Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 92, No. 3, June 1983, pp 271-278.

Copolymers and their polychelates derived from hydroxy benzoic (*o*- and *p*-) acids as antifungal and antibacterial agents

M M PATEL*, R MANAVALAN and K PRABHAKARAN
Department of Chemistry, Sardar Patel University,
Vallabh Vidyanagar 388 120, India

MS received 13 September 1982; revised 29 January 1983

Abstract. A series of complexes of salicylic acid-thiourea-trioxane polymer and *p*-hydroxybenzoic acid-thiourea-trioxane polymer respectively were isolated and characterised by elemental analysis and infrared data. The antimicrobial activity of these complexes against various bacteria and fungi is studied, which indicates that in several cases, the complexes possess fairly highly anti-microbial activity. A probable mechanism for the toxic action of these complexes against various organisms is discussed. IR spectra of complexes are also discussed.

Keywords. Antimicrobial activity; metal complexes; salicylic acid; *p*-hydroxybenzoic acid; *Penicillum islandicum; Xanthomonas citri.*

1. Introduction

Metal complexes of sulphur donor ligands have received great attention owing to their versatile use as antifungal and antibacterial agents (Satpathy *et al* 1981). It has been revealed that compounds containing both C = O and C = S groups possess fairly good fungicidal activity (Horsfall and Rich 1951). In spite of the fact that a number of sulphur donor ligands have been studied (Akbar Ali and Livingstone 1974) for the synthesis of metal complexes it appears that salicylic acid-thiourea-trioxane (STT) copolymer and *p*-hydroxybenzoic acid-thiourea-trioxane (PHBTT) copolymer having both C = O and C = S groups have not been used. In view of the biological importance of sulphur donor ligands we have attempted to prepare a few metal complexes with both STT and PHBTT polymeric ligands and to evaluate their antifungal and antibacterial activities. The results of our study on the metal complexes of STT and PHBTT are presented in this paper.

2. Experimental

All the chemicals used were of AnalaR or chemically pure grade.

2.1. *Preparation of STT polymeric ligand*

A mixture of salicylic acid (0.1 M), thiourea (0.1 M) and trioxane (0.2 M) in the presence of 2 M HCl was heated at 130 ± 2° C for 4 hr in an oil bath. The

*To whom all correspondence should be made.

separated yellow coloured polymer was washed with hot water and finaly with ether to remove excess of acid monomer. The polymer was purified by dissolving in 10% NaOH and reprecipitating by dropwise addition of 1 : 1 (V/V) HCl/water. The copolymer thus obtained was washed with boiling water and dried (Patel and Manavalan 1982).

2.2. *Preparation of PHBTT polymeric ligand*

PHBTT copolymer was prepared as described above by taking *p*-hydroxy-benzoic acid instead of salicylic acid.

2.3 *General method for preparing complexes*

The metal nitrate solution in dimethyl formamide (DMF) was mixed with the solution of STT or PHBTT in DMF. To the resulting clear solution, a concentrated solution of sodium acetate in DMF was added, and the product which separated out was digested, filtered, washed with DMF, hot water and dried in an oven at 60° C. In the case of VO (II) chelate, vanadyl sulphate salt was used. The metal-to-ligand ratio was 1 : 1. Metal, nitrogen and sulphur were estimated by standard methods.

2.4 *Physical measurement*

IR spectra were recorded by Carl-Zeiss UR 10 spectrophotometer in KBr disc.

2.5 *Antimicrobial activity*

To test the fungicidal and bactericidal ability of the compounds at 1000 ppm concentration *in vitro*, various plant pathogenic organisms were used *viz. Penicillium islandicum, Botrydiplodia theobromae, Scytalidium lignicolm, Glyocladium roseum, Erwinia chrysanthemi* Pv. *chrysanthemi, Erwinia caratorora* var *caratorora, Erwinia caratorora* var *atroseptica, Xanthomonas vesicatoria, Xanthomonas citri* and *Corynebacterium sepotonicum.* The fungi were grown on potato dextrose agar medium (PDA) having the following composition (Horsfall 1945).

