

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME ENAMINONES METAL COMPLEXES WITH Ca(II) AND Mg(II)

Tariq Mahmud, Javed Iqbal, Ahsan Amin Bhatti,
Asma Rashid Tariq, Aqsa Gulzar

Institute of Chemistry, University of the Punjab, Lahore-Pakistan.

Author to whom correspondence may be addressed

Email: tariqm06@yahoo.co.uk

Abstract: New enaminones 4-*N,N*-diethylamine-pent-3-ene-2-one[HL¹], 4-*N,N*-di-n-propylamine-pent-3-ene-2-one[HL²], 4-*N,N*-dicyclohexylamine-pent-3-ene-2-one[HL³] and their metal complexes were synthesized by reacting the equimolar amounts of ethanolic solutions of the ligands (HL¹, HL² and HL³) with ethanolic solution of Ca (II) and Mg (II) ions. All the complexes were characterized by infrared, electronic and atomic absorption spectroscopy. Enaminone-metal complexes thus prepared, showed square planar and octahedral geometries and were tested for their antibacterial activity against *E. coli* and *Staphylococcus aureus* bacteria.

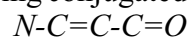
KEYWORDS: Enaminones, Spectroscopy, Antibacterial activity.

Introduction

Enaminones are versatile, readily obtainable reagents and their chemistry has received considerable attention in recent years [1]. Enaminones, also known as enamino ketones, are versatile synthetic intermediates [2]. The reactivity of enaminones and dienaminones makes them attractive intermediates for the preparation of heterocycles, such as oxazoles, pyridinones, quinolines, dibenzodiazepins, and tetrahydrobenzoxazines. Tetraoic acids, tetrahydrophenanthridines and aza steroids have all been synthesized from enaminones [3, 4]. The enaminone obtained from acetylacetone and diethylamine are used as a universal cation locating agent because they show characteristic colors with different transition metals [5]. Enaminones were also presented as a source of new azo compounds with linear optical properties [6]. Dannhardt and Bauer prepared series of pyrrolidino enaminones from 2-methoxy-1-pyrroline or 2-pyrrolinones and appropriate benzoylacetates or

benzoylacetone nitriles which show low or no antibacterial activity [7]. The anticonvulsant activity of methyl 4-[(*p*-chlorophenyl) amino]-6-methyl-2-oxocyclohex-3-en-1-oate (enaminone), was reported and compared with the phenytoin and carbamazepine [8]. The mechanism of anticonvulsant action for benzyl amino enaminones has also been reported [9]. The synthesis of anticonvulsant derivatives was done which represent a new and potentially active series of compounds for the treatment of generalized tonic, clonic and complex partial seizures [10]. The chemical reactivity and pharmacological activity of these analogues may bring a valuable addition to the present knowledge. These new structural classes of compounds can be useful for the design of future targets and development of new drugs [11].

The term enaminone is in fact used to indicate any compound containing the following conjugated system.



Enaminones were prepared by condensing 1,3-diketones with appropriate diammines [12,13], in which reactants were directly heated together. It was found that the use of *p*-toluene sulfonic acid complicates the isolation of the pure products [14]. Simple, common and successful procedures were applied for the synthesis of enaminones, i.e.; the reactions between formamide dimethyl acetates and ketones [15, 16]. Similarly, a reaction between β -chloro vinyl ketones and amines may also be employed for enaminone formation [17]. The catalytic hydrogenation of 1,2- and 1,3-diketo derivatives employing a variety of catalysts, including copper, nickel, cobalt, platinum, palladium and other metals, has been well documented [18]. Khouzani et al prepared and characterized certain enaminones such as 1,1-Difluoro-3-(1H-quinolin-2-ylidene)propan-2-one, 1,1,1-trifluoro-3-(1H-quinolin-2-ylidene)propan-2-one, 1,1,1-trifluoro-3-(4-chloro-1H-quinolin-2-ylidene)propan-2-one and 1,3-dibromo-1,1-difluoro-3-(2-quinoly)propan-2-one, by various spectroscopic techniques [19]. ^{17}O . NMR spectra of primary and secondary enaminones were studied by Zhou. Evaluation of substituent increments and intramolecular hydrogen bonding with various substituents at the C-1, C-3 and N positions were reported. Analyses of the ^{17}O chemical shifts of these enaminones give the values of the increments for the substituent at these positions [20]. The present research work comprises the synthesis of 4-*N,N*-diethylamine-pent-3-ene-2-one [HL^1], 4-*N,N*-di-n-propylamine-pent-3-ene-2-one [HL^2] and 4-*N,N* dicyclohexylamine-pent-3-ene-2-one [HL^3] ligands and their complexes with Ca(II) and Mg(II) ions. These ligands were prepared with the procedure reported in literature [21,22]. The complexes of Ca(II) and Mg(II) ions with these ligands were synthesized and

characterized by infrared and ultraviolet spectroscopy. The ligands and their metal complexes were also examined for their antibacterial activity using disc diffusion method.

