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FUNGICIDAL AND BACTERICIDAL ACTIVITY OF METAL DIETHYLDITHIOCARBAMATE FUNGICIDES

Synthesis and characterization

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Copper diethyldithiocarbamate, cadmium diethyldithiocarbamate, lead diethyldithiocarbamate, nickel diethyldithiocarbamate and zinc diethyldithiocarbamate have been synthesized. They have been characterized using TG, DTA, IR spectroscopy, X-ray and atomic absorption spectrophotometry. The thermal conversion of the compounds is 54.36–88% at 1000°C. Their solubility in sodium hydroxide, mineral acids, organic solvents, distilled water and salts solution has been measured. Fungicidal activity of the dithiocarbamates has been tested by well or cup diffusion method using five fungi species. Their activity has also been tested by broth dilution method using six bacterial species. Minimum Inhibitory Concentration (bactericidal) is 6.25–25.00 $\mu g\ mL^{-1}$.

Keywords: *diethyldithiocarbamate, DTA, fungicide, IR, synthesis, TG, X-ray diffraction*

Introduction

The plant protection has become necessary in order to increase the food production. Many organometallic compounds may have important biological activities. This work is continuation of our previous research on organometallic compounds including their thermal, spectral, structural and biological activities [1–14]. Among the commercially available current fungicides, the group of dithiocarbamate is of special interest. Zinc dithiocarbamate (zineb, ziram), manganese dithiocarbamate (maneb), iron dithiocarbamate (ferbam) are well known fungicides [15] which are used as protective fungicides [16] in grapes, cauliflower, potatoes, chillies, apples, groundnut, paddy etc. for the last many years. Their effectiveness has somewhat reduced and their large doses are required for fungal control. Thus there is a growing interest in synthesizing new dithiocarbamates, which can be applied at low concentration to control the fungal growth.

Therefore, now some new dithiocarbamate such as diethyldithiocarbamate of copper, cadmium, lead and nickel have been synthesized and characterized. The already known fungicide zinc diethyldithiocarbamate [17, 18] has now been prepared again and its properties have been included for the sake of comparison.

Experimental

Reagents and chemicals

Agar-agar (Hi media Laboratories Ltd, India); sodium diethyldithiocarbamate trihydrate, lead nitrate and cadmium nitrate tetrahydrate (CDH, India); copper nitrate pentahydrate, zinc nitrate hexahydrate and nickel chloride hexahydrate (E. Merck, India); fungi namely *Aspergillus niger*, *Chrysosporium tropicum*, *Rhizopus oryzae*, *Candida albicans*, *Saccharomyces cerevisiae*, *Biopolaris sorokinina*, bacteria namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Bacillus sphaericus*, *Chromobacterium violaceum* (Institute of Microbial Technology, Chandigarh) were used. All other chemicals and reagents used were of AR or LR grades.

Preparation of solutions

The solutions of cadmium nitrate (0.1 M), copper nitrate (0.1 M), nickel chloride (0.1 M), lead nitrate (0.1 M), zinc nitrate (0.1 M) and sodium diethyldithiocarbamate (0.1 M) were prepared in distilled water. The readymade potato dextrose agar (PDA) medium (39 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium was autoclaved at 15 lb $inch^{-2}$ for 20 min.

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Synthesis of metal dithiocarbamate

Copper diethyldithiocarbamate

Solution of sodium diethyldithiocarbamate (500 mL of 0.1 M) was added drop wise into a solution of copper nitrate (250 mL of 0.1 M) with continuous stirring of 1 h. The precipitate thus formed was kept overnight. The supernatant was removed by decantation, 200 mL distilled water was added, shaken for 15 min. The precipitate was allowed to settle and the supernatant was decanted. The process was repeated two times and then the precipitate was filtered on Buckner funnel. The precipitate was washed again three times with distilled water; dried initially at room temperature (20°C) and then dried at 60±5°C till constant mass. Finally the product was desiccated over calcium carbonate for a week.

