SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF A MANNICH BASES, AND ITS Cu (II) COMPLEX.

M.B.Swami
Department of Chemistry, B.S.College,
Basmathnagar-431512, [M. S] India.

Abstract : The synthesis of Cu(II) complex derived from Mannich base N-[l-ethyl, 1-piperidino (4hydroxyphenyl)]semicarbazide(EPHPS)andN_1[1-(4-hydroxyphenyl)-1-morpholinopropyl] semicarbazide (HPMPS) derived by the condensation of 4-hydroxy propiophenone, morpholine and semicarbazide. Its structure has been confirmed on the basis of analytical, magnetic and spectral studies. The Cu(II) complex exhibit square-planar geometry. The antibacterial activity of synthesized ligand (EPHPS) ,HPMPS) and its Cu(II) complex was studied by the usual cup-plate-agar-diffusion method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The qualitative and quantitative antimicrobial activity test results proved that the Cu(II) complex is very active than the free ligand. The increase in antibacterial activity is due to faster diffusion of metal complex as a whole through the cell membrane or due to the combined activity effect of the metal and ligand.

Keywords: Mannich-base, Copper (II) complex, Anti-bacterial activity.

I. INTRODUCTION

Metal complexes of Mannich bases have been studied extensively in recent years due to the selectivity and sensitivity of the ligands towards various metal ions.[1-2] Much work has been done so far on isolation of solid complexes of different aromatic aldehydes or ketones, semicarbazides with transition metals.[3] The ready accessibility, diverse chemical activity, and pharmacological properties.[4] Azomethines constitute one of the most important classes of biologically active ligands providing potential binding sites through nitrogen and oxygen donor atoms.

The present poster reports the synthesis of N-[l-ethyl, 1-piperidino (4-hydroxyphenyl)]semicarbazide(EPHPS), N_1[1-(4-hydroxyphenyl)-1-morpholinopropyl] semicarbazide and its Cu(II)complex. The bidentate ligand coordinates with the metal ion through the oxygen atom of the carbonyl group and nitrogen atom of semicarbazide group.

II. EXPERIMENTAL

All the reagents used were of AR grade and solvents were purified by standard method. Copper contents of the complexes were estimated complexometrically with EDTA using murexide and erichrome black T as an indicator after decomposing the complexes with concentrated

H_2SO_4 and H_2O_2 , ^{1}H-NMR spectra of the ligand measured in $CDOD_3$ at IICT Hyderabad. The IR spectra were recorded in KBr pellet using Shimadzo spectrophotometer at YM Nanded. The UV-Visible spectra of the complexes were recorded on a Shimadzu UV-1601 spectrophotometer. Magnetic moments were measured by the Gouy method using $Hg[Co(CNS)_4]$ as the calibrant. The molar conductivity was measured on equiptronic conductivity meter using 10-3M solution of complexes in DMSO.

The antibacterial activities of the investigated compound were studied by the usual cup-plate-agar-diffusion method. The compounds were screened for their antibacterial activity against the following microorganisms: (a) gram positive staphylococcus aureus (S aureus), (b) gram negative E coli. The cup-plate-agar-diffusion method comprises of the following steps.

(1) Preparation of media, sterilization, and tubing.
(2) Sterilization of the cleaned glass apparatus.
(3) Pouring of the seeded medium into sterilized Petri dishes and cutting of the cups.
(4) Pouring of the dilute solution of the compounds into the tubs.
(5) Incubation at a particular temperature.
(6) Determination of the "zones of inhibition."

The composition of the test media is the factor, which often exerts the greatest effect upon the drug activity. This is particularly true for thiosemicarbazones, since inhibitors of these compounds appear to be present in the common bacteriological culture medium. Efficient media of known chemical composition are available for many species such as S aureus and E coli. In addition to the composition of the test media, its pH is a factor which may directly or indirectly influence the activity of a drug. The pH of the test media taken for S aureus and E coli was adjusted in the range 7.6 ± 0.1.

The composition of the basal media used in the experiments was (i) sodium chloride = 6.0 gm, (ii) peptone = 10.0gm, (iii) beef extract = 3.0 gm, (iv) yeast extract = 2.0gm, (v) sucrose = 1.5 gm, (vi) agar-agar = 3.0%, and (vii) distilled water = 1.0 litre.