Potato 200 g; dextrose 20 g, Agar 20 g, water 1 litre (pH 5) and the bacteria were grown on nutrient agar medium (NA) having the following composition:

Beef extract 1 g, yeast extract 2 g, peptone (bacteriological) 5 g, sodium chloride 5 g, Agar 15 g, distilled water 1 litre (pH 7).

For the fungicidal and bactericidal activity of the compounds, 7 and 3 day old culture was used. PDA and NA were prepared with the compounds (1000 ppm) and autoclaved at 110° C for 15 min at 15 atm pressure. These media were poured into sterile petriplates and the organisms were inoculated after cooling. The percentage inhibition for fungi and bacteria was calculated after 3 days and 7 days respectively using the formula given below.

Table 1. Analytical and IR data of STT polymeric ligand and its polychelates.

Compound	Decomp. temp. °C.	Colour	Found (calculated) %			IR frequencies (cm^{-1})						MW of repeating unit (g/mol)
			Metal	Nitrogen	Sulphur	C=S	C=N	C=O	(M–N)	$-CH_2$	–OH stretching	
STT	240	Yellow	–	7.57 (11.77)	12.41 (13.44)	856(s)	1616(w)	1656(b)	–	2800–3300	2800–3200	238
Cu. STT H_2O	120	Light green	19.72 (20.01)	7.80 (8.81)	8.01 (9.54)	816(s)	1600(s)	1570(s)	530(w)	2850–3300	3200–3400	318
Ni. STT H_2O	160	Pale yellow	19.48 (18.77)	7.89 (8.95)	6.85 (9.68)	808(s)	1606(s)	1580(s)	542(b)	2900–3300	3200–3350	313
Co. SST H_2O	100	Light pink	16.93 (18.45)	8.15 (8.94)	8.92 (9.68)	810(w)	1605(w)	1550(s)	540(w)	2800–3000	3200–3000	313
Zn. STT H_2O	140	Grey	14.32 (17.46)	8.27 (8.76)	9.21 (10.86)	810(s)	1595(s)	1564(w)	536(b)	3000–3200	3250	319
Fe. STT NO_3	140	Violet	17.99 (15.78)	10.03 (13.56)	7.97 (9.04)	832(s)	1600(s)	1548(s)	546(w)	3100–3300	3200–3300	354
Cr. STT NO_3	120	Green	18.12 (15.84)	7.89 (12.1)	7.74 (9.87)	816(w)	1604(b)	1550(s)	530(w)	3100–3300	3200–3300	350
UO_2. STT	180	Rosy red	41.71 (39.36)	6.69 (8.41)	6.66 (8.41)	816(s)	1600(s)	1570(s)	548(w)	3050–3300	3200–3400	606
VO. STT	120	Green	18.56 (16.81)	8.95 (9.24)	9.04 (11.13)	808(s)	1590(s)	1564(w)	548(w)	3100–3300	3200	303

s: sharp; w: weak; b: broad.

Table 2. Analytical and IR data of PHBTT polymeric ligand and its polychelates.

Compound	Decomp. temp. °C	Colour	Found (calculated) %			IR frequencies (cm^{-1})						MW of repeating unit (g/mol)
			Metal	Nitrogen	Sulphur	C=S	C=N	C=O	(M–N)	$-CH_2$	–OH stretching	
PHBTT	245	Yellow	–	11.23 (11.76)	11.88 (12.41)	860(s)	1620(s)	1636(s)	–	2800 – 3300	2750 – 3200	238
Cu. PHBTT $2H_2O$	120	Light green	18.84 (18.93)	7.91 (8.34)	9.01 (9.53)	820(s)	1606(s)	1575(s)	524(s)	2860 – 3300	3100 – 3400	335
Ni. PHBTT $2H_2O$	165	Pale Yellow	16.92 (17.75)	8.09 (8.46)	9.12 (9.67)	824(s)	1600(s)	1588(s)	532(s)	2950 – 3300	3100 – 3450	331
Co. PHBTT $2H_2O$	125	Light pink	16.94 (17.81)	7.94 (8.46)	9.99 (9.66)	808(s)	1560(w)	1560(w)	542(w)	2900 – 3300	3000 – 3400	331
Zn. PHBTT $2H_2O$	150	Grey	18.65 (19.37)	8.87 (8.29)	8.98 (9.49)	832(b)	1612(s)	1570(s)	536(s)	2900 – 3200	3100 – 3300	337
Fe. PHBTT NO_3. H_2O	120	Violet	14.89 (15.01)	10.12 (11.29)	8.05 (8.61)	816(s)	1604(w)	1550(s)	536(s)	3000 – 3200	3150 – 3400	372
Cr. PHBTT NO_3. H_2O	120	Green	14.92 (14.12)	10.66 (11.41)	7.98 (8.69)	824(s)	1600(s)	(1590(s)	542(w)	2950 – 3300	3000 – 3350	368
UO_2. PHBTT H_2O	180	Rosy red	44.68 (45.45)	4.87 (5.34)	5.41 (6.11)	808(b)	1592(s)	1564(s)	542(b)	3100 – 3300	3200 – 3400	524
VO. PHBTT H_2O	125	Green	15.61 (15.87)	9.22 (8.72)	9.23 (9.97)	816(s)	1608(s)	1572(w)	548(w)	3000 – 3200	3200 – 3400	321

