Ultra Chemistry Vol. 9(3), 345-352 (2013).

Synthesis, Characterization and anti-inflammatory activity of zinc (II) and cadmium (II) chloro complexes of a new bioactive mannich base Diethyl Amino Methyl Maleic Hydrazide(DEAMMH)

P.R. SANTHI[a], G. SELVANATHAN[a] and G. POONGOTHAI

[a]Department of Chemistry, A.V.C. College (Autonomous), Mannampandal-609305 (INDIA)
[b]Department of Chemistry, Government Arts College for Men (Autonomous), Nandanam,Chennai-35 (INDIA)
E-Mail: prsroopa@gmail.com, Mobile:9487442853

(Acceptance Date 18th October, 2013)

Abstract

In this study ,a new bioactive mannich base N-diethyl amino methyl maleic hydrazide has been synthesized via the Mannich reaction of formaldehyde,maleichydrazide and diethylamine. The zinc(II) and cadmium(II) chloro complexes of the mannich base diethyl amino methyl maleic hydrazide have been synthesized.The structural characterization studies ranging from quantitative analysis to various spectral tools confirm the formation of the d^{10} complexes. Anti-inflammatory study was carried out by using SRBC and HRBC membrane stabilization.

Key words: DEAMMH,d^{10}, Anti-inflammatory activity, SRBC, HRBC.

1. Introduction

The present objective is to synthesize a new derivative of maleic hydrazide by incorporating amino methyl group and their d^{10} metal ion chloro complexes. Maleic hydrazide is one of the best- known pyridazine derivatives and has been the subject of several theoretical investigations.[1] The pyridazine system act as a potential substitute for aromatic moieties in antithrombotic,antiviral and antitumour agents.[2] Maleic hydrazide was found to be a potent inhibitor of leukemia[3] its derivatives are used as novel bioactive agents[4] and especially Mannich N-bases were proved to be pharmacologically more active than maleic hydrazide.[5,6] Derivatives of maleic hydrazide can act as purine or pyrimidine analog forming base pair with uracil and thymine by nucleoside formation through 'O' or with adenine through 'N'. Somereview articles have appeared in which several applications of mannich bases in the pharmaceutical

field[7-10] and in other industries[11,12] such as those connected with macromolecular chemistry[13] are described.In this present work the interaction of d^{10} metal ions with the above derivative is studied.

2. Experimental

All the reagents used for synthesising the ligand and its metal complexes were of analytical or reagent grade and the solvents were purified by the standard methods of distillation.

The elemental analysis (C,H & N) were carried out using LECO-CHN analyser. Metal contents in the complexes wereanalysed using standard procedures; conductance measurements were made using 10^{-3} m solutions of the complexes in DMF with the help of systronic Direct Reading Digital Conductivity meter. The IR spectra of the compounds were recorded as KBr discs using perkin Elmer -1430 ratio recording spectrophotometer.NMR spectra were recorded using JEOLGSX400NB, 400MHz spectrometer using TMS as internal standard and DMSO-d_6 as solvent at ambient temperature[14].

2.1 Synthesis of zinc(II) and cadmium (II) chloro complexes of DEAMMH. The d^{10} metal chloro complexes of DEAMMH (N-diethylamino methyl maleic hydrazide) were isolated using 2-propanol as the solvating medium.A (1:1) metal:ligandmol ratio of the hot 2-propanolic solutions of the ligand and the metal salt were mixed and stirred well whereupon the respective complex is precipitated. The product obtained was washed thoroughly with hot ethanol and dried at 110^0c. Both complexes are stable in solid state. The complexes aremostly insoluble in common organic solvents.Both zinc(II) and cadmium(II) chloro complexes are soluble in DMF and DMSO.

