted biguanide complexes. Metal complexes of p-phenylenedibiguanide were dissolved in DMSO using the concentration which was most effective from Dilution test method. The inoculation of fungus was done in PDA & SDA medium at 25°C and checked daily for a week. The fungi are *Aspergillus niger* & *Aspergillus versicolor*. Both are from ascomycetes group. The MIC was then calculated i.e. the minimum inhibition concentration of the fungus. During this process it was found that the complexes was effective in controlling 100% of the fungal growth if the concentration was raised to 800 $\mu g/ml$ to 1000 $\mu g/ml$. The solutions was used in the ratio 1:10 and the concentration of the solution was 400 $\mu g/ml$, 200 $\mu g/ml$ and 100 $\mu g/ml$.

It was found that the complexes are more effective in controlling the growth of *Aspergillus versicolor*. If the growth is compared it was concluded that the growth inhibition is more in PDA medium. The chromium complex was more effective in fungal inhibition as compared to nickel and copper.

IV. TABLE A

Observations of the test

Table I Percentage inhibition of growth of fungus *Aspergillus versicolor* at indicated dose

Metal complex	% Inhibition of fungal growth		
	Concentration ($\mu g/ml$)	PDA Medium	SDA Medium
Tris(p-phenylenedibiguanidine) chromium (III)	400 $\mu g/ml$	100%	99.20%
	200 $\mu g/ml$	88%	86%
	100 $\mu g/ml$	63.40%	57.4%
p-Phenylenedibiguanidium nickel (II) sulphate	400 $\mu g/ml$	98.5%	95.20%
	200 $\mu g/ml$	81.20%	79.25%
	100 $\mu g/ml$	59.51%	56.31%
p-Phenylenedibiguanidium Copper (II) sulphate	400 $\mu g/ml$	95.00%	90.12%
	200 $\mu g/ml$	82.00%	79.45%
	100 $\mu g/ml$	52.57%	50.91%

Table II. Percentage inhibition of growth of fungus *Aspergillus niger* at indicated dose

Metal complex	% Inhibition of fungal growth		
	Concentration ($\mu\text{g/ml}$)	PDA Medium	SDA Medium
Tris(p-phenylenedibiguanidine) chromium (III)	400 $\mu\text{g/ml}$	96.97%	94.30%
	200 $\mu\text{g/ml}$	72.43%	68.14%
	100 $\mu\text{g/ml}$	51.57%	49.91%
p-Phenylenedibiguanidium nickel (II) sulphate	400 $\mu\text{g/ml}$	95.80%	84.11%
	200 $\mu\text{g/ml}$	70.25%	69.59%
	100 $\mu\text{g/ml}$	50.73%	48.45%
p-Phenylenedibiguanidium Copper (II) sulphate	400 $\mu\text{g/ml}$	89.15%	87.45%
	200 $\mu\text{g/ml}$	67.21%	65.08%
	100 $\mu\text{g/ml}$	52.70%	47.07%

Figure 1 Percentage inhibition of fungal growth of *Aspergillus niger* in PDA & SDA medium by Tris(p-phenylenedibiguanidine)chromium (III)

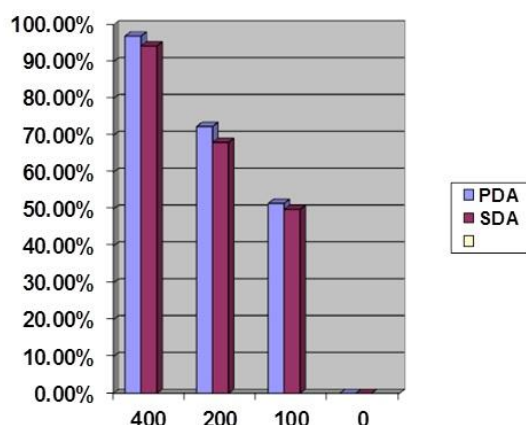
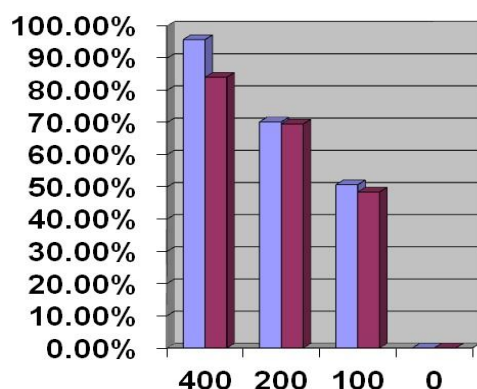
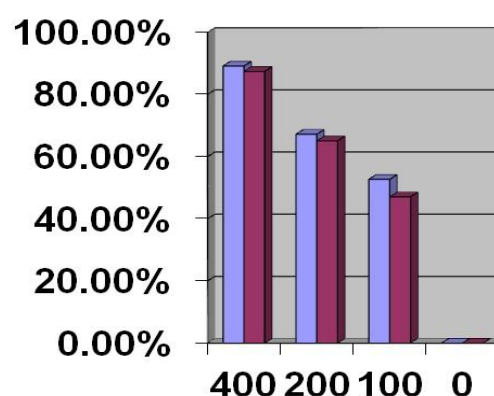


Figure 2 Percentage inhibition of fungal growth of *Aspergillus niger* in PDA & SDA medium by Nickel (II)p-phenylenedibiguanidium sulphate



*Concentration of the solution of the complex in $\mu\text{g/ml}$

Figure 3 Percentage inhibition of fungal growth of *Aspergillus niger* in PDA & SDA medium by Copper (II) p-phenylenedibiguanide sulphate



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