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Biological Evaluation of Some Acylated Derivatives of D-mannose

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Abstract: A number of D-mannose derivatives were evaluated for *in vitro* antibacterial activity against eleven human pathogenic bacteria. These compounds were also screened for *in vitro* antifungal activity against six plant pathogenic fungi. It was observed from the study that the tested acylated derivatives of D-mannose exhibited moderate to good antibacterial and antifungal activities. The acylated D-mannose derivatives were found to be more effective against fungal phytopathogens than those of the bacterial strains. For comparative study, the biological activity of two standard antibiotics (Ampicillin and Nystatin) were also measured. It was found that the antimicrobial functionality of D-mannose increased with the introduction of various acyl substituents and in some cases, this functionality was greater than or comparable to the antibiotics studied.

Key words: Antibacterial activity, antifungal activity, standard antibiotic, food poisoned technique, basal medium, incubation, mycelial growth, micro-organism, inhibition, stimulation

INTRODUCTION

Over the years, works have been done in the field of antimicrobial screening studies of chemical compounds[1]. Acylated glycosides were considered in this field as very important test chemicals due to their effective biological activity[2]. Literature survey revealed that a large number of biologically active compounds possess aromatic, heteroaromatic and acyl substituents[3]. It is also known that if an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity[3]. The benzene and substituted benzene nuclei play important role as common denominator for various biological activities. Results of an on-going research work on selective acylation of monosaccharides[4-6] and also evaluation of antimicrobial activities[7,8] it was observed that in many cases the combination of two or more aromatic and acyl substituents enhanced the biological activity many fold than its parent nuclei. Guided by these observations, we synthesized some acylated derivatives of D-mannose containing various substituents in a single molecular framework. The antibacterial activities of these products were carried out using a number of human pathogenic bacteria. Antifungal activities of these derivatives were also measured against a number of plant pathogenic fungi. Present study reported here the results of the antimicrobial screening.

MATERIALS AND METHODS

The test tube cultures of the bacterial and fungal pathogens were collected from the Microbiology Laboratory, Department of Microbiology, University of Chittagong and are listed below:

Bacterial cultures

Gram-positive bacteria: i) *Bacillus cereus* BTCC 19, ii) *Bacillus subtilis* BTCC 17, iii) *Staphylococcus aureus* BTCC 43 and iv) *Bacillus megaterium* BTCC 18,

Gram-negative bacteria: v) *Escherichia coli* BTCC 12, vi) *Vibrio cholerae* CRL (ICDDR, B), vii) *Salmonella typhi* AE 14612, viii) *Salmonella paratyphi*-A CRL (ICDDR, B), ix) *Pseudomonas species* CRL (ICDDR, B), x) *Shigella sonnei* CRL (ICDDR, B) and xi) *Shigella dysenteriae* AE 14396.

Fungal cultures: I) *Colletotrichum corchori* (Ikata and yoshida), ii) *Fusarium equiseti* (corda) Sacc, iii) *Alternaria alternata* (Savulescu and Sandu ville), iv) *Curvularia lunata* (wakker boedijin), v) *Botryodiplodia theobromae* (pat) and vi) *Macrophomina phaseolina* (Maubi) ashby.

Used test chemicals: Some partially protected derivatives of D-mannose (1-10) (Fig. 1) were used as test chemicals.

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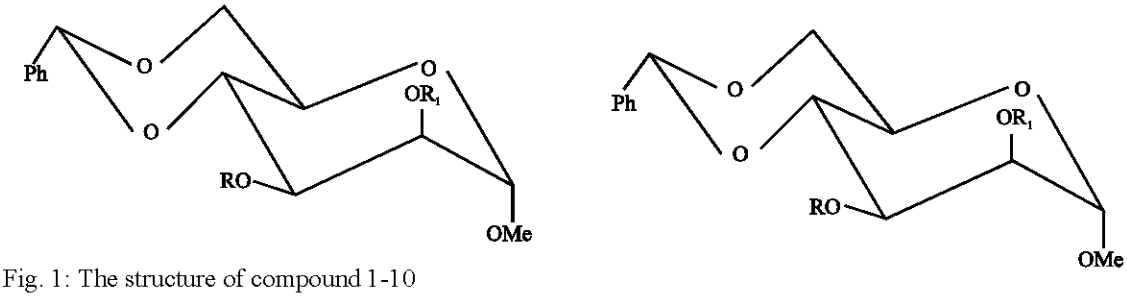

