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Comparison of Bactericidal and Fungicidal Activities of Cu (Ii) and Ni (Ii) Complexes of *Para*-Methoxy and *Para*-Hydroxy Benzoic Acid Hydrazide

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Abstract: Bactericidal and fungicidal were carried out on Cu(II) and Ni(II) *para*methoxy and *para*hydroxy benzoic acid hydrazides activities of the synthesized compounds were screened against 12 microorganisms viz: *Yersinia* sp., *Klebsilla* sp., *Saccharomyces cerevisae*, *Candida albicans*, *Rhodosporium* sp., *Proteus vulgaris*, *Staphylococcus aureus* and *E. coli*. The biological properties of the metal complexes revealed that in general complexation led to enhanced activity. The Cu(II) complexes appears to be much more potent antibacterial agents that the Ni(II) complexes *in vitro*; the three fungi used in this research *Rhodosporum* sp., *Saccharoymces cerevisae* and *Candida* sp. are completely resistant to all the copper complexes. They are susceptible to at least one of the nickel complexes. *E. coli* and *P. vulgaris* are completely resistant to the nickel complexes.

Key words: Hydrazide, bacteria and fungi, complexes, biological properties, anti bacterial agents

INTRODUCTION

The chemistry of hydrazides has been intensively investigated in recent years. The reasons are manifold; First is the coordinating ability of these compounds to chelate metal ions particularly transition and lanthanide ions. (Fox and Gibas, 1953) A considerable number of hydrazides have been reported to demonstrate tuberculostatic antibacterial and antifungal activities (Gursoy *et al*., 1997; Dodoff *et al*., 1995; Tabakova and Dodoff, 1995; Rando *et al*., 2002; Mamolo *et al*., 2001; Rollas *et al*., 2002). Application of Benzoic acid-[(5-Nitro-thiophenyl-l)-methylene]-hydrazide on *Mycobacterium tuberculosis in vitro* has indicated a new lead for potential antituberculosis activity (Dodoff *et al*., 1995). Isonicotinic acid hydrazide is highly selective for *Mycobaterium tuberculosis*.

In vitro studies on the biological activities of cyanoaliphatic acid *hydrazides* and their derivatives showed that these are active against *Tubercele bacilli* (Fox, 1952). Extension of the-CN group by methylene group retains appreciably the antibacterial activity of cyanoacetic acid hydrazide while modification of the cyano group into carboxy, amidino ammomethyl and other easily derivable groups reduces the antibacterial activity of cyanoaliphatic hydrazides. Dibasic acid hydrazides e.g., monoalkyl succinic acid dihydrazide inhibit the growth of *tubercele bacilli* in 200 τ cm^{-3} concentration and there is an appreciable difference in action due to difference in alkyl chain (Buu-Hoi *et al*., 1953). In our earlier research we reported the synthesis and biological activities of some transition metal complexes of dithiocarbazate ion (Adeoye *et al*., 2005).

We report here the synthesis of various Cu(II) and Ni(II) complexes of *p*-methoxy and *p*-hydroxybenzoic acid hydrazides and their bactericidal and fungicidal activities.

MATERIALS AND METHODS

Reagents and solvents: *p*-Methoxybenzoic, *p*-Hydroxybenzoic, Hydrazine hydrate and Ethanol were purchased from Aldrich. All the chemicals and solvents used were of reagent grade or purer and were used without further purification.

Synthesis of the ligands and complexes: The ligands and the complexes were synthesized according to the literature method (Odunola *et al*., 2002, 2003).

Preparation of *p*-methoxy benzoic acid hydrazide PMBAH: *p*-methoxybenzoic acid (0.1314 moles) was added to 50 mL of ethanol and 2 mL of H_2S0_4 and then refluxed for 5 h. The excess ethanol was distilled off and the solution was left to cool. The ester was separated from the aqeous layer by adding 250 mL of water in a separating funnel.

