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Antimicrobial activity studies of syn-2,4-diaza-1,5-dicarbonyl-3-pentanethione

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ABSTRACT: The synthesis of 2,4-diaza-1,5-dicarbonyl-3-pentanethione ligands and their nickel(II) and cobalt(II) complexes are reported. The antimicrobial effects of these compounds on selected bacterial and fungal species were carried out by *in vitro* experiments. Two standard strains of bacterial species – *Staphylococcus aureus, Pseudomonas aeruginosa* – and two fungi – *Aspergillus niger* and *Rhoma eupyrena* – were used for the investigation. The percentage inhibition was evaluated using algae diffusion technique for all compounds using water as control. The activities were compared with that of a standard antibacterial drug (Septrin). It can be established that the compounds possess both antifungal and antibacterial properties which compare favourably with the standard antibacteriaal drug (Septrin).

Key Words: Antimicrobial agents; Fungicides; Bactericides; Urea compounds.

Introduction

The lethal action of a chemical compound depends upon the concentration of the active compounds and the time of exposure [1]. In the same manner- species of fungi and bacteria exhibit great variation in their ability to resist the action of certain fungicides and bactericides [2].

Urea compounds have been cited to process both antifungal and antibacterial properties [3]. Some have been patented as herbicides and growth regulators. These include trialkyl ureas that contain simple and complex hydrocarbon radicals such as Diuron 3-(3,4-dichlorophenyl) - 1,1-dimethyl urea; Linuron3-(3,4-dichlorophenyl)-1-methoxyl-1-methyl urea; Metabromuron, 3-methyl-3-(4-bromophenyl)- 1-methoxy -1-methyl urea; Phenobenzuron, N-benzyl-N-(dichloro-3-4-phenyl) -N-N-dimethyl-urea [3]. Onaleye *et al* [4[prepared 1,5-dicinnamyl-2,4-diaza-1,35-pentanetrione compounds and established them to be tridentate in their mode of complexation to metal ions.

However, in this research work, investigation if focused on the synthesis and antimicrobial activity studies of 2,4-diaza-1,5-dicarbonyl-3-pentanethione ligands and complexes.

Materials and Methods

Materials and Reagents

The reagents used for Research work were purchased from Aldrich Chemical Company. They are of analytical grade and were used directly without further purification. The solvents were dried in molecular sieves and distilled to obtain the anhydrous solvents used for the refluxing. The chemicals used include

cinnamoyl chloride, crotonoyl chloride, acetyl chloride, benzoyl chloride, urea, calcium chloride and solvents such as benzene, toluene, acetone, chloroform, ethanol, methanol, acetonitrile, petroleum ether and diethyl ether.