3. Results and Discussion

3.1. Quantitative analysis and Electrical conductance

The results of the Quantitative analysis and the molar conductance values for the complexes isolated are given in Table 1

Table 1

Quantitative Analysis(%)			
Compound	Metal obs (cal)	Anion obs (cal)	ΩM^{*}
$ZnCl_2$.DEAMMH	18.92 (19.61)	20.87 (21.27)	19.4
$CdCl_2$.DEAMMH	27.69 (28.22)	16.92 (17.79)	53.1

DEAMMH=N-Diethylaminomethylmalei-chydrazide, $\Omega^{-1}cm^2\ mol^{-1}$ for 10^{-3} M DMF solution of the complex.The analytical and conductance data of zinc (II) and cadmium(II) complexes are in good agreement with the proposed composition as $ZnCl_2$.DEAMMH and $CdCl_2$.DEAMMH.

3.2 FT-IR spectral analysis

The IR spectrum of zinc(II) and cadmium(II) chloro complexes are shown in Fig. I and II

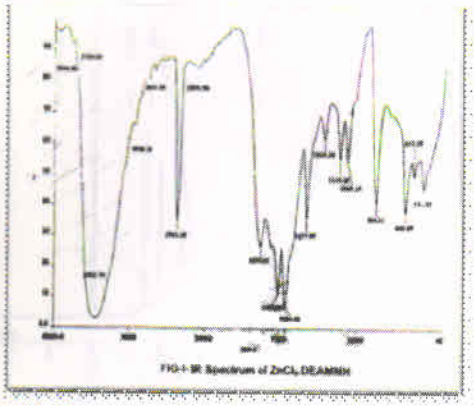


Fig. I

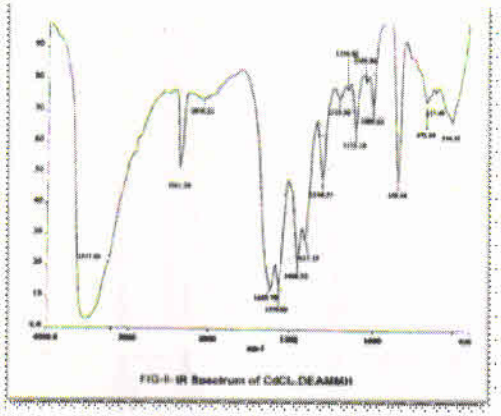


Fig. II

The important IR frequencies of d^{10} chloro complexes is given in Table 2 IR Absorption Bands(cm^{-1}) of Zn(II) and Cd(II) complexes of DEAMMH

Table 2.

Compound	γOH	γCH	γCO	dOH	γCNC
DEAMMH	3441.25	2934.94	1663.10	1491.92	1118.64
$ZnCl_2$	3443		1633	1464	1115
$CdCl_2$	3517		1623	1456	1115

A comparison of the IR absorption bands of the ligand and zinc(II) chloro complex shows that the γ_{CO} of the complex decreased from 1663 cm^{-1} to1633 cm^{-1}. In case of zinc(II) chloro complex the d_{OH} also undergoes major shift of nearly 27 cm^{-1} when compared with that of the free ligand.On comparing with the IR spectrum of DEAMMH,a new sharp and intense band at 1115 cm^{-1} is seen which can be assigned to the γ_{CNC} of the diethyl amine moiety.

The IR spectra of cadmium(II) chloro complex record negative shift in case of γ_{CO} and d_{OH} . The comparison of IR spectra of cadmium(II) chloro complex with that of the free ligand γ_{CO} is decreased from 1663 to 1623 cm^{-1}. The cadmium(II) chloro complex experience negative shift of 40 and 36 cm^{-1} respectively in γ_{CO} and d_{OH} values.On comparing with the IR spectrum of DEAMMH, a new sharp and intense band at 1115 cm^{-1} is seen which can be assigned the γ_{CNC} of the diethylamine moiety.

3.3 ^1H NMR spectral analysis.

