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Synthesis, Characterization, and Antifungal Studies of Transition Metal Complexes of ω-Bromoacetoacetanilide Isonicotinylhydrazone

K. P. Deepa and K. K. Aravindakshan*

Department of Chemistry, University of Calicut, Kerala 673635, India,
E-mail: aravindkuttamath@yahoo.com

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Abstract

Isonicotinic acid hydrazide or isonicotinylhydrazide, commonly known as isoniazid, is an antibacterial agent that has been used to treat tuberculosis. It interacts with microbial cell walls. Schiff's bases or anils are the compounds having >C=N–N< linkages, which have immense applications as catalysts, stabilizers, pigments, dyes, and drugs. They have good ability to form chelates with many metal ions. Isoniazid can form Schiff's bases with diketones such as acetoacetanilide. Acetoacetanilide isonicotinylhydrazone and its metal chelates exhibit anticancer activity. Our studies on *N*-methylacetoacetanilide isonicotinylhydrazone and its metal chelates revealed that they are active against pathogenic fungal strains. Hence, it is worthwhile to synthesize new complexes of ligands having different substituents on the acetoacetanilide moiety. We synthesized five new metal chelates of ω-bromoacetoacetanilide isonicotinylhydrazone. The ligand behaved as a tridentate monoanion or as a tridentate dianion in the complexes. These compounds were characterized mainly by elemental analysis; conductivity measurements; and electronic, infrared, and nuclear magnetic resonance spectral studies. We also carried out antifungal studies of these compounds against four selected pathogenic fungal strains using a cup-plate technique. Both the ligand and its metal chelates were active against all fungal strains investigated. However, the chelates were found to be more active than the ligand.

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*Author to whom all correspondence and reprint requests should be addressed.

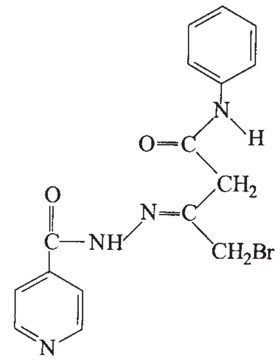

Fig. 1. Suggested structure of ligand.

Index Entries: Metal chelates; ω-bromoacetoacetanilide isonicotinylhydrazone; synthesis; characterization; antifungal studies.

Introduction

Schiff's bases or anils and the compounds that contain the triatomic linkage >C=N–N< have immense applications *(1,2)*. Isonicotinic acid hydrazide is an important antibacterial agent, which can form Schiff's bases with aldehydes and ketones. However, Schiff's bases derived from isoniazid and diketones are very rare. Acetoacetanilide, an important diketoanilide, can form Schiff's bases with isoniazid and was found to have good chelating ability *(3)*. It behaved as a tridentate dianion or tridentate monoanion in most cases. Our own investigations on isonicotinylhydrazones of β-diketo derivatives and their metal chelates revealed their antitumor activity *(4)*. Studies of *N*-methyl- and *N*-ethylacetoacetanilide isonicotinylhydrazone also revealed that both the ligands and their metal chelates possessed antifungal activity *(5)*. Hence, it is worthwhile to synthesize complexes of a new ligand having a different substituent on the acetoacetanilide moiety. We describe here the synthesis, characterization, and antifungal activity of some typical transition metal complexes of ω-bromoacetoacetanilide isonicotinylhydrazone, LH_2 (Fig. 1).

Materials and Methods

Chemicals

The chemicals used were of BDH Analar quality. ω-Bromoacetoacetanilide was prepared according to a reported method *(6)*.

Synthesis of Ligand

A solution of isonicotinylhydrazide in ethanol (0.1 mol, 250 mL) was added to a solution of ω-bromoacetoacetanilide in methanol (0.1 mol, 250 mL) and refluxed under boiling conditions for 2 h. The resulting solution was evaporated to dryness, cooled, and stirred several times with petroleum ether until a brown solid product separated out. The product was dried, recrystallized from acetone, and kept in a desiccator under reduced pressure over anhydrous calcium chloride for 3 to 4 d (yield = 60%).

