ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis, Spectroscopic Characterization and Antibacterial Activity of 2-[2- Hydroxyphenylazo]-1-naphthol-4-sulphonicacid and its Fe (III) Co (II) and Cu (II) complexes.

Swati[1], Manu Gupta[2], Romila Karnawat[3], IK Sharma[4], PS Verma*[5]

[1]Deptt. of Chemistry, Univ. of Rajasthan, Jaipur
[2]Deptt. of Chemistry, Univ. of Rajasthan, Jaipur
[3] Head, [2]Dept. of Chemistry, Vedic Kanya P.G. College, Jaipur
[4]Professor Dept. of Chemistry, Univ. of Rajasthan, Jaipur
[5]Professor (Former), Dept. of Chemistry, Univ. of Rajasthan, Jaipur.

ABSTRACT

2-[2- Hydroxyphenylazo]-1-naphthol-4-sulphonicacid (HPANS) and its metal complexes with iron, cobalt and copper were synthesized and characterized by IR, H^1NMR and C^{13} NMR spectral techniques. HPANS and its metal complexes were screened for their in-vitro antibacterial (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter cloacae*, *Enterococcus faecalis*) and antifungal (*Candida albicans*) activities by disc diffusion method. Minimum inhibitory concentrations (MIC) were also determined by the broath dilution technique. It was observed that HPANS was highly active against all microorganisms and the presence of metal enhanced the antimicrobial activity in comparison with the original compound.

Keywords: 2-[2- Hydroxyphenylazo]-1-naphthol-4-sulphonicacid, Antibacterial Activity, Antifungal activity, Disc diffusion, Broath dilution.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Azo compounds constitute one of the largest class of industrially synthesized organic compounds. These compounds have been used for a long time as dye in industry [1, 2]. The compounds having azo linkage are used in analytical chemistry as indicators in pH, redox or complexometric titrations [2, 3]. In addition azo compounds show a variety of interesting biological activities. Biological importance of azo compounds is well known for their use as antineoplastics, antidiabetics, antiseptics, anti HIV and other useful chemotherapeutic agents [4]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticidal activity [1, 5]. The complexes of azo compounds also exhibit bacteriostatic and other biochemical activities [6]. Since compounds with azo moiety and naphthalene moiety have been reported to exhibit biological activities independently therefore it is obvious that azo compounds of 4-hydroxy-1-naphthalenesulphonic acid will show a higher biological activity.

In this paper we report the synthesis of 2-[2- Hydroxyphenylazo]-1-naphthol-4-sulphonicacid (HPANS) and its transition metal complexes with iron, cobalt and copper and their inhibitory effect on the biological activities of some bacteria.

MATERIALS AND METHODS

Experimental

All the chemicals and solvents used were of AR grade. The compound HPANS and its metal complexes with iron, cobalt and copper were synthesized according to the procedure reported in the literature [6, 7, 8, and 9]. IR spectra were recorded on Schimadzu FTIR spectrophotometer model 8400S in KBr wafer and the H^1 NMR, ^{13}CNMR spectra have been obtained on JEOL AL 300, 300.4 MHz FT NMR spectrometer.

Preparation of Ligand

2-Aminophenol (0.016 mol) was dissolved in aqueous acidic medium to get a clear solution. This solution was cooled to 0-5˚C and diazotized with dropwise addition of sodium nitrite solution maintaining the temperature below 5˚C. The resulting mixture was stirred for an additional 15 minutes in an ice bath and was then buffered with solid sodium acetate trihydrate.

4-hydroxy-1-naphthalenesulphonic acid (0.016 mol) was dissolved in 10% NaOH solution and cooled to 0-5˚C in an ice bath. This solution was then gradually added to the cooled 2-hydroxybenzenediazonium chloride solution and the resulting mixture was stirred at 0-5˚C for additional 60 minutes. The crude red-brown precipitate was filtered with the help of a suction pump and washed with cold water for several times and recrystallized from hot methanol-water mixture 60:40 (v/v) after drying. (M.P.- 300˚C, Yield - 70%).

ISSN: 0975-8585

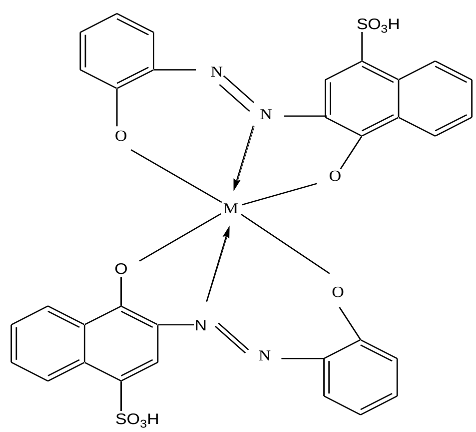
Synthesis of Complexes

An aqueous solution of appropriate metal salt (0.5 mole) was added dropwise to a solution of ligand i.e. azo compound (1.0 mole) in 20 dm^3 DMF. Anhydrous ferric chloride ($FeCl_3$), cobalt chloride dihydrated ($CoCl_2.2H_2O$) and cuprous chloride dihydrated ($CuCl_2.2H_2O$) were used to prepare iron, cobalt and copper metal complexes of azo compounds respectively. The pH of the solution was adjusted to 4.5 - 5.0 by addition of sodium acetate. This mixture was refluxed for 20-22 hrs. After cooling the mixture to room temperature it was poured into ice cold aqueous NaCl solution and then kept for 1 hr in an ice bath. The crude precipitate was isolated by filtration, washed several times with hot water to remove unreacted metal salt and then dried in a dessicator over anhydrous calcium chloride.

