Antibacterial and Antifungal Activity of Metal Derivatives of Salicylates

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Summary: The chelates of 2-hydroxy-, 2-acetoxy-, 2-hydroxy-4-amino-, 2-hydroxy-5-sulphonic-, and 2-hydroxy-3,5-dinitrobenzoic acids synthesized using transition metal cations like Cu^{++}, Ag^{+}, Zn^{++}, Cd^{++}, Hg^{++}, Ni^{++} and UO_2^{++} have been tested for their antibacterial and antifungal activities against *Staphylococcus aureus* [6571(16)NCTC] and *Aspergillus niger* strains of bacteria by cylinder plate diffusion method. Some of these metal derivatives were also tested against two strains of bacteria: *Staphylococcus aureus* (ATCC, 6538) and *Escherichia coli* (ATCC 8739) by agar plate diffusion method. Out of all the metal chelates investigated only mercury complexes of the salicylates were found to possess worthwhile antibacterial and antifungal activities. Moreover, the mercurial derivatives of these salicylates proved better antifungal agents as compared with their antibacterial behaviour against the strain tested. The antifungal activities of cadmium salicylates were observed to be lower than those of the corresponding mercury complexes.

Introduction

The literature survey reveals that considerable work has been carried out on the pharmacological and other medicinal/cosmetics aspects of salicylates [1-34]. Their metal derivatives have not been thoroughly investigated for their biological activity. However, copper salicylates have been patented as disinfectants [35]. Antimicrobial activity of mixed ligend complexes of Cu(II) has also been reported [36]. Moreover, ternary complexes of Zn(II) have been claimed as more toxic agents against various organisms than the binary zinc salicylates [37]. Therefore, metal complexes of nickel, copper, zinc, mercury, cadmium, silver and uranyl cations have been synthesized for 2-hydroxy-, 2-acetoxy-, 2- hydroxy-4-amino-, 2-hydroxy-5-sulphonic-, and 2-hydroxy-3,5-dinitrobenzoic acids for the investi-gation of their activity as antibacterial and antifungal agents.

Results and Discussion

Antibacterial activity

The antibacterial activity exhibited by 2-hydroxybenzoic acid (sod. salt) and its six derivatives has been investigated against *Staphylococcus aureus* by the cylinder plate diffusion method. Results are reported in Table 1. The mercury derivative of 2-hydroxybenzoic acid (sod. salt) exhibited the maximum activity followed by silver, copper and cadmium derivatives. Activity of the uranyl derivative of the salicylate was also assayed by the agar plate method both for *Staphylococcus aureus* and *Escherichia coli* (Table 6). These metals, with the exception of the uranyl group, generally enhance antibacterial activity in comparison with that of the original compounds.

The antibacterial activity shown by 2-acetoxybenzoic acid and its six derivatives against *Staphylococcus aureus* is indicated in Table 2. It may be seen that, as compared with the parent compound, the mercury derivative exerted greater antibacterial activity followed by the nickel derivative. Its nickel and zinc derivatives also indicated bacteriostatic behaviour while cadmium derivative was only bacteriostatic. The antibacterial activity of silver and mercury derivatives of aspirin against *Staphylococcus aureus* and *Escherichia coli* was also assayed by the agar plate method and results are shown in Table 6. It is clear that the activity of these compounds against *Staphylococcus aureus* was quite comparable with the results

Table1: Antibacterial activity of cylinder plate diffusion method exhibited by 2-hydroxybenzoic acid and its metal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Hydroxybenzoic acid	20 mg/mL	0.5 mL	25[x]
$(C_7H_5O_3)Ag(H_2O)$	"	"	30[x]
$(C_7H_5O_3)_2Hg(H_2O)1$ x 1/2	"	"	45[x]
$C_7H_5O_3)_2Cd(H_2O)_3$	"	"	26[x]
$C_7H_5O_3)_2Zn(H_2O)_7$	"	"	