Procedure

The measured quantity of the culture of the test organism (0.5 ml) was added to each heated (nearly 55^0C) agar media tubes. The tubes were shaken well, and the inoculated media were poured on to the sterilized Petri-dishes and then allowed to set in a refrigerator maintained at 4–8^0C. The test solutions of 500 g/ml and 1000 g/ml dilutions of the respective thiosemicarbazones were prepared in a mixture of DMF and H_2O (3: 7, v/v). Five cups of 5mm diameter were cut in the culture media on the pert-dishes. A compound solution of particular dilution (500 g/ml or 1000 g/ml) was put in the outer four cups of one of the Petri-

dishes, and the second solution was put in the four cups of other Petri-dishes. The central cups of all the Petri-dishes were filled with the
Controlled solution and all the Petri-dishes were allowed to remain in the refrigerator maintained at 10^0C for 1hr to allow diffusion of the solution. The Petri-dishes were then transferred to an incubator maintained at 35^0C and kept for nearly 30 hrs. The zones of inhibition formed were measured with calipers. The control of DMF and H_2O (3: 7, v/v) Showed no activity. The activity of the compounds is represented by size of the diameter in mm. The biological screening effects of the investigated compounds were tested by the well diffusion method, using Muller Hinton agar as the nutrient medium.

Synthesis Of Mannich Base

1] Mannich base was synthesized by reported procedure.[5] Semicarbazide hydrochloride (1.11 g, 10 m mol) in 20 mL of ethanol was neutralized with ammonia. To this solution, morpholine (0.9 mL, 10 m mol) was added drop wise with constant stirring under ice-cold condition. Then, 2-chlorobenzaldehyde (1.3 mL. 10 m mol) was added drop wise under the same condition. After 5 min colorless solid obtained was filtered and recrystallised from ethanol.
2] Morpholine (10 m mol) was added drop wise to the Semicarbazide hydrochloride (10 m mol) in 20 mL of ethanol with constant stirring under ice-cold condition. Then, 4-hydroxy propiophenone (10 m mol) was added drop wise under the same condition. After 24 hours, brown solid obtained, was filtered and re-crystallized from ethanol.

COMPOUND	M.P ^{0}C	YIELD in %
EPHPS	225	90
HPMPS	162	67

Synthesis Of Metal Complexes

A solution of 5 mmol of $CuCl_2$ and the Mannich base (10 m mol) in ethanol was boiled under reflux for about 3h. The pH of the resulting solution was adjusted to optimum pH 7.0 by drop wise addition of NH_3 solution. The precipitated complexes were filtered, washed with water and dried it.

III. RESULTS AND DISCUSSION

The Cu (II)complex is green colored and stable at room temperature. The elemental data show 1:2 (metal: ligand) stoichiometry. The absence of chloride is evident from Volhard's test. The low conductance value (1.8) of the complex supports their non-electrolytic nature.[6]
1] The IR spectrum of the ligand shows bands in the regions 3435 and 1695 cm^{-1}, which are assigned to V(N-H) and V(C=O) of semicarbazone, respectively[15-16]. In the spectra of complexes, the V(C-O) mode of the free ligand is not observed indicating the enolisation of C=O followed by deprotonation and complexation with metal ions. The vC=N) mode of ligand is found to shift to lower wave number suggesting coordination of azomethine nitrogen to the central metal ion.
The band at 3435 cm-1 observed in the ligand, is absent in the complexes, suggesting deprotonation of-NH of the ligand prior to the coordination to the metal.
2] The IR spectrum of the ligand shows bands in the regions 3420-3380 and 1703 cm^{-1}, which are assigned to υ(N-H) and V(C=O) of semicarbazone, respectively.[7] In the spectra of complexes, the V(C-O) mode of the free ligand is not observed indicating the enolisation of C=O followed by deprotonation and complexation with metal ions.
The stretching at 1599 cm^{-1} of *ν*(C=N) of ligand is found to shift to lower wave number suggesting coordination of azomethine nitrogen.[7]
The band at 3380 cm^{-1} observed in the ligand, is absent in the complexes, suggesting deprotonation of-NH of the ligand prior to the coordination to the metal.