Table 1 Yield and characterization data for HPANS and its metal complexes.

Compound	F.W.(g/mol)	Colour	M.P. (˚C)	Yield (%)	μ_{eff} (B.M.)	λ_m'$\Omega^{-1}$$cm_2$ mol^{-1}
HPANS $C_{16}H_{12}N_2O_5S$	341	orange	300	70	-	-
[Fe(HPANS)$_2$].Na ($C_{32}H_{20}N_4O_{10}S_2NaFe$)	761	Deep brown	329	47	4.69	47
[Co(HPANS)$_2$].2Na ($C_{32}H_{20}N_4O_{10}S_2Na_2Co$)	785	Reddish black	349	61	4.43	59
[Cu(HPANS)$_2$] .2Na ($C_{32}H_{20}N_4O_{10}S_2Na_2Cu$)	789	Light brown	342	73	1.73	84

The physical properties, magnetic susceptibility and molar conductance values of ligand and metal complexes are summarized in table 1 and the proposed structure of metal complexes are shown in fig 1.



M = Fe(III),Co(II), Cu(II)

Fig:1 (Structure of metal complex of HPANS)

ISSN: 0975-8585

SPECTROSCOPIC CHARACTERIZATION

IR Spectra

In the IR spectra of ligand O-H stretching vibrations appear at 3310 cm^{-1}. Such a low value of O-H stretching vibrations and presence of N-H stretching vibrations can be attributed to –O-H···N tautomeric shift and intramolecular hydrogen bonding [10] and their absence in the metal complexes indicates deprotonation of naphthyl –OH groups, thereby showing its coordination to the metal ions.

In the IR spectra of metal complexes stretching vibrations due to N=N, C-N, and C-O appear at lower values in comparison to free ligands indicating that it has been affected upon coordination to the metal ions.

Characteristic absorption bands for (M◄— N) and (M ◄— O) bonds also appear in the IR spectra of metal complexes [11] in the spectral region of 690-770 cm^{-1} and 440-510 cm^{-1} respectively and which were not found in the spectra of free ligand.

Important IR peak values of ligand and it metal complexes are given in table 2.

Table 2 IR spectral data of MPANS and its metal complexes

Compound	ν_{O-H}	ν_{N-H}	$\nu_{N=N}$	ν_{C-N}	ν_{C-O}
HPANS	3300	2800	1450	1330	1150
$[Fe(HPANS)_2].Na$	_	_	1420	1280	1120
$Co(HPANS)_2].2Na$	_	_	1410	1300	1130
$Cu(HPANS)_2].2Na$	_	_	1430	1300	1120

NMR Spectra

H^1 NMR spectral data of HPANS showed singlets at 11.56 ppm and 12.40 ppm and at 3.70 ppm due to one naphthyl O-H proton, one phenolic proton and one O-H proton of sulphonic acid group respectively. While the aromatic hydrogen peaks resonated as multiplates at 7.50-6.88 ppm.

In examining the ^{13}C NMR spectrum of HPANS, signals for all aromatic carbon atoms were observed in the range 117.7-151.5 ppm .

Magnetic Susceptibilty

The iron and cobalt metal chelates exhibit μ_{eff} values 4.69 and 4.43B.M. respectively, which are in the range of values corresponding to high spin octahedral structure of these complexes. The value of μ_{eff} for copper metal complex is 1.73 B.M. which offers a possibility of high spin distorted octahedral structure [12].

ISSN: 0975-8585

Molar Conductance

Molar conductance of complexes was measured in 1mM dimethylformamide solution. High value of λ_m shown in table 1 for iron metal complex indicates its ionic behavior [13].

ANTIMICROBIAL ACTIVITY

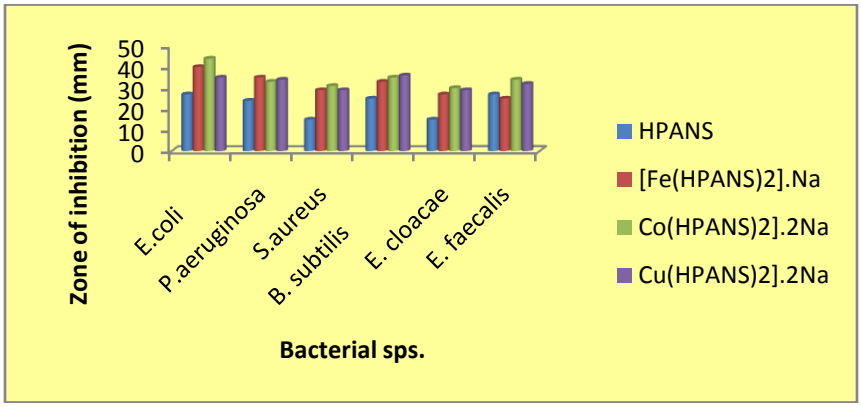


Fig 2. Statistical representation for biological activity of HPANS and its complexes

Table 3 Antimicrobial screening data (zone of inhibition in mm) of HPANS and its metal complexes.