EXPERIMENTAL WORK

Materials and Methods:

All volumetric apparatus used for dilution or making up volume was calibrated within ± 0.01 mL. Gallen Kamp melting point apparatus was used for the determination of melting points. Conductance was recorded by pre calibrated conductivity meter (Model Cyber Scan 500). The liquid state Labomed UV-Visible spectrophotometer (Model UVD-3500 with wavelength range 200-800nm) was used to record the electronic spectra of the compounds in various solvents. The solid state IR spectra of the ligands and their metal complexes were recorded on FT-IR spectrophotometer (Model Perkin-Elmer spectrum-RX-I) using Nujol mull with a full scan wavelength range of 4000-500 cm^{-1} . The amount of Ca(II) and Mg(II) ions in the complexes was estimated by atomic absorption spectrophotometer, (Model Varian AA-1275 spectrophotometer) using air-acetylene flame.

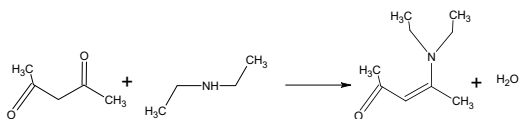
All the chemicals and reagents used were purchased from Fluka and Fisher Scientific (USA).

SYNTHESIS OF LIGANDS

a).Synthesis of 4-*N,N*-diethylamine-pent-3-ene-2-one (HL^1).

Acetylacetone 10.5 mL (0.1mole) was taken in a conical flask placed in an ice bath. Subsequently, diethylamine 10.4 mL (0.1mole) taken in a dropping funnel was added drop wise with continuous stirring. Yellow tiny crystals were formed during the addition of the diethylamine to the acetylacetone in about 15 minutes.

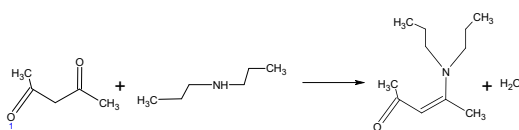
The needle like crystals thus formed were purified by sublimation and were stored in the refrigerator at 4°C.



b). Synthesis of 4-N,N-di-n-propylamine-pent-3-ene-2-one (HL²).

I. Distillation using Dean-Stark apparatus

Acetylacetone 61.7 mL (0.6 mole), di n-propylamine 83.9mL (0.6 mole) and *p*-toluene sulphonic acid (2.0 g) and benzene (150mL) were taken in a round bottom flask and were refluxed using a Dean-Stark apparatus, till the complete removal of water. Benzene was then removed under reduced pressure in a rotary evaporator. The contents of the flask were cooled using an ice bath. The yellow liquid thus obtained was shaken with cold saturated ethanolic sodium bicarbonate solution and extracted with ether, thrice. The combined ethereal extract was taken into a distillation flask and the fraction boiling at 95°C was collected.

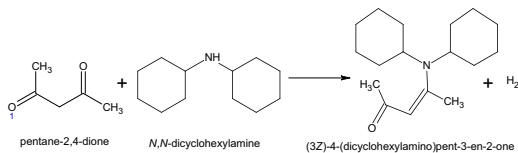


II. Anhydrous sodium sulphate method.

A mixture of di-n-propylamine 83.9 mL (0.6 mole) and acetylacetone 61.7 mL (0.6 mole) was taken in a round bottom flask. It was followed by the addition of anhydrous sodium sulphate (2.0 g). The contents of the flask were shaken well, stoppered tightly and placed in a refrigerator at 4°C for 48 hours. The reaction mixture was then filtered off and distilled under reduced pressure. The liquid product thus obtained was used for further analysis.

C). Synthesis of 4-N,N-dicyclohexylamine-pent-3-ene-2-one (HL³)

Acetylacetone 31.0 mL (0.3 moles), dicyclohexylamine 58.0 mL (0.3 moles), *p*-toluene sulphonic acid (1.0 g) and benzene (150mL) were refluxed on heating mantle at 85°C for five hours. The water produced in reaction mixture was removed using a Dean-Stark apparatus. Benzene was removed by distillation under reduced pressure and the viscous oily liquid obtained yielded shiny needle like crystals on cooling. The product was re-crystallized with ethanol.