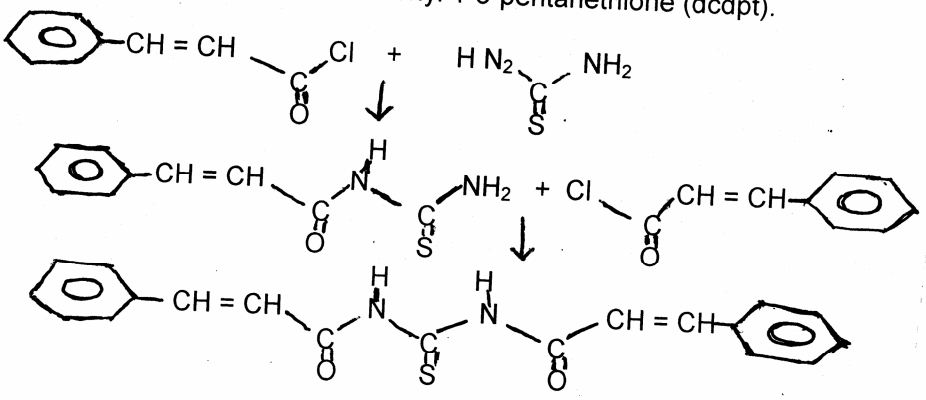
Experimental Procedure

Synthesis

Preparation of 2,4-diaza-1.5-dicarbonyl-3-pentathione ligands

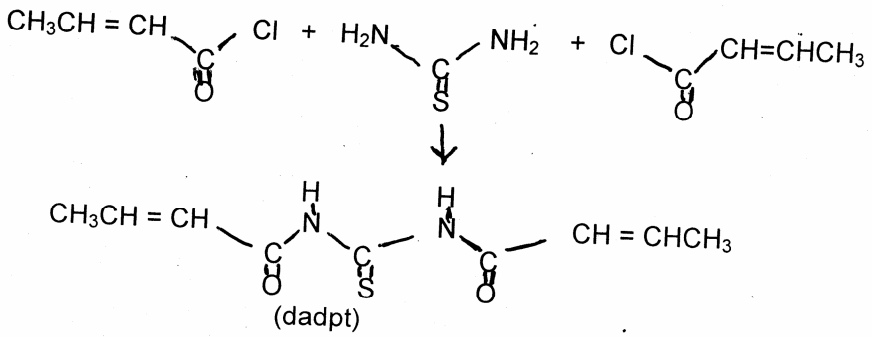
The ligands (dcdpt, dadpt, dbdpt and dmdpt) were prepared by employing the techniques of previously reported method [3,4]. This involves the refluxing of 7.6g (100mmoles) of thriorea with 16.8g (100 mmoles) of cinnamoyl chloride in dry benzene for a period of 4 hours until the evolution of HCI gas series. The solution was cooled in an ice bath over night and the white solid product formed was collected by filtration. The product (cinnamoyl urea) which was washed with petroleum ether, $NaHCO_3$ solution and recrystallized from aqueous ethanol was subsequently used in the preparation of the ligand by further acylation with the appropriate acyl chloride in 1:1 mole ratio. The equation of the reactions are as follows:

1.) 1,5-dicinnamyl-2,4-diaza-1,5-dicarbonyl 1-3-pentanethione (dcdpt).



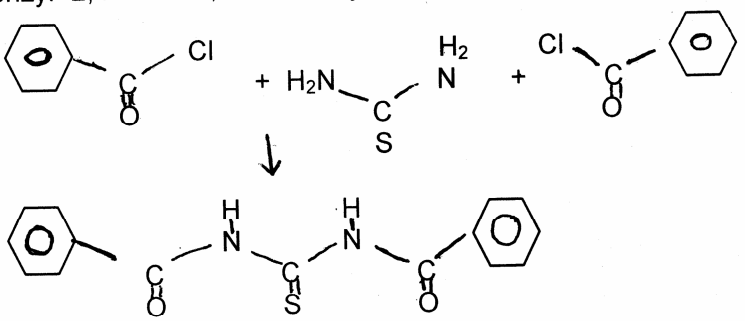
1,5-dicnnamyl-2,4-diaza-1,5-dicarbonyl -3-pentanethione (dadpt).

2.) 1,5-diallyl -2,4-diaza-1,5-dicarbonyl-3-pentanethione (dadpt).



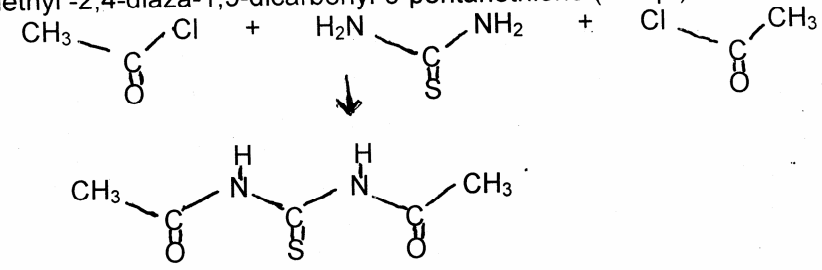
(dadpt)

3.) 1,5-dibenzyl -2,4-diaza-1,5-dicarbonyl-3-pentanethione (dbdpt).



(dbdpt)

4.) 1,5-dimethyl -2,4-diaza-1,5-dicarbonyl-3-pentanethione (dmdpt).