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Hydrazine hydrate (6.373 mL) was then added to the ester in 50 mL of ethanol and refluxed for 6 h. The ethanol, water and excess hydrazine hydrate were removed leaving the residual solid, (Yield 18.0 g).

Preparation of *p*-hydroxy benzoic acid hydrazide PHBAH: 0.2713 moles of *p*-hydroxybenzoic acid was added to 80 mL of ethanol with 2 mL of H_2SO_4 and then refluxed for 6 h. The excess ethanol was distilled off and the solution was left to cool. The ester was separated from the aqueous solution as before. 10.54 mL of hydrazine hydrate was added to the ester in 60 mL of ethanol and was refluxed for 6 h. The solid was recystallised from excess ethanolic solution of *p*-hydroxybenzoic acid hydrazide.

Preparation of $Cu[MBAH]_2Cl_2$: $CuCl_2.2H_2O$ (1.30 g, 7.6 mmole) dissolved in 10 mL of 50% methanol was added in drops to 20 mL hot methanolic solution of *p*-methoxybenzoic acid (2.25 g, 15 mmole) in a 100 mL beaker while stirring at room temperature. After stirring for 1 h, the green precipitate formed was filtered under suction, washed with water and methanol and dried in vacuum over anhydrous calcium chloride. Copper (II) sulfate and nitrate and acetate derivatives of *p*-methoxybenzoic acid and *p*-hydroxybenzoic acid hydrazides were similarly prepared.

Physical measurements: The infrared spectra of the synthesized ligands and complexes were recorded on Perkin-Elmer 1000 spectrometer between 350-4000 cm^{-1} using KBr discs. Melting point of the compounds were determined using a Buchi (B-540) melting point apparatus.

ANTIMICROBIAL ACTIVITY ASSAY

The antimicrobial activity of the synthesized complexes was assayed using twelve microorganisms collected from the Biology Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria using the disc diffusion method. The organisms are *Yersinia* sp. *Streptococcus pyogenes*, *Serratia marcenses*, *Bacillus subtilis*, *Bacillus licheniformis*, *Klebsiella* sp. *Saccharomyces cerevisae*, *Candida* sp. *Rhodosporum* sp. *Proteus vulgaris*, *Staphylococcus aureus* and *E. coli.*

Eighteen-hour-old broth culture of the test organisms was inoculated onto the surface of already prepared nutrient agar and potato dextrose agar plates using sterile swab sticks. Sterilized paper discs (5 mm diameter) were then impregnated with stock solutions (0.1 mg mL^{-1}) of the test compounds. The discs were then placed equidistant apart on the surface of the previously inoculated plates. These were then incubated overnight at 37°C after which the zones of inhibition were measured.

QUALITATIVE ANTIMCROBIAL ASSAY

Two organisms were selected for the assay of bactericidal and fungicidal activity of the complexes. *S. marcenses* was chosen for the bactericidal activity assay because it was most sensitive to the Cu(II) complexes, while *Rhodosporum* sp. was selected for the fungicidal activity assay because the action of $Ni(NO_3)_2$*p*-MBAH against it was sustained for more than 3 days. One milliliter of the stock solutions (0.1 mg mL^{-1}) of $CuCl_2$*p*-HBAH and $Ni(NO_3)_2$*p*-MBAH were mixed with 9 mL of a broth culture of the test organisms in screw cap test tubes. The broth cultures were previously prepared by inoculating 9.8 mL of sterile broth with 0.2 mL of an 18 h old broth culture of the test organism. The whole solution

