Synthesis, Characterization and Biological Properties of *p*-Nitrosalicylidene Aniline and its Complexes with Ni(II), Co(II), Cu (II), and Zn(II)

[1]MUHAMMAD IMRAN*, [2]JAVED IQBAL AND [2]SHOOMAILA LATIF
[1]*Undergraduate Block,* [2]*Institute of Chemistry, University of the Punjab Lahore, Pakistan.*

(Received 30th June 2007, revised 23rd April 2007)

Summary: Some biologically active Ni (II), Co (II), Cu (II) and Zn (II) complexes of *p*-nitrosalicylidene aniline have been synthesized and characterized by elemental analysis, measurement of conductance and magnetic moments, and spectral data. The ligand and its complexes have also been screened for their antibacterial activity against three bacterial strains such as *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) values were determined at 37 °C for a period of 24 h. The metal complexes were found to be more antibacterial as compared to un-complexed ligand.

Introduction

Many studies [1-3] have demonstrated the antitumour [4-8] and antibacterial characteristics of some coordination complexes. While increased carcinogenic and bacteriostatic properties of biologically active compounds have also been reported upon chelation [9]. Presently, number of coordination compounds are being used for therapeutic purposes as antibacterial, antiviral, and anticancer drugs [10-14]. Kirshner *et al.*, [15] suggested that the transfer of metal ion, from ligand to cancer causing virus, was an important mechanism for designing new anticancer therapies.

Schiff bases of *p*-substituted anilines have become focus of intense research regarding their behavior of forming complexes, chelating abilities and diagnostic applications [16-18].

Many ethanopharmacological properties, including antibacterial [19], anti-inflammatory and antiulcer [20], of these compounds have been highlighted previously. Biological properties of these Schiff bases have been attributed to azomethine linkage of these Schiff bases [21]. Anti-inflammatory and antiulcer activity of the Schiff bases derived from *p*-substituted anilines has been reported elsewhere [22-24]. As many aldehydes have been claimed to be potential inhibitors for DNA synthesis; therefore, if a salicylaldehyde moiety is coupled with *p*-nitro aniline, the resulting compound *p*-nitrosalicyliden aniline may show enhanced biological activity. These factors prompted us to carry out a study for synthesis of *p*-nitrosalicyliden aniline (a Schiff base) and its complexes with Ni (II), Co (II), Cu (II), and Zn (II) metal ions.

The aim of this study was to synthesize the *p*-nitrosalicylidene aniline to study its complex formation and antibacterial activity. Antibacterial activity of ligand and complexes was studied employing *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*, as bacterial strains.

Results and Discussion

p-nitrosalicylidene aniline was prepared by refluxing an equimolar amount of *p*-nitroaniline and salicylaldeyde in ethanol (Scheme-I). The structure of the ligand was established with IR, ^{1}H-NMR and analytical data.

All the complexes were colored, except that of Zn (II) complex. These complexes decompose above 180 °C. They are soluble in organic solvents the solubility of ligand and complexes in different solvents has been depicted in Table-1. Molar conductance values of soluble complexes in DMF have been compiled in Table-3, indicating their non-electrolytic nature [25].

Characteristic bands due to stretching of carbonyl $\bar{\nu}$(C=O) at ~1735 cm^{-1} in salicylaldehyde (reactant) and $\bar{\nu}$ (NH_2) 3315 cm^{-1} in *p*-nitro aniline (reactant) disappeared in IR spectra of ligand (Table-2), rather a new strong band appeared at ~ 1629 cm^{-1},

*To whom all correspondence should be addressed.

assigned to azomethine $\bar{\nu}$ (HC=N) linkage of ligand (product) [26,27], showing that amino and aldehyde moieties of the reactants are absent and have been converted into the ligand *i.e. p*-nitrosalicylidene aniline.

IR spectra of pure ligand and its metal complexes indicated that the ligand is principally coordinated to the metal ion in two ways; revealing monoanionic bidendate nature of the ligand.