(dmdpt)

Preparation of metal complexes

The nickel(ll) and cobalt (ll) complexes of the ligands dcdpt, dbdpt, dadpt and dmdpt were prepared by refluxing 50 mmoles of he nickel(ll) and cobalt (ll) salts in dry ethanol with 100 mmoles of the respective ligand in dry acetone for 24 hours. The solution was cooled afterwards, filtered and concentrated to about one half the content and stored overnight in refrigerator. A green and pink coloured solid compound precipitated from solution for the nickel(ll) and cobalt(ll) complexes respectively. The deepness of the colour increase with the length of the ligand. This was filtered, washed several times with diethyl ether and dried in C_aC1_2 pellets in a decicator. The general equation for the reaction be represented as follows:

$$MC1_2.\ 6H_2O + 2L\ [MLC1_2]_2\ + 6H_20$$

where L = dcdpt, dbdpt, dadpt and dmdpt.

Antimicrobial Activity Studies

The antimicrobial activity studies of the sythesized compounds were investigated using the agar diffusion techniques [3] Nutrient agar purchased from Aldrich was used for the culture media. Specifically about 96g of the nutrient agar was weighed and dissolved in 250ml of distilled water contained in a conical flask and incubated. The flask was clogged with non-absorbent cotton and wrapped with aluminium foil to make air tight. The mixture was then melted on the bunsen flame and later sterilized in the autoclave at 15 Ib/Jn^2 pressure at a temperature of 120^oC for 15 minutes. The sensitivities of the test organisms to the chemical compounds were verified using the agar diffusion technique [3]. The sterilized plates were half-filled with agar aseptically and allowed to solidify. Inoculation was spread in the solid agar after which a cork borer was used to dig a well around the plate. Inside the wells were later added a drop of the chemical compounds aseptically using a dropping pipette appropriately labelled.

The plates were then subsequently set in the incubators and growth rate was observed on daily basis, that is, the prepared solutions of each compound was introduced into separate wells by means of pipette. The incubation of the plates was done at 37°C for 24 to 48 hours. Wells with clear zones around indicate antimicrobial effects. The inhibition of growth expressed in % was determined on the growth medium compared to the respective controls. This is expressed by the relation;

$$\% \text{ inhibition} = \frac{(C-T)\ X\ 100\%}{C}$$

Where C = average diameter of bacterial or fungal growth on the control plate
and T = average diameter of bacterial or fungal growth on the test plate.

Results and Discussion

Physical and Spectroscopic Studies

The physical spectroscopic properties of the ligands and complexes are presented in Table 1 and 2. The melting of the ligands are quite distinct from that of the starting reagents and increases in the order dbdpt < dcdpt < dmdpt. This trend can be related to the length of chain and degree of unsaturation in the ligands. The complexes in turn have higher melting points than the corresponding free ligand and were found to decompose within the temperature range 200-282°.

The selected I.R bands for characterization are mainly due to v(N-H), v(C=O),δ(N-H),δ(N-C=S) and p(C=S) stretching deformation bands (Table 2) which have been assigned according to the procedure by Jensen *et al* [5]. In the complexes, the coordination of the ligands to the central metal ions were through the sulphur and oxygen donor atoms as supported by the following observations:

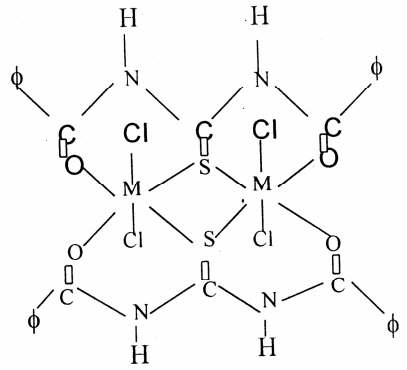
(i) A significant red shift in v(C=S) at 790 cm $^{-1}$ in free ligands to lower wave numbers in the complexes indicates the reduced double bond of the C=S group due to complexation.

(ii) A significant red shift in v(C=O) mode around 1751 cm $^{-1}$ of the free ligands to lower frequency n the complexes including the reduced double character of the C=O group due to complexation.

(iii) A blue shift in v(N-H) modes of the free ligand on complexation.

(iv) Formation of a six-membered stable chelate ring as against 4-membered ring if coordination was through the two nitrogen donor atoms.