SYNTHESIS OF METAL COMPLEXES

a). Synthesis of Ca(II) and Mg(II), complexes of HL¹.

An ethanolic solution of anhydrous calcium (II) chloride 1.10 g (0.01 moles) was added drop wise to ethanolic solution of (HL¹) enaminone (0.01 moles). The precipitates formed in the reaction mixture were allowed to stand for about five hours. The product obtained was filtered off and washed first with small amount of ethanol and then with n-hexane and re-crystallized in ethanol. It was then dried at room temperature and stored in a desiccator.

The Mg (II), complex of the ligand (HL¹) was also prepared by adopting the same procedure. The shiny crystals of Mg (II) complex were obtained.

b). Synthesis of Ca(II) and Mg(II), complexes of HL².

Solution of ligand (HL²) was prepared by dissolving the ligand (1.83g, 0.01 moles) in ethanol (20.0 mL). An ethanolic solution of anhydrous calcium(II) chloride (1.10 g, 0.01 moles) was added

drop wise to ethanolic solution of (HL²) enaminone. After 25 minutes continuous stirring, the reaction mixture was allowed to stand for five hours. The product obtained was filtered off and washed first with small amount of ethanol and then with n-hexane and re-crystallized in ethanol. It was then dried at room temperature and stored in a desiccator.

The Mg(II) complex of the same ligand (HL²) was also prepared by adopting the same procedure. The fluorescent crystals of Mg(II) complex were obtained.

c). Synthesis of Ca(II) and Mg(II), complexes of HL³.

An ethanolic solution of anhydrous calcium(II) chloride (1.10 g, 0.01 moles) was added drop wise to ethanolic solution of (HL³) enaminone (2.63 g, 0.01 moles). The contents were stirred for about 20 minutes to ensure the complete precipitation and allowed to stand for about five hours. The product obtained was filtered off and washed first with small amount of ethanol and then with n-hexane and re-crystallized in ethanol. It was dried at room temperature and stored in a desiccator.

The Mg(II) complex of the ligand (HL³) was also prepared by adopting the procedure described above. The fluorescent crystals of Mg(II) complex were obtained.

Antibacterial Studies

The ligands and its metal chelates were evaluated for antibacterial activity against the following bacterial species.

- *E.coli*
- *Staphylococcus aureus*

The discs were prepared by adopting the standard method [23]. The ligand complex, (30 µg in DMF 0.01ml) was applied to a paper disc with the help of micropipette. The discs were placed in an

incubator for 48 hrs at 37°C and then applied to the bacteria grown on agar plates. Inoculation was performed with the help of a platinum wire loop, which was made red hot in flame, cooled and then used for the application of bacterial strains. Sterilized forceps were used for the application of the paper disc on the inoculated agar plates. When the discs were applied, they were incubated at 37°C for 24hrs. The zone of inhibition (diameter in mm) was then measured around the disc.

Results and Discussion

The method of preparation of enaminones invariably involved the removal of water to complete the equilibrium of the reaction. Anhydrous sodium sulphate, sodium carbonate and anhydrous magnesium sulphate were generally used as water removing agents for this purpose. *p*-Toluene sulphonic acid was used for the removal of water in the preparation of some enaminones. These are 4-*N,N*-diethylamine-pent-3-ene-2-one [HL¹], 4-*N,N*-di-n-propylamine-pent-3-ene-2-one [HL²] and 4-*N,N*-dicyclohexylamine-pent-3-ene-2-one [HL³]. The removal of *p*-toluene sulphonic acid from the reaction mixture was difficult in case of the oily products. It was neutralized by treatment with the aqueous solution of sodium hydroxide or sodium bicarbonate. But the presence of water involved in these reactions may decompose the resulting enaminones. Some enaminones were highly susceptible to hydrolysis by water. Three different ligands were prepared in accordance with the methods reported in literature. Two of them were obtained as colorless crystalline solids (mp. [HL¹]=35°C, [HL³]=154°C) and one [HL²] was a viscous liquid with boiling point 95°C. The conductance of these ligands ranged from 22-159µS. These physicochemical parameters have been listed in Table-1.

Three enaminone ligands were prepared

Table-1: Physico-chemical characteristics of Ligands and their metal complexes.