x = Bacteriocidal

Table-2: Antibacterial activity by cylinder plate diffusion method exhibited by 2-acetoxybenzoic acid and its metal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Acetoxybenzoic acid	20 mg/1.0 mL	0.5 mL	22[x]
$(C_9H_7O_4)_2Cu(H_2O)_2$	"	"	16[x]
$(C_9H_7O_4)Ag(H_2O)_2$	"	"	16[x]
$(C_9H_7O_4)_2Zn(H_2O)_3$	"	"	22[xx]
$(C_9H_7O_4)_2Cd(H_2O)_3$	"	"	12[xx]
$(C_9H_7O_4)_2Hg(H_2O)_7$	"	"	28[x]
$(C_9H_7O_4)_2Ni(H_2O)_2$	"	"	23[x],26[xx]

xx = Bacteriostatic,
x = Bacteriocidal

Table-3: Antibacterial activity by cylinder plate diffusion method exhibited by 2-hydroxy-4-aminobenzoic acid (sod. salt) and its metal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inihibition zone DIA (mm)
2-Hydroxy-4-amino benzoic acid	20 mg/1.0 mL	0.5 mL	20[x]
$(C_7H_6O_3N)_2Cu(H_2O)$	"	"	20[x], 26[xx]
$(C_7H_6O_3N)Ag(H_2O)$	"	"	15[x]
$(C_7H_6O_3N)_2Zn(H_2O)_4$	"	"	22[xx]
$(C_7H_6O_3N)_2Cd(H_2O)_4$	"	"	27[xx]
$(C_7H_6O_3N)_2Hg(H_2O)_3$	"	"	29[x]
$(C_7H_6O_3N)_2Ni(H_2O)_2$	"	"	26[x]

x = Bacteriocidal, xx = Bacteriostatic

Table-4:Antibacterial activity by cylinder plate diffusion method exhibited by 2-hydroxy-5-sulphonicbenzoic acid and its metal derivatives.

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inhibition one DIA (mm)
2-Hydroxy-5-sulphobenzic acid	20 mg/1.0mL	0.05 mL	32[x]
$(C_7H_4O_6SK)_2Cu(H_2O)_2$	"	"	16[x]
$(C_7H_4O_6SK)Ag(H_2O)$	"	"	12x
$(C_7H_4O_6SK)_2Cd(H_2O)$	"	"	22[xx]
$(C_7H_4O_6SK)_2Hg(H_2O)_2$	"	"	40[x]
$(C_7H_4O_6SK)_2Ni(H_2O)_4$	"	"	25[xx]

x = Bacteriocidal, xx = Bacteriostatic

obtained by the cylinder plate diffusion method as shown in Table 2. Antibacterial activity shown by 2-hydroxy-4-aminobenzoic acid (sod. salt) and six derivatives against *Staphylococcus aureus* are listed in Table 3. In this case the mercury and nickel derivatives of this salicylate indicated significant activity, followed by the copper derivative. The other derivative did not demonstrate much difference over the activity of the uncomplexed salicylate. In addition, Cd and Zn derivatives showed bacteriostatic activity. In addition, Cd and Zn derivatives showed bacteriostatic activity, while the Cu derivative indicated both bacteriostatic and bacteriocidal activity. It is worth mentioning that antimicrobial activity was investigated again for the silver and mercury derivatives against *Staphylococcus aureus* and *Escherichia coli* by the agar plate method. The results are given in Table 6. Its uranyl derivative did not show any activity.

The antibacterial activity shown by 2-hydroxy-5-sulphonicbenzoic acid and its five complexes against *Staphylococcus aureus* is given in Table 4. It is again noted that its mercury derivative showed pronounced activity in comparison with the parent compound, while nickel and cadmium derivatives were still bacteriocidal, their activity was found to have been reduced on complexation.

Both the cylinder plate diffusion and the agar plate methods failed to indicate any activity for the silver and uranyl derivatives (Table 4-6). Similarly, antibacterial activity of 2-hydroxy-3,5-dinitrobenzoic acid and its six metal derivatives are shown in Table 5. It is again evident that only its mercury derivative exhibited high activity in comparison with the parent compound, while the other derivatives were generally inactive with the exception of the copper derivative. In the latter case the activity was also less than that of the parent compound. Moreover, silver and uranyl derivatives when tested by the agar plate method against *Staphylococcus aureus* and *Escherichia coli*, did not indicate much activity (Table 6).

