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SYNTHESIS CHARACTERIZATION AND STUDY OF MICROBIAL ACTIVITY ORGANOMATALIC COMPOUNDS OF La, Ce, Nd, Gd (III) WITH OF 5,6-o-ISOPROPYLIDINE-L- ASCORBIC ACID.

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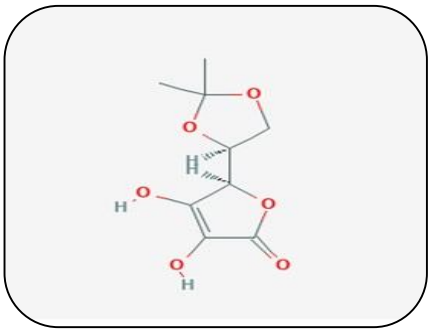
ABSTRACT :-

Complexes of La(III), Ce(III), Nd(III) and Gd(III) with Ligand of 5, 6-o- iso-propylidide–L-ascorbic acid derivative were prepared in hot aqueous ethanol. Complexes were characterized using UV- Visible, IR, NMR, Mass spectroscopic methods as well as magnetic susceptibility and conductivity measurements. In addition biological activity of the synthesised metal complexes against against Salmonella typhi, Escherichia coli ,Staphylococcus aureus and Klebsiella pneumonie bacteria and Aspergillus niger, Aspergillus flavus, Fusarium oxisporium and Trichoderma viride fungi respectively were examined in-vitro. Some of the metal complex displayed pounced biological activity.



KEYWORDS : *Complexes, Ligand, iso-propylidide–L-ascorbic acid, spectra, Antimicrobial Activities.*

INTRODUCTION:-

The coordination chemistry of lanthanide (III) ions is rapidly increasing, owing to the relevance of these compounds in basic and applied research in different scientific areas ranging from chemistry to material science to the life science [1–8]. Lanthanide coordination compounds are the subject of intense research efforts owing to their unique structures and their potential applications in advanced materials such as Ln-doped semiconductors [9], magnetic [10, 11], catalytic [12], fluorescent [13, 14], and nonlinear optical materials [15, 16]. It has been shown that ligands containing both nitrogen and oxygen donor atoms are good building blocks for the formation of various lanthanide coordination compounds [17– 18]. In view of this, we have designed a series of organometalic compounds which can enhance biological properties of lanthanide ions.

The most synthetically useful and well studied class of modified *L*-ascorbic acid is the 5,6-*O*-isopropylidene-*L*-ascorbic acid derivatives (Ketal of *L*-ascorbic acid). These derivatives (5,6-*O*-Ketal & 5,6-*O*-acetal) are significant in organic synthesis for protection of the 5,6- hydroxyl functions, which makes them more soluble in organic solvents and also limits the interference of the protected hydroxyl group from reactions involving the C2- and C3- enol-hydroxyls[19-21], consequently, most synthesis began with 5,6-*O*-isopropylidene- *L*-ascorbic acid as the starting material, which is cheaply and easily made from *L*-ascorbic acid [22]. Metal complexes of ascorbic acid have been prepared and characterized.

Microbial infection remains one of the most serious complications in several areas, particularly in medical devices, drugs, health care and hygienic applications, water purification systems, hospital and dental surgery equipment, textiles, food packaging, and food storage. Antimicrobials gain interest from both academic research and industry due to their potential to provide quality and safety benefits to many materials [23-24]. However, low molecular weight antimicrobial agents suffer from many disadvantages, such as toxicity to the environment and short-term antimicrobial ability [25-26]. To overcome problems associated with the low molecular weight antimicrobial agents, antimicrobial functional groups can be introduced into polymer molecules [27]. The use of antimicrobial polymers offers promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity, and prolonging the lifetime of the antimicrobial agents [28-30].

Instrumentation

1. Infra-red spectra between (400-4000 cm-1) were performed with (FT-IR) 8300 Shimadzu spectrophotometer. The NMR spectra were recorded on a Bruker NMR spectrometer (300 MHz).
2. The electronic spectra were recorded on the U V Visible spectrophotometer type (spectra 190-900) nm CECIL, using DMSO as a solvent.
3. The melting point was recorded on "Gallen kamp melting point Apparatus".
4. The conductance measurements were recorded on W.T.W. conductivity meter.
5. Elemental analysis for carbon, hydrogen was using a Euro Vector EA 3000 A Elemental Analysis .
6. Metal analysis. The metal contents of the complexes were determined by atomic absorption technique. Using a Shimadzu PR-5, ORAPHIC PRINTER atomic absorption spectrophotometer.