Lead diethyldithiocarbamate

Solution of lead nitrate (250 mL of 0.1 M) was taken in place of solution of copper nitrate and copper diethyldithiocarbamate was followed.

Cadmium diethyldithiocarbamate

Solution of cadmium nitrate (250 mL of 0.1 M) was taken in place of solution of copper nitrate and copper diethyldithiocarbamate was followed.

Nickel diethyldithiocarbamate

Solution of nickel chloride (250 mL of 0.1 M) was taken in place of solution of copper nitrate and Copper diethyldithiocarbamate was followed.

Zinc diethyldithiocarbamate

Solution of zinc nitrate (250 mL of 0.1 M) was taken in place of solution of copper nitrate and copper diethyldithiocarbamate was followed.

Apparatus and instruments

Atomic absorption spectrophotometer (GBC 902, Australia), Autoclave (Narang Company, India), Fourier transformed IR spectrometer (NEXUS 670, Nicolet Incorporation, USA), magnetic stirrer (Sunvic, UK), melting point apparatus (Reichert thermovar, Austria), temperature controlled electric oven (Tempo, India), TG-DTA instrument (Mettler Toledo 851^e Switzerland.), powder X-ray diffractometer (Bruker, Scotland) were used.

Analysis of sample

Solubility

The powdered sample (10 mg) was shaken with 1 mL of solution/solvent in a test tube, the contents were placed at room temperature (22°C) for 24 h and then the changes in the appearance of dithiocarbamates were noted.

Melting point

The melting point was recorded by Reichert thermovar.

Atomic absorption spectrophotometry

The dried and powdered sample (100 mg) was dissolved in 10 mL of nitric acid (1.0 M) and then it was diluted with distilled water to obtain a solution of the required concentration.

Infrared

The infrared spectra were taken using KBr disc method in the range of 4000–500 cm^{-1}.

X-ray

X-ray diffraction of powdered samples was recorded by powder X-ray diffractometer.

TG-DTA

The TG-DTA analysis of the samples was carried out at a constant heating rate up to 1000°C in air atmosphere.

Fungal activity test

The synthesized metal dithiocarbamates under study were tested for fungicidal activity by agar cup bioassay method [19] using five test organisms namely *Aspergillus niger*, *Chrysosporium tropicum*, *Rhizopus oryzae*, *Candida albicans*, *Saccharomyces cerevisiae*, as follows:

- Cup diffusion method: Agar cup bioassay was employed for testing antifungal activity. The potato dextrose agar (PDA) medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber when the medium in the plates solidified; 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone and different concentrations were made (50, 100 μg mL^{-1}). After inoculation, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentration of test solutions

(50, 100 μg mL^{-1}) were added. Controls were maintained with acetone and clotrimazole (100 μg mL^{-1}). The treated and the controls were kept at room temperature for 48 h. Inhibition zones were measured and diameter was calculated in millimeter. Three to four replicates were maintained for each treatment.

- Effect of dithiocarbamates fungicides on the spore germination: The fungicidal effect of synthesized metal dithiocarbamates was tested on spore germination of *Bipolaris sorokinina*. The test was based on the slide-germination technique [20] using a concentration 2000 ppm mL^{-1} of dithiocarbamates under study.

Bacterial activity test

The synthesized metal dithiocarbamates were tested for bactericidal activity by broth dilution method [21] using six test organisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Bacillus sphaericus*, *Chromobacterium violaceum*. The broth dilution method is as follows:

Broth dilution method

The test was used to determine the minimal inhibitory concentration (MIC). Serial dilutions of a stock solution of compounds were incorporated into 0.5 mL of nutrient broth in glass tubes to get required concentration. All the tubes were inoculated with standardized inoculum of organism. Control was maintained with acetone in the last tube. The whole setup was incubated at 25–30°C for 8 days. Examine tubes for visible signs of bacterial growth. The lightest dilution without growth is the minimal inhibitory concentration (MIC).