S.No	Compound	Physical State	M.P/B.P°C	% yield	Conductance (μ S)
1	HL ¹	Colorless, needlelike, shiny crystals	35	66-68	159(D.W)
2	HL ²	Golden yellow viscous liquid with fishy smell	95	65-67	22(ETHANOL)
3	HL ³	Colorless needle like shiny crystals	154	64-65	120(D.W)
4	Ca(HL ¹)	White, shiny crystals with an irritating odor	>230	58.07	42(DMF)
5	Mg(HL ¹)	White, shiny crystals with an irritating odor	>230	67.02	103(DMSO)
6	Ca(HL ²)	White amorphous solid	>230	65.03	66(D.W)
7	Mg(HL ²)	White amorphous solid	>230	64.10	297(DMF)
8	Ca(HL ³)	White, shiny crystals	>230	58.2	141(D.W)
9	Mg(HL ³)	Light green, shiny crystals	>230	62.76	116(D.W)

and each ligand formed a complex with different metals i.e. Ca(II), Mg(II). About 58 to 65% yield of each complex was obtained. Most of these complexes were crystalline solids with shiny appearance and only few were amorphous solids. Their melting points are above 230°C and conductance ranged from 42-297 μ S respectively. The conductivity of the ligands and metal complexes in their 0.001M solutions were measured at room temperature in various solvents such as distilled water (D.W), ethanol, methanol,

dimethylsulfoxide (DMSO) and dimethylformamide (DMF).

The solubility of the ligands and their complexes were determined in fifteen different solvents in both cold and hot states. All the ligands and the complexes were insoluble in n-hexane. Most of these were soluble in water and methanol with the exception of ligand [HL²]. The description of their solubility is given in Table-2

Table-2: Solubility of ligands HL¹, HL², HL³ and their Ca (II), Mg (II) complexes.

Solvent	HL ¹	HL ²	HL ³	Ca(HL ¹)	Mg(HL ¹)	Ca (HL ²)	Mg(HL ²)	Ca(HL ³)	Mg(HL ³)
Dist. Water	Sol	In.S	Sol	Sol	Sol	S.Hot	Sol	S.Hot	S.Hot
Methanol	Sol	Sol	Sol	Sol	Sol	Sol	S.Hot	Sol	Sol
Ethanol	Sol	Sol	Sol	Sol	Sol	Sol	S.Hot	S.Hot	S.Hot
Dimethylformamide	Sol	Sol	Sp.S	Sol	Sol	S.Hot	Sol	In.S	S.Hot
DMSO	Sol	Sol	S.Hot	Sol	S.Hot	S.Hot	S.Hot	In.S	In.S
Carbon tetrachloride	Sol	Sol	Sol	In.S	In.S	Sp.S	In.S	In.S	In.S
Chloroform	Sol	Sol	Sol	In.S	In.S	In.S	In.S	In.S	In.S
Acetonitrile	Sol	Sol	Sol	In.S	In.S	Sol	In.S	In.S	In.S
n-Hexane	In.S	In.S	In.S	In.S	In.S	In.S	In.S	In.S	In.S
Benzene	Sol	Sol	Sol	In.S	In.S	In.S	In.S	In.S	In.S
Diethylether	Sol	Sol	Sol	In.S	In.S	In.S	Sp.S	S.Hot	S.Hot
Ethylacetate	Sol	Sol	S.Hot	Sp.S	S.Hot	In.S	In.S	In.S	In.S
Acetone	Sol	Sol	S.Hot	Sp.S	Sp.S	Sp.S	Sp.S	S.Hot	In.S
Tetrahydrofuran	Sol	Sol	Sol	Sp.S	S.Hot	In.S	In.S	Sol	In.S
Toluene	S.Hot	S.Hot	S.Hot	In.S	In.S	In.S	In.S	In.S	In.S

Note: Soluble = Sol, Insoluble =In.S, Sparingly Soluble=Sp.S and Soluble in hot=S.Hot

Electronic Spectra of the Compounds

The spectral data obtained from the UV-visible spectrophotometer has been summarized in Table-3. These spectra were recorded in different solvents depending on the solubility of the compounds. The spectra for each enaminone ligand yielded broad band in the wavelength range of 290-310nm. The λ_{max} values were recorded as: 310nm, 310 nm and 290 nm for HL¹, HL² and HL³

respectively. This was attributed due to π - π^* transitions. For Ca(II) and Mg(II) complexes with HL¹ red shifts were observed at 300nm and 305nm respectively. This is due to the bond formation between nitrogen/oxygen atom of the ligand, and respective metal ion. Similar red shifts were also observed for the metal complexes with ligands HL² and HL³.