Table-5: Antibacterial activity by cylinder plate diffusion method exhibited by 2-hydroxy-3,5-dinitrobenzoic acid and its metal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Hydroxy-3,5-di nitrobenzoic acid	20 mg/1.0 mL	0.5 mL	35x
$(C_7H_2O_7N_2)_2Cu(H_2O)_2$	"	"	30^x
$(C_7H_2O_7N_2)Ag(H_2O)$	"	"	20^x
$(C_7H_2O_7N_2)_2Cd(H_2O)_2$	"	"	18^x
$(C_7H_2O_7N_2)_2Hg(H_2O)$	"	"	40^x
$(C_7H_2O_7N_2)_2Ni(H_2O)_2$	"	"	19^x

x = Bacteriocidal

Table-6: Antibacterial activity by agar plate method

Name of the compound	Inhibition zone *S. aureus* (ATCC, 6338)	DIA (mm) *E.coli* (ATCC, 8739)
$(C_7H_5O_3)Ag(H_2O)$	13.4	13.0
$(C_9H_7O_4)Ag(H_2O)$	14.6	12.0
$(C_7H_6O_3N)Ag(H_2O)$	13.9	10.0
$(C_7H_4O_6SK)Ag(H_2O)$	10.0	10.0
$(C_7H_2O_7N_2)Ag(H_2O)$	17.8	09.0
$(C_7H_5O_3)_2Hg(H_2O_2)1x1/2$	24.0	14.0
$(C_9H_7O_4)_2Hg(H_2O)_7$	28.0	22.0
$(C_7H_6O_3N)_2Hg(H_2O)_3$	22.0	12.0
$(C_7H_4O_6SK)_2Hg(H_2O)_2$	24.0	15.0
$(C_7H_2O_7N_2)_2Hg(H_2O)_2$	24.0	18.0
$(C_7H_5O_3)_2UO_2(H_2O)_2$	07.0	06.0
$(C_7H_6O_3N)_2UO_2(H_2O)_2$	00.0	00.0
$(C_7H_4SO_6)_2UO_2(H_2O)$	10.0	06.0
$(C_7H_2O_7N_2)_2UO_2(H_2O)_2$	05.0	0.00

Table-7: Antifungal activity by cylinder plate diffusion method exhibited by 2-hydroxybenzoic acid (sod. salt) and its metal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assy	Inhibition zone DIA (mm)
2-hydroxybenzoic acid	20 mg/1 mL	0.5 mL	25^x
$(C_7H_5O_3)_2Cu(H_2O)_2$	"	"	22^x
$(C_7H_5O_3)Ag(H_2O)$	"	"	30^x
$(C_7H_5O_3)_2Zn(H_2O)_7$	"	"	21^x
$(C_7H_5O_3)_2Cd(H_2O)_3$	"	"	32^x
$(C_7H_5O_3)_2Hg(H_2O)1x1/2$	"	"	60^x

x = Fungicidal

Table-8: Antifungal activity by cylinder plate diffusion method exhibited by 2-acetoxybenzoic acid and its medtal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Acetoxybenzoic acid	20 mg/mL	Assay 0.5 mL.	35^x
$(C_9H_7O_4)_2Cu(H_2O)_2$	"	"	20^x
$(C_9H_7O_4)Ag(H_2O)_2$	"	"	22^x
$(C_9H_7O_4)_2Zn(H_2O)_2$	"	"	18^x
$(C_9H_7O_4)_2Cd(H_2O)_3$	"	"	$25^x, 30^{xx}$
$(C_9H_7O_4)_2Hg(H_2O)7$	"	"	45^x
$(C_9H_7O_3)_2Ni(H_2O)$	"	"	18^x

x = Fungicidal, xx = Fungistatic

Antifungal activity

The antifungal activity has been assayed against *Aspergillus niger* for all the compounds tested. In the case of 2- hydroxybenzoic acid (sod. salt) and its metal derivatives, this activity has been reported in Table 7. It may be inferred from these results that the activity was improved in case of the mercury derivative, followed by cadmium and silver derivatives. Copper and zinc derivatives were less active as compared with the unchelated ligend. The antifungal activity shown by 2- acetoxybenzoic acid and its six derivatives is given in Table 8. The maximum activity is shown again by the mercury derivative and is quite high as compared with that of the original compound. On the other hand, the remaining derivatives were found to be less active as compared with the original compound. However, cadmium complex behaved as a fungiostatic.