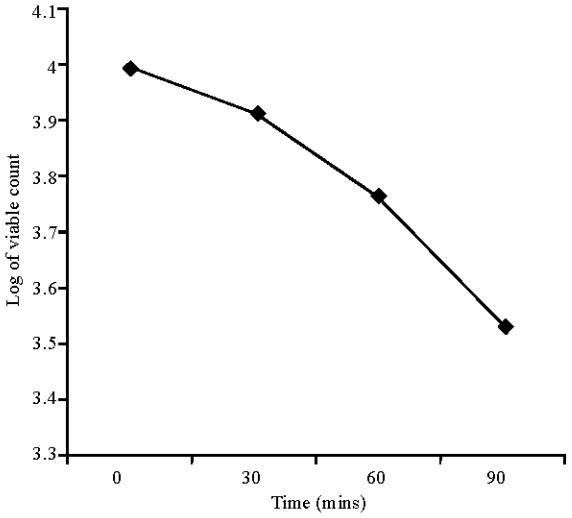

Fig. 1: Bactericidal activity of the Cu(II) complex against *S. marscences*

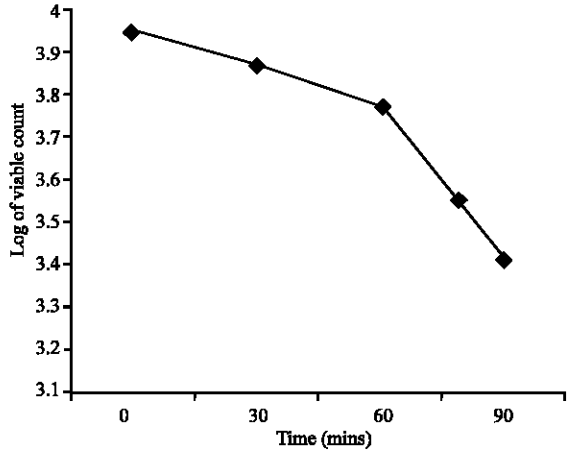

Fig. 2: Fungicidal activity of the Ni(II) complex against *Rhodosporum* sp.

was thoroughly mixed by inverting the test tubes rapidly several times after which 0.5 mL was withdrawn aseptically at 30 min intervals, serially diluted and 0.5 mL of appropriate dilutions plated in duplicates on already prepared agar plates. The plates were then incubated overnight at 37°C and the number of colony forming units estimated and reported as the bactericidal activity (Fig. 1 and 2).

RESULTS AND DISCUSSION

The colour analytical data percentage yield elemental analysis and are given in Table 1. The *para*methoxy and *para*hydroxy benzoic acid hydrazides were obtained from the reaction of their respective *para*methoxy and *para*hydroxy benzoate esters and anhydrous hydrazine in fairly good yield (58-64 %) according to Eq. 1 and 2.

$p\text{-}OCH_3C_6H_5COOCH_3 + NH_2NH_2 \rightarrow p\text{-}OCH_3C_6H_5CONHNH_2 + CH_3OH$ *1*

$p\text{-}OHC_6H_4COOCH_3 + NH_2NH_2 \rightarrow p\text{-}OHCH_3C_6H_4CONHNH_2 + CH_3OH$ *2*

The ligands and the complexes were obtained in reasonable yields, the formation of the Cu(II) and Ni(II) *para*methoxy and *para*hydroxy benzoic acid hydrazides can be represented according to the following Eq. 3 and 4.

$MX_2.xH_2O + 2\ p\text{-}OCH_3C_6H_5CONHNH_2 \rightarrow M[p\text{-}OCH_3C_6H_5CONHNH_2]_2\ X_2 + H_2O$ *3*

$M\ X_2.xH_2O + 2p\text{-}OHC_6H_4CONHNH_2 \rightarrow M\ [\ p\text{-}OH\ C_6H_4CONHNH_2]_2\ X_2 + H_2O$ *4*