Table-3: UV-Visible data of ligands and their metal complexes

S.No.	Compound	π - π^* transitions	
		λ_{max} (nm)	Abs.
1.	HL ¹	310	1.722
2.	HL ²	310	1.315
3.	HL ³	290	1.039
4.	Ca(HL ¹) Complex	300	2.887
5.	Mg(HL ¹) Complex	305	1.987
6.	Ca(HL ²) Complex	310	4.448
7.	Mg(HL ²) Complex	295	2.102
8.	Ca(HL ³) Complex	310	1.113
9.	Mg(HL ³) Complex	290	0.190

IR spectra of some enaminones or enamino ketones were reported by Dabrowski and Kamienska [24]. The spectra were carried out as thin film in tetrachloroethane, methanol, dioxane and D₂O. The characteristic absorption bands in the range 1572-1670 cm⁻¹ and 1427-1542 cm⁻¹ were reported. Some enaminone also showed absorption at 1612-1620cm⁻¹. The IR spectra recorded for the present enaminones were also of same order. The IR data given in the Table-4 depicted strong absorption bands for =C-H stretch, C=C, stretch, C=O stretch and C-N stretch with high absorption intensities, indicative of the formation of enaminones. These peaks were shifted to lower wave numbers in case of almost all the synthesized metal complexes.

In order to determine M-L ratio, the calculated and observed values for different geometries of different metal complexes were done. Finally by comparing these values, appropriate geometries of these metal complexes were proposed. The experimental HL¹ to Ca(II) ratio matches with theoretical ratio corresponding to 2:1. The experimental HL² to Ca(II) ratio matches with theoretical ratio corresponding to 3:1. The experimental HL³ to Ca(II) ratio matches with theoretical ratio corresponding to 3:1. The experimental HL¹ to Mg(II) ratio matches with theoretical ratio corresponding to 2:1. The experimental HL² to Mg(II) ratio matches with theoretical ratio corresponding to 2:1.

The experimental HL³ to Mg (II) ratio matches with theoretical ratio corresponding to 2:1. The synthesized complexes were proposed to exhibit square planar and octahedral geometries.

Antibacterial Studies

The antibacterial activities of the synthesized ligands and their metal complexes were determined against two

strains of bacteria i.e. *E. coli* and *Staphylococcus aureus*, using disc-diffusion method. All the three ligands were found to be inactive against both strains of bacteria. All of the metal complexes were found to be active against these two bacterial strains with the exception of Ca(II) complex with HL¹, and Mg(II) complex with HL³.

Table-4: Characteristic IR band absorption frequencies of Ligands and their metal complexes

Bond frequencies cm ⁻¹	HL ¹	Ca(HL ¹)	Mg(HL ¹)	HL ²	Ca(HL ²)	Mg(HL ²)	HL ³	Ca(HL ³)	Mg(HL ³)
v(C-H) str	3048	3012.5	3023.2	3076	3047	3068.6	3030.6	3025.1	3023
v (C-H) str.	2974	2885.3-	2928.9	2875	2825.2	2918.9	2850.7	2916.2	2922
v (C-C) str.	1483	1454	1405.5	1406.4	1461.1	1413.6	1461	1480.9	1544.2
vC=O str.	1685.6	1661	1678	1698	1685.4	1688	1715	1701.8	1709.4
v(=C-H) bend	976.8	916	-	958	916.3	929.5	958	918.1	964.3
v (C-N) str.	1041.4	1014	1017.2	1255	1198.3	1261	1257.7	1312.1	1376.7
v (C-H) rock, sym	1380	1376	-	1365	1391.9	1431	1447.9	1377.5	1310.4
v (C-H) bend	1405.3-	1451	1436.1	1437	1450.9	1466	1447	1465.6	1460.5
v (C-H) rock, unsym		722	794.7	776	721.3	722	795.2	722.8	723

In general, the complex Mg (HL²) was found to have highest activity against the *E. coli* and *Staphylococcus aureus* bacteria studied under the test conditions, showing that it has a good activity as bactericide. The other complexes

showed some activity against *E. coli* and *Staphylococcus aureus* bacteria. The Ca (HL¹) and Mg (HL³) complexes did not show any activity against the same bacteria strains. This is shown in Table-5.

Table-5: Antibacterial activity of Ligands and their metal complexes

S.No.	Compound	Bacteria Species	
		<i>E.coli</i>	<i>Staphylococcus Aureus</i>
1.	Ligand I	-	-
2.	Ca(HL ¹) Complex	-	-
3.	Mg(HL ¹) Complex	+	+
4.	Ligand II	-	-
5.	Ca(HL ²) Complex	+	+
6.	Mg(HL ²) Complex	+	++
7.	Ligand III	-	-
8.	Ca(HL ³) Complex	+	+
9.	Mg(HL ³) Complex	-	-

* + Active, ++ Very active, - Inactive

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