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EFFECTS OF SOME NOVEL ASCORBIC ACID-METAL COMPLEXES ON SELECTED BACTERIAL AND FUNGAL SPECIES

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Abstract

The antimicrobial activity of some novel ascorbic acid-metal complexes [bisascorbate complexes of copper(II), zinc(II), manganese(II), iron(III), cobalt(II), lead(II) and cadmium(II)] was investigated. Four standard strains of bacterial species - *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsiella pneumonia* and four fungal species - *Trichophyton* sp. *Penicillium* sp., *Aspergillus flavus* and *Aspergillus niger* were used for the investigation. The activities of pure ascorbic acid are compared with the complexes. Generally, percentage inhibition of ascorbic acid on fungal species was the greatest among all compounds tested. The complexes have little or no activity on the bacterial species studied. The latter suggests that ascorbic acid complexation can perhaps be a possible mechanism for the prevention of heavy metal poisoning in biological systems.

Introduction

Ascorbate (Vitamin C) complexes of some transition metal ions have been prepared and satisfactorily characterized [1-4]. Ascorbic acid has been reported to act in a number of ways. It acts as a biological hydrogen carrier for redox enzyme systems in cell metabolism [5, 6], as a food preservative by oxidative rancidity of fatty oily foods or to prevent discoloration of preserved fruits and vegetables [7]. Although ascorbic acid has a wide range of antimicrobial effects, some of its oxidative products are toxic [8, 9, 10].

In 1975, Richardson *et al.* [11] suggested complexation with monodentate thiamine (vitamin B_1) as a possible mechanism for prevention of cadmium, manganese and mercury poisoning, but ascorbic acid being a bidentate ligand may prove a better substitute for thiamine. There are several other areas where complexation might be an important facet to the chemistry of vitamins. Vitamins complexed with metal ions at the active sites are known to function as coenzymes; these include thiamine, biotin, flavin and pyridine nucleotide enzymes [12]. Interest in ascorbic acid has increased greatly because it occurs naturally in many foods. It is an essential nutrient generally recognized as safe.

Recently, platinum(II) complexes of vitamin C have been synthesized and patented as new platinum-based antitumor agents [13]; they have been found to be a better substitute than cis-platin in the treatment of breast, lung and colon cancers.

Relatively few studies have been reported on the application of ascorbic acid-metal complexes [13] in spite of numerous reports in the literature on vitamin-metal complexes [14, 15].

In this paper, we investigated the effect of newly synthesized and characterized bisascorbate complexes of copper(II), zinc(II), manganese(II), iron(III), cobalt(II), lead(II) and cadmium(II) metal ions [1-4] on a range of microorganisms.

Materials and Methods

All chemicals and solvents used were of analytical

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grades and were used without further purification. They were obtained from B.D.H. Ltd., England. The ascorbic acid-metal complexes were prepared by established methods [1-4; 16]. A 4 to 1 mixture of methanol and water (v/v) was used in the preparation of the complexes. 20 mmol of ascorbic acid and 10 mmol of the metal chloride salts were measured, and their solutions separately prepared in 15 ml of solvent mixture. The two solutions were mixed and the pH adjusted to 8.0 using 0.10 molar sodium hydroxide solution. The mixture was then refluxed at 60°C for 6 hours with continuous stirring with a Gallenkamp magnetic stirrer. The interference of dissolved atmospheric oxygen and moisture with the reaction system was prevented by absorption in cotton-wool packed with calcium chloride and alkaline pyrogallol.

The coloured solid compounds formed were filtered, washed thoroughly with methanol-water mixture and dried over silica gel in a desiccator. The solid compounds were purified by re-crystallization in a 4 to 1 mixture of methanol and water (v/v).

The antimicrobial activity of the compounds was investigated using the agar diffusion technique [17]. The antibacterial activity was determined on the seeded nutrient agar (Oxoid) on which 0.9 cm diameter wells were punched. Different concentrations (0.1% and 1.0% w/v) of sterile filtered solutions of the ascorbic acid and the complexes were made using dimethylsulphoxide (DMSO) as solvent, 0.1 ml of each concentration was applied into the wells and incubated at 37°C for one to three days. DMSO was used as control.

The antibacterial activity of the ascorbic acid and the complexes was estimated on the basis of the size of the inhibition zone formed around the wells on the seeded nutrient agar. The bacterial species used for this test included standard strains of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and a clinical isolate of *Klebsiella pneumonia.*

To investigate the antifungal activity of the compounds, known concentrations (0.1% and 1.0% w/v) were incorporated into sabour and maltose agar (Oxoid) using four fungal species viz. *Trichophyton* sp., *Penicillium* sp., *Aspergillus flavus* and *Aspergillus niger,* respectively. The plates were point inoculated. Plates which were not incorporated with any of the compounds but equally inoculated with the respective organisms served as controls. Incubation (in dark) was done at room temperature (25°C - 27°C) for one to three days. Each medium (35 ml) was incorporated with one concentration in each plate for each test. The bacterial and fungal species tend to thrive better in the selected media [17].