The ^1H NMR spectrum of zinc(II) and cadmium(II) chloro complexes are shown in Fig. III and IV

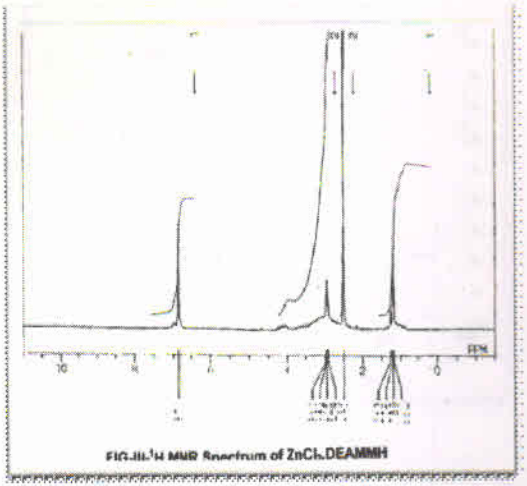


Fig. III

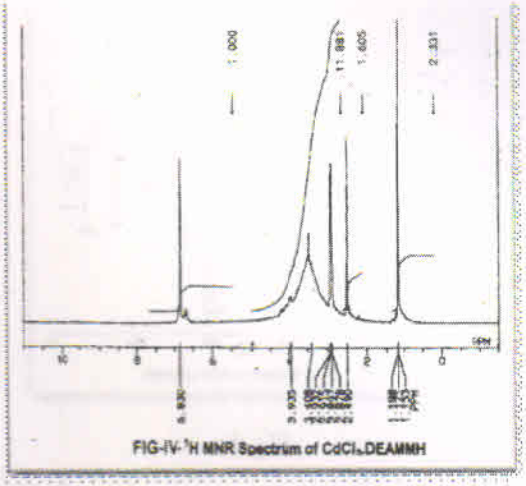


Fig. IV

The ^{1}H NMR spectrum of zinc(II) and cadmium(II) chloro complexes various resonance signals are given in Table-3 and Table-4.

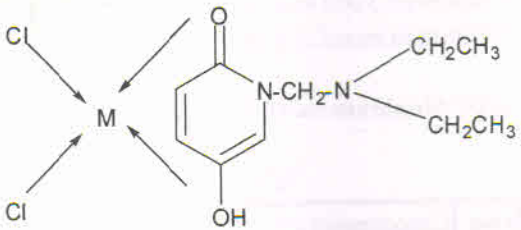
Table 3.

Type of Proton	DEAMMH	$ZnCl_2$.DEAMMH
-CH=CH-	6.91	6.87
>C-OH	-	3.40
-N-CH_2-N-	3.00	3.70
-N(CH_2)$_2$	3.5	2.92
(CH_3)$_2$	1.13	1.18

Table-4

Type of proton	DEAMMH	$CdCl_2$DEAMMH
-CH=CH-	6.91	6.83
>C-OH	-	3.40
-N(CH_2)$_2$	3.50	3.50
-N-CH_2-N-	3.00	2.89
(CH_3)$_2$	1.13	1.11
Solvent Peak	2.49	2.49

The various resonance signals exhibited by zinc(II) and cadmium(II) chloro complex is compared to free ligand DEAMMH. The ^1H NMR spectra of both complexes show electron drift from CNC group, the $>N\text{-}CH_2\text{-}N<$ and $-N(CH_3)_2$ signals suffer downfield shift on complexation of the ligand with the metal atom. Based on the analytical, conductivity, IR and NMR spectral study, tetra coordinate structures are assigned to the zinc(II) and cadmium(II) chloro complexes respectively as shown Fig. V



M=Zn(II) (or) Cd(II)

Tetra coordinate structure Fig. V

4. Anti-inflammatory activity :

4.1 SRBC and HRBC Membrane Stabilization.

Inflammatory reaction is a basic defensive response to a variety of stimuli, which may be biological, chemical or physical. The term inflammation originates from latine 'inflammare'= to burn.The clinical sign that inflammation evokes are heat, redness, swelling and loss of function.

Inflammation a haemostatic phenomenon is one of the fundamental protective responses of the cells and tissues to injuries caused by various noxious and infectious agents.[14] The inflammation may be broadly classified under categories viz acute and chronic inflammation. Theacute inflammation is the response of tissues to serve but transient stimuli. The chronic inflammation occurs when a stimulus is persistent.[15] Lysosomal enzymes play an important role in the development of acute and chronic inflammation.

