

RESEARCH ARTICLE



SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF A MANNICH BASE AND ITS IRON (III), ZINC (II), CADMIUM (II), MANGANESE (II) OXOMOLYBDENUM (V) COMPLEXES

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Abstract

Mannich base was synthesized and characterized by elemental analyses and spectral studies. Its complexes with Fe (III), Zn (II), Cd (II), Mn (II) and Oxomolybdenum (V) were prepared and characterized by elemental analyses, molar conductance, magnetic susceptibility and spectral studies. The ligand has been behaved in a bidentate manner bonding through carbonyl O of acetamide and CNC Nitrogen of morpholine. Based on these studies octahedral geometry has been proposed for all the complexes. The antimicrobial activities of the ligands and its metal complexes the comparison shows that the ligand is less potent antibacterial than its metal complexes.

Keywords: Complexes, stoichiometry, Multiplet, Inhibition, Antimicrobial activity.

Introduction

Mannich bases for an interesting group of ligands. A No. of mannich bases prepared and their complexation behaviour have studied (Kasim et al., 1999; Gandhi and Kulkarani, 1999; Raman and Ravichandran, 2003; Pelczar et al., 1998; Haidne, 1990). The complexes of mannich bases find extensive applications in a variety of field (Vogel, 2004). It has been observed that the transition metal complexes of Mannich bases are of particular interest because of their synthetic flexibility, structure features, bonding interactions, antimicrobial properties and their multiplication. Keeping these facts in view the Mannich base -N-[morpholino(phenyl) methyl] acetamide was prepared and characterized its metal complexes with metals Fe (III), Zn (II), Cd (II), Mn (II), Oxomolybdenum (V), have been prepared (1:2) molar ratio and characterized the antibacterial activities of the ligand its complexes has also been carried out.

High purity acetamide (Merk), benzaldehyde (Merk), morpholine (Merk) were used as supplied. All other

solvents and metal salts used were of A.R. grade and used as received.

Synthesis of the Ligand

N-[Morpholino(phenyl) methyl] acetamide (MBA) was prepared by Mannich synthetic route (Tramontini and Angiolini, 1990). Acetamide (5.90 g, 0.1 mol) was dissolved in minimum quantity of ethanol. To this solution, benzaldehyde (10 mL., 0.1 mol) followed by morpholine (9 mL, 0.1 mol) were added in small quantities with constant stirring in an ice bath. After 28 days, a yellow solid was obtained. It was washed with water and acetone. The compound was dried in air and then at 60°C in an air oven and recrystallised from ethanol. The percentage yield of the compound was 73 and its melting temperature was 148-150°C.

Synthesis of the Complexes

The hot methanolic solution of the metal salt was added slowly with constant stirring to the hot

ethanolic solution of the ligand in 2:1 mol ratio. The insoluble complexes (Singh et al., 1985) formed, were filtered, washed with methanol and ethanol to remove the unreacted metal and ligand, dried in air and then in an air oven at 80°C.

Instruments

Micro elemental (C, H and N) data were obtained with Carlo Erba 1108 elemental analyzer at RSIC, CDRI, Lucknow. Metal contents were estimated by usual procedure, after digesting the complexes with conc. HNO₃. Sulphate was estimated gravimetrically as BaSO₄ and chlorides were estimated volumetrically by Volhard's method. The conductance data were obtained in ~10⁻³ M DMF solutions of the complexes at room temperature using a Systronics direct reading digital conductivity meter-304 with dip type conductivity cell. IR spectra were recorded using a spectrum-one Perkin Elmer FT-IR spectrometer employing KBr pellets. The UV-Visible regions were recorded in DMF solutions using double beam UV-Visible spectrometer, Perkin EZ-301 of working range 1100-190 nm. The ¹H and ¹³C NMR of the ligand were recorded on a Bruker instrument and on a JEOL-GSX 400 spectrometer employing TMS as internal reference and DMSO-d₆ as solvent. The FAB mass recorded for the ligand was carried out using a JEOL-GC mate mass spectrometer. Electrochemical studies were performed on a Bio-Analytical System CV-50W electrochemical analyzer with three-electrode system of a glassy carbon electrode as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. The room temperature magnetic susceptibility measurements of the complexes were made by using a Gouy magnetic balance calibrated using mercury (II) tetrathiocyanatocobaltate (II).