s: sharp; w: weak; b: broad.

Table 3. Antifungal activity of STT polymeric ligand and its complexes.

Compound	Zone of inhibitions at 1000 ppm				
	Fungi	1	2	3	4
STT	...	...	35.7	...	42.1
Cu. STT H_2O	...	...	...	...	...
Ni. STT H_2O	...	...	...	...	...
Co. STT H_2O	...	...	...	23.0	37.0
Zn. STT H_2O	...	...	...	...	...
Fe. STT NO_3	...	...	62.0	23.8	29.2
Cr. STT NO_3	...	...	19.8	17.6	11.9
UO_2 STT	...	...	43.0	...	...
VO STT	...	...	100.0	16.9	67.2

1. *Botrydiplodia theobromae*; 2. *Penicilliumislandicum*; 3. *Scytalidium lignicola*; 4. *Gliocladium roseum*

Table 4. Antifungal activity of PHBTT polymeric ligand and its complexes.

Compound	Zone of inhibitions at 1000 ppm				
	Fungi	1	2	3	4
PHBTT	...	100.0	83.7	100.0	...
Cu. PHBTT $2H_2O$	...	...	...	...	...
Ni PHBTT $2H_2O$	...	...	...	11.5	...
Co. PHBTT $2H_2O$	...	...	48.0	51.6	49.2
Zn. PHBTT $2H_2O$	...	...	...	...	...
Fe. PHBTT NO_3H_2O	...	...	...	...	...
Cr. PHBTT NO_3H_2O	...	...	58.0	...	...
UO_2. PHBTT H_2O	...	...	...	...	...
VO. PHBTT H_2O	...	...	100.0	43.4	32.7

1. *Botrydiplodia theobromae*; 2. *Penicillium islandicum*; 3. *Scytalidium lignicola*; 4. *Gliocladium roseum*

Table 5. Antibacterial activity of STT polymeric ligand and its complexes.

Compound	Gram negative					Gram positive
	1	2	3	4	5	
STT	100	100.0	100.0	100.0	100.0	100.0
Cu. STT H_2O	100	98.0	73.0	87.5	86.0	93.1
Ni. STT H_2O	42	93.8	92.0	83.2	87.2	92.0
Co. STT H_2O	53	91.0	97.3	93.8	77.9	94.8
Zn. STT H_2O	100	78.3	91.0	94.7	95.6	87.1
Fe. STT NO_3	100	82.8	90.0	96.3	97.8	91.3
Cr. STT NO_3	100	89.1	96.5	95.4	88.0	100.0
UO_2. STT	100	62.0	91.0	82.9	89.2	93.1
VO. STT	100	91.2	65.0	72.7	73.1	87.3

1. *Erwinia caratovora* var *caratovora*; 2. *Erwinia chrysanthemi* Pv *chrysanthemi*; 3. *Erwinia caratovora* var *atroseptica*; 4. *Xanthomonas citri*; 5. *Xanthomonas vesicatoria*; 6. *Corynebacterium sepodonicum.*

Table 6. Antibacterial activity of PHBTT polymeric ligand and its complexes.

