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ANTIMICROBIAL EVALUATION OF METHYL 4-*O*-ACETYL-α-L-RHAMNOPYRANOSIDE DERIVATIVES

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ABSTRACT

A number of 2,3-di-*O*-acyl derivatives (6-11) of methyl 4-*O*-acetyl-α-L-rhamnopyranoside (5) obtained by using various acylating agents were screened for *in vitro* antifungal activity against four plant pathogenic fungi, viz., *Alternaria alternata, Curvularia lunata. Fusarium equiseti* and *Macrophomina phaseolina*. These compounds were also screened for *in vitro* antibacterial activity against ten human pathogenic bacteria, viz., *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, INABA ET (*Vibrio*), *Pseudomonas* species, *Salmonella paratyphi*, *Shigella dysenteriae* and *Salmonella typhi*. The study reveal that these 4-*O*-acetyl-α-L-rhamnopyranoside derivatives are more prone towards antifungal activities than that of antibacterial activities.

Key words: Rhamnopyranoside, Acyl derivatives, Antimicrobial activities.

INTRODUCTION

During the last few decades, considerable works have been done in the field of antimicrobial activities (Singh *et al*. 1987) of chemical compounds. It must, however, be admitted that a lot of the reports on the benefits of one or the other chemicals were based on empirical knowledge. Different classes of chemicals have been screened all over the world. Monosaccharides, especially acylated glycoses and glycosides, are very important due to their effective biological activity (Ishii *et al*. 1980, Andry *et al*. 1982).

Results of an ongoing research project on selective acylation of carbohydrates (Kabir and Matin 1987, Matin and Azad 2006) and nucleosides (Kabir *et al*. 1993) and also evaluation of microbial activities (Kabir *et al*. 1998a, Kabir *et al*. 1998b) revealed that in many cases the combination of two or more acyl, aromatic or heteroaromatic nuclei (Gupta *et al*. 1997) enhanced the biological

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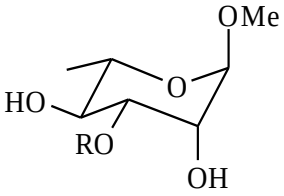
activity many fold than its parent nuclei. It was also observed that, methyl 3-*O*-decanoyl-α-L-rhamnopyranoside (2, Figure 1) obtained from methyl α-L-rhamnopyranoside (1) by using dibutyltin oxide method (Nashed and Anderson 1976) exhibited better antimicrobial activity than that of standard antibiotic (Kabir *et al*. 2002, Kabir *et al*. 2003). L-Rhamnose, an important member of the monosaccharide series, is wide spread in nature, being a component of some plant glycosides and bacterial polysaccharides of immunological importance (Schafer 1972). Therefore, we became interested to extend studies of 4-*O*-acetyl derivative of methyl α-L-rhamnopyranoside (1) instead of acyl group at position 3. This might provide important information about positional effects of the acyl group in its role as antimicrobial functionality. The 4-*O*-acetylation of 1 was performed by blocking-deblocking technique and a number of 2,3-di-*O*-acyl derivatives containing various groups (e.g. methanesulfonyl, pivaloyl, 2-chlorobenzoyl, benzenesulfonyl, octanoyl, and decanoyl) with 4-*O*-acetylated methyl α-L-rhamnopyranoside molecular framework were also prepared (Matin and Ibrahim 2007) to obtain newer derivatives of biological importance and to evaluate their antifungal and antibacterial activities using a variety of fungal and bacterial strains.



1: R = H
2: R = Dec

FIGURE 1:

MATERIALS AND METHODS

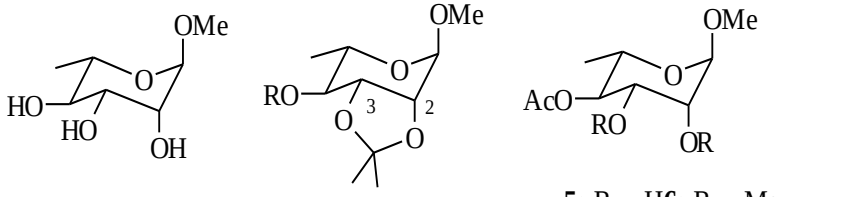
Collection of organisms

The test tube cultures of the fungal and bacterial pathogens were collected from the Microbiology laboratory, Department of Microbiology, University of Chittagong, and are listed as below:
Fungi: (i) *Alternaria alternata* (Fr) Keissler, (ii) *Curvularia lunata* (wakker boedijin), (iii) *Fusarium equiseti* (corda) Saec and (iv) *Macrophomina phaseolina*

(Tassi) Goid.
Gram-positive bacteria: (i) *Bacillus subtilis* BTCC 17, (ii) *Bacillus cereus* BTCC 19, (iii) *Bacillus megaterium* BTCC 18 and (iv) *Staphylococcus aureus* ATCC 6538.
Gram-negative bacteria: (i) *Escherichia Coli* 25922, (ii) INABA ET (*Vibrio*) AE 14748, (iii) *Pseudomonas* species CRL (ICDDR, B), (iv) *Salmonella paratyphi*-A CRL (ICDDR, B), (v) *Shigella dysenteriae* AE 14396 and (vi) *Salmonella typhi* AE 14632.

Chemicals used

Methyl 4-*O*-acetyl-α-L-rhamnopyranoside (5) and some of its 2,3-di-*O*-acyl derivatives (6 - 11) as shown in Figure 2 were used as test chemicals. The chemicals were synthesized, isolated and characterized in the Organic Research Laboratory, Department of Chemistry, University of Chittagong (Matin and Ibrahim 2007). For comparison, antimicrobial activities of methyl α-L-rhamnopyranoside (1), 2,3-*O*-isopropylidene derivatives 3 and 4 were also measured. Solutions of the compounds in chloroform (2%, W/V) were used for determination of antimicrobial activities.



