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Synthetic, characterization, evaluation of enzyme inhibition activity and biocidal studies of some dithiophosphamate complexes

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ABSTRACT

Dithiophosphamates are the sulphur containing ligands which have tendency to form complexes with Tungsten metal. The Multinuclear compounds of Tungsten have been synthesized and characterized by different methods. Physical and Analytical measurements like conductivity measurement, TLC Technique used to check the purity of synthesized compounds. Enzyme inhibition activity of compounds has been carried out by cup plate method results show that the enzyme activity of ligands has considerably increased in the form of their metal complexes. Biocidal studies of synthesized compounds against certain Bacterial and Fungal species has been carried out by serial dilution method to evaluate the MIC (Minimum inhibitory concentration) value on the basis of MIC value it is interpretated that metal complexes are most active as compared to ligand fragments.

Keywords: Synthesis, spectra, Enzymatic, Biocidal.

INTRODUCTION

Dithiophosphamates, the derivative of Dithiophosphamate acids are important on account of their wide applications it contain sulphur donor atoms and constitute an important series of ligands which show an interesting versatility in their bonding modes (e.g uni as well as bidentate chelating/bridging) towards different metals [1-4].

O,O'-alkylene dithiophosphoric acids, the cyclic analogues of O,O'-dialkyl dithiophosphoric acids have been described and their metal and organometal complexes have been studied extensively.The dithiophosphamate ligands form bridging complexes with Tungsten which are used as pesticides.

EXPERIMENTAL SECTION

All the chemicals and reagents were of reagent grade solvents and chemicals were purified and dried by standard methods. The ligands and tetrathiometallate of Tungsten were prepared by the reported procedures. FTIR were recorded on Thermo Nicolet Avater 370. Electronic spectra on Varian carry 5000 UV-Visible-NIR Spectrophotometer. NMR spectra on Bruker Avance III, 400 MHz spectrometer. The conductance measurements were carried out in a Toshniwal CL-01-06 conductivity Bridge using 1×10^{-3} M DMF solution. M.P was determined on a Toshniwal Melting point apparatus C and H were analyzed on Carlo-Erba micro analyser model 1106. Metal and sulphur were estimated by standared procedures [5].

Synthesis of Metal Complexes

0.1 mole of O,O-di-isopropyl/ O,O-diethyl phosphamate was dissolved into minimum quantity of water. The Tetrathiometallate of Tungsten was dissolved in minimum amount of water at 60^0C then phosphamate was slowly added to it. This solution mixture was kept over water bath for 10-15 minutes. The resulting solution was concentrated and cooled to get the orange red/yellow solid. The resulting coloured precipitate was washed with ethanol followed by ether and then dried under reduced pressure over anhydrous $CaCl_2$. (Yield 60-70%)

Enzymatic Activity

Enzymatic activity is carried out by cup plate method. All the plates were incubated at 30^0C for 48 hrs in BOD incubator. The activity zone of the produced enzyme after incubation was developed by flooding the plates with an appropriate developing agent. The total diameter of zone of inhibition in mm was measured and the activity was calculated [6-11].

Biocidal Studies

All the compounds were tested for their Biocidal studies by using serial dilution method against Bacterial species Viz. Escherichia coli (g-ve) and staphylococcus aureus (g+ve) (incubation period for Bacteria 24 hrs at 37^0C) Fungal species Viz Aspergillus niger and candida albicans (incubation period for Fungi 96 hrs at 28^0C) using BOD incubator. All operation must be conducted in aseptic experimental condition in order to avoid the atmospheric external contamination [12-16].

From Figure-1 it is clear that if Logarithm of the cell counts is plotted against times, "S" shaped curve "ABCD" is obtained AB is called period of Lag phase, BC the logarithmic phase and CD the stationary phase if an inhibitor is supplemented in the medium the period of Lag phase shifts to AB^1. That of logarithmic phase to B^1C^1 and that of stationary to C^1D^1.

RESULTS AND DISCUSSION

All the complexes are colored solids, air stable and insoluble in water and common organic solvents but found soluble in DMF, DMSO and Acetonitrile etc. Their Molar conductance values (0.82-5.2$\Omega cm^2 mol^{-1}$) indicate their Non Electrolytic nature. The analytical data reveal 1:1 metal ligand stoichiometry (Table 1) compounds were routinely checked for their purity on silica gel-G TLC plates and spots were visualized by iodine vapours.