Comparison of IR spectra of pure ligand and its complexes shows, the characteristic band appearing at ~ 1629 cm^{-1} due to azomethine is shifted to lower frequency ~ 5 to 15 cm^{-1} in all complexes, indicating participation of azomethine nitrogen in interaction with metal ion. A broad band appearing at 3415cm^{-1} assigned to $\bar{\nu}$(OH) in ligand, was no longer found in the spectra of the metal complexes, and indicates deprotonation and coordination of hydroxyl oxygen to the metal atom. Moreover all the complexes exhibited new bands at 565-570 cm^{-1} and 554-560 cm^{-1} in the far-infrared region. These were assigned to $\bar{\nu}$(M-N) and $\bar{\nu}$M-O) in the metal complex rather than in ligand. Further more in the spectra of Ni (II), Co (II), Zn (II) complexes, the broad band at ~3375 cm^{-1} together with new band at 883 cm^{-1} confirms the presence of coordinated water [28]. The disappearance of these bands in Cu (II) complex suggest square planar geometry for Cu(II) complex (Fig. 1) and an octahedral geometry for Ni(II), Co(II), Zn (II) complex (Fig. 2).

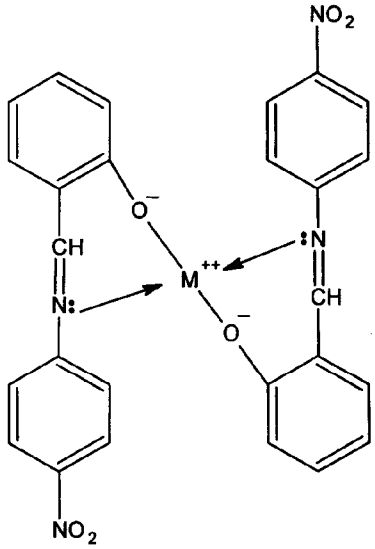


Fig. 1: Proposed structure of Cu (II) complex.

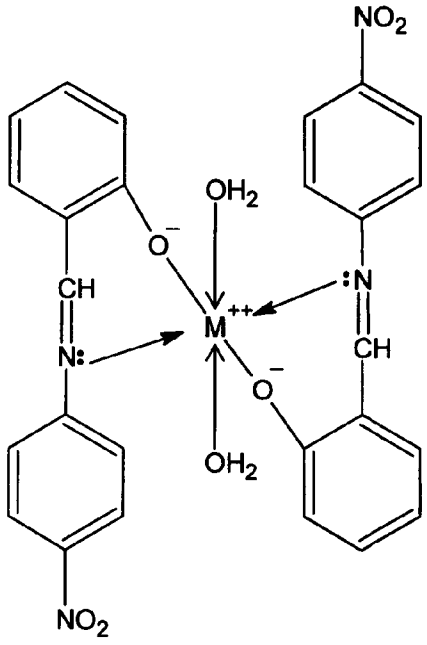


Fig. 2: Proposed structure of Metal (II) complexes. Where M (II)= Ni (II), Co (II) and Zn (II).

The ^{1}H-NMR spectra of the Schiff base and its complex with Zn (II) taken in DMSO-d_6 is listed in (Table-2).The Schiff base showed signal due to all expected protons in their expected region and were compared with the reported signal of known identical compounds [29].Comparisons of chemical shift of the uncomplexed Schiff base with Zn(II) complex showed that some of the signals experience shift upon complexation. The protons due to azomethine undergo a shift towards downfield by 0.9 ppm in the complex, indicating coordination of this group with metal ion. Also, a proton due to hydroxyl group (OH) was found to be absent in the spectra of Zn (II) complex. The absence of this signal suggested the deprotonation of hydroxyl group and the involvement of oxygen atom in complex formation [30]. The metal to ligand ratio of all these complexes was 1:2 according to the elemental results (Table-1).

The data obtained from the electronic spectroscopy is tabulated in (Table-3).Two bands appeared in the ligand, one at 37878 cm^{-1}, attributed to benzene $\Pi \rightarrow \Pi^*$ transition and the second appeared at 26,666 cm^{-1} which is assigned to $n \rightarrow \Pi^*$ involving non bonding electron present on nitrogen

Table-1: Physico-analytical properties of the ligand and metal complexes.