Synthesis of Complexes

To an ethanolic solution of the ligand (0.01 mol in 50 mL), a methanolic solution of the metal acetate (0.005 mol in 25 mL) was added and stirred for 1 h. A 1:1 ratio of metal:ligand gave a good yield in the case of the Cu(II) complex. The solid complexes that formed readily were filtered off and washed several times with methanol. They were dried in a desiccator over anhydrous calcium chloride.

Analytical Methods

The complexes were analyzed by standard methods to determine the percentages of the metals present *(7)*. Bromine was determined as silver bromide. Carbon, hydrogen, and nitrogen were determined by microanalysis using Hitachi CHN-O rapid analyzer. The molar conductances of the complexes were determined using a 10^{-3} *M* solution in dimethyl sulfoxide (DMSO) at 28 ± 3°C on a Systronic conductivity bridge and a dip-type cell (cell constant = 1.083) calibrated with AnalaR potassium chloride. The magnetic moments were determined by the Gouy-method using $Hg[Co(NCS)_4]$ as a calibrant after applying necessary diamagnetic corrections *(8)*. The electronic spectra of the complexes were recorded in solid state on a Shimadzu UV-Visible 1601 spectrophotometer using the Nujol mull technique *(9)*. The infrared (IR) spectra were recorded using KBr discs on a Shimadzu FTIR 8101 spectrophotometer. The ^1H nuclear magnetic resonance (NMR) spectra of the ligand and the Zn(II) complex were recorded in DMSO-d_6 on a Varian 300 NMR spectrometer.

Antifungal Studies

Antifungal studies were carried out using four pathogenic fungal strains: *Aspergillus niger*, *Candida albicans*, *Trichosporon* sp., and *Penicillium* sp. The cultures were maintained on a Sabouraud's glucose agar medium and kept at 0°C. Other materials used for the preparation of agar medium, peptone and agar-agar, were of bacteriological grade. DMSO was used as a solvent for preparing solutions of the ligand and the metal complexes. Other chemicals, such as NaCl and D-glucose, were of AnalaR grade.

The antifungal activity of the compounds under study against four pathogenic fungal strains was evaluated using a cup-plate technique *(10)*. The fungi were grown in a Sabouraud's glucose agar medium. Suspensions of the spores of *A. niger*, *Trichosporon* sp. and *Penicillium* sp. were prepared in normal saline (0.9%). In the case of *C. albicans*, the suspension was made using the cells from the slope of Sabouraud's agar medium. About 25 mL of Sabouraud's agar (1 g of peptone, 4 g of D-glucose, 2 g of agar-agar, and

100 mL of distilled water; the pH was adjusted to 5.7 using 10% HCl, autoclaving at 15 lb of pressure for 15 min) was poured into sterilized Petri plates (9 cm) and allowed to cool. To this solid agar medium, the suspension of fungal strain in normal saline was added. After swirling the plates properly, for uniform distribution of the spores on the surface, excess suspension was decanted. The plates were allowed to dry in an incubator at 37°C for 1 h. Using an agar punch, wells of 10 mm diameter were made on the plates. In each well 200 μL of 500-ppm solutions of the compounds in DMSO were added. Each plate has a well for the control, i.e., in which the solvent DMSO alone was poured. The wells were properly labeled for each sample, and the plates were prepared in triplicate and incubated at room temperature for 3 to 4 d. Antifungal activity was detected by measuring the diameter of the inhibition zone around each well.

Results and Discussion

The data from analytical and physicochemical studies were correlated to explain the properties and natures of the complexes (Table 1). All the complexes were colored, nonhygroscopic, and air and photo stable. The ligand and the complexes were found to be soluble in ethanol, methanol, and DMSO. The molar conductance values in DMSO proved that the complexes are nonelectrolytes *(11)*. The analytical data of the complexes correspond to the formula $[M(LH)_2]$, in which M = Mn(II), Co(II), Ni(II), or Zn(II) and LH = tridentate monoanion of LH_2 and $[CuL(H_2O)]$, in which L = tridentate dianion of LH_2.