Results and discussion

Copper diethyldithiocarbamate

Copper diethyldithiocarbamate under study is dark brown powdered substance. It is soluble in 1 M HCl, 1 M HNO_3, 1 M H_2SO_4 and benzene and insoluble in distilled water, deep well water, 1 M NaOH, 1 M $K_2Cr_2O_7$ (oxidizing agent) and 1 M ascorbic acid, 1 M $Na_2S_2O_4$ and 1 M hydroxyl amine (reducing agent). The results are recorded in Table 2. It melts at 210°C (Table 3). IR spectrum shows the peak (cm^{-1}) at 1675.56w, 1506.00s, 1431.71sh, 1376.66sh, 1351.59sh, 1299.59w, 1271.72s, 1205.00w, 1145.09w, 1098.25w, 1070.99w, 993.10m, 910.07w. X-ray diffraction data are reported in Table 5. DTA and TG curves show two peaks at 247 and 320°C and only one plateau corresponding to 73.63% conversion (241.48–349.72°C). The results are recorded in Table 6. It shows good fungicidal and bactericidal activity as it inhibits the growth of both fungi and bacteria (Tables 7–9).

Lead diethyldithiocarbamate

The lead diethyldithiocarbamate under study is off white powdered substance. It is soluble in 1 M HCl, 1 M HNO_3, 1 M H_2SO_4 and benzene and insoluble in distilled water, deep well water, 1 M NaOH, 1 M $K_2Cr_2O_7$ (oxidizing agent) and 1 M ascorbic acid, 1 M $Na_2S_2O_4$ and 1 M hydroxyl amine (reducing agent). The results are recorded in Table 2. It melts at 224°C (Table 3). IR spectrum shows the peak (cm^{-1}) at 1483.07s, 1456.04w, 1417.57s, 1376.62w, 1353.09w, 1298.86w, 1265.85s, 1204.07w, 1137.14m, 1070.81sh, 980.85sh, 905.35w. The X-ray diffraction data are reported in Table 5. DTA and TG grams show only one peak at 345°C and only one plateau corresponding to 52.78% conversion (241.48–349.72°C). The results are recorded in Table 6. It shows good fungicidal and bactericidal activity as it inhibits the growth of both fungi and bacteria (Tables 7–9).

Cadmium diethyldithiocarbamate

The cadmium diethyldithiocarbamate under study is white powdered substance. It is soluble in 1 M HCl, 1 M HNO_3, 1 M H_2SO_4 and benzene; slightly soluble in 1 M NaOH and insoluble in distilled water, deep well water, 1 M $K_2Cr_2O_7$ (oxidizing agent) and 1 M ascorbic acid, 1 M $Na_2S_2O_4$ and 1 M hydroxyl amine (reducing agent). The results are recorded in Table 2. It melts at 280°C (Table 3). IR spectrum (Table 4) shows the peak (cm^{-1}) at 1502.64s, 1454.26w, 1430.29s, 1377.28w, 1352.37w, 1297.91w, 1271.61s, 1206.89s, 1145.24m, 1096.62w, 1071.67m, 991.34s, 910.34m. The X-ray diffraction data are reported in Table 5. DTA and TG curves show only one peak at 361°C and only one plateau corresponding to 81.84% conversion (241.48–349.72°C). The results are recorded in Table 6. It shows good fungicidal and bactericidal activity as it inhibits the growth of both fungi and bacteria (Tables 7–9).

Nickel diethyldithiocarbamate

The nickel diethyldithiocarbamate under study is green powder. It is soluble in 1 M HCl, 1 M HNO_3, 1 M H_2SO_4 and benzene and insoluble in distilled water, deep well water, 1 M NaOH, 1 M $K_2Cr_2O_7$ (oxidizing agent) and 1 M ascorbic acid, 1 M $Na_2S_2O_4$ and 1 M hydroxyl amine(reducing agent). The results are

