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In vitro antimicrobial-activity studies on the mixed ligand complexes of Hg(II) with 8-hydroxyquinoline and salicylic acids

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Abstract. A series of mixed ligand complexes of Hg(II) with the general formula Hg (OX) (SA) (where OX : 8-hydroxyquinoline, SA : salicylic, 5-chloro-, 3,5-dibromo, 3,5-diiodo, 3,5-dinitro, acetyl thiosalicylic acids) are isolated in pure state and characterised by elemental analysis and infrared data. The low molar conductance of the complexes in dimethylformamide indicates non-electrolyte nature. The antimicrobial activity of these complexes against various bacteria and fungi is studied which indicates that in several cases, the mixed ligand complexes possess fairly highly antimicrobial activity than the binary mercury-oxinate. The lipophilic tendency of these complexes and its influence on the antimicrobial activity is critically examined. A probable mechanism for the toxic action of these complexes against various organisms is discussed.

Keywords. Antimicrobial activity ; mixed ligand complexes of Hg(II) ; 8-hydroxyquinoline and salicylic acids.

1. Introduction

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. Albert *et al* (1953) explained the antimicrobial activity of copper-oxinate assuming that the *bis*-chelate due to its high liposolubility penetrates the cell, reaches the site of action and there it undergoes dissociation into 1 : 1 complex and free 8-hydroxyquinoline. The 1 : 1 charged complex thus formed will become the toxic entity by combining with and blocking the metal-binding sites on enzymes. The same mechanism may equally apply well for explaining the antimicrobial activity of all divalent metal oxinates.

It has been observed that the cost factor can be minimised by replacing one oxine molecule in the divalent metal oxinates with low cost fungicides like salicylic acid or substituted salicylic acids. We have taken up a programme to isolate

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and study the antimicrobial activity and physico-chemical properties of a series of mixed ligand complexes of divalent metals with oxine and salicylic acids as hetero ligands. The results of our study on the mixed ligand complexes of Hg(II) with 8-hydroxyquinoline and salicylic acids are presented in this paper.

2. Experimental

All the chemicals used are analytical grade (BDH) reagents.

2.1. *General method for the preparation of the complex*

Equimolar solutions of salicylic acid or substituted salicylic acids (0·2 M), 8-hydroxyquinoline (0·2M) and Hg(II) acetate (0·2 M) in 80% aqueous methanol are mixed. After stirring for half an hour, the product is removed by filtration, washed with several volumes of water and boiled in acetone and filtered. The complexes are dried at 70° C for 12 hr. Metal and nitrogen are estimated by standard methods.

2.2. *Physical measurements*

Infrared spectra are recorded by using Perkin Elmer model 577 spectrophotometer (4000 cm^{-1} to 200 cm^{-1}) by KBr disc technique. The conductivity of the complexes in DMF (10^{-3} M) is measured at 27° C by systronics conductivity bridge 305.

2.3. *Antimicrobial activity*

The antimicrobial activity of the compounds in dimethyl formamide (DMF) are examined *in vitro* by serial dilution method (Schaub *et al* 1958) against various bacteria and by paper disc method (Jasper *et al* 1958) against fungi. All the stock cultures were supplied by the Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India. Peptone water and saline water is used for making the inoculum for bacteria (18 hr culture) and fungi respectively. Nutrient broth and Saboround's dextrose agar (M/s. Hindustan Dehydrated Media, Bombay) are used as test media for bacteria and fungi respectively. The minimum inhibition concentration (MIC μg/ml) of the compounds against bacteria and average zone of inhibition (mm) of the compounds at 1000 μg/ml against fungi is given in tables 2 and 3. All the tests are carried out in duplicate.

3. Results and discussion

With $5d^{10}$ configuration Hg(II) forms tetrahedral complexes using $6s\,6p^3$ hybrid orbitals for bonding leaving a completely non-bonding shell ($d_\gamma^4\, d_\epsilon^6$) which can cause least perturbation to preferred stereochemistry. The elemental analyses of these complexes (table 1) show that Hg(II) forms mixed ligand complexes which can be represented as shown below (figure 1).

Table 1. Analytical, conductometric and IR data of the mercury complexes with 8-hydroxyquinoline and substituted salicylic acids.