Compound	Gram negative					Gram positive
	1	2	3	4	5	
PHBTT	100.0	100.0	100.0	100.0	100.0	100.0
Cu. PHBTT $2H_2O$	93.0	61.0	72.3	76.4	81.2	79.7
Ni. PHBTT $2H_2O$	100.0	78.0	77.8	77.0	79.0	78.1
Co. PHBTT $2H_2O$	85.3	98.9	92.8	91.3	94.0	96.3
Zn. PHBTT $2H_2O$	100.0	97.8	91.7	88.0	90.4	89.8
Fe. PHBTT NO_3H_2O	84.8	100.0	99.2	88.7	91.7	100.0
Cr. PHBTT NO_3H_2O	81.2	100.0	98.2	94.6	93.1	95.6
UO_2. PHBTT H_2O	59.0	98.6	97.3	97.0	96.8	94.8
VO. PHBTT H_2O	100.0	95.4	92.1	91.0	92.3	93.5

1. *Erwinia caratovora* var *caratovora*; 2. *Erwinia chrysanthemi* Pv *chrysanthemi*; 3. *Erwinia caratovora* var *atroseptica*; 4. *Xanthomonas citri*; 5. *Xanthomonas vesicatoria*; 6. *Corynebacterium sepodonicum.*

Percentage of inhibition = $[(X - Y)/X] \times 100$

where X = area of colony in control plate; Y = area of colony in test plate. The fungicidal and bactericidal activity displayed by various compounds is summarised in tables 3 – 6.

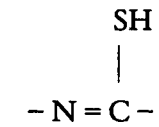
3. Results and discussion

Owing to the insolubility of metal chelates, none of the polychelates was recrystallized and the molecular weight of the repeating unit was estimated from analytical data as shown in tables 1 and 2.

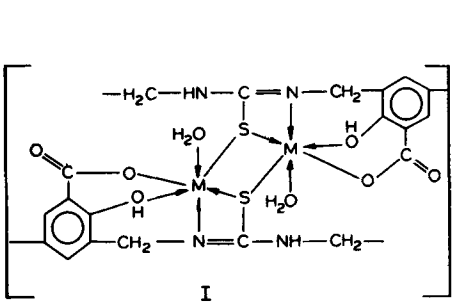
3.1 *IR data*

The important IR absorption bands of the polymeric ligands STT and PHBTT and their complexes are given in tables 1 and 2. In all the complexes the symmetric and asymmetric vibrations of (O – C – O) group (Bellamy 1956) of salicylic acid and *p*-hydroxybenzoic acid are observed at ~ 1400 and 1560 cm^{-1} respectively, while the carbonyl stretching frequency which appeared in the free salicylic acid and *p*-hydroxybenzoic acid between 1650-1670 cm^{-1} disappeared. Thus, it is obvious that there is metal-carboxylate linkage in all the complexes.

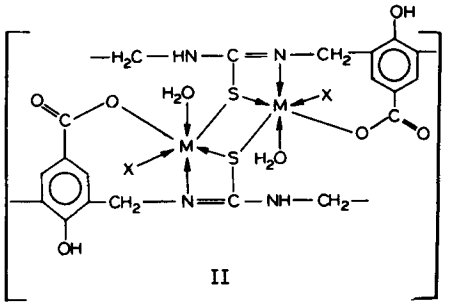
The sharp strong band which appeared at ~ 1616 cm^{-1} in the ligands due to $\nu_{C=N}$ shifts to lower frequencies at ~ 1600 cm^{-1} showing the coordination through the nitrogen atom of



(Mahto 1981). The strong band at ~ 856 cm^{-1} in STT and PHBTT respectively may be assigned to $\nu_{C=S}$ (Campbell and Grazeskowiak 1967). The weak band observed at ~ 2520 cm^{-1} is due to the presence of a small thioenolic form even in the free ligands (Satpathy *et al* 1979). However, this ν_{SH} band disappears in complexes showing that the M-S bond is formed due to deprotonation of (– SH) group. Besides this, very clear bands between 1010 – 1030 cm^{-1} due to $\nu_{C=S}$ are observed in all the complexes. Therefore, it is obvious that thioenolisation is more favoured during complex formation. Considering



I



II

$X = H_2O$; $M = Cu^{2+}$, Ni^{2+}, Co^{2+}, Zn^{2+}

$X = NO_3^-$; $M = Fe^{3+}$ and Cr^{3+}

the position of the potential donor atoms and from IR spectral studies and elemental analyses the dimeric 'S' bridge structures (I and II) for STT complexes and PHBTT complexes respectively have been suggested.

