ORIGINAL ARTICLE

Synthesis and study of anti-bacterial activity of complexes of diallyldisulphide from garlic

Shashi Prabha Singh, Meenakshi Bajpai, Bal Kishen Razdan[1]

Department of Pharmaceutics, Uttarkhand Technical University, Government Girls Polytechnic, Post Office Chandanwadi, Sudhowala, [1]29-A, Circular road, Dalanwala, Dehradun, Uttarakhand, India

Background: The development of bacterial resistance to available antibiotics and increasing incidence of multiresistant bacterial infections in hospitals and in the community has necessitated the search for new antibacterial agents to treat the bacterial infection. It has long been known that metal ions are involved in biological processes of life through bonding to the heteroatoms of the heterocyclic residues of biological molecules i.e., proteins, enzymes and nucleic acids etc. The behavior of the disulfide group as a donor in transition metal complexes has not been subjected to such detailed study as a number of other donor groups. **Aim:** The Diallyldisulphide is one of the main constituents of *Allium sativum* (Garlic). The antibacterial and antifungal activity of the Garlic is due to the presence of sulphur containing compounds. The aim of the present work was to synthesize metal complexes with Diallyldisulphide. **Materials and Methods:** Complexation reactions between the Diallyldisulphide and the metal ions were carried out at three different pH i.e. acidic (pH 3), neutral (pH 7) and alkaline (pH 10) using three different ratios of metal ligand namely 1:1, 1:2, 1:3, respectively. Complex formation with the ligand and all the metals took place at pH 10, indicating that complexes were stable at this pH. Studies with different metal: ligand ratio showed that in case of silver the complexation took place at 1:1 ratio. In case of divalent metals, the appropriate ratio of metal: ligand was 1:2. The structures of the new complexes obtained were determined by spectroscopic methods. **Results and Discussion:** Synthesized complexes were investigated for antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and E.coli*. Diallyldisulphide-silver complex exhibited significant antibacterial activity (MIC 100 µg/mL), and was found to be effective against selected organisms. The results concluded that the metal complexes are better antibacterial agents as compared to the silversulphadiazine.

Key words: Antibacterial activity, complexes, diallyldisulphide, garlic, minimum inhibitory concentration

INTRODUCTION

Search of the new ligands is perhaps the most important step in the development of metal complexes, which exhibit unique desired properties and novel reactivity. The inclusion of biologically-active ligand into organometallic complexes offers much scope for the design of novel drugs with enhanced, targeted activity. Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases such as, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes.[1] It is also effective against bacterial as well as fungal infections.[2] Garlic has different active constituents among which Diallyldisulphide shows antibacterial and antifungal activity. Considering the biological activities of Garlic and the fact that transition metals such as silver possess antimicrobial properties, it is proposed to complex the biologically active ligand of garlic such as Diallyldisulphide with metals such as silver.

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.104927

Transition metal complexes having disulfide ligands S_2^{2-} and organic disulfide ligands RSSR (R = alkyl, aryl, …) are of significant importance in bioinorganic chemistry as well as in some transition metal assisted and homogeneously catalyzed reactions such as C–H bond activation, C–S bondformation and hydrodesulfuration reactions.[3-6] The most common coordination mode of RSSR ligand is the bridging coordination where the two metals may be additionally linked by a metal–metal bond, (I) In Figure 1 furthermore, the RSSR ligand may be monodentately coordinated (II) And few examples are known where RSSR acts as bidentate ligand coordination (III) Finally, there are few reports in which coordination (IV) for an RSSR ligand is assumed.[7] Crystallographic evidence for type II (M = Ag, R = Ph;[8] M = Cu, R = $CH_2CH_2NH_3$;[9] M = W, R_2 =-CH_2CH = $CHCH_2$–)[10,11] and III (M = Nb, R = Me,i-Pr)[12] complexes is rare.

Keeping the diverse therapeutic activities of Diallyldisuphide in view, it was contemplated to

Address for correspondence: Mrs. Shashi Prabha Singh, Uttarkhand Technical University, Government Girls Polytechnic, Post Office Chandanwadi, Premnagar, Sudhowala, Uttarakhand, India. E-mail: Shashi_pharm2005@yahoo.co.in

Received: 07-04-2012; **Accepted:** 16-07-2012

synthesize a series of complexes with transition metals and screening them for their *in vitro* antibacterial activity.