where X = Cl^-, NO_3^-, CH_3COO^-, or $½SO_4^{2-}$; M= Cu ,Ni

Infrared spectra: The IR spectra bands of the ligands and complexes of the Cu(II) and Ni(II) salts are summarized in Table 2. The spectra obtained are consistent with the structural characteristics of hydrazides. The spectra also revealed that two out of the possible three binding sites are actually used in coordination. These are the 'amide 1' carbonyl stretching mode ν_s(C = O); which occurred in the ligands at 1600 cm^{-1} but appeared in lower regions (1601-1604 cm^{-1}) in the complexes suggesting the coordination of the carbonyl oxygen to the metal. The 'amide II' bands consists of three different bands which may appear as distinct bands and in some cases coupling may occur between the in-plane bending δ(N-H) and ν(C-N) stretching mode and the stretching frequency for the amino ν(NH_2). In the ligands, the amino stretching vibrations ν(NH_2) occurs between 3395cm^{-1} and this band becomes weaker and lowered to region between 3120-3485 cm^{-1} in the complexes. This shift indicates that the-NH_2 group is involved in coordination to the metal.. The amide II bands in the ligands and complexes occurring in the region 1534-1570 cm^{-1} remain relatively unchanged and we can safely conclude that the $\nu(C\text{-}N)_{enol}$ and $\nu(C\text{-}N)_{keto}$ forms are not involved as a binding site in coordination to the metal. This has been noted in previous studies on similar systems (Rollas *et al*., 2002; Fox, 1952).

Bioactivity of the compounds: Preliminary screening for antimicrobial activities of the stock solutions of the complexes in DMSO (100 µL) were performed using disc diffusion assay.

Overall, the Cu(II) complexes appears to be much more potent antibacterial agents than the Ni(II) complexes *in vitro* (Table 3 and 4). While the 3 fungi used in this

Table 1: Analytical data of the complexes

Compound	F.W	Colour	M.Pt °C	%M expected	%M observed
$Cu[p\text{-}MBAH]_2SO_4$	523.85	Lt green	148[d]	12.13	12.18
$Cu[p\text{-}MBAH]_2Cl_2$	498.78	Ash green	158[d]	12.74	13.04
$Cu[p\text{-}MBAH]_2(CH_3COO)_2$	545.87	Deep green	150[d]	11.64	11.94
$Cu[p\text{-}MBAH]_2NO_3$	489.86	Bluish-green	174[d]	12.97	12.95
$Cu[p\text{-}HBAH]_2SO_4$	495.81	Blue	100	12.82	12.70
$Cu[p\text{-}HBAH]_2Cl_2$	470.73	Blue	110	13.5	13.2
$Cu[p\text{-}HBAH]_2(CH_3COO)_2$	517.89	Brownish-green	98	12.27	12.16
$Cu[p\text{-}HBAH]_2NO_3$	461.81	Green	86	13.76	13.70
$NiMBAH]_2SO_4$	519.01	Blue	138	11.3	11.05
$Ni[p\text{-}MBAH]_2Cl_2$	493.94	Green	110	11.88	12.10
$Ni[p\text{-}MBAH]_2(CH_3COO)_2$	541.10	Purple	190	10.85	11.00
$Ni\ [p\text{-}MBAH]_2NO_3$	485.02	Purple	170	12.10	12.00

Table 2: Relevant Infrared spectra bands

[Cu MBAH]2Cl2	3345s	3058s	1602s	1534m,1517m	1300w	549vs 504s
Cu[MBAH]2(NO3)2	3163s	3064s	1600s	1577vs1516s	1312m	699s 616s
Cu[MBAH]2SO4	3261s	3055s	1600s	1570vs	1320m	550s 520s
Cu[MBAH]2(CH3COO)2	3155s		1600s	1542vs	1340s	567s
Ni[MBAH]2Cl2	3120s	2984s	1605s	1578vs	1305s	616s
Ni[MBAH]2(NO3)2	3256s	2901s	1601s	1578vs	1290s	617s
Ni[MBAH]2SO4	3340s	3050s	1602m	1550s	1360s	599s
Ni[MBAH]2(CH3COO)2	3489s	3057s	1604s	1540s	1350	544s