Compound	Color	Solubility	M.P (°C)	Yield (%)	Calculated · (Found) (%) C	H	N	M
LH	Orange Red	Ethanol DMF, Acetone	148	73	62.8(62.2)	3.93(3.857)	12.2(11.97)	–
$NiL_2(H_2O)_2$	Brown	DMF , DMSO	223	55	55.81(55.72)	3.48(3.37)	5.42(5.39)	11.2(11.12)
$CoL_2(H_2O)_2$	Pale Yellow	Ether, DMF , DMSO, Methanol	245	57	55.92(55.85)	3.49(3.40)	10.87(10.82)	11.06(10.98)
CuL_2	Bright Yellow	Ether , DMF DMSO, Methanol	180	62	55.27(55.22)	3.45(3.40)	10.74(10.70)	12.09(12.02)
$ZnL_2(H_2O)_2$	Off-white	DMF , DMSO	193	68	55.06(55.00)	3.44(3.39)	10.70(10.65)	12.42(120.49)

LH= *P* –nitrosalicylidene aniline

Table-2: I. R and ^{1}H NMR spectral data of ligand and metal complexes.

Compound	I.R (cm^{-1}) ν(OH)	ν (CH=N)	ν (M-N)	ν (M-O)	^{1}H NMR (ppm) OH(s),	Phenyl(m),	CH=N(s)
LH	3415	1629	-----	------	11.2	6.4-7.3	5.6
$NiL_2(H_2O)_2$	3375,883	1623	566	557	—	—	—
$CoL_2(H_2O)_2$	3375,883	1620	567	557	—	—	—
CuL_2	——	1618	565	556	—	—	—
$ZnL_2(H_2O)_2$	3375,883	1616	570	560	----	6.3-7.1	6.5

LH= *P* –nitrosalicylidene aniline
s, singlet. m, multiplet
------ disappeared
– Not determined

Table-3: Magnetic moment, conductance and electronic spectral data of the ligand and metal complexes.

Compound	μ_{eff} (B M)	λ m mho cm^{-2} mol^{-1}	λmax (cm^{-1})
L H	–	5.6	37878; 26666
$NiL_2(H_2O)_2$	3.17	8.40	6,312; 15,433; 9789
$CoL_2(H_2O)_2$	3.79	16.9	7,373; 17,213; 20,613
CuL_2	1.79	11.7	31055; 19820
$ZnL_2(H_2O)_2$	–	9.6	27,123

LH= *P* –nitrosalicylidene aniline

atom of azomethine group in the ligand LH. The electronic spectrum of Cu (II) complex showed two bands at 31055 cm^{-1} and 19820 cm^{-1} and are assigned to charge transfer and $E_g \leftarrow {}^2T_{2g}$ transition. These two bands are in agreement with those usually observed for square planar complexes of Cu (II) [31]. The magnetic moment value for Cu(II) complex (1.79B.M) also favors its square planar geometry (Fig. 1). The electronic spectrum of Co(II) complex showed three bands observed at 7,373 cm^{-1}, 17,213 cm^{-1} and 20,613 cm^{-1} and are assigned to $^4T_{1g}$ (F) → $^4T_{2g}$(F), $^4T_{1g}$(F)→ $^4A_{2g}$(F) and $^4T_{1g}$ (F)→ $^4T_{2g}$(P) transitions respectively which correspond to octahedral geometry for Co(II) complex. The magnetic moment value for Co (II) complex (3.79B.M) provides a clue for its octahedral geometry (Fig. 2). The Ni (II) complex exhibited three spin allowed bands at 26,312 cm^{-1}, 15,433 cm^{-1}, and 9789 cm^{-1}, assignable respectively to $^3A_{2g}$(F) → $^3T_{2g}$ (F) , $^3A_{2g}$ (F) →$^3T_{1g}$(F), $^3A_{2g}$(F) →$^3T_{2g}$(P) transitions consistent with well defined octahedral geometry. The magnetic moment value (3.17B.M) showed two unpaired electrons per Ni (II) ion and also confirm the octahedral environment for Ni (II) complex. The Zn (II) complex was found to be diamagnetic. The diamagnetic Zn (II) complex did not show any d-d transition and its spectrum was dominated only by the charge transfer band at 27,123 cm^{-1}

The ligand that is *p*-nitrosalicylidene aniline and its metal complexes were evaluated for antibacterial activity against three bacterial species namely *Escherichia coli, Staphylococcus aureus, Pseudomonas aerugionosa*. The antibacterial activity data (Table-4) have shown that metal ions on coordination significantly increase the antibacterial activity of uncomplexed ligand against all bacterial species. Such increased activity of metal complexes can be explained on the basis of chelation theory, electrostatic metal binding ability, formation constants and cell permeability [19].

