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RESEARCH PAPER

Synthesis and Biocidal Activity of Some Metal Chelates of M-Toluidnyl Oxamic Acid

Part 1: Bis- (M-Toluidinyl Oxamic Acid) - Zn (Ii) Chelate

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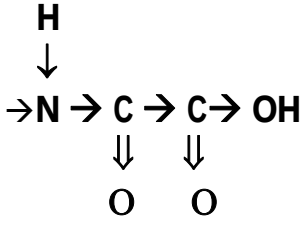
ABSTRACT

In the present study, effect of Zn-chelation on the efficiency of m-toluidinyl oxamic acid has been studied. First m-toluidinyl oxamix acid was obtained by the reaction of m-toluidine and oxalic acid and then its Zn-chelate was synthesized by complexation of m-toluidinyl oxamic acid zinc. The compounds thus obtained by elemental analysis and IR spectral studies. These products were further screened against two much common species each of bacteria and fungi and it was concluded that chelation with Zn enhances the bacterial and fungicidal activity of m-toluidinyl oxamic acid.
Key words: Biocidal activity, m- toluidinyl oxamic, IR spectra, Bacterial and fungicidal activity.

INTRODUCTION

Most of the ligands are good antimicrobial agents. It appears that the activity of those ligands depends upon their complexing nature. It has also been observed that the activity of some of the ligands is increased in the form of metal complex on being coordinated to a suitable metal ion. Oxamic acids are N-amine derivatives of aliphatic dibasic acids, they possess the characteristics oxamic acid function grouping:-

Oxamic acids are generally prepared by condensing aromatic amines with alky derivates of aliphatic dibasic acids (Aschon, 1980).



MATERIAL AND METHODS

All the starting chemicals were of extra pure grade as and were used as such. The solvents were distilled and dried before use by prescribed procedures (Vogel, 1975). Decomposition points are determined in open capillaries in sulfuric acid bath.

Both the compounds, ligand and its metal chelate, were characterized by their elemental analysis and IR spectral data; the metal was estimated satisfactory with the stoichiometry shown in the structure.

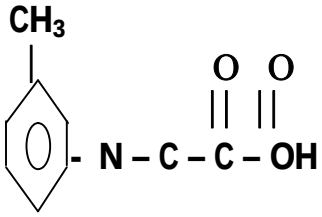
SYNTHESIS OF M-TOLUIDINYL OXAMIC ACID

10.80 ml. (0.1M) freshly distilled m-toluidine and 9.0 gm. (0.1M) of anhydrous oxalic acid (obtained by keeping oxalic acid in an air oven at 100^0 C for 3 days) were hated on an oil bath at 120^0 C – 130^0 C for 3 hours. The resulting crystalline product was dissolved in 200 ml. of boiling water, stirred and filtered while hot. A crystalline product was obtained on cooling the solution. The obtained solid was filtered and repeatedly washed with 10 ml. of dil H_2SO_4 (5% by v/v). The ethereal extract containing m-toluidinyl oxamic acid was separated and the solvent either containing m-toluidinyl oxamic acid was separated or the solvent ether was evaporated on water bath to get the crystalline m-toluindinyl oxamic acid. The acid was purified by repeated recrystallisation with water and finally with alcohol.

Compound obtained: m-toluidinyl oxamic acid.

Molecular formula: $C_9H_9NO_3$

Structure



Synthesis of bis- (m-toluidinyl oxamic acid) – Zn (II) Complex

10 ml. alcoholic solution 1.79 gm. (0.01 M) m-toluidinyl oxamic acid was added to 10 ml. aqueous solution of 1.1 gm. (0.005 M) Zinc acetate in a round bottom flask. The solution was stirred for 10 minutes. After adding ammonia solution to maintain the pH of the solution between 6-7, it was refluxed over a water condenser. The solution was filtered hot and the filtrate was kept at room temperature for two days to get crystals of bis-(m-toluidinyl oxamic acid)- Zn (II) complex. The crystals were washed with distilled water and alcohol. Finally, the crystals were dried in vaccum over P_4O_{10}.

Compound obtained: bis(m-toluidinyl oxamic acid) –Zn (II) complex

Molecular formula $(C_9H_8No_3)_2$ Zn.

CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

Characterization of m-toluidinyl oxamic acid bis(m-toluidinyl oxamic acid)- Zn (II) complex was done by determination of physical properties, elemental analysis and IR spectral studies and the results are summarized in Table I and Table II.

Table 1. Observations and analytical data.

S. No	m-toluidinyl oxamic	Bis-(m-toluidinyl oxamic acid)-Zn (II) complex
1. Molecular formula	$C_9H_9NO_3$	$(C_9H_8NO_3)$ Zn.
2. Colour	White	Off-
3.Decomposition Point (^{0}C)	141	216
4. Soluble in	Ethyl alcohol, ether, propylene, glycol and sparingly soluble in water.	Ethyl alcohol, benzene and propylene glycol.
5. Analytical	F C	F C
%C	60.45 60.33	51.00 51.26
%H	5.20 5.03	3.52 3.80
%N	7.64 7.89	7.00 6.65
%Zn	…… ……	14.91 15.52

F= Found C= Calculated

INTERPRETATION OF IR SPECTRA

In the IR spectra to the ligand and its complex certain peaks are observed which are common in both the cases. For the sake of brevity, only important peaks have been explained which are either altogether new in the IR spectra of complex or show a remarkable shift in their frequencies indicating the presence of co-ordination.

The strong absorption band at 3220 cm^{-1} in the spectra of m-toluidinyl oxamic acid may be attributed to the coupled stretching vibrations due to-OH and –NH groups which shows due to which lowering of frequency occurs. This band is further shifted towards the lower frequency region in the complex bis-(m-toluidinyl oxamic acid)-Zn (II) showing the coordination through –NH and –OH groups.

In the complex, an absorption peak in the region between 2920-2845 cm^{-1} may be attributed to the aromatic C-H stretching.

A strong band corresponding to carbonyl group is observed between 1760-1745 cm^{-1} in the ligand which is shifted towards lower frequency region in the complex confirming the c0-ordination of this group. The presence of aromatic ring may also be responsible for the lowering of this frequency.

The broad medium band in the region of about 1680 cm^{-1} in the spectra of m-toluidinyl1 carboxylic frequency which is lowered in the spectra of the complex indicating co-ordination of metal through carboxylic group. In the case of complex, this band appears in the region between 1655-1615 cm^{-1}.

Table 2. IR spectral data in $cm^{-1.}$

S.No	Possible assignments	Frequency in Cm-1	
		m-TOA	Bis(m-TOA)-Zn (II) Complex
1.	OH and NH coupled stretching	3220 (st)	-------
2.	Aromatic carbonyl stretching	1760 (st)	1720 (m) 1675 (st)
3.	Aromatic C=C stretching	1600 (m)	1545 (vw)
4.	Aromatic C-H stretching	2950 (st)	2920 (m)
5.	Mixing of C-O and NH bond	1530 (m)	1500 (w)
6.	Aromatic C-C multiple bond	1480 (st)	1465 ()st
7.	C-N Stretching	1370 (mb)	1360 (st)
8.	In plane C-H deformation	1000 (m)	940 (vw) 900 (w)
9.	Out of plane C-H deformation	780 (st) 715 (m) 670 (m)	720 (mb) 690 (m) 610 (w)
10.	Out of plane ring C-C bond	580 (mb) 430 (m)	------- -------
11.	M-O bond	-------	450 (st)

St=strong m=medium m b=medium broad w=weak v w=very weak

The presence of some peaks in the region 1600-1560 cm^{-1} in the free ligand may be attributed due to the presence of aromatic C=C Stretching (Dyer, 1974).

The characteristics –NH frequency at 1530 cm^{-1} is quite prominent in the case of m-toluidinyl oxamic acid ligand. It, is however, lowered in the case of complex indicating the probable co-ordination through nitrogen.