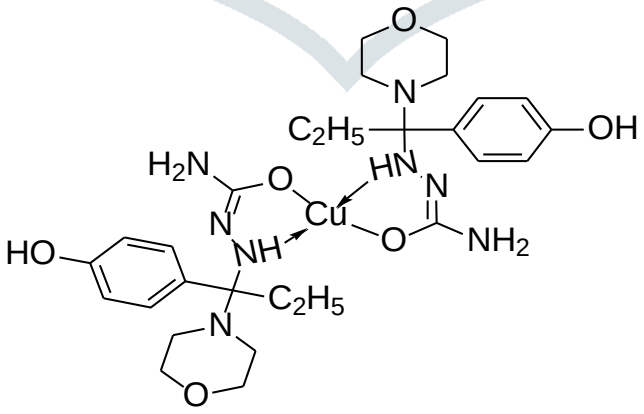

Figure 1 Cu[HPMPS]$_2$ complex

ANTIBACTERIAL ACTIVITY

1] [EPHPS] The UV-Vis spectrum of copper complex in CH_3COOH solution displays a broad band at 14598 cm-1 attributable to $^2B_{1g} \rightarrow {}^2A_{lg}$ transition which strongly favours the square-planar geometry around the central metal ion17-19. This is further supported by the magnetic susceptibility value (1.7B.M.).

2] [HPMPS] The UV-Vis spectrum of copper complex in DMSO solution displays a broad band at 14440cm^{-1} attributable to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition which strongly favours the square-planar geometry.[8] This is further supported by the magnetic susceptibility value (1.77 B.M.).

The ligand EPHPS, HPMPS and its Cu (II) complexes were tested for antibacterial activity. Mueller-Hinton agar was used for testing the susceptibility of microorganism by well diffusion method[9] using DMSO as solvent, at a concentration of 0.1 M against Gram-positive (S.aureus) and Gram-negative (E.coli) bacteria. The zones of inhibition against the growth of microorganisms were determined at the end of an incubation period of 24 h at 37° C and the results are presented in Table 1. It was found that the metal complexes are more active than the free ligand. The increase in antibacterial activity is due to faster diffusion of metal complexes as a whole through the cell membrane or due to the combined activity effect of the metal and ligand.[10]

Table 1. Antibacterial activity of ligand and its Cu (II) complex

Compound	Zone of inhibition	
	S.aureus	E.coli
EPHPS/MBSC	7.0	6.0
$Cu(MBSC)_2$	14.0	13.0
$Co(MBSC)_2$	10.0	11.0
$Ni(MBSC)_2$	11.0	10.0
$Zn(MBSC)_2$	9.0	12.0
Ampicillin	8.0	7.0
HPMPS	7.0	6.0
$[Cu(HPMPS)_2]$	13	11

IV. CONCLUSIONS

Copper (II) complex have been synthesized from the Mannich base N-[l-morpholino(2-chlorobenzyl)semicarbazide]andN_1[1-(4-hydroxyphenyl)-1-morpholinopropyl] semicarbazide and characterized by elemental data, magnetic and spectral analyses. The metal complexes exhibit higher activity than the free ligand.

REFERENCES

1. K. S. Nimavati, et al. *J. Indian Chem. Soc.*, 80, 711(2003).
2. J.N.Gadre, M.Mulay and C.Vaze, *Indian J. Het. Chem*., **13,** 335 (2004).
3. N. Raman and S. Ravichandran, *Polish. J. Chem.*, 78, 2005 (2004).
4. A.R.Emelda et al., *Int.J.Chem.Sci*.,5(1), 2007
5. N.Raman and D.Anbuthilagam., *Int. J. Chem. Sci*., 3(1), 100,(2005).
6. W. J. Geary, *Coord. Chem. Rev.*, 7, 81 (1971).
7. B.R.Havinale and I. B.Pujar, *Indian Chem.*, A24, 1042 (1985).
8. A. B. P. Lever, "*Inorganic Electronic Spectroscopy*", Elsevier, NY (1968).
9. C. H. Collin and P. M. Lyne, "*Microbiological Methods*", 3rd. Edn., Butterworth, London (1970)
10. Y. Anjaneyuluetal. *Synth. React. Inorg.Met.Org.Chem.*, 16, 257 (1986) .