Table 3: Response of the test organisms to the cu (ii) complexes

Organisms	Zones of inhibition (mm)							
	$CuSO_4$ *p*-HBAH	$CuSO_4.5H_2O$ *p*-MBAH	$Cu(NO_3)_2$ *p*-HBAH	$Cu(NO_3)_2.H_2O$ *p*-MBAH	$CuCl_2.H_2O$ *p*-MBAH	$CuCl_2$ *p*-HBAH	Cu(OAC)2 *p*-HBAH	$Cu(OAC).H_2O$ *p*-MBAH
P. vulgaris	R	10	11	12	11	R	16	R
E. coli	17	12	15	10	16	11	15	11
Candida sp.	R	R	R	R	R	R	R	R
Klebsiella sp.	R	R	R	R	R	13	11	R
S. pyogenes	16	15	R	12	R	R	R	R
S. marcenses	15	R	11	R	13	25	13	R
B. licheniformis	12	R	15	R	R	11	R	R
Yersinia sp.	13	10	R	17	R	10	10	12
Rhodosporum sp.	R	R	R	R	R	R	R	R
S. cerevisae	R	R	R	R	R	R	R	R
S. aureus	12	R	13	R	12	10	R	R
B. subtilis	10	13	15	11	11	13	10	R

Table 4: Response of the test organisms to the ligands and Ni (ii) complexes

Organisms	Zones of inhibition (mm)					
	p-HBAH	*p*-MBAH	$NiSO_4$ *p*-MBAH	$NiCl_2$ *p*-MBAH	$NiNO_3$ *p*-MBAH	$Ni(OAC)_2$ *p*-MBAH
P. vulgaris	R	14	R	R	R	R
E. coli	15	R	R	R	R	R
Candida sp.	R	R	R	R	R	18
Klebsiella sp.	R	R	R	10	10	R
S. pyogenes	16	15	R	R	15	17
S. marcenses	12	16	R	R	11	11
B. licheniformis	11	R	R	R	R	16
Yersinia sp.	12	14	15	20	10	R
Rhodosporum sp.	R	R	15	R	19	20
S. cerevisae	R	R	R	R	14	R
S. aureus	12	11	R	17	R	12
B. subtilis	15	13	15	R	14	13

research, *Rhodosporum* sp., *S. cerevisae* and *Candida* sp. are completely resistant to all the Cu complexes, they are susceptible to at least one of the Ni complexes. The two organisms, *E. coli* and *P. vulgaris* completely resistant to the Ni complexes are bacterial species. The largest zone of inhibition of 25 mm was recorded against *S. marcences* followed by 20 mm each recorded against *Yersinia* sp. and *Rhodosporum* sp. The activity of $Ni(NO_3)_2$ *p*-MBAH against *Rhodosporum* sp. was sustained for the longest period of over 72 h after exposure this is the reason why it was selected as the test agent for fungicidal activity. *E. coli* appears to be the most susceptible to the Cu complexes being sensitive to all the complexes tested followed by *B. subtilis*, which is sensitive to seven of the eight complexes tested. Among the bacterial species, the resistance of *Klebsiella* sp. and *S. pyogenes* to the Cu complexes are remarkable with resistance to six and seven of the tested complexes, respectively. However, the Ni complexes appears to be more potent antifungal agents, as the highest zone of inhibition of 20 mm was recorded against *Rhodosporum* while *S. cerevisae and Candida* sp. are both sensitive to $Ni(NO_3)_2$ *p*-MBAH and $Ni(OAC)_2$ *p*-MBAH with zones of inhibition of 14mm and 18mm respectively. This weak antibacterial activity of the Ni complexes is consistent with observations of Adeoye *et al*. (2005) who discovered that Ni complexes have the weakest activity when the biological activity of dithiocarbazate complexes of Zn, Pb and Ni were tested against seven bacterial isolates.