A medium broad band between 170-1320 cm^{-1} is definitely due to the presence of C-N stretching vibrations. A remarkable lowering in the spectra of the complex clearly indicates the involvement of nitrogen atom in the spectra of m-toluidinyl oxamic acid which may be due to aromatic C-C multiple bands (Palm and Werbin, 1953). In the IR spectra o free ligand as well as its complex some absorption bands are common in the region 1170-1000 cm^{-1} which are assigned to the In-Plane C-H deformation (Bellamy, 1970). Similarly few bands in the region between 790-670 cm^{-1} in the IR spectra of ligands and complex assigned to the out-of plane C-H deformation (Jones and Sandor, 1956). As a result of complex formation certain new peaks are found in the IR spectra of metal complex which are caused by the formation of new bonds between metal and electron donating atoms. The absorption bands in the region of 520-420 cm^{-1} are the characteristics of metal-nitrogen bands.

BIOASSAY

Both of the compounds, the ligand and its Zn-chelate, thus synthesized and characterized were screened agains two species each of bacteria and fungi. Antibacterial screening was carried out against Fungi *Aspergillus niger* and *Candida albicans*. Maximum concentrations tested: 100 μg/ ml in Propylene glycol (AR grade). Minimum inhibitory concentration (MIC), at which growth of the test organism was completely inhibited, was determined for the compounds and expressed in terms of μg/ ml. 100% solutions were obtained by dissolving the compound in propylene glycol (AR grade). For the growth of micro-organism, a proper temperature, pH, necessary nutrients and growth media free from other micro-organism was provided for the preparation of culture of pathogenic bacteria and fungi using aseptic techniques (Harrigan and Mc Cance, 1996; Crunch, 1975). The culture media used for slant and broth was sterilized by moist heat sterilization method (Donald & Williams, 1955) (by autoclaving at 121 ^{0}C using 15 lbs. pressure for 15 minutes). All the utensils used were also sterilized by their usual methods. The incubation period for the bacteria was kept 24 hours at 37 ^{0}C temperature and for fungi 96 hours at 28 ^{0}C.

Culture media for bacteria (Spooner and Skyes, 1972)

Peptone	0.6%
Yeast	0.3%
Beef extract	0.15%
Dextrose	0.1%
Agar (only for slant)	1.5%

Total volume of water added to make 250 ml.

pH adjusted 6.5-6.6

Culture media for fungi (Spooner and Sykes, 1972)

Peptone 1.0%
Dextrose 2.0%
Agar (only for slant) 2.5%
Total volume of water added to make 250 ml.
pH adjusted 5.4

Subcultures of the above mentioned micro-organism were prepared monthly from the principal culture and broth culture weekly from the subculture. All the cultures were stored at 4 ^{0}C. The inoculation process was carried out in a well-cleaned inoculation chamber having UV lamp. Seeded broth for the test to be conducted was prepared by diluting the broth culture of the desired organism in 1:100 times and already kept for overnight at their optimum temperature.

The results obtained after antimicrobial screening are summarized in Table 3.

Table 3. Antimicrobial Screening.

S.No	Test organism	m-toluidinyl oxamic acid	bis-(m-toluidinyl oxamic acid)-Zn (II)
1.	*S. aureus*	50	25
2.	*E. coli*	50	12.5
3.	*A. niger*	>100	25
4.	*C. albicans*	100	6.25

It is observed from the MIC values of the ligand and complex that the biological efficacy of m-toluidinyl oxamic acid increases on complexation. It may be due to following facts.

1. On complexation the lipo-soluble nature of the biologically active ligand or metal is increased (Sharma, Parashar and Mohan, 1987).
2. The metal ion present in metal enzyme of the biological system is displaced by foreign metal ion of the more lipo-soluble metal complex. This is only possible when the foreign metal has a stronger affinity for the apo-protein molecule of the metal enzyme in biological system (Rhode, Shafer, Idriss and Levin-son, 1979).
3. By the displacement of protein molecule from the metal enzyme by the foreign lignad of the more lipo-soluble nature, thus the rupturing of enzyme affects a biological system.
4. Due to the combined activity effect of both the metal and the ligand in metal chelates (Levinson, Idriss and Jackson, 1979).

However, among all the above possible factors, the more rapid penetration of the metal complex as a whole through the wall of cells of the micro-organism may be one of the other important factors. The active constituents may also acnt on protein synthesis ans nucleic acids, DNA or RNA (Mikelens, Levinson, 1978; Rhode, Cordell and Webster, 1977).

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