MATERIALS AND METHODS

The uncorrected melting points were taken in open glass capillaries. The IR spectra were recorded on a Perkin Elmer system FT spectrophotometer in KBr with wave number given in cm^{-1}. The ^1H NMR spectra have been recorded on Brucer 400 Avance Fourier Transform Spectrometer operating at 400 mega hertz in deutirated dimethylsulfoxide (DMSO) with all shifts referred to internal tetramethylsilane (TMS). The mass spectra were recorded on a LCMS Agilent Technology model 6520. Most of the spectra were recorded at IIT, Delhi and IPC, Ghaziabad. Elemental analysis was performed on a Perkin Elmer series C, H, N, S analyzer 2400. Diallyldisulfide were purchased from sigma Aldrich and used as supplied. All chemicals used were of analytical grade (AR) and of highest purity available.

General Method for the Preparation of Metal Complexes

Complexation reactions between the ligand and the metal salts (silver nitrate, copper nitrate, manganese chloride, magnesium nitrate and zinc nitrate) were carried out to investigate the coordination capability of the ligand. The complexes were prepared by reaction of the ligand with metal salts in 2:1 molar ratios in ethanol except silver. The complex of silver with Diallyldisulphide was formed in 1:1 ratio. Complex formation was confirmed by Thin Layer Chromatography and test for metal ions. Thin Layer Chromatography was carried out on Silica Gel G using solvent system n-Butanol: Isopropanol: Acetic acid: Water (3: 1: 1: 1). For comparison the ligand diallyldisulphide was used. A negative chemical test indicated the formation of a complex.

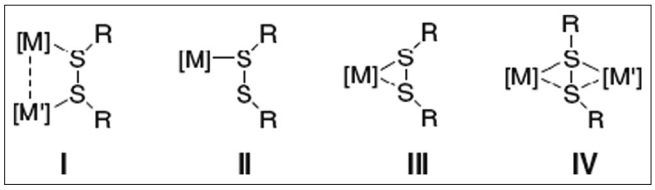

Figure 1: Coordination types of organic disulphide RSSR ligands

The ligand diallyldisulphide (2 mmol) was dissolved in 20 ml of absolute ethanol and a solution of the metal salts (1 mmol) in 20 ml of ethanol was added drop wise to the ligand solution with continuous stirring, the pH of the solutions was adjusted to 10 adding a NaOH suspension in ethanol. The mixture was refluxed for 6 hours. The solid was filtered, washed with cold ethanol and allowed to air-dry and recrystallised from hot ethanol.

Antibacterial Activity

Newly synthesized complexes were screened for their antibacterial activity against *Staphylococcus aureus* (ATCC 6538*), Pseudomonas aeruginosa* (ATCC 9027*), Klebsiella pneumoniae* (ATCC 10031*) and E.coli* (ATCC 8739). The antibacterial activity was performed by cup-plate method. Silver sulphadiazine was used as standard. Nutrient agar was employed as culture medium. DMSO was used as solvent control for antibacterial activity.

RESULTS

A series of complexes have been synthesized between Diallyldisulphide and transition metals at pH 10. Among the different pH used, pH 10 is most favorable for complexation. Complexes of Diallyldisulphide and transition metals are optimized in 2:1 ratio. For silver complex 1:1 ratio is optimized. All the metal complexes are colored, solid and stable towards air and moisture at room temperature. Metal complexes of Diallyldisulphide were analyzed for their elemental composition. Color change of the complexes along with decomposition point greater than 200°C shows characteristic differences between ligand and metal complexes. Color change of the complexes is observed due to decomposition of the complexes above 200°C. Synthesized complexes are soluble in ethanol, methanol and DMSO. Analytical data of the complexes, together with their physical properties are consistent with molecular ion peak are given in Table 1.

Table 1: Analytical data and physical properties of the ligands and complexes

S. No.	Compounds	Color	M.P°C	Yield %	Found (Calc.) % C H S	Mol.wt found (M^+ Peak)
1	$Mn(L)_2Cl_2$	Blue	>300	67	39.62 5.44 38.46	417
2	$Zn(L)_2(NO_3)_2$	Black	>300	76	(39.15) (5.32) (38.42)	480
3	$Cu(L)_2(NO_3)_2$	Black	>300	65	40.27 5.63 35.83	478
4	$Mg(L)_2(NO_3)_2$	White	235-	57	(40.14) (5.43) (35.50)	440
5	$Ag(L)NO_3$	Light	240	62	40.47 5.66 36.02	314
		brown	186-		(40.39) (5.40) (35.60)	
			190		45.49 6.36 40.48	
					(44.97) (6.26) (40.32)	
					28.36 3.97 25.23	
					(28.26) (3.66) (24.89)	

Infrared Spectra

FTIR spectra of the complexes were compared with those of the free ligand to determine the co-ordination sites that may have been involved in the bonding. Characteristic peaks in the spectra of the ligand and complexes were considered and compared [Table 2].