Sl. No.	Compound	Decomp. temp. °C	Colour	Found (calculated)%		Molar conductance (in Mho-cm²/g)	IR freq encies (cm^{-1}) in mixed liand complexes				
							Oxine			Salicylic acids	
				Metal	Nitrogen		ν (C–O)	ν (M–O)	ν (M–N)	ν (O–C–O) asy.	ν (O–C–O) sy.
1.	Hg (OX) (SA)	193	yellow	41·30 (41·63)	2·86 (2·91)	3·0	1110 (b)	390 (w)	540 (b)	1580 (b)	1460 (s)
2.	Hg (OX) (Cl–SA)	192	yellow	38·62 (38·85)	2·63 (2·71)	2·4	1110 (b)	390 (w)	540 (w)	1580 (b)	1470 (b)
3.	Hg (OX) (2Br–SA)	232	yellow	31·20 (31·36)	2·10 (2·19)	4·0	1110 (s)	390 (w)	560 (b)	1570 (w)	1480 (s)
4.	Hg (OX) (2I–SA)	190	orange yellow	27·01 (27·36)	1·82 (1·91)	3·7	1110 (s)	390 (w)	550 (w)	1580 (b)	1460 (s)
5.	Hg (OX) ($2NO_2$–SA)	198	yellow	34·93 (35·00)	7·24 (7·34)	3·2	1110 (s)	390 (w)	540 (w)	1580 (s)	1460 (s)
6.	Hg (OX) (Ace–SA)	201	yellow	38·18 (38·29)	2·58 (2·67)	4·5	1110 (s)	390 (w)	540 (w)	1580 (s)	1460 (s)
7.	Hg (OX) (Thio–SA)	205	white	41·54 (41·63)	2·83 (2·93)	2·1	1110 (s)	400 (w)	540 (b)	1580 (s)	1460 (s)

(OX) : 8-hydroxyquinoline; (SA) : salicylic acid; (Cl–SA) : 5-chlorosalicylic acid ; (2Br–SA) : 3,5-dibromosalicylic acid; ($2NO_2$–SA) : 3,5-dinitrosalicylic acid ; (2I–SA) : 3,5-diiodosalicylic acid ; (Ace–SA) : Acetyl salicylic acid ; (Thio-SA) : thiosalicylic acid : (s) : sharp ; (w) : weak ; (b) : broad.

Table 2. Antibacterial activity MIC (μg/ml) of mercury complexes with 8-hydroxyquinoline and substituted salicylic acids at 37° C after 18 hours in nutrient broth.

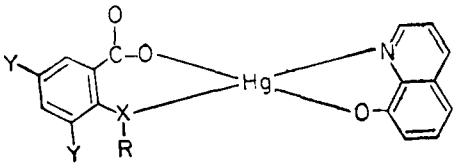
Sl. No.	Compound	Gram-positive		Gram-negative							
		1	2	3	4	5	6	7	8	9	10
1.	$Hg(OX)_2$	3·1	50	25	50	50	50	25	100	12·5	25
2.	Hg (OX) (SA)	6·2	50	25	50	50	50	25	50	25	25
3.	Hg (OX) (Cl-SA)	12·5	50	3·1	12·5	6·2	100	12·5	100	12·5	50
4.	Hg (OX) (2Br-SA)	50	12·5	12·5	12·5	25	100	25	50	25	25
5.	Hg (OX) (2I-SA)	100	50	100	100	100	>100	>100	>100	>100	>100
6.	Hg (OX) ($2NO_2$-SA)	100	100	25	12·5	25	100	25	100	25	50
7.	Hg (OX) (Ace SA)	6·2	12·5	12·5	3·1	6·2	25	12·5	50	12·5	12·5
8.	Hg (OX) (Thio-SA)	50	12·5	6·2	6·2	12·5	100	12·5	100	6·2	6·2

(1) *Staphylococcus albus*, (2) *Staphylococcus aureus*, (3) *Schigella schmitzi*, (4) *Pseudomonas pyogenes*, (5) *Shigella sonnei*, (6) *Klebsilla aerogenes*, (7) *Shigella flexneri*, (8) *Vibriocholerae*, (9) *Salmonella typhi*, (10) *Salmonella paratyphi*-B.

Table 3. Antifungal activity of the mercury complexes with 8-hydroquinoline and substituted salicylic acids at 1000 μg/ml after 48 hours at 30°C.

Sl. No.	Compound	Zone of inhibitions at 1000 μg/ml in mm				
	Fungi	1	2	3	4	5
1.	$Hg(OX)_2$	10	9	9	10	8
2.	Hg (OX) (SA)	9	8	10	10	12
3.	Hg (OX) (Cl-SA)	8	10	9	9	10
4.	Hg (OX) (2Br-SA)	7	12	..	9	9
5.	Hg (OX) (2I-SA)	8	7	10	8	8
6.	Hg (OX) ($2NO_2$-SA)	8	9	8	8	12
7.	Hg (OX) (Ace-SA)	8	..	..	9	11
8.	Hg (OX) (Thio SA)	..	..	..	..	7

(1) *Penicillium* spp., (2) *Aspergillus niger*, (3) *Trychophyton rubrum*, (4) *Aspergillus fumigatus*, (5) *Candida albicans*.