Fig. 1: The structure of compound 1-10

1: R=R_1=H 2: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=H 3: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=Ac
4: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=Ms 5: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=Bz 6: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=Pv
7: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=4-$CH_3C_6H_4CO$- 8: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=4-$NO_2C_6H_4CO$- 9: R=R_1 =3,5$(NO_2)_2C_6H_3CO$-;
10: R=3,5$(NO_2)_2C_6H_3CO$-; R_1=2-$COOHC_6H_4CO$-

The chemicals were synthesized, isolated, purified and characterized in the Organic Research Laboratory, Department of Chemistry, University of Chittagong and reported earlier[9] In all the cases, a 1% solution (w/v) in chloroform of the chemicals were used.

Preparation of Nutrient Agar (NA) medium, stock culture, bacterial suspension and preservation of stock culture: Three gram of beef extract, 5 g of peptone and 15 g of agar were taken in a beaker and then distilled water (1000 mL) was added. The mixture was boiled and mixed thoroughly with a glass rod After complete dissolution of agar, the medium was dispensed into several conical flasks of 250 mL volume. The conical flasks were closed with cotton plug and rapped with aluminum foil. Then the medium was autoclaved for 15 min at 121°C and 15 psi. After autoclaving, the medium was used for culturing different micro-organisms.

In a hard glass screw cap test tube, sterile slants of nutrient agar (NA) were prepared. Old cultures from Microbiology Laboratory were transferred to the freshly prepared NA slants separately for each species with the help of sterilized bacterial loop In such a way, four test tubes were freshly prepared for each bacterial pathogen. These test tubes of inoculated slants were incubated at (35±2)°C in an incubator. Two days-old culture was used for antibacterial screening.

For preservation of the stock culture, one set of culture slants were kept in polythene bag, properly tied and preserved as stock culture at 10°C Occasional sub-culture (3 to 4 weeks, intervals) was maintained to keep the culture in active condition with character unimpaired.

About 10 mL of distilled water was taken in a clean screw cap test tube. A number of test tubes with water were sterilized in an autoclave. From two days-old bacterial culture, one loop of bacterial culture was transferred to the sterilized distilled water and mixed it properly These bacterial suspensions of the test tube were used to the pour plate during sensitivity test.

Antibacterial activity test: The antibacterial activities of the synthesized chemicals were detected by disc diffusion method[10] as described below:

Paper discs of 4 mm in diameter and glass petriplate of 90 mm in diameter were used throughout the experiment Paper discs were sterilized in an autoclave and dried at 100°C in an oven. Then the discs were soaked with test chemicals at the rate of 20 μg (dry weight) per disc for antibacterial analysis. One drop of bacterial suspension was taken in a sterile petridish and then approximately 20 mL of sterilized melted NA (~45°C) was poured into the plate, then mixed thoroughly with the direction of clockwise and anticlockwise. After solidification of the seeded NA medium, paper disc after soaking with test chemicals (1% in chloroform) were placed at the centre of the inoculated pour plate. A control plate was also maintained in each case with chloroform. Firstly, the plates were kept for 4 h at low temperature (4°C) and the test chemicals diffused from disc to the surrounding medium by this time.

The plates were then incubated at (35±2)°C for growth of test organisms and were observed at 24 h interval for two days. The activity was expressed in terms of inhibition zone diameter in mm each experiment was repeated thrice. The standard antibiotic, Ampicillin, was used as a positive control and compared with tested chemicals under identical conditions.

Evaluation of chemicals against fungi: The antifungal activities of the synthesized carbohydrate derivatives (1-10) were investigated against six plant pathogenic fungi. The investigation was based on food poisoned technique[11] and the technique in some modified condition[12].

Potato Dextrose Agar (PDA) was used as basal medium for test fungi. Chloroform was used as a solvent to prepare the desired solution (1%) of the compounds initially. Proper control was maintained with chloroform.