Table 1 Abbreviation used

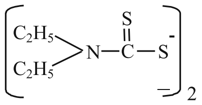
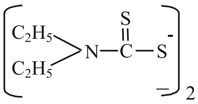
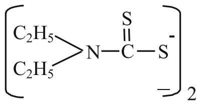
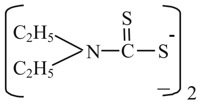
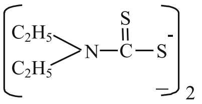
Compound	Colour	Empirical formula	Stoichiometric formula	Abbreviation
Copper diethyldithiocarbamate	Dark brown	$C_{10}H_{20}N_2S_4$ Cu	 Cu	HSR_1
Lead diethyldithiocarbamate	Off white	$C_{10}H_{20}N_2S_4$ Pb	 Pb	HSR_2
Cadmium diethyldithiocarbamate	White	$C_{10}H_{20}N_2S_4$ Cd	 Cd	HSR_3
Nickel diethyldithiocarbamate	Green	$C_{10}H_{20}N_2S_4$ Ni	 Ni	HSR_4
Zinc diethyldithiocarbamate	White	$C_{10}H_{20}N_2S_4$ Zn	 Zn	HSR_5

Table 2 Solubility of metal dithiocarbamates

Solvent	Compound				
	HSR_1	HSR_2	HSR_3	HSR_4	HSR_5
H_2SO_4	Soluble	Soluble	Soluble	Soluble	Soluble
HNO_3	Soluble	Soluble	Soluble	Soluble	Soluble
HCl	Soluble	Soluble	Soluble	Soluble	Soluble
NaOH	Insoluble	Insoluble	Slightly Soluble	Insoluble	Soluble
$K_2Cr_2O_7$	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
$Na_2S_2O_4$	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Ascorbic acid	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Hydroxylamine	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Distilled water	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Tap water	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Benzene	Soluble	Soluble	Soluble	Soluble	Soluble

Table 3 Melting point and analysis of metal dithiocarbamates

Compound	Metal ion	Melting point/°C	Metal ion/%	
			Experi-mental	Calculated
HSR_1	Cu(II)	210	16.0	17.7
HSR_2	Pb(II)	224	40.0	41.15
HSR_3	Cd(II)	280	22.0	27.5
HSR_4	Ni(II)	259	17.6	16.5
HSR_5	Zn(II)	181	16.0	18.1

Table 4 Selected Infrared frequencies of metal dithiocarbamates

Compound	IR frequencies/cm^{-1}		
	$\nu_{C=S}$	$\nu_{C–N}$	$\nu_{C=N}$
HSR_1	993.10m	1271.72s	1506.00s
HSR_2	980.85m	1265.85s	1483.07s
HSR_3	991.34m	1271.61s	1502.64s
HSR_4	990.35w	1273.33s	1517.11s
HSR_5	985.09m	1267.62s	1499.32s

Table 5 Powder X-ray data of metal dithiocarbamates

HSR_1		HSR_2		HSR_3		HSR_4		HSR_5	
d-values α^1/ Å	Relative intensity/ %	*d*-values α^1/ Å	Relative intensity/ %	*d*-values α^1/ Å	Relative intensity/ %	*d*-values α^1/ Å	Relative intensity/ %	*d*-values α^1/ Å	Relative intensity/ %
8.87417	100.00	9.23565	100.00	9.00871	100.00	8.17901	100.00	8.97330	100.00
6.21947	60.34	4.58347	45.70	7.50056	75.00	4.14338	41.40	7.54648	60.90
4.20927	56.89	7.38302	40.00	6.27025	56.30	5.13414	32.80	6.32067	58.69
7.54482	53.45	3.78773	35.40	4.31021	46.87	3.20287	27.60	4.45697	58.69
3.71644	43.10	3.42565	31.40	4.46810	37.50	5.43244	22.40	3.82459	58.69
3.10290	36.20	4.27434	30.90	3.82056	37.50	4.44252	22.40	2.85437	56.52
4.58892	34.50	3.17592	29.70	2.28056	15.60	6.15493	20.70	4.90894	54.35