The antifungal activity demonstrated by 2-hydroxy-4-aminobenzoic acid (sod. salt) and its six derivatives is shown in Table 9. It is to be noted that as compared with the uncomplexed ligend, only the activity of its mercury derivative indicated improvement and was almost equal to that of the cadmium derivative, which was fungiostatic as well. The antifungal activity of 2-hydroxy-5-sulphonicbenzoic acid and its metal derivatives is shown in Table 10. Here, its mercury derivative showed considerably higher potential than the parent molecule. In case of 2-hydroxy-3, 5-dinitro-benzoic acid and its metal derivatives, the activity is shown in Table 11. It is obvious that on comparison with the uncomplexed ligend, only its mercury derivatives showed improved activity.

One may observe that in the entire spectrum of metal cations tried for complexation with different salicyclate, only mercury complexes of all salicylates showed greater antibacterial and anti-fungal activity than the uncomplexed salicylate. The descending order of bacteriocidal activity of mercury derivatives of salicylates was 2-hydroxy-benzoic acid > 2-hydroxy-5-sulphonic benzoic acid > 2-hydroxy-3,5-dinitrobenzoic acid > 2-hydroxy-4-aminobenzoic acid and > 2-acetoxy-benzoic acid. Similarly, the order of antifungal activity exhibited by mercurated salicylates was 2-hydroxybenzoic acid (sod. salt) > 2-hydroxy-3,5-dinitrobenzoic acid > 2-acetoxybenzoic acid > 2-hydroxy-4-amino-

Table-9: Antifungal activity by cylinder plate diffusion method exhibited by 2-hydroxy-4-aminobenzoic acid (sod.salt) and its metal derivatives

Name of the compound	Concentration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Hydroxy-4-amino-benzonic acid (sod. salt)	20 mg/1 mL	0.5 mL	45[x]
$(C_7H_6O_3N)_2Cu(H_2O)$	"	"	22[x]
$(C_7H_6O_3N)Ag$	"	"	18[x]
$(C_7H_6O_3N)_2Zn(H_2O)_4$	"	"	18[x]
$(C_7H_6O_3N)_2Cd(H_2O)_4$	"	"	44[x],45[xx]
$(C_7H_6O_3N)_2Hg(H_2O)_3$	"	"	45[x]
$(C_7H_6O_3N)_2Ni(H_2O)_2$	"	"	19[x]

x = Fungicidal, xx = Fungistatic

Table-10: Antifungal activity by cylinder plate diffusion method exhibited by 2-hydroxy-5-sulphonicbenzoic acid and its metal derivatives.

Name of the compound	Concen-tration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Hydroxy-4-sulpho-benzoic acid (Pot. salt)	20 mg/1 mL of	0.5 mL	35[x]
$(C_7H_4O_6SK)Cu(H_2O)_2$	"	"	23[x]
$(C_7H_4O_6SK)_2Zn(H_2O)$	"	"	18[x]
$(C_7H_4O_6SK)_2Cd(H_2O)$	"	"	32[x]
$(C_7H_4O_6SK)_2Hg(H_2O)_2$	"	"	48[x]
$(C_7H_4O_6SK)Cd(H_2O)_2$	"	"	20[x]

x = Fungicidal

Table-11: Antifungal activity by cylinder plate diffusion method exhibited 2-hydroxy-3,5-dinitrobenzoic acid and its metal derivatgives

Name of the compound	Concen-tration mg/mL of DMF	Volume used for the assay	Inhibition zone DIA (mm)
2-Hydroxy-3,5-dinitrobenzoic acid	20 mg/1 mL	0.5 mL	40[x]
$(C_7H_2O_7N_2)_2Cu(H_2O)_2$	"	"	30[x]
$(C_7H_2O_7N_2)Ag$	"	"	18[x]
$(C_7H_2O_7N_2)_2Zn(H_2O)2$	"	"	14[x]
$(C_7H_2O_7N_2)_2Cd(H_2O)_2$	"	"	25[x]
$(C_7H_2O_7N_2)_2Hg(H_2O)_2$	"	"	50[x]
$(C_7H_2O_7N_2)_2Ni(H_2O)2$	"	"	14[x]

x = Fungicidal

benzoic acid (sod. salt). The mercurial derivatives of these salicylates were better antifungal agent as compared with their antibacterial behaviour against the strain tested. After mercury, cadmium complexes were also found to be antifungal.