Similarly, the electronic spectra of the compounds gave two absorption peaks at 220-222 nm and 272-294 nm which have been assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the 1,5-dicarbonyl-2,4-diaza-3-pentanethione system. Additional peaks were observed in the cases of the complexes due to d→d transitions. In the nickle(II) complex, the two absorption peaks around 404-412 and 715-754 nm have been assigned to the transitions from $^3A_2g \rightarrow {}^3T_1g$, ^3T_2g respectively. The only absorption peak around 525-530 nm in the cobalt (II) complexes is assigned to $^4Tig(F) \rightarrow {}^4Tig$ (p) which is a characteristics property of cobalt (II) complexes. The Binuclear structure (I) is being proposed for the complexes based on the i.r., spectra, electrical conductivity and metal analysis data.



here M = Co (II) or Ni (II)

ϕ = $C_6H_5CH = CH, C_6H_5$, $CH_3CH = CH, CH_3$

The structure was supported by the chemical spot test for chloride ion which was negative indicating the inclusion of chloride ion inside the coordination sphere.

Antimicrobial Activity Studies

The antimicrobialactivity of the compounds was estimated on the basis of average size of inhibition zone formed around the wells on the seeded agar plate. The inhibition growth expressed in % was determined on the basis of the average diameter of bacterial or fungal colony on the growth medium compared to the respective controls and standard antimicrobial drug (septrin) (7). The result is presented in Table 3. The result shows that all the compounds process both antibacterial and antifungal activity on the tested organisms. This result represents the maximum inhibition of the compound at 1% concentration which was observed after 48 hours in most cases. The values of the % inhibition were found to persist even several days after 4 days showing that the effect of the compound on the organisms are bactericidal and fungicidal respectively. The antimicrobial activity of the complexes may not be unconnected with the incorporation of the ligands (8,9). Both the ligands and complexes possess the 1,5-dicarbonyl -2,4-diaza-3-pentanetrione system of which the chromphores are well suited for electron-transfer and ion-exchange process with the cell components (10,11). Although, the activity of the complexes may be lower than that of the ligands but its overriding importance should not be ignored (9). Menavathu *et al* (9) stated that the high molecular weight of the complexes enhances retentive period in biological system. Hence thier application in the formation of slow release drugs and suppositories cannot be undermined.

Table 1: Physical properties of the ligands and complexes.

Compound	Colour	M.pt (^{o}C)	% Yield	Rf-value	% M	A^{-1} (cm^2 mol^{-1})
Dcdpt	Brownish white	171	74	0.60		5
Dbdpt	Brownish white	153	72	0.62		5
Dadpt	Brownish white	187*	70	0.61		5
Dmdpt	Brownish white	236	71	0.63		6
$(Co(dcdpt)Cl_2)_2$	pink	221*	64	0.45	6.1(6.32)	7.0
$(Ni(dcdpt)Cl_2)_2$	green	222*	65	0.44	6.1(6.32)	7.4
$(Co(dbdpt)Cl_2)_2$	pink	200*	63	0.48	6.9(7.12)	8
$(Ni(dbdpt)Cl_2)_2$	green	201*	64	0.45	6.9(7.09)	7.5
$(Co(dbdpt)Cl_2)_2$	Pink	232*	64	0.46	8.2(8.61)	8
$(Ni(dadpt)Cl_2)_2$	Green	234*	64	0.45	8.3(8.58)	7.6
$(Co(dmdtp)Cl_2)_2$	Pink	281*	63	0.45	9.71(10.16)	10
$Ni(dmdpt)Cl_2)_2$	Green	282*	64	0.46	9.8(10.12)	9.8

*Compounds decomposed at the stated temperature.

Values in parenthesis are theoretical values.

Table 2: Spectroscopic properties of the compounds.