Magnetic Behavior

The magnetic moment values of the complexes are given in Table 1. The Mn(II) complex showed a magnetic moment value of 5.22 BM, which is slightly lower than the spin-only value (5.90 Bohr magnetons [BM]). The magnetic moments of a number of bivalent Mn(II) complexes of Schiff's bases have been found to be appreciably lower than the spin-only value *(12)*. The Co(II) complex showed a magnetic moment value of 5.11 BM. This value, together with the brown color of the complex, indicates octahedral geometry around the Co(II) ion. A magnetic moment value of 2.95 BM for the Ni(II) complex indicates its octahedral geometry. Although the magnetic moment value of 2.34 BM cannot give an indication of the geometry of the Cu(II) complex, it eliminates the possibility of antiferromagnetism in the complex.

Electronic Spectra

The electronic spectra of the complexes were recorded using the Nujol mull technique. The Mn(II) complex showed two bands at 395 and 426 nm. These bands may be assigned, respectively, to ${}^6A_{1g} \rightarrow {}^4E_g(G)$ and ${}^6A_{1g} \rightarrow {}^4T_{2g}(G)$ transitions characteristic of the spin-free d^5 configuration of Mn(II) ion *(13)*. The Co(II) complex showed bands at 428, 620, and 1012 nm. These may be assigned, respectively, to ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_1), ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ (ν_2),

Table 1
Analytical Data of Ligand and Complexes

Serial no.	Compound	Color	% Found (calculated)					μ_{eff} B.M.
			M	C	H	N	Br	
1	LH_2	Brown	—	50.71 (51.19)	4.12 (4.03)	14.96 (14.93)	21.15 (21.31)	—
2	$[Mn(LH)_2]$	Dark brown	6.56 (6.84)	47.29 (47.82)	3.48 (3.52)	13.92 (13.95)	19.67 (19.90)	5.22
3	$[Co(LH)_2]$	Brown	7.18 (7.30)	47.17 (47.58)	3.49 (3.50)	13.85 (13.88)	9.75 (9.80)	5.11
4	$[Ni(LH)_2]$	Brown	7.04 (7.28)	47.12 (47.58)	3.52 (3.50)	13.86 (13.88)	9.78 (9.81)	2.95
5	$[CuL(H_2O)]$	Greenish brown	13.42 (13.98)	41.92 (42.24)	3.16 (3.33)	12.30 (12.32)	17.56 (17.58)	2.34
6	$[Zn(LH)_2]$	Brown	8.15 (8.04)	47.17 (47.21)	3.40 (3.47)	13.75 (13.77)	19.62 (19.64)	—

and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_3) transitions characteristic of octahedral geometry. The ratio ν_3/ν_1 is also found in agreement with the expected value of this ratio for a Co(II) complex with octahedral geometry *(14)*. The Ni(II) complex showed bands at 410, 538, 668, and 1031 nm. These bands may be assigned, respectively, to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_1), $^3A_{2g}(F) \rightarrow {}^3A_{1g}(G)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2), and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_3) transitions characteristic of an octahedral Ni(II) complex. The ratio ν_2/ν_1 is also found to be characteristic of an Ni(II) complex with octahedral geometry. The Cu(II) complex showed bands at 432 and 630 nm. These bands may be assigned, respectively, to $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions characteristic of a square-planar Cu(II) complex.