Antimicrobial Studies

Antimicrobial activities of MBA (Chohan et al., 2005) and their metal complexes, such as Zn(II), Mn(II) and Fe(II) were tested in vitro against six bacterial species *E. coli*, *P. aeruginosa*, *S. typhi*, *B. subtilis*, *S. pyogenes* and *S. aureus* and the fungal species *A. niger* and *A. flavus* by disc diffusion method using agar nutrient as medium and gentamycin as control. The paper disc containing the compound (10, 20 and 30 µ/disc) was placed on the surface of the nutrient agar plate previously spread with 0.1 mL of sterilized culture of microorganism. After incubating this at 37°C for 24 h, the diameter of

inhibition zone around the paper disc was measured.

Results and discussion

Characterization of the Ligand

Infrared spectrum of MBA shows a sharp peak at 3290 cm⁻¹, which may be assigned to the νNH of the secondary amide group (Mishra and Srivastava, 1994). The strong band at 1647 cm⁻¹ may be attributed to the νC=O stretching mode. The other strong bands appearing at 1490, 1445, and 1205 cm⁻¹ are indicative of bending vibrations of the methylene (δ_{CH₂}) group and stretching vibration of the morpholine ring (V_{ring}). The medium absorption band at 1116 cm⁻¹ suggests the presence of new C-N-C bond pertaining to the formation of Mannich base by the insertion of morpholinobenzyl group on acetamide. The absorption band present at 1140 and 1072 cm⁻¹ may be assigned to the C-N-C frequency of morpholine. The strong bands at 1048 and 1034 cm⁻¹ may be attributed to νC-O-C frequency of morpholine group. The band at 748 cm⁻¹ indicates the presence of monosubstitution of morpholine in MBA.

The UV-Visible spectrum (Deshmuck, 1995) in DMF registers two intense split bands centered at 286 nm and 242 nm, which are presumably due to ν→π* transition of the carbonyl group and π→π* transition of the carbonyl group and the benzene ring.

The ¹H NMR signal at δ=8.44 ppm may be assigned to the secondary amide NH proton. The methine proton shows a signal at δ=5.61 ppm. The multiplet in the range δ=7.44 - 7.28 ppm (7.44 ppm for C at the position 2&6 and the peaks at δ=7.36 and δ=7.28 ppm for C at 3&5 and 4, respectively) attributed to the protons of the benzene ring (Pandit, 1995). The chemical shift of the protons of N(CH₂)₂ group of morpholine ring occurs at δ=2.51 ppm. The chemical shift of the protons of O(CH₂)₂ group of morpholine occurs at δ=3.58 ppm.

The ¹³C NMR spectrum (Seigel and Martin, 1982) shows the carbonyl carbon at δ=170.13 ppm. The signals observed between δ=139.71 - 127.76 ppm are due to aromatic carbons of benzamide. The resonance signals at δ=139.71, 128.64, 127.93 and 127.76 ppm are assigned to the carbons of the phenyl group at 1,2 & 6, 3&5 and 4 positions

respectively. The signals due to the C₁ carbon of benzene ring can be differentiated by its decreased peak height of $\delta=139.71$ ppm. The signal at $\delta=66.70$ ppm is due to the O(CH₂)₂ group and that at $\delta=48.98$ ppm is due to N(CH₂)₂ carbon of morpholine. The mass spectrum (Yongsheng Hu et al., 2006)¹ of MBA was obtained on electron ionization mode, showing a very weak molecular ion peak at $m/z = 234$. This confirms the already assigned molecular mass to the Mannich base under study. Thereupon, on fragmentation, intense signals at $m/z = 143$ & $m/z = 114$ are recorded. They are due to the removal of C₆H₅CH₂⁻ and CHO⁻ groups respectively. The next m/z signal at 86 is due to morpholine ion.

Characterization of the Complexes

To find out the stoichiometry (Kasuga et al., 2006) of the complexes, the percentage of the metal ions, anions and CHN were determined. The molar conductance values reveal that the complexes of Zn (II), Cd (II) and Mn (II) are non-electrolytes. The CHN analyses are also in good agreement with the calculated values Table.1).

In the IR spectra (Nakamoto, 1997) of all the MBA complexes (Table. 2) the stretching frequencies of C=O and C-N-C bonds are found lowered showing that both carbonyl oxygen and CNC nitrogen atoms are coordinated to the metal ions. So the ligand acts as ON donor. The IR spectrum of the sulphato complexes shows the presence of coordinated sulphato group. The bands at the ranges of 1150, 1000 and 900 cm⁻¹ are due to 'SO' stretching mode, ν_3 of sulphato group. The triply degenerate 'OSO' bending mode, ν_4 splits up into its components at about 650, 600 and 580 cm⁻¹ in the complexes. The frequencies at 750(ν_1) and 500(ν_2) are also observed. These are due to the bidentate chelation (Savinkina et al., 2007) associated with the coordinated sulphato group.