The spectral data confirm the formation of desired product (IR at 3081-3422 cm^{-1} for C-H Stretch, 1623-1634 cm^{-1} for C = C-Stretch and 406-418 cm^{-1} S-S Stretch), further confirmed by the Mass spectra. Complexes show molecular ion peak in good agreement with the empirical formula suggested by elemental analysis [Table 1].

^{1}H NMR spectra of the complexes

The ^{1}H NMR spectra of the complexes were obtained in d_6-DMSO at room temperature using TMS as internal standard. The ^{1}H NMR data is presented in Table 3.

In ligand and its complexes, the proton peak of-CH_2 were not affected by chelation, thus the NMR results further supports the IR inferences.

Table 2: Characteristic IR bands (cm^{-1}) of the ligands and its complexes

Compounds	C-H Stretch	-C = C- Stretch	S-S Stretch
L	3075	1652	579
1	3081	1623	406
2	3012	1634	411
3	3081	1623	413
4	3011	1634	418
5	3022	1630	416

Table 3: The ^{1}H NMR spectra of the complexes in d_6-DMSO (δ,ppm)

Complex	Data
1	3.3 ~ 3.8 (m,8 H,CH_2); 5.24 ~ 5.96 (m,4 H,CH_2); 4.8 ~ 5.13 (m,8 H,CH_2)
2	3.19 ~ 3.51 (m,8 H,CH_2); 5.20 ~ 5.94 (m,4 H,CH_2); 4.7 ~ 5.14 (m,8 H,CH_2)
3	2.8 ~ 3.21 (m,8 H,CH_2); 5.19 ~ 5.92 (m,4 H,CH_2); 4.7 ~ 5.17 (m,8 H,CH_2)
4	2.4 ~ 3.5 (m,8 H,CH_2); 5.19 ~ 5.94 (m,4 H,CH_2); 5.0 ~ 5.16 (m,8 H,CH_2)
5	2.0 ~ 3.4 (m,4 H,CH_2); 5.13 ~ 5.96 (m,2 H,CH_2); 4.8 ~ 5.12(m,4 H,CH_2)

Antibacterial Activity

The synthesized complexes 1-5 were screened for their *in-vitro* antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and E.coli* by measuring the zone of inhibition in mm. The zone of inhibition and MIC of the complexes against above bacterial strains are summarized in Table 4. The results obtained showed that most of complexes possess high to moderate activity. The higher inhibition zone of the metal complexes can be explained on the basis of overtone's concept and chelation theory. On chelation the polarity of the metal ion will be reduced to greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganism.

DISCUSSION

Among the synthesized complexes under investigation, complex 2, 3 and 5 showed significant activity. Complex 5 in particular showed remarkable antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E.coli* which were closer to silver sulphadiazine. The better antibacterial results of the synthesized complex can be attributed due to complexation. The results also indicated that the metal complexes are better antibacterial agents as compared to Diallyldisulphide.

CONCLUSION

Based on the antimicrobial activity of Garlic Diallyldisulphide was taken up as ligand for synthesis of complexes. The corresponding salts used for complexation were silver nitrate, zinc nitrate, manganese chloride, copper nitrate and magnesium nitrate. Complexation reactions between the ligand and the metal ions were carried out at three different pH i.e. acidic (pH 1), neutral (pH 7) and alkaline (pH 10) using three different ratios of metal ligands namely 1:1, 1:2, 1:3, respectively. It was found that complex formation takes place at pH 10. Metal: ligand