Preparations of Ligand and their complexes

Synthesis of 5, 6-O-isopropylidene-L-ascorbic acid (IPAA): The Ligand (IPAA) was synthesised by refluxing a solution congaing Powdered L-ascorbic acid (1) 10 gm and acetone 40 ml while stirring about 30 minutes. After addition of benzyl chloride (1.5 ml), the reaction mixture was stirred for 2-3 hours at ambient temperature in a 250 ml flask. The precipitate thus obtained, was washed several times with acetone- hexane mixture, cooled at 0^0C and then dried to give (2) 93% as a white crystalline residue M.P. 206-208 ^{0}C, Soluble in water, ethanol, DMF and DMSO. Anal. Cald for $C_9H_{12}O_6$: C 50.00 %,H 5.50 %, found C 48.96 %,H 5.47; IR: 3242(–OH),2910 (-C-H), 1753 (-C=O), 1662(-C=C-) 1436 (-C-H), 1141, 898 (C-O; ^{1}HNMR: δ 1.8 ppm (s, for the isopropylidene methyl proton) δ 2.4 – 2.9 ppm (m, H_4, H_5, H_6), δ 3.1 ppm (s, for the -OH); ^{13}CNMR: δ 171.11 (-C=O), δ 152.26, 108.89 (=C-OH), δ 74.12, 64.73,(-CH-CH-CH_2), δ 25.70, 25.30 (-CH_3) ppm ; Mass (FAB) :216 (M^+), .

Synthesis of Binary Complex:

The general procedure for the synthesis of M (III) Ligand complexes: La(III), Ce(III), Nd(III), Gd(III) binary metal complexes with 5, 6-O-isopropylidene-L-ascorbic acid. The binary metal complexes were synthesized by mixing 10 ml solution of metal salts (0.01 mol) with 10 ml of L-ascorbic acid (0.01 mol) in

hot ethanol by keeping the metal-Ligand ratio (1:2 v/v). The mixture was refluxed for about 4 to 6 hours on a water bath with continuous stirring. The pH of the solution was adjusting about 5 to 6 by adding an acidic buffer solution in ethanol. The volume of the solution was reduced to half. The solid coloured products thus obtained were filtered, washed with distilled water and cold ethanol and then dried in vacuum over anhydrous calcium chloride a dedicator.

Metal Complexes **3(a-d)** was synthesized in the similar manner using compound 1 and various selected Metal Chlorides. Characterization data are presented in **Table-1 to 5** respectively**.**

Metal complexes were tested for in vitro antibacterial activity against some bacterial strains using spot on lawn on Muller Hinton Agar (MHA). Four test pathogenic bacterial strains, viz., *Bacillus cereus* (MTCC 1272), where MTCC—Microbial type culture collections, *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 739), and *Staphylococcus aureus* (MTCC 1144), *Klebsiella pneumonia* (MTCC 1377) bacteria and *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi were considered for determination of minimum inhibitory concentration (MIC) of selected complexes. The test pathogens were sub-cultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37°C (24 h). Working cultures were stored at 4°C in Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at –70°C in BHI broth containing 15% (v/v) glycerol (Qualigens, Mumbai, India). The organism was grown overnight in 10 ml of BHI broth, centrifuged at 5.000 g for 10 min, and the pellet was suspended in 10 ml of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD-545) was adjusted to obtain 108 cfu/ml followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

Determination of MIC. MIC is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. Antimicrobial activity of the compounds was evaluated using spot_on_lawn on MHA (HiMedia, Mumbai, India). Soft agar was prepared by adding 0.75% agar in Muller Hinton Broth (HiMedia, Mumbai, India). Soft agar was inoculated with 1% of 108 Cfu/ml of the test pathogen, and 10 ml were overlaid on MHA. From 1000X solution of compound (1 mg/ml of DMSO) 1, 2, 4, 8, 16, 32, 64, and 128X solutions were prepared. Dilutions of standard antibiotics (streptomycin and griseofulvin) were also prepared in the same manner: 5 µl of the appropriate dilution was spotted on the soft agar and incubated at 37°C for 24 h. Zones of inhibition of compounds were considered after subtraction of the inhibition zone of DMSO. Negative control (with no compound) was also observed.