Nujolmull electronic spectra of compounds could not be recorded as the compounds do not form a good mull and hence the reflectance spectra of compounds were recorded. The compounds being insoluble in common solvents, the solution electronic spectra also could not be recorded. The complexes of Fe (III) exhibits three bands at 12715, 19590 & 25010 cm⁻¹ due to ${}^6A_{1g} \longrightarrow {}^4T_{1g}(G)$, ${}^6A_{1g} \longrightarrow {}^4T_{2g}(G)$ and ${}^6A_{1g} \longrightarrow {}^4A_{1g}(G)$ transitions, respectively, in an octahedral symmetry (Lever, 1984). The complex of Mn (II) compound exhibits three bands at 18440, 22985 and 25230 cm⁻¹ due to

${}^6A_{1g} \longrightarrow {}^4T_{1g}(G)$, ${}^6A_{1g} \longrightarrow {}^4T_{2g}(G)$ and ${}^6A_{1g} \longrightarrow {}^4A_{1g}(G)$ transitions, respectively, in an octahedral environment (Lever, 1984). It is of interest to note that although both Mn (II) and Fe (III) have ${}^6A_{1g}$ ground state, all the bands occur in Fe (III) compound at lower energy than those of Mn (II) compound. This is due to the lower value of Racah parameters (B and C) in the Iron (III) compound in comparison to those of Mn (II) compound (Lever, 1984). The electronic spectra of oxomolybdenum (V) complexes were best considered as octahedral with a strong tetragonal distortion resulting from molybdenum oxygen bond. The complex exhibit three distinct absorption bands in the ligand field region (Ballhausen and Gray, 1962). The low intensity absorption peaks in the long wave length region were possibly due to first crystal field transition ${}^2B_2 \longrightarrow {}^2E$ (dxy, dxz, dyz). This transition were observed at 12820 - 16000 cm⁻¹. The second crystal field transition were observed at 18750 - 21000 cm⁻¹ due to transition ${}^2B_2 \longrightarrow {}^2B_1$ (dxy, dx²-y²). The third peak appeared at 22000 - 30000 cm⁻¹ and was due to transition ${}^2B_2 \longrightarrow {}^2A_1$ (dxy, dz²). The spectral band at 15150 cm⁻¹ indicates octahedral geometry for the complexes in Ballhausen Gray scheme (Ballhausen and Gray, 1965).

Antibacterial Activity

A comparison of diameters of the inhibition zones of the compounds investigated and listed in Tables 3 and 4 shows that Zn(II) complex exhibits highest antibacterial and antifungal activity against all the bacterial and fungal species studied. This is because, they have larger diameters of inhibition zones than even the control gentamycin at the same concentration and identical conditions. The complexes have more antibacterial and antifungal activities than the ligand against all the bacteria and fungi studied. This observation clearly indicates that the chelation increases the activity. The higher activity of the Zn (II) complex may be due to the fact that Zn(II) is an essential micronutrient during transcription and transformation of nucleic acids. Zn (II) complexes were shown to inhibit cellular protein and RNA synthesis. In Zn(II) complex, the unsaturated metal center present, achieves higher coordination number by binding with some of the functional groups of the protein. This leads to the increased uptake of the compound by the bacterium and thereby inhibiting its growth. Steric constraints are less for a tetrahedral complex than for an octahedral complex. So the tetrahedral complexes

Table 1. Physical Data of Ligand and its Complexes

Sl. No.	Molecular Formula of Ligand and complex	Elemental Analyses						M.P. °C	Colour	Magnetic Moments in B.M.
		% of C	% of H	% of N	% of S	% of M	% of Cl			
1.	Ligand - C ₁₃ H ₁₈ N ₂ O ₂ (MBA)	66.66 (66.72)	7.69 (7.73)	11.96 (12.01)	-	-	-	150	Yellow	-
2.	ZnSO ₄ (C ₁₃ H ₁₈ N ₂ O ₂) ₂	49.57 (49.64)	5.72 (5.79)	8.89 (8.92)	5.08 (5.17)	10.39 (10.51)	-	185	Cream	dia magnetic
3.	Cd(NO ₃) ₂ (C ₁₃ H ₁₈ N ₂ O ₂) ₂	44.29 (44.38)	5.11 (5.19)	11.92 (11.99)	-	15.95 (16.02)	-	196	Light yellow	dia magnetic
4.	MnSO ₄ (C ₁₃ H ₁₈ N ₂ O ₂) ₂	50.40 (50.51)	5.81 (5.87)	9.04 (9.12)	5.16 (5.23)	8.88 (8.92)	-	182	Brown	5.92
5.	ZnSO ₄ (C ₁₃ H ₁₈ N ₂ O ₂) ₂ .2H ₂ O	36.16 (36.24)	5.09 (5.19)	6.49 (6.56)	7.41 (7.50)	15.15 (15.32)	-	163	Yellowish Brown	dia magnetic
6.	FeCl ₃ (C ₁₃ H ₁₈ N ₂ O ₂) ₂	49.48 (49.61)	5.70 (5.79)	8.88 (8.96)	-	8.88 (8.98)	16.89 (16.97)	186	Dark Brown	5.97
7.	[MoO(C ₁₃ H ₁₈ N ₂ O ₂) ₂ .Cl ₃]	45.45 (45.52)	5.24 (5.32)	8.15 (8.23)	-	13.98 (14.06)	15.51 (15.70)	188	Pale yellow	1.65