Anti-inflammatory agent is a drug that inhibits any facet of inflammation of an experimentally induces nature of as a part of clinic syndrome.It has been reported that the structure of red blood corpuseles(RBC) is similar to lysosomal membrane components. Since lysosomal membrane resembles sheep RBC membrane, its stabilization effects have been studied by SRBC.When the RBC is subjected to hypotonic stress, the release of haemoglobin from RBC is prevented by anti-inflammatory agents because of membrane stabilization.So the stabilization on SRBC membrane by drugs against hypotonicity-induced haemolysis serves as a useful in vitro method for assessing the anti inflammatory activity of various compounds.

4.2 Experimental

Fresh blood was collectedand mixed with equal volume of sterilized alsever solution (2% dextrose,.87% sodium citrate,0.05% citric acid and0.42% NaCl). It is used within 5h. Hyposaline (0.25%, 2ml), phosphate buffer (0.15ml, pH=7.4, 1ml) and SRBC instead of HRBC were taken in seven tubes.Solutions of different concentration of the drug were added to six of the control in which instead of the drug, isosaline (0.85%, 1ml) was added. The contents in all the seven tubes were

incubated at 37^{0}C of 30 minutes and then centrifuged.The intensityof colour of the supernatant, which is due to haemoglobin was measured at 560 nm.

4.3 Material and Methods

(a) Collection of Blood: Freshblood was collected and mixed with equal volume of sterilized alsever solution (containing 2% dextrose,0.8%sodium citrate, 0.05% citric acid and 0.43% sodium chloride) and stored at 4^{0}C .Sheep blood was collected separately and used for the purpose.

(b) Saline: Saline at different concentration was prepared (isosaline 0.85% and hyposaline 0.6%).

(c) Preparation of SRBC suspension: The blood was centrifuged at 3000 rpm and the packed cells obtained were washed with isosaline (0.85%,pH 7.2) 3 times and a 10% (v/v) suspension was made with isosaline.

(d) Determination of SRBC membrane stabilization: Solution of different concentrations of metal ion complexes were prepared. Assay mixture contained the drug (metal ion complexes are mentioned in Table-5 and 6 and the Figure VI and VII.

1 ml of phosphate buffer (0.15%, pH 7.4) 1ml of hyposaline (0.36% and 0.5ml of 10% SRBC suspension, in another tube instead of 2ml of hyposaline, 2 ml of distilled water was taken and this served as the control. All the tubes were incubated at 37^{0}C for 30 minutes. Then they were centrifuged and the haemoglobin content in the supernatant was estimated using a photoelectric colorimeter at 560 nm.

4.3 Results and Discussion

The SRBC is used as a screening study for the anti-inflammatory activity of the DEAMMH. It is given in Fig-VI and Table 5. From the Fig. VI it is evident that the concentration of $ZnCl_2$. DEAMMH increased from 25µg the capability is also increased with increase in concentration.Thus initially it was observed that the capacity is a dose dependent one. But after reaching an optimum stabilization at 50µg the trend declines for further increase in concentration.That is beyond the concentration of 50µg only hypotonicity induced haemolysis is observed.Such kind of biphasic property is common in metal complexes.

SRBC Membranestabilization $ZnCl_2$.DEAMMH

Table 5

S.No.	Concentration of Drug(µg)	Transmittance
1	0.25	0.17
2	0.50	0.19
3	0.75	0.18
4	1.00	0.17
5	2.00	0.16

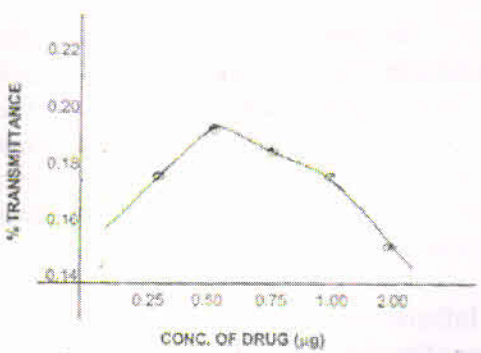


Fig-VI

From the Figure.VII and Table 6

it is proved that $CdCl_2$. DEAMMH was used for SRBC membrane stabilization studies the capacity of the drug increases as in the case of previous one. The drug reach in optimum value at 75µg beyond this concentration once again haemolysis is noted.