IR Spectra

The ligand spectrum showed (Table 2) bands at 3649 cm^{-1} together with weak bands at 3450, 3210, 3180, and 3150 cm^{-1}. These may be assigned to ν(N-H) and ν(C-H). In the spectra of all the complexes, this region remains as a broad band and may be assigned to ν(N-H) and ν(C-H) *(15,16)*

The ligand spectrum showed a medium band at 1698 cm^{-1} and may be assigned to ν(C=O) (anilide). In the spectra of all the complexes, except that of the Cu(II) complex, this band shifted to lower frequency, indicating the participation of anilide carbonyl oxygen in coordination. In the spectrum of the Cu(II) complex, this band disappeared and a new band was observed at 1157 cm^{-1}. This may be owing to the enolization of $–CH_2$-C=O to –CH=C-OH and subsequent coordination through deprotonated oxygen. The ν(C=O) (isonicotinyl) appeared as a strong band at 1684 cm^{-1} in the ligand spectrum. In the spectra of all the complexes, this band disappeared and a new band was observed at ~1250 cm^{-1}. This observation indicates the enolization of =N-NH-C=O to =N-N=C-OH and subsequent coordination through deprotonated oxygen.

The azomethine stretching band appeared at 1636 cm^{-1} in the ligand spectrum. In the spectra of the complexes, this band was found to be shifted to a lower frequency by ~40 cm^{-1}, indicating the participation of azomethine nitrogen in coordination. A medium band observed at 1020 cm^{-1} in the ligand spectrum may be assigned to ν(N-N). A shift of this band to higher frequency was observed in the spectra of all the complexes. This observation confirms the participation of azomethine nitrogen in coordination. In the spectrum of the Cu(II) complex, a broad structure at 3500 cm^{-1} together with bands at 840–950 and 650 cm^{-1} may be assigned, respectively, to stretching, rocking, and wagging modes of coordinated water molecule *(17)*. A medium band at about 507 cm^{-1} in the spectra of the ligand and the complexes may be assigned to ν(C-Br). The nature of the metal-ligand bonding is confirmed by the new bands at approx 530 and 425 cm^{-1} in the spectra of all the complexes. These may be assigned to ν(M-N) and ν(M-O), respectively.

Table 2
Significant IR Spectral Bands (cm^{-1}) of Compounds and Their Assignments[a]

Compound	ν(N-H) and ν(C-H)	ν(C=O) anilide	ν(C=O) (isonicotinyl)	ν(C=N)	ν(C-O) (isonicotinyl)	ν(C-O) (anilide)	ν(N-N)	ν(M-N)	ν(M-O)
LH_2	3649 m 3450 b 3210 m 3180 m 3150 m	1698 m	1684 m	1636 m	—	—	1020 m	—	—
$[Mn(LH)_2]$	3210 s, b 3061 m 3020 m	1660 m	—	1599 m	1257 s	—	1030 m	520 w	427 s
$[Co(LH)_2]$	3650 m 3180 m 3080 sh	1670 m	—	1599 m	1250 m	—	1028 m	540 w	420 w
$[Ni(LH)_2]$	3580 m 3450 b 3080 m	1650 m	—	1597 m	1219 m	—	1028 m	518 w	432 w
$[CuL(H_2O)]$	3600–2800 b	—	—	1599 m	1219 s	1157 m	1030 m	510 w	420 w
$[Zn(LH)_2]$	3480 s, b 3080 sh	1650 m	—	1597 s	1258 m	—	1028 m	520 w	424 m

[a] s = strong; m = medium; w = weak; b = broad; sh = shoulder.

Table 3
Significant ^{1}H NMR Spectral Assignments of LH_2 and Its Zn(II) Complex

Compound	δ (ppm)[a]	Proton
LH_2	11.16 (1H, s)	NH
	8.92–8.90 (2H, s)	α-Protons of pyridine ring
	8.27–6.97 (7H, m)	β-Protons of pyridine ring and protons of aromatic ring
	5.17 (2H, s)	ω-CH_2
	3.75 (2H, s)	α-CH_2
$[Zn(LH)_2]$	10.96 (1H, s)	NH
	8.81–8.79 (2H, s)	α-Protons of pyridine ring
	7.96–6.92 (7H, m)	β-Protons of pyridine ring and protons of aromatic ring
	5.17 (2H, s)	ω-CH_2
	3.92 (2H, s)	α-CH_2

[a] s = singlet; m = multiplet.