3.2 *Antimicrobial activity*

In many cases the toxic effect of the mercury-oxine-salicylic acid 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 (Anjaneyulu *et al* 1982). From the observations (tables 3 and 4) it is evident that the polymeric ligands (STT and PHBTT) and their chelates are capable of inhibiting fungal growth to a considerable extent both at high and low dilutions. Oxovanadium (IV) complexes of both STT and PHBTT cause 100% inhibition of *Penicillium islandicum.* Similarly, PHBTT polymeric ligand causes 100% inhibition of *Botrydiplodia theobromae* and *Scytalidium lignicola.* But STT polymeric ligand has no effect on the fungi. The fungicidal data reveal that all the complexes except Cu(II), Ni(II), Zn(II) complexes of STT and Cu(II), Zn(II) and Fe(III) complexes of PHBTT are physiologically more active. The reason may perhaps be due to the presence of C = O and C = S (C – S) groups in the ligands (Satpathy *et al* 1981) as well as their complexes. Hence, the complexes which inhibit may be used as antifungal agents against the said organisms. Almost all the compounds are active against the bacteria which have been mentioned earlier.

However, the polymeric ligands STT and PHBTT respectively show 100% inhibition against all the bacteria. It has been assumed 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 (the released salicylic acid from the chelates) cannot take place and toxic reactions within the spore do not occur. The released salicylic acid and *p*-hydroxybenzoic acid may play an important role in the antimicrobial activity through a different mechanism.

Penicillium islandicum causes watery rot of *Amla* (*Phyllanthus emblica*, L.), *Botrydiplodia theobromae*, causes soft rot in oranges; *Scytalidium lignicola* grows on wood and degrades the wood polysaccharides; *Glyocladium roseum* causes rotting in *Annanas* fruit (custard apple); *Erwinia chrysanthemi* causes stalk rot of *chrysanthemum*; *Erwinia caratovora* var *caratovora* causes Potato rot; *Erwinia caratovora* var *astroseptia* causes 'black leg' disease in potato plant; *Xanthomonas citri* initiates 'canker' in Citrus plants; *Xanthomonas vesicatoria* causes 'lead spot' disease in chillies and tomato and *Corynebacterium sepodonicum* causes 'ring rot' disease in potato. Most of the compounds inhibit the growth of the above organism which cause disease in many plants. As some substituted dithiocarbamates (Sharma *et al* 1981) have been used as potential insecticides and fungicides, our compounds may also be expected as important insecticides and agricultural and garden fungicides.

Acknowledgements

The authors thank Dr H C Dube for the supply of fungi and bacteriae for the experimental studies. They are thankful to Prof. S R Patel, for necessary facilities. The award of a teacher fellowship by UGC, India, to one of the authors (RM) is gratefully acknowledged.

References

Akbar Ali M and Livingstone S E 1974 *Coord. Chem. Rev.* **13** 101

Anjaneyulu Y, Prabhakar Rao R, Swamy R Y, Eknath A and Narasimha Rao K 1982 *Proc. Indian Acad. Sci. (Chem. Sci.)* **91** 157

Bellamy L J 1956 *The infrared spectra of complex molecules* (London: Methuen) 279

Compbell M J M and Grazeskowiak R 1967 *J. Chem. Soc.* **A** 396

Horsfall J G 1945 *Bot. Rev.* **11** 357

Horsfall J G and Rich S 1951 *Contrib. Boyce Thompson Inst.* **16** 361

Mahto C B 1981 *J. Indian Chem. Soc.* **58** 1173

Patel M M and Manavalan R 1983 *Indian J. Chem.* **22A** 117

Satpathy K C and Mahana T D 1979 *J. Indian Chem. Soc.* **56** 1173

Satpathy K C, Mishra H P and Pradhan G C 1981 *J. Indian Chem. Soc.* **58** 844

Sharma S K, Sharma A K and Chaurasia M R 1981 *J. Indian Chem. Soc.* **58** 687