Experimental

Analytical reagent grade ethanol was used after distillation. All other chemicals and solvents were of reagent grade and procured from Sigma Aldrich or E.Merck unless stated otherwise. Metal chlorides were used as metal (II) salts for synthetic purposes. IR spectra were recorded on Philips Analytical PU 9800 FTIR using KBR disc method.

Table-4: Antibacterial data of the ligand and metal complexes.

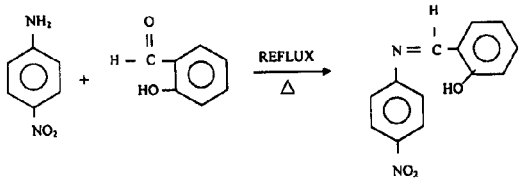
Ligand/ complex	Microbial species		
	a	b	c
L H	++	+++	++
$NiL_2(H_2O)_2$	++	++++	+++
$CoL_2(H_2O)_2$	+++	+++	++
CuL_2	++	+++	++++
$ZnL_2(H_2O)_2$	+++	+++	+++

LH= *P* –nitrosalicylidene aniline
a = *Pseudomonas aeruginosa* b = *Staphylococcus aureus* c = *Escherichia coli*
Inhibition zone measured in diameter
+ 5-10 mm ++ 10-15 mm +++ 15-20 mm ++++ 20-25mm

UV/VIS spectra were obtained in DMF by SPECORD-200 using software ACUTA710. While ^{1}H-NMR spectra were recorded on a Bruker 250 MHz NMR spectrometer in DMSO-d_6. Conductance of the metal complexes were determined in DMF on a Hitachi YSI-32 model conductometer. Percentage of metals was determined by atomic absorption spectrophotometer using calibration curve method. Magnetic measurement was done on the solid complexes using the Gouy method. M.P. was determined by Gallenkamp apparatus.

Synthesis of p-Nitrosalicylidene *Aniline*

I0ml of salicylaldehyde (0.01M, 1.2g, 1.1ml) in ethanol was added to 10ml ethanolic solution of *p*-nitro aniline (0.01M, 1.38g) and mixed. 2–3 drops of concentrated H_2SO_4 was added and then it was refluxed for 2 hours. On cooling, a solid colored product formed which was filtered, washed with ethanol and ether, followed by drying. Crystallization with hot ethanol gave the required Schiff base (scheme I).



(Scheme I)

Synthesis of Metal Complexes

The metal complexes were prepared with 1:2 ML ratio by refluxing quantities of ligand (0.02 M) in warm alcohol (10 ml) and respective metal chloride (0.01 M) in 10 ml alcohol for 2-3 hours, followed by cooling to room temperature. On cooling, colored precipitates were formed, which were filtered, washed with ethanol, acetone and dried.

Antibacterial Studies

Paper Disc Diffusion method [19,32] was used to study antibacterial activity against three bacterial strains namely *Escherichia Coli, Staphylococcus aureus,* and *pseudomonas aeruginosa.*

The nutrient agar medium (peptone, beef extract, NaCl and Agar-Agar) and 5 mm diameter paper discs (Whatman No.1) were used. The compounds *i.e.* ligand & complexes, were dissolved (30 µg) in DMF (0.01ml). The filter paper disc were soaked in solutions of ligand as well as complexes, dried, and then placed in Petri plates previously seeded with the test organisms. The plates were incubated for 24 h at 37° C and the inhibition zone around each disc was measured.