Preparation of the medium: Two hundred gram of sliced potato was boiled in distilled water (300 mL). After proper boiling, the extract was decanted and was transferred into a 1000 mL beaker and the solution was made upto the mark with distilled water. Then the solution was taken in a pot when 20 g of dextrose and 16 g of agar was added slowly to the solution with gentle heating and stirring with a glass rod. The medium was boiled for 15 min and then transferred into four 250 mL conical flasks. The conical flasks were closed with cotton plug. Then the medium in the conical flasks were autoclaved for half an hour at 120°C and 15 psi. The sterilized medium was then used for culturing different micro-organisms under investigation.

Maintenance of fungal cultures: Test tube slants of PDA medium were prepared for the maintenance of cultures. Small portions of mycelia of the test pathogens were transferred to the test tubes separately from old cultures with the help of sterilized needles. A number of test tubes were freshly prepared for each fungal pathogen. The inoculated slants were incubated at room temperature under laboratory condition and 4 to 6 days-old cultures were used for antifungal screening.

Preparation of cultures: A number of glass petridishes were cleaned and sterilized in an autoclave. Then sterilized and melted (~45°C) PDA was poured into each plate at the rate of 10-12 mL After a few minutes the medium solidified. Then small portions of mycelium of each fungal pathogen were placed at the centre of each PDA plate with the help of sterilized needles. In such a way, each fungal species was transferred into a number of petri plates. After a few days, the mycelium grow in the whole petriplate and were ready for the antifungal activity tests of the synthesized chemicals.

Mycelial growth test: A required amount of medium (PDA) was taken in conical flasks separately and was sterilized in autoclave (at 121°C and 15 psi) for 15 min. After autoclaving, calculated amount of test chemical (1%) was added to the sterilized medium in conical flask and the flask was shaken thoroughly to mix the chemical with the medium before pouring. The medium with definite concentration (1%) of chemical was then poured at the rate of 10 μL in sterilized glass pertridishes individually Proper control was maintained separately with sterilized PDA medium without chemical and three replications were prepared for each treatment. After solidification of medium, the fungal inoculum (5 mm mycelial block) was placed at the centre of the petri plates at inverted position.

All the plates were incubated at room temperature on the laboratory desk for three days. The inoculated plates were incubated at (25±2)°C. The experiment was replicated three times. After three to five days of incubation, the diameter of fungal mycelial growth were measured. The average of two measurement was taken as mycelial colony diameter of the fungus in mm. The percentage inhibition of mycelial growth of the test fungus was calculated by a formula given below:

$$I = \left(\frac{C - T}{C} \right) \times 100$$

Where, I = Percentage of inhibition
C = Diameter of the fungal colony in control ($CHCl_3$)
T = Diameter of the fungal colony in treatment

The antifungal results were compared with that of the standard antibiotic, Nystatin.

RESULTS AND DISCUSSION

The test chemicals (1-10) in the present investigation contained a variety of functional groups such as methyl, phenyl, acetyl, mesyl, benzoyl, nitro, pivaloyl and carboxyl groups. The test chemicals (2-10) were prepared from a common precursor namely, methyl 4, 6-O-benzylidene-α-D-mannopyranoside (1)[9]. We deliberatly introduced the above mentioned functional groups into the D-mannose molecule in order to evaluate their effectiveness against a number of human pathogenic bacteria and plant pathogenic fungi. For comparison, the antimicrobial activity of the precursor diol (1) and two antibiotics, Ampicillin and Nystatin, were also measured.

The results of antibacterial activity studies of the selected test chemicals (1-10) are presented in Table 1 and 2. In case of gram positive organisms, most of the test chemicals were found to be active against *Bacillus subtilis* and *Bacillus megaterium* against *Bacillus cereus* and *Staphylococcus aureus*, a number of chemicals showed some activity and the rest showed no activity. However, compound 10 showed high activity, almost comparable to ampicillin, against the four gram-positive bacterial strains studied. In case of gram-negative organism, maximum test chemicals showed moderate to good inhibition and some of the chemicals were unable to show inhibition against the test bacterial pathogens. Interestingly, compound 10 was again found to be very effective against all the seven gram-negative bacterial