RESULT AND DISCUSSION

Complexes of La(III), Ce(III), Nd(III) and Gd(III) with mixed Ligand of 2-aminopurene and L-ascorbic acid derivative were prepared in aqueous ethanol. All complexes were characterized on the basis of elemental analysis, molar conductance, magnetic susceptibility measurements, IR, UV-vis, NMR, ESR and Mass spectral studies. IR spectra of these complexes reveal that the complex formation occurred through both nitrogen and oxygen atoms. On the basis of electronic spectral data and magnetic susceptibility measurement octahedral geometry has been proposed for the complexes. The ESR spectral data of the M (III) complexes showed that the metal-ligand bonds have considerable covalent character. The electrochemical behaviour of mixed Ligand M(III) complexes was studied which showed that complexes of IPAA appear at more positive potential as compared to those for corresponding AA complexes. Complexes were characterized using UV- Visible, IR, NMR, Mass spectroscopic methods as well as magnetic susceptibility and conductivity measurements. In addition biological activity of the synthesised metal complexes against *Salmonella typhi*, *Escherichia coli* ,*Staphylococcus aureus and Klebsiella pneumonia* bacteria and *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi respectively were examined *in-vitro*. Some of the metal complex displayed pounced biological activity.

Antimicrobial Studies

The antibacterial tests were prepared and characterized according to the standard method. All strains were isolated from laboratory of microbiology. The identity of all the strains was confirmed. A bacterial suspension was prepared and added to the Nutrient Agar, the fungi added to surrounded Agar, All this before medium solidification and under aseptic condition. Then different concentration of complexes were placed on the surface of the culture, The bacteria incubated at 37 °C for 24 h, The fungi incubated at 28 °C for 72 h.

Bacterial & Fungi Cultures

Plate cultures of nutrient agar medium were used for culture of bacteria. the medium was prepared by dissolving 14 g, and culture of fungi's 32.5 g of powder in 500 mL of sterile distilled water, Then the medium was sterilized by autoclaving at 121 °C for15 min.

Table -1: Physical parameters of $[M(IPAA)_2(H_2O)_2]^-$

Complexes	Colour	M.P. in ^0C	Elemental Analysis Calculated (Found)		
			C%	H%	M(III) %
$[La(IPAA)_2(H_2O)_2]^-$	White	256-260	35.82 (35.76)	3.98 (3.91)	23.19 (23.26)
$[Ce(IPAA)_2(H_2O)_2]^-$	Red	242-244	35.75 (35.69)	3.97 (3.94)	23.19 (23.11)
$[Nd(IPAA)_2(H_2O)_2]^-$	Brown	248-250	35.50 (35.43)	3.94 (3.91)	23.71 (23.68)
$[Gd(IPAA)_2(H_2O)_2]^-$	Pink	236-238	34.77 (34.69)	3.86 (3.84)	25.30 (25.22)

Table -2: Spectral analysis of Table -2: Spectral analysis of $[La(IPAA)_2(H_2O)_2]^-$

Code Of Complex : 3a	**Molecular Formula:** $[La(IPAA)_2(H_2O)_2]^-$ **Chemical Formula:** $[C_{18}H_{24}O_{14}La]^-$ **Chemical Name:** Di-aqua-bis-(5,6-o-isopropylidine-L-ascorbate)-lanthanate (III) ion
UV-Visible (λ max (in DMSO) in nm**)**	254
IR (in cm^-)	3382,3364, 841 (H_2O), 2996 (-C-H), 1754(C=O), 1662(C=C) 1436 (C-H), 1141, 898 (C-O), 3381,3362, 841 (H_2O), 457 (M-O), 1388 (=C-O).
^{1}HNMR	δ 2.4 – 2.9 ppm (m, H_4, H_5, H_6), δ 3.1 ppm (s, for the -OH), δ 4.6 ppm (s, for H_2O).
^{13}CNMR	δ 171.11 (-C=O), δ 152.26, 108.89 (=C-OH), δ 74.12, 64.73,(-CH-CH-CH_2), δ 25.70, 25.30 (-CH_3) ppm
Mass	**602(M^+)**

Table -3: Spectral analysis of Table -2: Spectral analysis of $[Ce(IPAA)_2(H_2O)_2]^-$