Table 6 Thermogravimetry of metal dithiocarbamates

HSR_1		HSR_2		HSR_3		HSR_4		HSR_5	
Temperature range/ °C	Conversion/ %	Temperature range/ °C	Conversion/ %	Temperature range/ °C	Conversion/ %	Temperature range/ °C	Conversion/ %	Temperature range/ °C	Conversion/ %
25.00	0.00	25.00	0.00	25.00	0.00	25.00	0.00	25.00	0.00
133.24	2.00	133.24	3.44	133.24	3.68	133.24	3.62	133.24	2.76
241.48	2.94	241.48	3.03	241.48	2.75	241.48	3.24	241.48	2.37
349.72	75.97	349.72	31.38	349.72	37.12	349.72	6.02	349.72	43.00
457.96	77.96	457.96	55.81	457.96	84.59	457.96	78.94	457.96	61.85
566.20	80.18	566.20	55.53	566.20	85.46	566.20	80.43	566.20	62.48
674.44	79.96	674.44	53.98	674.44	85.93	674.44	81.57	674.44	62.41
782.69	79.92	782.69	51.93	782.69	87.03	782.69	82.05	782.69	65.34
890.93	80.22	890.93	52.25	890.93	87.53	890.93	82.44	890.93	66.04
999.17	82.65	999.17	54.36	999.17	88.00	999.17	82.76	999.17	66.50

Table 7 Fungicidal activity of metal dithiocarbamates by cup diffusion method

Compounds	*Aspergillus niger*		*Rhizopus oryzae*		*Chrysoporium tropicum*		*Candida albicans*		*Saccharomyces cervisiae*	
	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg
HSR_1	8	12	–	–	8	11	7	9	7	10
HSR_2	6	9	–	–	6	8	8	11	6	9
HSR_3	7	11	–	–	8	12	6	9	7	9
HSR_4	6	9	–	–	6	9	7	9	8	11
HSR_5	9	12	–	–	7	11	8	11	6	9
Control		26		29		23		26		27

recorded in Table 2. It melts at 259°C (Table 3). IR spectrum shows the peak (cm^{-1}) at 1517.11s, 1443.15m, 1349.60sh, 1273.33m, 1203.81w, 1150.01w, 1077.54w, 990.35w, 910.23w. The X-ray diffraction data are reported in Table 5. DTA and TG grams show only one peak at 382°C and only one plateau corresponding to 75.70% conversion (241.48–349.72°C). The results are recorded in Table 6. It shows good fungicidal and bactericidal activity as it inhibits the growth of both fungi and bacteria (Tables 7–9).

Zinc diethyldithiocarbamate

The zinc diethyldithiocarbamate under study is white powder. It is soluble in 1 M HCl, 1 M HNO_3, 1 M H_2SO_4 and benzene, 1 M NaOH and insoluble in distilled water, deep well water, 1 M $K_2Cr_2O_7$ (oxidiz-

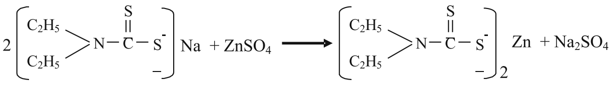
Table 8 Effect of dithiocarbamates fungicides on the spore germination of *Bipolaris sorokinina*

	R1	R2	Mean	SD
HSR$_1$				
Slide 1	58.8	80.0	69.4	
Slide 2	71.4	67.9	69.6	
Slide 3	90.0	76.2	83.1	
Slide 4	81.3	72.4	76.8	
Slide 5	76.7	87.5	82.1	
			76.2	6.6
HSR$_2$				
Slide 1	79.4	62.5	70.9	
Slide 2	72.7	75.0	73.8	
Slide 3	68.9	75.9	72.4	
Slide 4	66.7	82.4	74.5	
Slide 5	68.8	61.1	64.9	
			71.3	3.8
HSR$_3$				
Slide 1	84.6	85.4	84.9	
Slide 2	77.8	76.9	77.4	
Slide 3	56.7	78.6	67.6	
Slide 4	51.7	72.7	62.2	
Slide 5	76.5	67.6	72.0	
			72.8	8.8
HSR$_4$				
Slide 1	69.6	65.6	67.6	
Slide 2	66.7	52.9	59.8	
Slide 3	46.7	67.7	57.2	
Slide 4	45.9	38.5	42.2	
Slide 5	55.6	71.8	63.7	
			58.1	9.7
HSR$_5$				
Slide 1	64.5	73.3	68.9	
Slide 2	80.0	81.8	80.9	
Slide 3	66.7	74.4	70.5	
Slide 4	78.9	75.0	76.9	
Slide 5	77.2	71.4	74.4	4.9