Calculated values are given in parenthesis.

Table 2. Important IR Absorption Bands (cm⁻¹) of MBA and of Zn(II), Cd(II) and Mn(II) complexes.

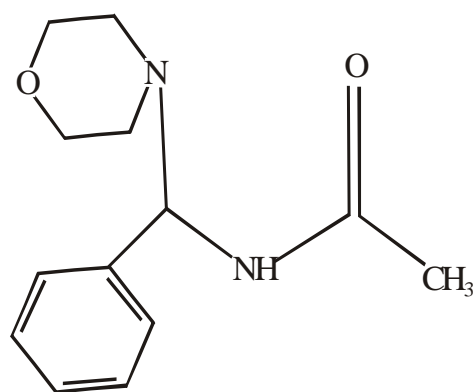
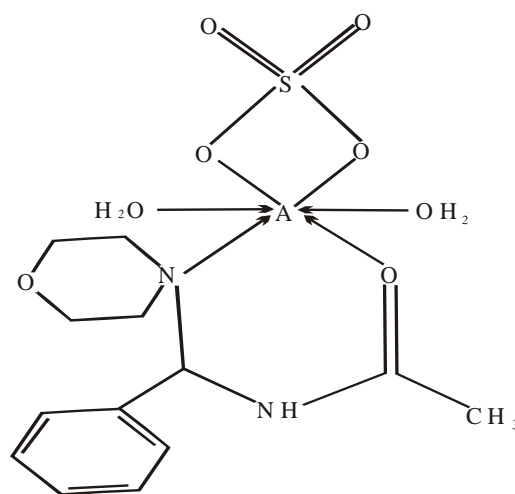
Compound	ν _{NH}	ν _{C=O}	ν _{CNC}	ν ₃	ν ₄	ν ₁₁	ν ₂	ν ₅	ν ₆
MBA	3298	1646	1106	-	-	-	-	-	-
ZnSO ₄ .2MBA	3339	1631	1090	1140 1095 984	755 634 576	853	462	-	-
Cd(NO ₃) ₂ .MBA	3293	1623	1093	-	-	1340	1026	1385	809
MnSO ₄ .MBA	3545	1626	1102	-	-	1310	1050	1385	803
ZnSO ₄ .MBA.2H ₂ O	3486	1630	1105	1150 1109 988	754 640 610	852	496	-	-

Table 3. Antibacterial Activity of Ligand and its Complexes

Compound	<i>E.coli</i>			<i>P. aeruginosa</i>			<i>S. typhi</i>			<i>B. subtilis</i>			<i>S. pyogenes</i>			<i>S. aureus</i>		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Conc. (g/disc)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2
MBA	2	5	0	0	3	8	4	7	2	1	4	8	0	2	6	2	7	0
ZnSO ₄ .2MBA	-	-	0	0	0	0	0	0	0	-	0	0	0	0	0	-	0	1
			8	4	8	9	4	6	6		6	9	4	4	6		7	0
Cd(NO ₃) ₂ .MBA ₂	1	1	2	1	1	2	1	1	2	1	1	2	1	2	2	1	2	3
	6	8	3	4	9	4	4	9	6	4	9	7	7	3	8	8	3	0
MnSO ₄ .(MBA) ₂	0	1	1	0	0	1	1	1	1	0	1	1	1	1	2	1	1	1
	9	2	7	6	9	5	0	2	0	8	4	8	4	9	2	0	0	8
ZnSO ₄ .MBA.2H ₂ O	1	2	2	1	1	2	1	1	2	1	1	2	1	2	2	1	1	2
	4	0	1	6	6	1	4	5	1	2	8	4	6	1	6	2	9	5
	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	5	9	2	5	8	2	1	6	2	0	9	1	5	8	5	6	8	4