Cmpounds	*E.coli* (Gram negative)	*P.aeruginosa* (Gram negative)	*S.aureus* (Gram positive)	*B. subtilis* (Gram positive)	*E. cloacae* (Gram positive)	*E. faecalis* (Gram positive)	*C. albicans*
HPANS	27	24	15	25	15	27	12
$[Fe(HPANS)_2].Na$	40	35	29	33	27	25	17
$Co(HPANS)_2].2Na$	44	33	31	35	30	34	21
$Cu(HPANS)_2].2Na$	35	34	29	36	29	32	18

Table 4 Minimum inhibitory concentrations (MIC) in µg/mL of MPANS and its metal complexes.

Compounds	E. coli (Gram negative)	P. aeruginosa (Gram negative)	S. aureus (Gram positive)	B. subtilis (Gram positive)
HPANS	25.0	25.0	50.0	50.0
$[Fe(HPANS)_2].Na$	6.25	12.5	12.5	12.5
$Co(HPANS)_2].2Na$	6.25	6.25	12.5	12.5
$Cu(HPANS)_2].2Na$	12.5	12.5	25.0	25.0

The synthesized ligand and its iron, cobalt and copper metal complexes were screened for the presence of antibacterial constituents against six strains of bacteria i.e. *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter cloacae*, *Enterococcus faecalis* and one species of fungi i.e. against *Candida albicans* by disc diffusion method. Nutrient agar was used as culture medium for bacterial growth while fungi were subcultured in potato dextrose agar medium. All compounds were dissolved in DMF. Ciprofloxacin (5 mcg/disc for bacteria) and ketoconozole (100 units/disc for fungi) was used as

reference antibiotic and DMF as control. The zones of inhibition were determined at the end if an incubation period of 24 hr at 35° C. During this period, the test solution diffused and the growth of inoculated microorganism was affected. The bacterial inhibition zone values are summarized in table 3 and its statistical presentation is shown in fig 2. Minimum inhibitory concentrations (MIC) were also determined by the broath dilution technique for four strains of bacteria and the results are shown in table 4.

RESULTS AND DISCUSSION

It has been observed from the results that the metal complexes have higher activity than that of the free ligand and the standard. Such increased activity of metal chelates can be explained on the basis of overtone and chelation theory.

According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials, due to which liposolubility is an important factor controlling the antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a great extent due to the overlap of the ligand orbit and the partial sharing of the positive charge of the metal ion with donor groups. Furthermore, it increases the delocalization of electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity leads to break down of the permeability barrier of the cell and thus retarts the normal cell processes.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Chemistry, University of Rajasthan, Jaipur and the Principal, S.M.S. Medical college, Jaipur, for the antimicrobial screening of the compounds. One of the authors, Ms. Swati, gratefully acknowledges CSIR, New Delhi, for providing Fellowship.

REFERENCES

[1] A Jarrahpour, M Zarei, Molebank , M377, 2004**.**
[2] Imahori, Seiichi, Himeno, Kiyoshi, Maeda, Shuichi, US Patent 4313731, 06/186372, 1982.
[3] A Eriksson, Acta universitatis. Comprehensive summaries of Uppasala, Dissertations from the faculty of science and technology**,** 638.43 pp. ISBN 91-554-5049-0,2001.
[4] Sanjay F Thakor, Dinesh M Patel, Manish P Patel, Ranjan G Patel. Saudi Pharmaceutical Journal 2007; 15C_1 Jan.
[5] AV Patel. E-journal of chemistry 2009; 6(4): 1247-1252.
[6] LV Gavali, PP Hankarep. J physical sciences 2007; 11: 147-155.
[7] E Yildiz, H Boztepe. Turk J Chem 2002; 26: 897-903.
[8] R Kilincarslan, E Erden. Transition Metal Chemistry, Springer, 2007; 32: 102-106.
[9] N Kabay, E Erdem, R Kilincarslan, E Yildirim Sari. Transition Metal Chemistry, Springer 2007; 32: 1068-1072.

[10] Kilincarslan R & Erdem E. Transition Met Chem 2007; 32: 102-106.
[11] Masoud MS, Khalil EA, Hindawy AM, Ali AE & Mohamed EF. Spectrochimica Acta Part A : Molecular and Bimolecular Spectroscopy 2004; 60: 2807-2817.
[12] Karipcin F & Kabalcilar E. Acta Chim Slov 2007; 5: 242–247.
[13] Kulkarni AD, Patil SA & Badami PS. Int J Electrochem Sci 2009; 4: 717 – 729.
[14] Raman N & Ravichandran S. Synthesis and reactivity in inorganic, metal-organic and nano-metal chemistry 2005; 35: 439-444.