ing agent) and 1 M ascorbic acid, 1 M $Na_2S_2O_4$ and 1 M hydroxyl amine (reducing agent). The results are recorded in Table 2. It melts at 181°C (Table 3). IR spectrum shows the peak (cm^{-1}) at 1499.32s, 1427.87m, 1347.62w, 1267.62s, 1199.46m, 1142.37w, 1068.88w, 985.09s, 907.57w. The X-ray diffraction data is reported in Table 5. DTA and TG grams show only one peak at 345°C and only one plateau corresponding to 59.48% conversion (241.48–349.72°C). The results are recorded in Table 6. It shows good fungicidal and bactericidal activity as it inhibits the growth of both fungi and bacteria (Tables 7–9).

Conclusion

When water soluble alkali salt, sodium diethyldithiocarbamate, reacts with metal salts (Cu, Pb, Cd, Ni, Zn), the corresponding salts of dithiocarbamic acid is formed. These salts are practically soluble in acids and organic solvents while insoluble in water and aqueous solvents. This observation is in line with those reported [16].



Relevant IR bands for the compounds under study are given in Table 4. The following regions of the spectra are particularly informative with respect to molecular structure of these compounds [22, 23].

Peak at 1250 cm^{-1} is due to the absorption caused by C–N stretching vibrations. The thiureide band, which arises as a consequence of delocalization of the electron pair on nitrogen to the $R_2N–CS_2$ bond (polar canonical form) appears at 1500 cm^{-1}. This bond increases the double bond character of the linkage and displaces the band from 1250 to 1500 cm^{-1}. This effect can be clearly noted by inspecting the values reported in Table 4 which ranges from 1517 to 1483 cm^{-1}.

The position of antisymmetric C=S stretching vibration of NCS_2 group of carbodithioate ligands appear to be in region 1050 to 1200 cm^{-1}. The electron donation from S atom to the metal causes a shift from its typical position of 1200 to 970 cm^{-1}. This effect is also apparent from the results reported in Table 4.

The *d*-values and relative intensity of X-ray diffraction data of the compounds under study are reported in Table 5.

DTA data shows that the copper diethyldithiocarbamate gives two peaks at 247 and 320°C. It is because, when copper dithiocarbamate heated in air, it gave cuprous sulphide around 245°C and latter it was partially oxidized to sulphate.

Table 7 shows the fungicidal activity of the compounds under study as they inhibit the fungal growth by forming an inhibition zone. Table 8 shows the effect of dithiocarbamates under study on the spore germination of fungi *Bipolaris sorakinina*. The results are quite satisfactory since standard deviation in the data is not more

Table 9 Bactericidal activity of metal dithiocarbamates by broth dilution method

Compounds	Microorganisms					
	Gram positive			Gram negative		
	Bacillus subtilis	*Bacillus sphaericus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*E. coli*	*Chromobacterium violaceum*
	Minimum inhibitory concentration/μg mL^{-1}					
HSR_1	25.00	6.25	12.50	25.00	25.00	25.00
HSR_2	25.00	12.50	25.00	25.00	25.00	25.00
HSR_3	12.50	25.00	12.50	12.50	12.50	25.00
HSR_4	6.25	12.50	25.00	25.00	6.25	12.50
HSR_5	6.25	12.50	25.00	25.00	6.25	12.50
Control	0.00	0.00	0.00	0.00	0.00	0.00

than 10 but out of these, nickel diethyldithiocarbamate seems to be promising. The results given in Table 9 show the minimum inhibitory concentration of the compounds using broth dilution method.

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