

Isolation, Characterisation and Antimicrobial Activity Studies of the Mixed Ligand Complexes of Cd(II) with 8-Hydroxyquinoline and Various Salicylic Acids

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A series of mixed ligand complexes of Cd(II) with the general formula CdL_1L_2 (where L_1 =8-hydroxyquinoline; L_2 =salicylic acid, 5-chloro, 3,5-dibromo, 3,5-diiodo, 3,5-dinitro, acetyl salicylic acids) are isolated in pure state and characterised by elemental analysis and infrared data. The low molar conductance of these complexes in DMF indicates non-electrolyte nature of these complexes. The antimicrobial activity of these complexes against 14 pathogenic bacteria and 5 fungi is studied under various conditions. Even though one 8-hydroxyquinoline molecule in the binary cadmium-oxinate is replaced by a low cost fungicide like salicylic acid in the formation of mixed ligand complexes, these mixed ligand complexes are found to possess fairly higher antimicrobial activity against several organisms than the binary cadmium-oxinate. The lipophilic tendency of these complexes is determined and its influence on the antimicrobial activity is critically examined. The probable mechanism of the toxic action of these complexes against various organisms is discussed.

THOUGH 8-hydroxyquinoline (oxine) and its divalent metal chelates are known to possess fungicidal and bactericidal properties, the high cost of 8-hydroxyquinoline limits their applicability. It has been observed by the present authors in the use of oxine chelates as antimicrobial agents that the cost factor can be minimised by replacing one oxine molecule in the divalent metal oxinates of Cu(II)¹, Hg(II)² and Cd(II) with a low cost fungicide like salicylic or substituted salicylic acids as heteroligands. A series of mixed ligand complexes of Cd(II) with oxine and salicylic acids are prepared and their antimicrobial activity is studied under various conditions. The relation between their antimicrobial activity and physico-chemical properties is examined critically to understand the mechanism of action. The results of these investigations are presented in this paper.

Experimental

All the chemicals used are of analytical grade (B.D.H.) reagents.

General method for the preparation of the complexes: Equimolar solutions of salicylic acids (0.2 M), Cd(II) acetate (0.2 M) and 8-hydroxyquinoline (0.2 M) in 80% aqueous methanol are mixed. After stirring for 0.5 hr, the product is removed by filtration, washed with several volumes of water, digested in acetone and filtered. The complexes are dried at 70° for 12 hr. Metal and nitrogen are estimated by standard methods.

Physical measurements: Infrared spectra are recorded on a Perkin-Elmer model 577 spectrophotometer (4000 cm^{-1} to 200 cm^{-1}) by KBr disc technique. The conductivity of the complexes (10^{-3} M) is measured at 27° by Systronics conductivity bridge 305 in dimethyl sulphoxide (DMSO).

Antimicrobial activity: The antimicrobial activity of the compounds in DMSO is examined *in vitro* by serial dilution method³ against various bacteria and by paper disc method⁴ against fungi. All the stock cultures are obtained from the Department of Microbiology, All India Institute of Medical Sciences, New Delhi. Peptone water and saline water are used for making the inoculum for bacteria (18 hr cultures) and fungi, respectively. Nutrient broth and Saborounds dextrose agar (M/s Hindustan Dehydrated Media, Bombay) are used as test media for bacteria and fungi, respectively. The minimum inhibition concentration (MIC; $\mu\text{g/ml}$) of the compounds against bacteria and the average zone of inhibition (mm) of the compounds at 1000 $\mu\text{g/ml}$ against fungi are given in the Tables 2 and 3, respectively. All the tests are carried out in duplicate.

Results and Discussion

The elemental analysis of these complexes (Table 1) show that Cd(II) forms mixed ligand complexes of general formula CdL_1L_2 (where L_1 =8-hydroxyquinoline and L_2 =salicylic or substituted salicylic acids). The low molar conductance

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TABLE 1—ANALYTICAL, CONDUCTOMETRIC AND IR DATA OF THE Cd-oxine-salicylate mixed ligand complexes

Sl. No.	Compounds	Colour	Decomp. temp. °C	% metal		% nitrogen		Δ mhos cm^{-2}	IR frequencies in mixed ligand complexes (cm^{-1})				
				cal	obs	cal	obs		Oxine		Salicylic acids		
									$\nu(\text{M}-\text{O})$	$\nu(\text{C}-\text{O})$	$\nu(\text{M}-\text{N})$	$\nu_{\text{asy}}(\text{O}-\text{C}-\text{O})$	$\nu_{\text{sy}}(\text{O}-\text{C}-\text{O})$
1.	Cd(OX)(SA)	Greenish yellow	>300	28.55	28.50	3.56	3.52	8.1	380 b	1110 s	500 s	1570 b	1420 s
2.	Cd(OX)(Cl-SA)	Greenish yellow	>300	26.25	26.28	3.27	3.25	8.6	390 b	1110 s	500 s	1570 b	1420 s
3.	Cd(OX)(2Br-SA)	Greenish yellow	>300	20.38	20.36	2.54	2.55	8.4	380 b	1110 s	500 s	1570 s	1420 s
4.	Cd(OX)(2I-SA)	Greenish yellow	292	17.57	17.58	2.18	2.14	8.3	380 b	1110 s	500 s	1570 s	1420 s
5.	Cd(OX)(2NO ₂ -SA)	Golden yellow	298	23.24	23.21	8.69	8.66	8.2	390 b	1110 s	490 s	1570 s	1420 s
6.	Cd(OX)(Ace-SA)	Greenish yellow	>300	25.79	25.81	3.27	3.28	8.4	380 b	1110 s	490 s	1570 s	1420 s