Experimental

Antibacterial and antifungal activities of 2-hydroxy-, 2-acetoxy-, 2-hydroxy-4-amino-, 2-hydroxy-5-sulphonic-, and 2-hydroxy-3, 5-dinitro-benzoic acids and their metal derivatives reported here have been determined following the method of Malik *et al.*, 1980 [38]. The synthesis of these metal salicylates along with their analytical and other related .data has been taken from recent research work [39] and is being published separately. However, a brief account of the synthetic procedure is given here.

In this connection aqueous solutions of sodium salts of respective aromatic acids and different salts in appropriate molar amounts were mixed together with stirring for about one hour. The products formed were filtered off, washed several times with hot water to remove unreacted materials and finally washed with alcohol. Different products obtained were usually kept under vacuum for 48 hours and characterized by their infrared spectra and elemental analysis. Following this general procedure 2- hydroxy-, 2-acetoxy-, 2-hydroxy-4-amino-, 2-hydroxy-5-sulphonic-, and 2-hydroxy-3,5-dinitrobenzoic acids on reaction with copper(II) chloride, silver(I) nitrate, zinc(II) chloride, cadmium(II) chloride, mercury(II) acetate, nickel(II) acetate and uranyl nitrate afforded respective metal salicylates in fairly good yield. After necessary purification, these chelates were used in the present investigations.

Assay for antibacterial activity by the cylinder plate diffusion method

Cylinder plate diffusion method was used to test the bacteriostatic activity [40]. Molten nutrient agar (22.0 mL) Bacto beef extract, 0.3%; Bactopeptone, 0.5%; sodium chloride, 0.5 %; Bactogar, 2.0 %) was poured to petri plate (100 mm dia) and allowed to harden as a base layer. Then 5.0 mL. of nutrient agar kept at 45°C was seeded with the test organism, *Staphylococcus aureus* (6571(16) NCTC, obtained from Central Public Health Laboratory, Colindale Avenue (London). This was poured and spread uniformly over the base layer. After solidification of the seeded layer, three stainless steel cylinders (6 x 10 mm) were held firmly onto the agar surface. The cylinders filled with test fluid bacterial plates were incubated at 37° for 24 hours. The results are shown in Table 1-5. The values given are the means of quadruplicate determinations.

Assay for antibacterial activity by the agar plate method

Some of the complexes were tested by the agar plate method by placing filter paper disc (6.0 mm in dia.) in the centre of the plates. The disc was treated with a solution of 5.0 mg of complex in 2.5 mL of aqueous DMSO. The plates were then

inoculated with growing cultures of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739), and organisms were incubated at 37°C for 24-48 hours. Inocula from the cultures were used without dilution. Trypticase soy agar was used for the plates. The zone of inhibition around the discs was then measured; the measurements include the 6.0 mm of the discs. A compound may be considered quite active if the zone of inhibition is between 20 mm and 30 mm. A zone of inhibition of less than 20 mm is indicative of slight activity. The results are shown in Table 6.

Assay for antifungal activity by the cylinder plate diffusion method

Cylinder plate diffusion method was used to test the bioactivity, Molten nutrient agar (22.0 mL) (Bacto beef extract, 0.03 %; Bactopeptone, 0.5 %; Sodium chloride, 0.5 %, Bactoagar, 2.0 %) was poured into a petri plate (100 mm dia) and allowed to harden as a base layer, then 5.0 mL of nutrient agar kept at 45°C was seeded with the test organism. *Aspergillus niger* (isolated from a different source at NIAB, Faisalabad) was poured and spread uniformly over the base layer. After solidification of the seeded layer, three stainless steel cylinders (6 x 10 mm) were dropped over the seeded agar so that the cylinders were held firmly onto the agar surface. The cylinders were filled with test fluids. Fungal plates were incubated at 28°C for 72 hours. The results are shown in Table 7-11. The values indicated are the means of quadruplicate determinations.

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