References

1. D. R. Williams, The Metals of Life, Van Nostrand , London, (1971).
2. D. H. Brown, W. E. Smith, J. W. Teape and A. J. Lewis, *J. Med Chem.*, **23**, 729 (1980).
3. a) A. Sozzafava, L. Menabuoni, F. Mincione, G. Mincione and C. T. Supuran, *Bioorg. Med. Chem.Lett.*, **11**, 575 (2001).
b) A. Scozzafava and C. T. Supuran, *J. Med. Chem.*, **43**, 3677 (2000).
4. a) R. J. P. William, *Quart. Rev.*, **24**, 331 (1970).
b) R. J. P. William, Bio Inorganic Chemistry, American Chemical Society, Washington, (1971).
5. B. Rosenberg and L. V. Camp, *Cancer Res.*, **30**, 1799 (1970).
6. M. J. Clare and J. D. Heeschele, *Bioinorg. Chem.*, **2**, 187 (1973).
7. V. L. Narayanan, M. Nasr and K. D. Paull, Tin-based Antitumour Drugs, M. Gielen (Ed), NATOASI Series, Springer Verlag-Berling, (1990).
8. A. J. Crowe, Metal Based Antitumour Drugs, M. Gielen (Ed), Freund, London, (1988).
9. R. S. Srivastava, *Ind. J. Chem.*, **29 (A)**, 1024 (1990).

10. A. Y. Louie and T. J. Meade, *Chem. Rev.*, **99,** 2711 (1999).
11. a) D. H. Brown, W. E. Smith , J. W. Teape and A. J. Lewis, *J. Med.Chem.*, **23,** 729(1980). (b) R. D. Gillard , *Inorg. Chim. Acta . Rev.*, **1,** 60 (1961).
12. C. Walsh, *Science.*, **409**, 226 (2001).
13. I. Bertini, H. B. Gray, S. J. Lippard and J. S. Valentine, *Bioinorganic Chemistry*, Univ Science Books, CA, USA, (1994).
14. R. K. Watt and P. W. Ludden, *Cell Mol. Life Sci.*, **56,** 604 (1999).
15. S. Kirschner, Y. K. Wei, D Francis and D. Bergman, *J. Med. Chem.*, **9**, 369 (1966).
16. A. E. Marteil, *Pure and Appl.Chem.*, **12,** 129 (1968).
17. M. Y. Khuhawar, *J. Chem. Soc. Pak.*, **6**, 225 (1984).
18. K. Lieno and A. E. Marteil, *J. Phys. Chem.*, **41**, 257 (1957).
19. M. Hassan, A. Scozzafava, Z. H. Chohan and C. T. Supuran, *J. Enzy. Inhib. Med. Chem.*, **18**, 495, (2003).
20. R. K. Parashar, R. C. Sharma and G. Mohan, *Biol. Trace Elem. Res.*, **23**, 150, (1990).
21. P. Phatak, V. S. Jolly and K. P. Sharma, *Orient. J. Chem.*, **16**, 493 (2000).
22. S. A. Sadeek, M. S. Refat and S. M. Teleb, *Bulletin of the Chemical Society of Ethiopia.*, **18**, 149, (2004).
23. F. Arod, M. Gardon, P. Pattison and G. Chapuis, *Acta Cryst.*,. **C61**, o317, (2005).
24. J. Burgess, J. Fawcett, V. Palma and S. R. Gilani, *Acta Cryst.*, **C57**, 277, (2001).
25. D. J. Finney, Probit Analysis, Cambridge University Presss, Cambridge, (1971).
26. K. Burger, I. Rauff and F. Ruff, *J. Inorg. Nucl. Chem.*, **27**, 179, (1965).
27. M. Mashima, *Bull. Chem. Soc. Japan.*, **35,** 1882 (1962).
28. K. Nakamoto, Infrared and Raman spectra of Inorganic and Coordination Compounds, Wiley. New York, (1978).
29. D. J. Pasto and C. R. Johnson, Organic Structure Determination, 4th Ed. Prentice-Hall, International, Inc, U.K (1969).
30. Z. Hong-Yon, C. Dong-Li, C. Pei-Kun, C. De-Ji, C. Guang-Xia and Z. Hong-Quan, *Polyhedron.*, **11,** 2313 (1992).
31. A. B. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdom (1968).
32. J. Iqbal, S. A. Tirmizi, F. H. Wattoo, M. Imran, M. H. S. Wattoo, S. Sharfuddin and S. Latif, *Turk. J. Biol.*, **30**, 1 (2006).