Results of the bactericidal and fungicidal activity shows a progressive decrease in the population of cells in the test medium with time for the 2 organisms tested (Fig. 1 and 2). There was a 65.5% reduction in the population of *S. marcences* after 1 h 30 min exposure to $CuCl_2$ p-HBAH, while there was a reduction of 74.7% in the population of *Rhodosporum* sp. after the same period of exposure to $Ni(NO_3)$ p_7MBAH. There was also a reduction in the colony size of the test organisms with time, this could be as a result of protein leakage from the cells or the physiological stress imposed on growth by exposure to the metal complexes. Microorganisms have been known to exhibit abnormal growth behaviour under adverse growth conditions.

CONCLUSION

The results obtained in this research indicates that the complexes investigated possess bactericidal and fungicidal activity.

REFERENCES

Adeoye, I.O., O.O. Adelowo, F.A. Adekunle and M.A. Oladipo, 2005. Synthesis and biological activities of some transition metal complexes of some dithiocarbazate ion. Sci. Focus, 10: 180-183.

Buu-Hoi, N.P. N.D. Xuong, N.H. Nam, F. Binon and R. Roger, 1953. Tuberculostatic hydrazides and their derivatives J. Chem. Soc. London, pp: 1354-1364.

Dodoff, N. K. Grancharov and N. Spassovska, 1995. platinum (II) complexes of 4-methoxy and 4-chlorobenzoic Acid Hydrazides synthesis characterization and cytotoxic effect, J. Inorg. Biochem., 60: 257-266.

Dodoff, N., K. Grancharov and N. Spassovska, 1994. Platinum (II) Complexes of B enzoic and 3-Methoxy benzoic Acid hydrazides synthesis characterization and cytotoxic effect, J. Inorg. Biochem., pp: 54 221-223.

Fox, H.H. and J.T. Gibas, 1953. Pyridine Carboxylic Acid Hydrazides and Benzoic Acid Hydrazides. J. Organic Chem. (Easton), 17: 1653-1660.

Fox, H.H., 1952. Synthetic Tuberculostats III. Amino and Hydroxy-Pyridine Carboxylic Acid Derivatives J. Organic Chem., (Easton), 17: 542-546.

Gursoy, A., N. Terzioglu, G. Otuk, 1997. Synthesis of new hydrazides-hydrazones, thiosemicarbazides and thiazolidines as possible antimi crobials Eur. J. Med. Chem., 32: 753-759.

Mamolo, M.G., V. Falagiani, D.V.O.L. Zamper and E. Banfi, 2001. Synthesis and antimicrobacterial activity of [5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-yl thio]acetic acid arylidene hydrazide derivatives Farmaco, 56: 58ol-592.

Odunola O.A., I.O. Adeoye and J.A.O. Woods, 2002. Synthesis and structural features of copper(II) complexes of Benzoic acid and methyl substituted Benzoic acid hydrazides and X-ray structure of Cu[C6H5CONHNH2]2 (NO3)2 Synth React Inorg Met-Org. Chem., 32: 801-817.

Odunola, O.A., I.O. Adeoye J.A.O. Woods and A.C. Gelebe, 2003. Synthesis and Characterization of Nickel (II) complexes of Benzoic Acid Hydrazides and X-Ray structure of Ni[C6H5CONHNH2]3 Cl2. 3CH3OH. Synthesis and reactivity in Inorganic and Metal-Organic Chem., 33: 205-221.

Rando, D.G., D.M. Sato, L. Signera, A. Malvezz C.Q. Leite, A. Amaral, E.I. Ferreira and L. Tavares, 2002. Potential Tuberculostatic Agents. Topliss Application on Benzoic acid [(S-Nitrothiophen-2-yl)-Methylene]-hydrazide series. Bio. Organic and Med. Chem., 10: 557-560.

Rollas, S., N.N. Gulerman and H. Erdenz. 2002. Synthesis and antimicrobial activity of some new hydrazones of 4-flourobenzoic acid hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines Farmaco, 57: 171-174.

Tabakova. S. and N. Dodoff, 1995. Effect of platinum (II) complexes of Benzoic and 3-methoxybenzoic Acid Hydrazides on saccharomyces cerevisae Z Naturforsch 50C, pp: 34-734.