Table 6

S.No.	Concentration of Drug(µg)	Transmittance
1	0.25	0.18
2	0.50	0.20
3	0.75	0.24
4	1.00	0.22
5	2.00	0.20

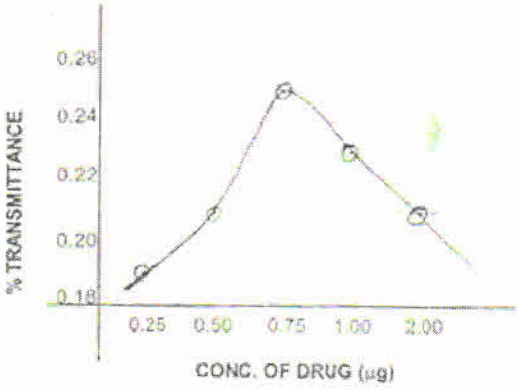


Fig.VII

5. Conclusion

The synthesis and characterization of zinc(II) and cadmium(II)complexes of DEAMMH showed that the metal ion complexes were isolated from non-aqueous media.The electrical conductance data of 10^{-3} m solutions of the complexes in DMF showed that allthe complexes are non electrolytes.The ligand behaves as ambidentate chelating ligand. The d^{10} complexes are colourless. The IR spectral data provide evidences for γ_{CO} and $\gamma_{C\text{-}N\text{-}C}$ bonds in complexes and the geometries of the complexes were proposed as tetra coordinated species.The zinc (II) and cadmium(II) chloro complexes were covalently hydrated.

To further ascertain ^1H NMR studies were also carried outfor the d^{10} complexes, registered a downfield shift for the protons, which were directly attached to the donor atom due to the electron shift from the atom involved in coordination to the metal. The screening study for the anti-inflammatory activity of the complexes were proved that the concentration increases the SRBC membrane stabilization is also increases but beyond certain concentrations ie 50µg and 75µg ,the hypotonicity induced haemolysis is observed.

6. References

1. Greenwood, Jeremy, R., Capper, Hugh, R, Allan, Robbin, *Internet J. Chem., 129*, Article **21 (Eng)** 27667S (1998).
2. Baraldi, pier Giovanni, Cacciari, Barbara, Naser, Abdel-Zaid, DeLas, Infantas, Mariajosepineda, Romagnoli, Romeo, Spalluto, *Giampiero, Actapharm. Hung;* 126, **66 (Suppl.)**, S 35 (Eng) (1996).
3. Heinisch, Gottfreid, *Actapharm. Hung.*, **66 (Suppl.)**,S9(Eng) (1996).
4. Strumillo, J., "Synthesis of Mannich N-bases with expected pharmacological Activity" *Acta pol. Pharm*, 32, 287 (1975); chem. Abstr., *84*, 15080 (1976).

5. Werner, W. and Fritzsche, H., "potentielle-cytostatikadu AminomethylierungNH acider Hypotika", *Arch.pharm., 302,* 188 (1969).
6. Dorgjham, C., Richard, B., Richard, M. and Lenzi, M., "Synthese de certainsderives mono-et dialkyles de I'acidecyanurique par reactions of d'aminomethylation," *Bull. Soc. Chim.* Fr., *414* (1991).
7. Varma, R. S., *Chem. Abstr., 85*, 45637, (1976).
8. Sweeney, T.R. and Pick, R.O., Handbook *Exp. Pharmacol., 68*, 363 (1984).
9. Bundgaard, H., *Methods Enzymol., 112,* 347 (1985).
10. Riera de Narvaez, A.J. and Ferrerira, E.I., Quim. *Nova Rio de janeiro,* 38 (1985); *Chem. Abstr., 107*, 198116 (1987).
11. Zalai, A., 5th Int. Koll. Addit. Schmicrst, *Arbeitsflussigkeitin,* Budapest, *2,* 9/4/1 (1986).
12. Fedtke, M., Makrom. Kem. Makrom. Symp., *7,* 153 (1987).
13. Tramontini, M., Angiolini, L. and Ghendini, *Polymer, 29,* 771 (1988).
14. Geary, W. J., *Coord, Chem. Rev., 7,* 81 (1971).
15. T.Y. Shen, *J. Med. Chem.*, 241 (1981).