Code Of Complex : 3b	**Molecular Formula:** $[Ce(IPAA)_2(H_2O)_2]^-$ **Chemical Formula:** $[C_{18}H_{24}O_{14}Ce]^-$ **Chemical Name:** Di-aqua-bis-(5,6-o-isopropylidine-L-ascorbate)-Cerate(III)-ion
UV-Visible (λ max (in DMSO) in nm**)**	256
IR (in cm^-)	3382,3364, 841 (H_2O), 2996 (-C-H), 1754(C=O), 1662(C=C) 1436 (C-H), 1141, 898 (C-O), 3381,3362, 841 (H_2O), 457 (M-O), 1388 (=C-O).
^{1}HNMR	δ 2.4 – 2.9 ppm (m, H_4, H_5, H_6), δ 3.1 ppm (s, for the -OH), δ 4.6 ppm (s, for H_2O)
^{13}CNMR	δ 171.11 (-C=O), δ 152.26, 108.89 (=C-OH), δ 74.12, 64.73,(-CH-CH-CH_2), δ 25.70, 25.30 (-CH_3) ppm
Mass	**608(M^+)**

Table -4: Spectrum analysis of $[Nd(IPAA)_2(H_2O)_2]^-$

Code Of Complex : 3c	**Molecular Formula:** $[Nd(IPAA)_2(H_2O)_2]^-$ **Chemical Formula:** $[C_{18}H_{24}O_{14}Nd]^-$ **Chemical Name:** Di-aqua-bis-(5,6-o-isopropylidine-L-ascorbate)-Neodymate(III)-ion
UV-Visible (λ max (in DMSO) in nm**)**	254
IR (in cm^-)	2996 (-C-H), 1754(C=O), 1662(C=C) 1436 (C-H), 1141, 898 (C-O), 3381,3362, 841 (H_2O), 457 (M-O), 1388 (=C-O)
^{1}HNMR	δ 2.4 – 2.9 ppm (m, H_4, H_5, H_6), δ 3.1 ppm (s, for the -OH), δ 4.6 ppm (s, for H_2O)
^{13}CNMR	δ 171.11 (-C=O), δ 152.26, 108.89 (=C-OH), δ 74.12, 64.73,(-CH-CH-CH_2), δ 25.70, 25.30 (-CH_3) ppm
Mass	**608(M^+)**

Table -5: Spectrum analysis of $[Gd(IPAA)_2(H_2O)_2]^-$

Code Of Complex :3d	**Molecular Formula:** $[Gd(IPAA)_2(H_2O)_2]^-$ **Chemical Formula:** $[C_{18}H_{24}O_{14}Gd]^-$ **Chemical Name:** Di-aqua-bis-(5,6-o-isopropylidine-L-ascorbate)-Gadolinate(III)-ion
UV-Visible (λ max (in DMSO) in nm**)**	254
IR (in cm^-)	2996 (-C-H), 1754(C=O), 1662(C=C) 1436 (C-H), 1141, 898 (C-O), 3381,3362, 841 (H_2O), 457 (M-O), 1388 (=C-O).
^{1}HNMR	δ 2.4 – 2.9 ppm (m, H_4, H_5, H_6), δ 3.1 ppm (s, for the -OH), δ 4.6 ppm (s, for H_2O).
^{13}CNMR	δ 171.11 (-C=O), δ 152.26, 108.89 (=C-OH), δ 74.12, 64.73,(-CH-CH-CH_2), δ 25.70, 25.30 ($-CH_3$) ppm
Mass	**621(M^+).**

General Properties

The colour of ligand was change from white colour of the free ligand to several different colours according to the type metal ions, this change mainly due to the effect the linkage between the ligand and the different electrons in 5f orbital's, where the attracting electrons between the ligand and the metal in f orbital's where the high and the less in energy, the magnetic frequency beam is proportion to the different in energy between the two states energy in atom. Some electrons rise into energy high level. The conductivity of the complexes depended mainly on the free electrons which non conjugation in the last orbital's, where the conductivity become less when conjugating occur between the metal and the ligand, this mean these electrons are bounded.

Molar Absorbance

A series of metal–ligand aqueous solutions were prepared with different [L]/[M] ratios. The absorptions of these solutions were measured using UV spectrophotometer at λ max of the expected complex MLx. It was observed that the absorption increase linearly as the ligand concentration increase, because of the formation of the complex, until the solution reaches the actual molar ratio of the investigated complex. At this point, all of the added materials were completely reacted, and the absorbance observed is the absorption of the investigated complex alone. After this point, the excess amount of the added ligand causes an inflection in the straight line, that is because the ligand has an absorptance value differ from that of the complex at λ max of the complex. [L]/[M] ratio corresponding to the inflection point in (ABS—[L]/[M] curve) indicates to the actual [L]/[M] ratio of the investigated complex, Referring to the Figure 5, The data showed that of the studied complexes in this investigation are able to be stable in the form ML, and ML_2 were geometric isomerism.