The inhibition growth expressed in percentage was determined on the basis of the average diameter of the fungal colony on the growth medium compared to the respective control culture. The average zone of inhibition was determined from the readings taken in triplicate.

Thus, percentage inhibition = $\frac{(C\text{-}T) \times 100}{C}$ where C = average diameter of the fungal growth on the control plate, and T = average diameter of fungal growth on the test plates.

Results and Discussion

Antimicrobial activity of the compounds was tested on the microorganisms using the agar diffusion technique [17]. There was no inhibition on the tested bacterial and fungal species at 0.1% concentration. At 1.0% concentration (w/v) inhibition was observed on the fungal species but not bacterial species. However, ascorbic acid was found to have a very slight activity against *Bacillus subtilis* at 1.0% concentration.

The fungistatic effect of the compounds are presented in Tables 1 and 2. The results show that ascorbic acid caused the greatest inhibition rate (Table 2). However, the inhibitory effects of the complexes varied with type of fungal species. For example, the complexes appeared to have a constant value of 7.1, 23.1 and 20.0% inhibition at the end of two days on *Trichophyton* sp., *Aspergillus flavus* and *Aspergillus niger,* respectively. The inhibitory effect has been attributed to the presence of ascorbic acid in each of the complexes.

The antimicrobial action of ascorbic acid may probably be due to its ability to promote the cyclic reduction of cupric ions which then combine with ion-containing sites within the cell [18] and/or the oxidative products [8-10]. This has also been attributed to some undefined processes involving the cell components [19, 20].

The inhibitory activity of pure ascorbic acid and bisascorbate lead(II) on the fungal species are represented in Figures 1 and 2, respectively. These show that the complexes have maximum average percentage inhibition at the end of two days but start dropping after two days. The two days may be described as the dormant or resting period during which the organisms undergo physiological adaptation to the environment.

Both the ascorbic acid and the complexes were found to have no activity on the *Trichophyton* sp. at the end of three days. Generally, the average percentage inhibition of the ascorbic acid and the complexes on *Aspergillus flavus* and *Aspergillus niger*

Table 1. Fungistatic effect of the compounds: average diameter (cm) of the fungal growth on the control plate (C) and test plate (T) at 1% concentration

	Trichophyton sp.						*Penicillium* sp.						*Aspergillus flavus*						*Aspergillus niger*					
Sample	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	6.0	6.2	6.3	7.0	7.0	7.0	2.9	3.0	5.0	6.0	6.2	7.0	1.9	2.3	3.5	6.0	7.0		1.2	1.5	3.8	5.0	6.0	7.0
2	6.0	6.2	6.5	7.0	7.0	7.0	3.1	3.2	5.2	6.2	6.5	7.0	2.0	2.3	5.0	6.5	7.0		1.4	1.7	3.8	5.0	6.0	7.0
3	6.0	6.2	6.5	7.0	7.0	7.0	3.2	3.4	5.6	6.0	6.7	7.0	2.0	2.3	5.0	6.5	7.0		1.5	1.7	4.0	5.0	6.5	7.0
4	6.0	6.2	6.5	7.0	7.0	7.0	3.4	3.6	5.6	6.1	6.7	7.0	2.0	2.2	5.0	6.5	7.0		1.5	1.7	4.0	5.0	6.5	7.0
5	6.0	6.2	6.5	7.0	7.0	7.0	3.2	3.4	5.6	6.0	6.7	7.0	2.0	2.2	5.0	6.5	7.0		1.5	1.7	4.0	5.0	6.5	7.0
6	6.0	6.2	6.5	7.0	7.0	7.0	3.2	3.4	5.6	6.0	6.5	7.0	2.0	2.2	5.0	6.5	7.0		1.5	1.7	4.0	5.3	6.5	7.0
7	6.0	6.2	6.5	7.0	7.0	7.0	3.2	3.6	5.6	6.0	6.5	7.0	2.0	2.2	5.0	6.5	7.0		1.5	1.7	4.0	5.0	5.0	7.0
8	6.0	6.2	6.5	7.0	7.0	7.0	3.2	3.4	5.6	6.0	6.5	7.0	2.0	2.2	5.0	6.5	7.0		1.5	1.7	4.0	5.0	6.5	7.0
9	6.0	6.2	6.5	7.0	7.0	7.0	3.2	3.6	5.6	6.0	6.5	7.0	2.0	2.2	5.0	6.5	7.0		1.5	1.7	4.0	5.0	6.5	7.0