Table 4. Antifungal Activity of Ligand and its Complexes

Compound	<i>A. niger</i>			<i>A. flavus</i>		
Conc. (g/disc)	10	20	30	10	20	30
MBA	04	04	05	04	05	05
ZnSO ₄ .2MBA	14	18	24	13	17	25
Cd(NO ₃) ₂ .(MBA) ₂	11	17	20	10	14	19
MnSO ₄ .(MBA) ₂	12	15	21	11	15	23
ZnSO ₄ .MBA.2H ₂ O	10	16	23	12	16	24

**Figure 1.** N-[Morpholino(phenyl)methyl]acetamide.**Figure 2.** A.SO₄. MBA.2H₂O (A=Zn)

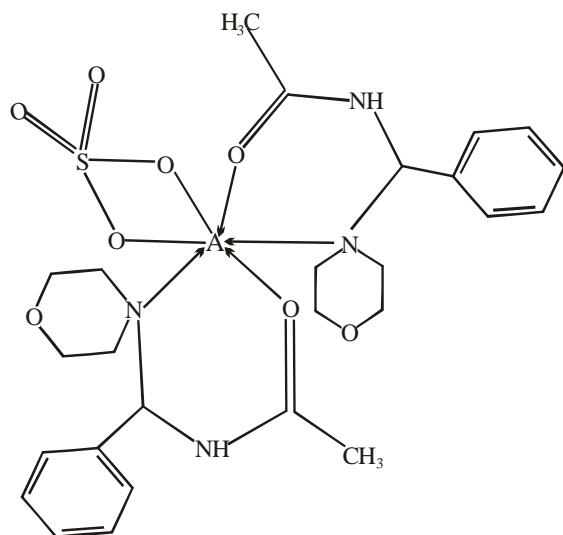


Figure 3. A.SO₄. 2MBA A=Zn, Mn

are biologically more active than the octahedral complexes (Filion et al., 2006).

Antifungal Activity

The fungi toxicity of the free ligand is less severe than that of the metal chelates. A possible mechanism of toxicity may be speculated in the light of chelation theory (Hamers and Casterman, 1972): Chelation reduces considerably the polarity of the metal ion, mainly because of partial sharing of its positive charge with donor groups and a possible delocalization of electron over the chelate ring. This increases the lipophilic character of the neutral chelate, which favours its permeation through lipid layers of fungus membranes. Furthermore, the mechanism of action of the compounds may involve the formation of hydrogen bond through the uncoordinated heteroatoms viz. O, S, and N with the active centers of the cell constituents, thus resulting in the interference with the normal cell process (Steiner and Koellner, 2001). These compounds have a greater chance of interaction with either the nucleotide bases or biologically essential metal ions present in the biosystem and also coordinatively unsaturated metal present in the metal complexes. The low activity of some of the complexes may be due to a mismatching of the geometry and charge distribution around the molecule with that around the pores of the fungal cell wall, preventing penetration and hence toxic reaction within the pores. As a corollary, the complex cannot reach the desired site of action on

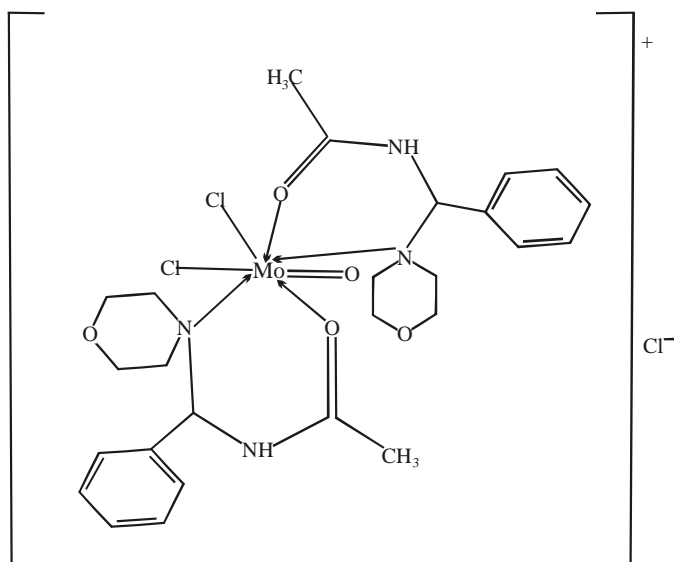


Figure 4 . Complex of Oxomolybdenum (V)

the cell wall to interfere with normal cell activity (Eichhorn, 1980).

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