Compound	Selected i.r. bands (cm^{-1})								U.V bands (nm)			
	v (N-H)		v (C=O)	δ (N-H)		v (N-C=S)		p(C=S	π→π*	π→π*		d→d
Dcdpt	3288s	3249s	1751s	1654s	1585s	1464s	1097m	790s	220	290		
Dbdpt	3288s	3200s	1750s	1651s	1584s	1464s	1075s	791s	220	280		
Dadpt	3288s	3200s	1749s	1645s	1583s	1466s	1095vs	792s	220	284		
Dmdpt	3285s	3205s	1750s	1640s	1585s	1464s	1095m	790s	220	272		
$(Co(dcdpt)Cl_2)_2$	3491s	3471s	1701s	1661s	1565m	1454m	1001w	712w	221	294	530	
$(Ni(dcdpt)Cl_2)_2$	3464s	3400s	1700s	1662s	1565m	1554m	1001w	721w	221	293	404	751
$(Co(dbdpt)Cl_2)_2$	3484s	3407s	1702s	1665s	1565m	1454m	1005w	715m	220	283	529	
$(Ni(dbdpt)Cl_2)_2$	3478s	3398s	1699s	1668s	1564m	1455m	1002w	714m	222	282	410	753
$(Co(dadpt)Cl_2)_2$	3464s	3402s	1701s	1655s	1564s	1454s	1001w	714m	221	287	528	
$(Ni(dadpt)Cl_2)_2$	3456s	3398s	1698s	1656s	1563m	1455m	1002w	715m	220	286	411	754
$(Co(dmdpt)Cl_2)_2$	3450s	3425s	1704s	1645s	1565s	1466m	1002w	724w	270	276	525	
$(Ni(dmdpt)Cl_2)_2$	3445s	3417s	1702s	1650s	1562s	1466m	1004w	712w	270	275	412	75

[a] s = Strong, m = medium. Vs = very strong, w = weak, v = stretching vibration, δ = in-plane deformation band, p = out of plane deformation.

Table 3: Result of activity of the compounds at 1 % concentration after 48 hours.

Compound	Bacterial Species		Fungal Species	
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Candida albicans*	*Penicilluim sp.*
Dcdpt	(+ 20mm) 143%	(+ 14mm) 70%	(+ 12mm) 86%	(+ 20MM) 75%
Dbdpt	(+ 20mm) 143%	(+ 13mm) 65%	(+ 11mm) 78%	(+ 20mm) 70%
Dadpt	(+ 16mm) 114%	(+ 12mm) 60%	(+ 10mm) 71%	(+ 12mm) 60%
Dmdpt	(+ 14mm) 100%	(+ 12mm) 60%	(+ 10mm) 71%	(+ 12mm) 60%
Septrin	(+ 14mm) 100%	(+ 20mm) 100%	(+ 14mm) 100%	(+ 20mm) 100%
$(Co(dcdpt)C1_2)_2$	(+ 16mm) 114%	(+ 12mm) 60%	(+ 10.5mm) 75%	(+ 13mm) 65%
$(Ni(dbdpt)C1_2)_2$	(+ 15mm) 107%	(+ 12mm) 60%	(+ 11mm) 78%	(+ 12mm) 60%
$(Co(dbdpt)C1_2)_2$	(+ 15mm) 107%	(+ 11.5mm) 58%	(+ 10mm) 71%	(+ 12mm) 60%
$(Ni(dbdpt)C1_2)_2$	(+ 15mm) 107%	(+ 11.5mm) 58%	(+ 10mm) 71%	(+ 12mm) 60%
$(Co(dadpt)C1_2)_2$	(+ 14mm) 100%	(+ 11mm) 55%	(+ 9.5mm) 68%	(+ 10mm) 50%
$(Ni(dadpt)C1_2)_2$	(+ 13.5mm) 96%	(+ 10mm) 50%	(+ 9.0mm) 64%	(+ 10mm) 50%
$(Co(dmdpt)C1_2)_2$	(+ 12mm) 86%	(+ 11mm) 55%	(+ 9.0mm) 64%	(+ 9.5mm) 48%
$(Ni(dmdpt)C1_2)_2$	(+ 12mm) 86%	(+ 10.5mm) 53%	(+ 9.0mm) 64%	(+ 10.0mm) 50%

Conclusion

It can be established from this work that the ligands dcdpt, dbdpt, dadpt and dmdpt as well as its nickel(II) and cobalt(II) and cobalt(II) complexes possess antifungal and antibacterial activity like the standard drug (Septrin). Hence they are potential antimicrobial agents although they are yet to be tried *in vivo.*

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