Table 1: Antibacterial screening studies against some gram-positive bacteria. Diameter of zone of inhibition in mm sample 20 µg dw/disc

Compound no.	*B. cereus*	*B. subtilis*	*S. tapaureus*	*B. megaterium*
1	-	-	-	-
2	7	*10	10	9
3	-	-	-	6
4	-	-	-	-
5	-	*11	7	8
6	6	6	-	6
7	-	6	-	-
8	7	-	10	8
9	-	-	-	6
10	*14	*15	*15	*12
**Ampicillin (20 µg dw/disc)	16	16	20	15

Table 2: Antibacterial screening studies against some gram-negative bacteria. Diameter of zone of inhibition in mm, sample 20 µg dw/disc

Compound no.	*E. coli*	*V. cholerae*	*S. typhi*	*S. paratyphi*	*P. seudomonus* species	*S. sonnei*	*S. dysenterei*
1	-	-	-	-	7	-	-
2	9	*17	7	8	9	8	8
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5	6	6	8	7	-	*12	6
6	8	7	6	6	-	7	-
7	-	7	-	-	-	6	-
8	*12	-	8	7	*12	*11	6
9	-	-	-	-	-	-	-
10	*11	*12	*15	*9	*11	*17	*11
**Ampicillin (20 µg dw/disc)	28	24	25	12	19	24	13

Table 3: Percent inhibition of fungal mycelial growth, sample 100 µg dw/mL PDA

Sample	*C. corchori*	*F. equiseti*	*A. alternata*	*C. lunata*	*B. theobromae*	*M. phaseolina*
1	2308	164	800	+909	-	769
2	2157	1667	1800	2457	2807	*3429
3	588	*4167	+200	351	1228	1143
4	-	-	+455	1000	2222	2000
5	1373	500	1200	2457	3333	2143
6	1176	833	-	700	2982	1571
7	*3725	2500	1200	3158	2982	1857
8	784	*3333	200	1404	+351	1000
9	784	1333	400	1930	-	2143
10	*4118	*6333	*4200	*4386	3860	3000
**Nystatin 100 µg dw/disc	4100	4500	5100	7000	7000	7600

NB "*"=Marked inhibition; "** "= Standard antibiotic "-"=No inhibition; "dw"=Dry weight, "+"= Stimulation

strains studied. From the antibacterial evaluation data presented in Table 1 and 2, it was observed that most of these D-mannose derivatives were found reasonably effective against the tested bacterial pathogens. This is the first antibacterial activity test report of our newly synthesized D-mannose derivatives against the selected human pathogenic bacteria. It is quite encouraging to find that some of these derivatives have shown reasonable inhibition, comparable to that of the standard antibiotic, Ampicillin.

The results of the percentage inhibition of mycelial growth due to treatment of test chemicals is shown in Table 3. The overall results indicated that chemical 10 was the most effective against all the organisms studied Chemical 10, however, showed the highest inhibition (63.33%) against *Fusarium equiseti*, which was higher than the standard antibiotic, Nystatin (45.00%). Most of the test chemicals showed moderate to good inhibition against the test fungi and some of the chemicals were unable to show inhibition against the test fungal pathogens. It was quite interesting to notice that some chemicals exhibited stimulation of radial growth rather than inhibition against the selected pathogenic fungi. Our newly synthesized test chemicals have not been tested before against the selected fungal pathogens. An important observation was that these acylated D-mannose derivatives were more effective against fungal pathogens than those of bacterial organisms. Again, these chemicals were more active against gram-positive organisms than those of gram-negative organisms. In general, a major number of the acylated D-mannose derivatives showed more inhibition than that of their precursor compound 1. That is, with the introduction of various acyl substituents, antimicrobial functionality of mannopyranoside derivatives increased and in some cases, this functionality was greater than or comparable to the standard antibiotics

employed. Thus, a comparative study of antimicrobial evaluation of chemical (1) and its acylated derivatives (2-10) was successfully carried out against a variety of human pathogenic bacteria and plant pathogenic fungi. The results of this investigation may create an opportunity for further evaluation of these test chemicals against other micro-organisms. It is also expected that this piece of work employing monosaccharide derivatives as test chemicals will help further work on the development of pesticides and medicines for human disease control.

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