Table 2. Fungistatic effect of the compounds: average % inhibition at 1% concentration after 1,2, 3 days

Compound	Sample	*Trichophyton*			*Penicillium* sp.			*Aspergillus flavus*			*Aspergillus niger*		
		1 day	2 days	3 days	1 day	2 days	3 days	1 day	2 days	3 days	1 day	2 days	3 days
Pure ascorbic acid	1	3.2	10.0	0	3.3	16.6	11.4	17.4	41.6	14.3	20.0	24.0	14.3
Bis-(ascorbate) cadmium(II)	2	3.2	7.1	0	3.1	16.1	7.1	13.0	23.0	7.1	17.6	24.0	14.3
Bis-(ascorbate) lead(II)	3	3.2	7.1	0	5.8	6.6	4.3	13.0	23.0	7.1	11.8	20.0	7.1
Bis-(ascorbate) diaquo-manganese(II)	4	3.2	7.1	0	5.5	8.2	4.3	9.1	23.1	7.1	11.8	20.0	7.1
Bis-(ascorbate) diaquo-iron(III) chloride	5	3.2	7.1	0	5.8	6.6	4.3	9.1	23.1	7.1	11.8	20.0	7.1
Bis-(ascorbate) diaquo-cobalt(II)	6	3.2	7.1	0	5.8	6.3	7.1	9.1	23.1	7.1	11.8	20.0	7.1
Bis-(ascorbate) diaquo-nickel(II)	7	3.2	7.1	0	11.1	6.6	7.1	9.1	23.1	7.1	11.8	20.0	7.1
Bis-(ascorbate) copper(II)	8	3.2	7.1	0	9.1	6.6	7.1	9.1	23.1	7.1	11.8	20.0	7.1
Bis-(ascorbate) zinc(II)	9	3.2	7.1	0	11.1	6.6	7.1	9.1	23.1	7.1	11.8	20.0	7.1

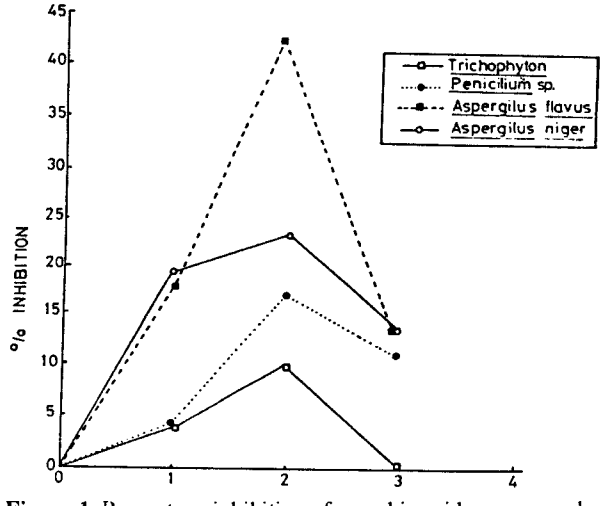

Figure 1. Percentage inhibition of ascorbic acid versus number of days

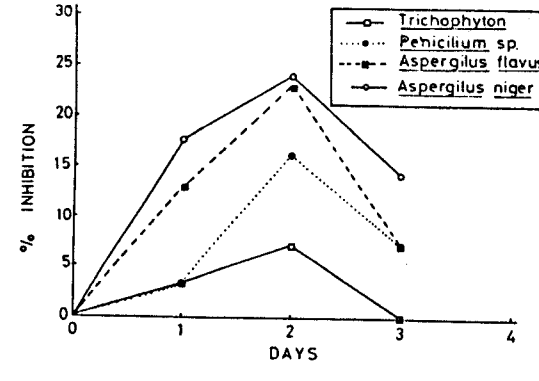

Figure 2. Percentage inhibition of bis (ascorbate) cadmium (II) versus number of days

are greater than those on *Trichophyton* sp. and *Penicillium* sp., respectively. This may be attributed to differences in metabolic rates, absorption rate and constitution of the cell wall of the various fungal species. The low inhibition rate of ascorbic acid and the complexes can be explained by the fact that the effect of the complexes is merely inhibitory as the organisms are resuming growth after prolonged and further incubation as a result of diminishing concentration. This agrees with Eddy *et al.* [21] who described the effect of ascorbic acid as being either stimulatory or inhibitory.

The fact that the complexes show no activity on the bacterial species tested but have very low activity on the fungal species suggests the application of ascorbic acid in the treatment of metal poisoning by cadmium, lead, manganese and nickel. This corroborates with the studies of Richardson *et al.* [11]. This may perhaps be a possible means for prevention of heavy metal poisoning in biological systems.

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