Table 1: Analytical and physical data of compounds

S. No.	Compound	Colour	M.P. (^{O}C)	Analysis % Found/(Calcd.)			
				C	H	S	M
1.	$C_4H_{10}O_2PS_2Na$	grey	92	23.00 (23.18)	4.50 (4.83)	30.20 (30.91)	-
2.	$C_6H_{14}O_2PS_2Na$	grey	102	29.50 (30.63)	4.82 (5.95)	27.00 (27.23)	-
3.	$W_2[C_8H_{20}O_4P_2S_8]$	orange red	287	10.90 (11.11)	2.00 (2.31)	29.50 (29.62)	42.00 (42.59)
4.	$W_2[C_{12}H_{28}O_4P_2S_8]$	yellow	296	14.90 (15.65)	2.96 (3.01)	27.00 (27.82)	39-20 (40.00)

Spectral Studies

The IR spectra of all the ligand fragments reveal sharp and prominent bands at 590-490 cm^1, 770-755 cm^{-1} and 1140-1100 cm^{-1}. These have been assigned to $\upsilon_{p\text{-}s}$ and $\upsilon_{p\text{-}o\text{-}c}$ stretching vibrations respectively. In the spectra of all the complexes a strong bands around 513-500 cm^{-1} indicate the presence of metal sulphur terminal bond υ_{W-S_t}. The additional important bands in the region 435-432 cm^{-1} suggest the presence of bridging sulphur atom υ_{W-S_b} The medium intensity stretching vibrations at 347-330 cm^{-1} confirms that coordination to metal has taken place through sulphur atom of the dithiophosphamate ligand and tungsten is reduced to (V) state from (VI) state by dithiophophamate group. The band due to υ_{P-S} vibrations show a chemical shift of 15-20 cm^{-1} as compared to their position in the spectra of the corresponding ligands which is suggestive of S→ W coordination in the metal complexes[17]. The Electronic spectra of tungsten complexes show two d-d- bands in the range 14700-13333 cm^{-1} and 12600-12400 cm^{-1} respectively which correspond to the transition $^3E \rightarrow {}^1A_1$ and $^1E \rightarrow {}^1A_1$ which indicate the presence of dithiophophamate bound to tungsten(V). The moderately strong bands in the region 25,800-25,500 cm^{-1} can be attributed to S→W ($a_2 \rightarrow e$) charge transfer band. Thus the above mentioned bands are probably a combination of the twin sulphur to metal transitions[18]. The ^{1}H NMR spectra of the tungsten complexes in $CDCl_3$ showed the signals at δ1.24-1.71 (20H, m, Me, CH_2), δ1.68 (14H. m, Me, CH) respectively. A singlet observed at δ3.12-3.48 in the parent dithiophosphoric acid and assigned for SH proton is found to be absent in the spectra of the corresponding complexes indicating the deprotonation of SH group and formation of W-S bond[19].

Enzyme inhibition Activity

A comparative study of enzyme activity of the ligands and their respective metal complexes (table 2) indicate that the enzyme activity of the ligands has considerably increased in the form of their metal complexes. The ligand/metal

complexes play an competitive role with the substrate required for their production and thus the free availability of the substrate is reduced inhibting their growth Ligands and their metal complexes on diffusion inside the cell affect their mitochondrial action causing a death to them. A combined activity effect of the metal and ligand in the metal complex causing a retardation in their growth .

Table 2: Enzyme activity (in mm) of synthesized ligands and their metal complexes at 30^0C After 48 hrs

S.N.	Compound	Enzyme		
		Cellulase	Protease	Amylase
1.	$C_4H_{10}O_2PS_2Na$	4.0	5.0	8.0
2.	$C_6H_{14}O_2PS_2Na$	3.0	4.0	7.0
3.	$W_2[C_8H_{20}O_4P_2S_8]$	1.0	3.0	4.0
4.	$W_2[C_{12}H_{28}O_4P_2S_8]$	1.0	2.0	3.0

Biocidal Studies

The Biocidal studies (Table 3) show that compound No. 1 is used as starting material for the synthesis of Tungsten complexes is biologically inactive against Both the Fungal and Bacterial species This is probably either due to lipid insoluble nature (water soluble character) or their instability in solution. The compounds (Nos. 2a, 3a) are more active as compared to the ligands (Nos 2,3) and the activity of the ligands has enhanced several folds on complexation because Tungsten complexes are fairly stable, water insoluble and so strong that it does not dissociate inside the cells in cellular fluid to release again free bioactive ligand, the metal and it can easily pass through the cytoplasmic membrane and become lipid soluble inside the cell causing bacteriostatic and fungitoxic effects [20-22].