Y = Cl, Br, NO_2, I, H
X = O or S
R = H or $COCH_3$

The low molar conductance values of these complexes in DMF indicate that they are of non-electrolyte type.

3·1. *IR data*

The important absorption peaks of the IR spectra of these complexes agree with their structure. In all the mixed ligand complexes the symmetric and asymmetric vibrations of (O–C–O) group (Bellamy 1956) of salicylic acids are observed at $\sim 1420\ cm^{-1}$ and $\sim 1570\ cm^{-1}$ respectively, while the carbonyl stretching frequency which appeared in the free salicylic acids between 1650–1670 cm^{-1} disappeared. This clearly indicates metal–carboxylate linkage. Charles *et al* (1956) reported that in several 8-hydroxyquinoline complexes of divalent metals, the $\nu_{(c-o)}$ appeared at $\sim 1120\ cm^{-1}$ region and the position of the band slightly varies with the metal. The $\nu_{(c-o)}$ 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 $1110\ cm^{-1}$ which clearly indicates the coordination of 8-hydroxyquinoline in the complexes. In all the mixed ligand complexes, the observed sharp peaks between ~ 540–$560\ cm^{-1}$ and ~ 340–$400\ cm^{-1}$ may be assigned to the M–N and M–O stretching frequencies respectively (Nakamato 1970).

3·2. *Antimicrobial activity*

In many cases the toxic effect of the mercury-oxine-salicylic or substituted salicylic acid mixed ligand complexes against various bacteria and fungi is found to be either equal or slightly greater when compared to the *bis* (8-hydroxyquinolinato) mercury(II) complex. Salicylic acid or substituted salicylic acids and their mercury chelates are found to have measurable activity against these bacteria and fungi at relatively very high concentrations (for bacteria $> 100\ \mu g/ml$, fungi $> 2000\ \mu g/ml$). This may be due to their higher water solubility.

In explaining the antimicrobial activity of *bis* (8-hydroxyquinolinato) copper(II), Albert *et al* (1953) believed that the 1 : 2 chelate due to its liposolubility is necessary to transport the toxic moiety, i.e., 1 : 1 chelate 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. Block (1955) proposed that the natural chelators within the cell were poisoned by removing copper from Cu(II)-oxine,

thereby releasing oxine which could then bind the metallic prosthetic groups from enzymes. Zentmyer *et al* (1960) 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 cystene 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 Fletcher (1961) proposed that the activity of copper(II)-8-hydroxyquinoline was due to the 1 : 1 complex which could bind with an enzyme site that was involved in the biosynthesis of pteridines. This was based on the reversal of inhibition by several pteridines and precursors. It was 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 lipoid membrane surrounding the cell favours the passage through that membrane of lipid-soluble materials and liposolubility is considered as one of the important factors which control the antimicrobial activity of any toxic agent. The partition of the toxic agent between olyl alcohol or chloroform and 7·4 pH phosphate buffer (pH of the biological medium) system is considered as a good model to understand the lipophobic or lipophilic tendency (Dweyer and Mellor 1964). So we have determined distribution of all these complexes in between chloroform and 7·4 pH buffer and the results are given in table 4. As expected the mercury-oxine-salicylic or substituted salicylic acid mixed complexes have lower partition coefficient in chloroform when compared to the binary mercury oxinate. However, in

Table 4. Percentage of extraction of metal into chloroform at 7·4 pH.

Sl. No.	Complex	% of mercury extracted into chloroform
1.	$Hg(OX)_2$	80
2.	Hg (SA) (OX)	23
3.	Hg (Cl-SA) (OX)	28
4.	Hg (2Br-SA) (OX)	76
5.	Hg (2I-SA) (OX)	38
6.	Hg ($2NO_2$-SA) (OX)	34
7.	Hg (Ace-SA) (OX)	44
8.	Hg (Thio-SA) (OX)	62

The extraction of mercury(II) with various salicylic acids into chloroform are found to be less than 20% at 7·4 pH.

many cases the mixed complexes have equal or slightly more toxic effect against various bacteria and fungi in comparison with binary complex. This indicates that in the mixed complexes not only the 1 : 1 mercury-oxine complex is acting as toxic agent but also the released salicylic acid may be playing an important role in the antimicrobial activity through a different mechanism. The salicylic acids or mercury salicylate chelates, though possess toxic effect, 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 mercury-oxine is the only toxic moiety then the antimicrobial activity of the mixed complexes should increase with increasing pk_1 values of the salicylic acids and mercury-oxine-diiodo-salicylic acid must have maximum activity. But no such relation is found to exist from their antimicrobial activity screening studies (tables 2 and 3). It is also believed that if the geometry and charge distribution around the molecules are incompatible with geometry and charge distribution around 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 one of the reasons for certain mixed ligand complexes showing less effective antimicrobial activity than the corresponding $Hg(OX_2)$ complex.

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