Table 4: Antibacterial activity of complexes 1-5

Complexes	*S. aureus*		*E. coli*		*P. aeruginosa*		*K. pneumonia*	
	zone of inhibition	MIC	zone of inhibition	MIC	zone of inhibition	MIC	zone of inhibition	MIC
1	NA	ND	NA	ND	NA	ND	NA	ND
2	18	500	15	500	17	500	19	500
3	13	500	14	200	14	500	14	500
4	NA	ND	NA	ND	NA	ND	NA	ND
5	18	100	16	100	18	100	19	100
Silver sulphadiazine*	17	100	16	100	16	100	16	100

*Silver sulphadiazine used as a referance. Zone of inhibition (mm) and minimum inhibitory concentration (μg/ml) NA–Not active; ND–Not determined

ratio for the complex was 1:1 in case of silver ion and 1:2 in case of other metal ions. The complexes so formed were further subjected to elemental analysis and spectroscopy namely IR, NMR and Mass spectroscopy. There is no report on a complex of Diallyldisulphide and silver. Complex 5 (Diallyldisulphide silver complex) in particular showed remarkable antibacterial activity against selected organisms. Moreover, the incorporation of silver metal into Diallyldisulphide enhances the biological activity of the ligand. Further synthesized silver complex can be formulated into suitable topical formulations.

ACKNOWLEDGMENT

We are thankful to Raj Kumar Goel Institute of Technology, Ghaziabad for providing basic facilities for laboratory work. We also thankful to I.I.T. Delhi for NMR analysis and IPC Ghaziabad for providing facilities for IR, Mass and microbiological studies.

REFERENCES

1. Ernst E. Cardiovascular effects of garlic *(Allium sativum)*: A review. Pharmatherapeutics 1987;5:83-9.
2. Tsao SM, Yin MC. *In-vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oils. J Med Microbiol 2001;50:646-9.
3. Tan JT, Bardwell JC. Key Players Involved in Bacterial Disulfide-Bond Formation. Chem Bio Chem 2004;5:1479-87.
4. Kang YJ. Metallothionein redox cycle and function. Exp Biol Med 2006;231:1459-67.
5. Matsumoto K, Sugiyama H. Organometallic-like CH bond activation and CS bond. Acc Chem Res 2002;35:915-26.
6. Bianchini C, Meli A, Vizza FJ. Role of single-site catalysts in the hydrogenation of thiophenes: From models systems to effective HDS catalyst. Organomet Chem 2004;689:4277-90.
7. Natile G, Bor G. Studies of Differences in Ligand Transfer, Stability, and Fragmentation on Electron-Impact of Some Organosulfur Derivatives of Cobalt and Iron Carbonyls. J Organomet Chem 1972;35:185-93.
8. Roesky HW, Gries T, Jones PG, Weber KL, Sheldrick GM. Synthesis and x-ray structure of tetrakis (diphenyl disulfide or diselenide) disilver bis (hexafluoroarsenate) six-membered silver-sulfur and silver-selenium rings. J Chem Soc Dalton Trans 1984;1781-4.
9. Louvain N, Mercier N, Kurmoo M, Cu^{I}–Br. Oligomers and Polymers Involving Cu–S (cystamine) Bonds. Eur J Inorg Chem 2008;1654-60.
10. Adams RD, Long JW, Perrin JL. A New Route to Dihydrodithiins by a Catalytic Reaction of Vinylthiiranes with W $(CO)_5$(NCMe). J Am Chem Soc 1998;120:1922-8.
11. Adams RD, Perrin JL. Catalytic Transformations of Vinylthiiranes by Tungsten Carbonyl Complexes. A New Route to 3, 6-Dihydro-1, 2-dithiins. J Am Chem Soc 1999;121:3984-91.
12. McKarns PJ, Heeg MJ, Winter CH. Synthesis, Structure, Hydrolysis, and Film Deposition Studies of Complexes of the Formula [NbCl(4)(S(2)R(2))(2)][NbCl(6)]. Inorg Chem 1998;37:4743-7.

How to cite this article: Singh SP, Bajpai M, Razdan BK. Synthesis and study of anti-bacterial activity of complexes of diallyldisulphide from garlic. Int J Green Pharm 2012;6:180-3.

Source of Support: Nil, **Conflict of Interest:** None declared.

Announcement

Android App

A free application to browse and search the journal's content is now available for Android based mobiles and devices. The application provides "Table of Contents" of the latest issues, which are stored on the device for future offline browsing. Internet connection is required to access the back issues and search facility. The application is compatible with all the versions of Android. The application can be downloaded from https://market.android.com/details?id=comm.app.medknow. For suggestions and comments do write back to us.