OX=8-hydroxyquinoline; SA=salicylic acid; Cl-SA=5-chloro salicylic acid; 2Br-SA=3,5-dibromo salicylic acid; 2NO₂-SA=3,5-dinitro salicylic acid; 2I-SA=3,5-diiodo salicylic acid; Ace-SA=acetyl salicylic acid; s=sharp; b=broad.

TABLE 2—ANTIBACTERIAL ACTIVITY MIC ($\mu\text{g}/\text{ml}$) OF THE CADMIUM COMPLEXES WITH 8-HYDROXYQUINOLINE AND SUBSTITUTED SALICYLIC ACIDS AT 37° AFTER 18 Hr IN NUTRIENT BROTH

Sl. No.	Compound	Gram-positive		Gram-negative													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1.	Cd(OX) ₂	12.5	6.2	12.5	25	50	12.5	50	12.5	100	25	12.5	12.5	12.5	>100		
2.	Cd(OX)(SA)	6.2	3.1	12.5	100	3.1	50	50	12.5	12.5	25	25	12.5	25	25		
3.	Cd(OX)(Cl-SA)	6.2	3.1	12.5	100	3.1	50	100	25	3.1	25	25	12.5	50	50		
4.	Cd(OX)(2Br-SA)	6.2	3.1	12.5	100	3.1	50	100	12.5	3.1	25	25	12.5	25	50		
5.	Cd(OX)(2I-SA)	12.5	6.2	12.5	100	6.2	50	100	25	25	25	25	25	50	50		
6.	Cd(OX)(2NO ₂ -SA)	6.2	3.1	6.2	100	3.1	50	100	12.5	6.2	25	25	25	50	50		
7.	Cd(OX)(Ace-SA)	12.5	3.1	25	100	6.2	50	100	25	50	50	25	12.5	25	25		

(1) *Staph. albus*; (2) *Staph. aureus*; (3) *Sch. schmitzi*; (4) *Pseudomonas pyocanes*; (5) *Shigella sonnei*; (6) *Klebsilla aerogenes*; (7) *Proteus morgani*; (8) *Shigella flexneri*; (9) *Vibrio cholerae*; (10) *Escherichia coli*; (11) *Salmonella typhi*; (12) *Salmonella paratyphi-A*; (13) *Salmonella paratyphi-B*; (14) *Shigella boydii*.

values of these complexes in DMSO indicate that they are of non-electrolyte type.

Infrared data: The asymmetric $\nu(\text{O}-\text{C}-\text{O})$ and the symmetric $\nu(\text{O}-\text{C}-\text{O})$, which appeared in all the salicylic acids around 1600 and 1440 cm^{-1} respectively are found to be shifted in the mixed complexes to lower values of 1570 and 1420 cm^{-1} , indicating metal carboxylate linkage^{5,6}. Charles *et al*⁷ reported that in several oxine complexes of metals, the $\nu(\text{C}-\text{O})$ is observed at 1120 cm^{-1} region but the position of the bands slightly varies with the metal. The $\nu(\text{C}-\text{O})$, which appeared in the free oxine molecule at 1090 cm^{-1} is found to be shifted in all the mixed complexes giving a strong absorption band at 1110 cm^{-1} indicating clearly the oxine coordination in the complex. In all the mixed ligand complexes, the observed band in the neighbourhood of 500 cm^{-1} may be identified as M-N stretching frequency⁸. A broad band at 380-400 cm^{-1} in all the complexes may be assigned to metal-oxygen stretching frequency⁸.

Antimicrobial activity: The toxic effect of the cadmium-oxine-salicylic or substituted salicylic acid mixed ligand complexes against various bacteria and fungi is found to be either equal or slightly greater than the cadmium-bis-8-hydroxyquinoline complex. Salicylic acid or substituted salicylic acids and their cadmium chelates are