Table 3 : Minimum Inhibitory Concentration (MIC) Values In Molar Concentrations ($X10^{-4}$) Of The Ligands And Complexes

S. No.	Compound	No.	Bacteria		Fungi	
			E.coli	*S. aureus*	*A. niger*	*C. albicans*
1.	$(NH_4)_2WS_4$	1	2.8735	2.1551	1.4367	1.4280
2.	$C_4H_{10}O_2PS_2Na$	2	0.1509	0.3019	0.1509	0.1509
3.	$C_6H_{14}O_2PS_2Na$	3	0.2659	0.2659	0.5319	0.2659
4.	$W_2[C_8H_{20}O_4P_2S_8]$	2a	0.0045	0.0045	0.0090	0.0090
5.	$W_2[C_{12}H_{28}O_4P_2S_8]$	3a	0.0169	0.0084	0.0169	0.0169

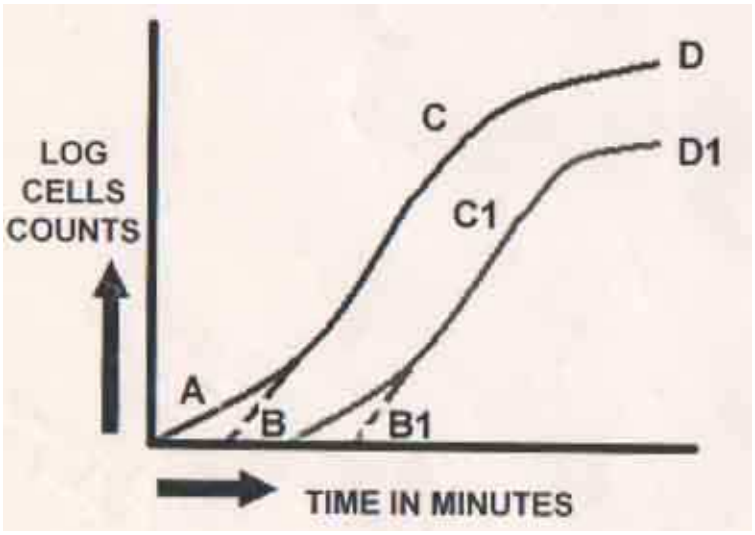

Fig.1 Growth curve of Microorganism

CONCLUSION

In this paper we investigate, synthesis, characterization of tungsten complexes with dithiophosphamate ligands. Enzyme inhibition activity and Biocidal studies of the synthesized compounds were examined and both studies revealed that the complexes are potentially much more active as compared to ligand fragments. Our findings may give a new application and open a new way in the design of more effective and useful Tungsten complexes for synthetic as well as medicinal chemists.

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REFERENCES

[1] JR Wasson; GM Wolterman; HJ stoklosa. Top Curr, Chem; **1973,** 35, 65-68
[2] C Fest; KJ Schmidt. The Chemistry of organophosphorous pesticides, springer-verlag Berlin, Heidlberg, New York, **1982.**
[3] M Eto. organophosphorous pesticides organic and Biological chemistry CRC Press Cleveland, ohio, USA, **1974.**
[4] HPS Chauhan; CP Bhasin; G Srivastava; RC Mehrotra, Phosphorous sulphur, **1983,** 15, 49.
[5] AI Vogel. A Text Book Quantitative Inorg Analysis, ELBS-Longman, London, **1978**
[6] CV Winder; Jwax; M welfore. J. Pharmacol Exp. Therap **1965,** 140, 442.
[7] PC Freeman; AC Goudie; FR Mangan; M Thomson. J Pharm. Pharmacol **1976,** 28, 865
[8] KB Khare; G Brompeix. Rev. de. Mycologie, **1976,** 40, 65
[9] WA Ayers; Go papavizas, AF Dein. Phytopathology, **1966,** 56, 1006
[10] JR sorenson. J. Med. Chem. **1976,** 19, 135
[11] AC satorelli; KC Agrawal; AS Tsftsoglou; EC Moore, Advances in Enzyme regulation **1977,** 15, 117
[12] Gray young. A text book of Microbiology Mcgraw Hill Book Company, Inc. New York, **1962.**
[13] MO William. Practical Hand Book of Microbiology; CRC press, **1989.**
[14] H Nfsika; A Barbara, A smith Antimicrob. Agents Chemotherap, **1987,** 31(1), 46-51
[15] DF sponer; G sykes. Methods in Microbiology, Academic Press London **1972.**
[16] E Goldman. Practical Hand Book of Microbiology, CRC Press, **2008.**
[17] LJ Bellamy. Advances in Infrared group frequencies, Methuen and Co Ltd. London, **1956.**
[18] ABP Lever.Inorganic Electronic spectroscopy Elsevier, Amsterdam, **1968.**
[19] RR Ernst; G Bodenhausess; A wokaun. Principle of NMR in one and two Dimensions Oxford science Publication, **1990.**
[20] ZR Zgoda; JR Porter. Pharm. Biol; **2001,** 39, 221.
[21] AL Barry. The antimicrobial susceptibility tests Principle and Practice, **1976,** 180.
[22] PK Mukherjee; P Balasubramanian; K saha; BP saha; M Pal. *Indian drugs*; **1995,** 32, 274-279.