Metal Complexes **2(a-d)** was synthesized in the similar manner using compound 1 and various selected Metal Chlorides.

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Table -6: Anti-bacterial Activity of Synthesised Compounds

Compound code	*Salmonella typhi*		*Bucillus Subsniss*		*Staphylococcus aureus*		*Klebsiella pneumonia*	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
3a	+	+	++	++	+	++	+	++
3b	++	+++	++	+++	++	+++	++	+++
3c	++	+++	++	++	++	++	++	+++
3d	+	++	+	++	++	++	++	++
SM	+++	++++	+++	++++	+++	++++	+++	++++

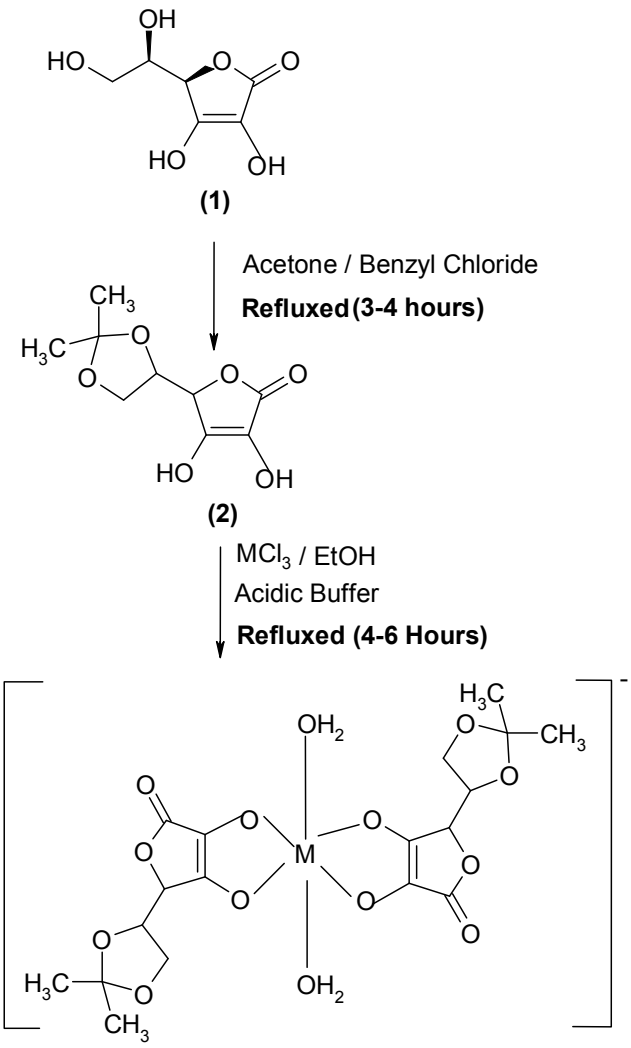
SM = streptomycin inhibition diameter in mm
Highly active = +++ (inhibition zoes > 15)
moderatively active= ++ (inhibition zone 10-15)
slightly active = + (inhibition 10)
inactive inhibition zone -6) for bacteria

Table 7: Antifungal activity of the synthesized compound derivatives

Compound code	Aspergillus niger		*Aspergillus flavus*		*Fusarium oxisporium*		*Trichoderma viride*	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
3a	+	++	+	++	+	++	++	+++
3b	++	+++	+	++	+	++	++	+++
3c	++	++	+	++	+	++	+	+++
3d	+	++	+	++	+	++	+	+++
GF	+++	++++	+++	+++	++++	++++	+++	++++

Std- Griseofulvin inhibition diameter in mm
Highly active = ++++ (inhibition zone > 20-25)
More Active = +++ (inhibition zone > 12-20)
Moderatively active= ++ (inhibition zone 6-12)
slightly active = + (inhibition Zone less than 6)
Inactive inhibition zone - for Fungi

SCHEME 1



(3 a-d)

M = La^{3+} , Ce^{3+}, Nd^{3+} and Gd^{3+}

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