found to have measurable activity against these bacteria and fungi only at relatively high concentrations (for bacteria >100 $\mu\text{g}/\text{ml}$, fungi >2000 $\mu\text{g}/\text{ml}$). This may be due to their higher water solubility. Albert *et al* explained the antimicrobial activity of copper(II)-bis-8-hydroxyquinoline by assuming that this complex first penetrates the cell and undergoes dissociation to a 1:1 copper-oxine complex and free oxine at the site of action. The 1:1 chelate will become the toxic entity by combining with and blocking metal binding sites on enzymes. Thus the 1:2 chelate due to its lipo solubility is necessary to take the toxic moiety i.e. the 1:1 charged complex to the site of action. The assumption was supported by the fact that antimicrobial activity of these complexes was reversed in the presence of excess of copper. This may be due to the inability of the ionically charged 1:1 chelate (which is produced in the presence of excess of metal) to penetrate the cell membrane. Block⁹ proposed that the natural chelates within the cell were poisoned by removing copper from Cu(II)-oxine, thereby freeing oxine which could then bind the metallic prosthetic groups of the enzymes. Zentmyer *et al*¹⁰ proposed a mechanism of detoxication of the 1:2 chelate by natural metabolites. The 1:2 chelate dissociates to the 1:1 chelate, thereby entering the aqueous phase of the cell. Histidine and cystine, which form more

stable complexes with copper than the 1 : 1 chelate of copper and 8-hydroxyquinoline, remove the copper and form lipid soluble chelates. Esposito and Fletcher¹¹ proposed that the activity of copper(II)-8-hydroxyquinoline was due to the 1 : 1 complex which could bind with an enzyme site involved in the biosynthesis of pteridines. This was based on the reversal of inhibition by several pteridines and precursors. It is also believed that a similar mechanism may be working well in explaining the toxic action of all other bivalent oxinates.

According to Overton's concept of cell permeability, the lipid membrane surrounding the cell favours the passage of lipid soluble materials through the membrane and lipo solubility is considered as one of the important factors that control the antimicrobial activity of any toxic agent. The partition of the toxic agent between oily alcohol or chloroform and pH 7.4 phosphate buffer system is considered as a good model to understand the lipophilic or lipophobic tendency¹². So we have determined the distribution of all these complexes between chloroform and pH 7.4 buffer and the results are given in Table 4. As expected, the cadmium-oxine salicylic acid or substituted salicylic acids

salicylic acids and cadmium-oxine-diiodosalicylic acid must have maximum activity. But no such relation is found to exist from their antimicrobial activity screening studies (Tables 2 and 3) which

TABLE 4—PERCENTAGE EXTRACTION OF METAL INTO CHLOROFORM AT pH 7.4

Sl. No.	Complex	% of cadmium extracted
1.	Cd(OX) ₂	45
2.	Cd(OX)(SA)	26
3.	Cd(Cl-SA)(OX)	37
4.	Cd(2Br-SA)(OX)	40
5.	Cd(2I-SA)(OX)	42
6.	Cd(NO ₂ -SA)(OX)	35
7.	Cd(Ace-SA)(OX)	35
8.	Cd(SA) ₂	7
9.	Cd(Cl-SA) ₂	3
10.	Cd(2Br-SA) ₂	9
11.	Cd(2I-SA) ₂	5
12.	Cd(2NO ₂ -SA) ₂	2
13.	Cd(Ace-SA) ₂	2

TABLE 3—ANTIFUNGAL ACTIVITY OF THE CADMIUM COMPLEXES WITH 8-HYDROXYQUINOLINE AND SALICYLIC ACIDS AT 1000 μg/ml IN SABOROUNDS DEXTROSE AGAR AFTER 48 Hr at 30°

Sl. No.	Compound	Zone of inhibition in mm at 1000 μg/ml				
		1	2	3	4	5
1.	Cd(OX) ₂	9	7	—	—	7
2.	Cd(OX)(SA)	—	7	—	—	9
3.	Cd(OX)(Cl-SA)	7	10	9	12	7
4.	Cd(OX)(2Br-SA)	9	—	—	8	9
5.	Cd(OX)(2I-SA)	—	8	—	9	9
6.	Cd(OX)(2NO ₂ -SA)	7	7	—	—	7
7.	Cd(OX)(Ace-SA)	—	8	—	7	7

Fungi : (1) *Penicillium spp.*, (2) *Asp. niger*, (3) *Tricophyton rubrum*, (4) *Asp. fumigatus*; (5) *Candida albicans*.

mixed complexes have lower partition coefficient in chloroform when compared to cadmium(II)-oxine complex. However, they have equal or slightly more toxic effect against various bacteria and fungi in comparison to cadmium(II)-bis-8-hydroxyquinoline complex. This indicates that in the mixed complexes not only the 1 : 1 cadmium oxine complex is having toxic activity but also the released salicylic acid may be playing an important role in the antimicrobial activity through a different mechanism. The salicylic acids or cadmium salicylate chelates, though possessing toxic effects due to their higher water solubility, cannot go to the site of action as much as the mixed complexes can penetrate. If in the mixed complexes also the 1 : 1 cadmium oxine is the only toxic moiety then the antimicrobial activity of the mixed complexes should increase with increasing pK₁ values of the

indicate that salicylic acids also play an important role in the toxic action. It is also believed that if the geometry and charge distribution around the molecule are incompatible with geometry and charge distribution around the peripheries of the pores of the fungal or bacterial cell wall, penetration through the wall by the toxic agent cannot take place and toxic reactions within the spore do not occur. This may be the one of the reasons for certain mixed ligand complexes showing less effective antimicrobial activity than the corresponding Cd(OX)₂ complex.

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