^{1}H NMR Spectra

In the spectrum of the ligand (Table 3), a peak observed at 11.16 ppm may be assigned to the NH proton of the anilide ring *(18)*. A singlet observed at 8.92–8.90 ppm may be assigned to α-protons of the pyridine ring. The multiplet observed at 8.27–6.97 ppm may be assigned to β-protons of the pyridine ring and protons of the aromatic ring. The singlets observed at 5.17 and 3.75 ppm may be assigned to ω-CH_2 and α-CH_2 protons, respectively.

In the spectrum of the Zn(II) complex, signals observed at 10.96 (s), 8.81–8.79 (s), 7.96–6.92 (m), and 5.17 (s) and 3.92 (s) ppm may be assigned to the NH proton of anilide moiety; α-protons of the pyridine ring; β-protons of the pyridine ring; and protons of the aromatic ring, ω-CH_2 and α-CH_2 protons, respectively.

Antifungal Studies

The antifungal studies showed that the ligand and the complexes exhibited considerable activity against all the pathogenic fungal strains (*see* Table 4). It was found that the chelates were more potent than the ligand. This was probably owing to the higher lipophilic nature of the complexes. Another important observation was that the Co(II) and Ni(II) complexes were found to be more active against the fungal strains than the Mn(II) and Cu(II) complexes. The exact mechanism of the mode of action of the compounds is unknown and its elucidation will require further studies. However, it may be owing to the destruction of the fungal cell walls by these compounds, thus leading to their death.

Table 4
Antifungal Activity of LH_2 and Complexes

Serial no.	Compound	Diameter of zone of inhibition (mm)			
		A. niger	*Penicillium* sp.	*C. albicans*	*Trichosporon* sp.
1	DMSO	10	10	14	10
2	LH_2	15	15	20	22
3	$[Mn(LH)_2]$	17	25	20	26
4	$[Co(LH)_2]$	25	27	25	30
5	$[Ni(LH)_2]$	20	28	28	28
6	$[CuL(H_2O)]$	18	28	24	22

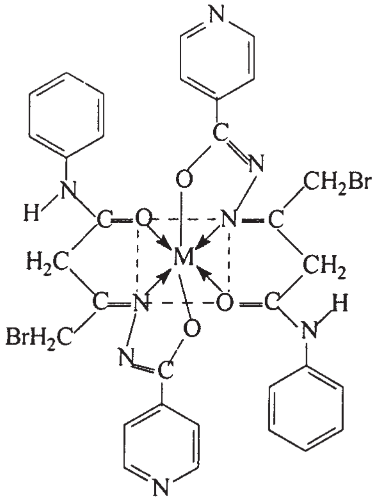

Fig. 2. Suggested structure of $[M(LH)_2]$ complexes.

Conclusion

The ligational behavior of a tridentate "ONO" donor ligand, ω-bromoacetoacetanilide isonicotinylhydrazone (LH_2), toward several typical divalent transition metal ions—Mn(II), Co(II), Ni(II), Cu(II), and Zn(II)—was studied. The analytical studies indicated the following structural formulae for the complexes: $[M(LH)_2]$, in which M = Mn(II), Co(II), Ni(II), or Zn(II) and LH = tridentate monoanion of LH_2 and $[CuL(H_2O)]$. Based on various physicochemical and structural investigations, it was concluded that the ligand acted as a tridentate monoanion forming octahedral complexes with Mn(II), Co(II), Ni(II), or Zn(II) ions (Fig. 2) In the case of the Cu(II) complex, the ligand acted as a tridentate dianion forming a square-planar complex (Fig. 3). All the compounds showed appreciable antifungal activity.

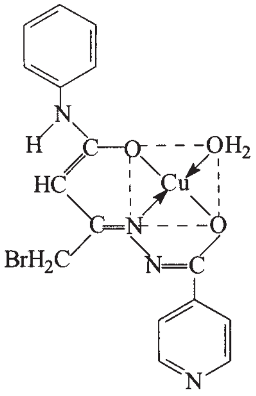

Fig. 3. Suggested structure of Cu(II) complex.

Acknowledgments

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