1

3: R = H
4: R = Ac

5: R = H, **6**: R = Ms
7: R = Piv, **8**: R = 2-Cl.Bz
9: R = Bs, **10**: R = Oct
11: R = Dec

FIGURE: 2. STRUCTURE OF TEST CHEMICALS

Procedure for antifungal activity test

Medium. Potato Dextrose Agar (PDA) medium was used as basal medium throughout the investigation. Sliced potato (200 g) was boiled in distilled water (1000 mL). After proper boiling, the extract was decanted and diluted to a 1000 mL of solution. The solution was then taken in a pot and 20 g of dextrose and 16 g of agar were added slowly to the above solution with gentle heating and stirring. The mixture was then boiled for 15 minutes 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 fungi under investigation.

Fungal cultures.

A number of glass petridishes were cleaned and sterilized in an autoclave. Then 10-12 mL of sterilized and melted (~45 °C) PDA was poured into each petridish. When the medium solidified, small portions of mycelium of each fungus pathogen were placed at the centre of each PDA plate with the help of sterilized needles. Each fungus species was transferred into a number of petri plates. After 5 days, the mycelia grew in the whole petri plate. Now these were ready for the antifungal activity tests of the synthesized chemicals.

For the maintenance of cultures, slants of PDA medium were prepared. A loop of mycelia of the collected pathogens was transferred to the test tubes separately from old culture 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.

Mycelial growth test: Food poisoned technique

The antifungal functionality test of the synthesized chemicals were tested by the mycelial growth tests (Grover and Moore 1962) which was based on "food poisoned" technique as modified by Miah *et al*. (1990).

Necessary amount of medium (PDA) was taken in conical flasks separately and was sterilized in autoclave (at more then 120 °C & 15 psi) for 15 minutes. After autoclaving, calculated amount of test chemical (2%) 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 (2%) 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 inoculums (5 mm mycelial block) were placed on 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 diameters of fungal mycelia growth were measured. The average of measurements 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 activities were compared with that of the standard antibiotic, Nystatin.

Procedure for antibacterial activity test

Medium

Standard NA (Nutrient Agar) medium was used throughout the study. In a beaker 15 g of agar, 5 g of peptone, 3 g of NaCl and 3 g of beef extract were added to 1 L distilled water. The mixture was boiled and mixed thoroughly. After complete dissolution of agar, the medium was dispensed into several 250 mL conical flasks. The conical flasks were closed with the cotton plug and rapped with aluminum foil. The medium was autoclaved for 15 minutes at 121 °C and 15 psi. After autoclaving, the medium was used for culturing different microorganisms.

Stock culture

In a hard glass screw cap test tube, sterile slants of Nutrient Agar (NA) were prepared. Old cultures were transferred to the freshly prepared NA slants separately for each species with the help of sterilized bacterial loop. Similarly, 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 for two days. Old culture was used for antibacterial screening. For preservation of 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.

Bacterial suspension

For each organism, 10 mL of distilled water was taken in a clean screw cap test tube and sterilized in an autoclave. From 48 h old bacterial culture, one loop of bacterial culture was transferred to sterilized distilled water and mixed well. These bacterial suspensions 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* (Bauer *et al*. 1966).

RESULTS AND DISCUSSION

In the present investigation, the acylated derivatives (4-11) of methyl α-L-rhamnopyranoside (1, Figure 2) containing various acyl groups, e.g. acetyl, methanesulphonyl, pivaloyl (trimethyl acetyl), 2-chlorobenzoyl, benzenesulphonyl, octanoyl and decanoyl groups were selected and screened *in vitro* for their antifungal and antibacterial activities. For structural comparison antimicrobial activities of methyl α-L-rhamnopyranoside (1) and 2,3-*O*-isopropylidene rhamnopyranosides (3 and 4) were also determined. Four plant pathogenic fungi and ten human pathogenic bacteria (four Gram-positive and six Gram-negative) were employed in this study. For comparative study, biological activities of standard antibiotics (Nystatin 100 μg/mL and Ampicillin 10 μg/disc) were also determined.

Antifungal potentiality of the acylated rhamnopyranosides

Alternaria alternata (Fr) Keissler, *Curvularia lunata* (wakker boedijin), *Fusarium equiseti (*corda*)* Saec and *Macrophmina phaseolina* were selected for mycelial growth test. The results of the percentage inhibition of mycelial growth of the four plant pathogenic fungi due to the effect of chemicals (1, 3-11) are presented in Table 1. The efficiency of the test chemicals against the selected fungi showed that incorporation of acyl groups in rhamnopyranoside 1 increased its antifungal potentiality. 2,3-Di-*O*-pivaloate **7** (60.5%) and 2,3-di-*O*-octanoate 10 (62.6%) showed marked inhibition against *Alternaria alternata*, while Nystatin exhibited 55.5% inhibition against the same organism. Compound 10 and 11 were found most active against *Curvularia lunata.* 4-*O*-Acetyl rhamnopyranoside 5, 6 and 9 was found to exhibit very effective toxicity against *Macrophomina phaseolina*. All the compounds were found to be less toxic to the *Fusarium equiseti* as compared to the standard antibiotic, Nystatin (45.8%).

TABLE 1: ANTIFUNGAL ACTIVITIES OF THE ACYLATED RHAMNOPYRANOSIDES (1, 3-11).

Compound no	% inhibition of fungal mycelial growth, sample 100 μg.dw./mL PDA			
	Alternaria alternata	*Curvularia lunata*	*Fusarium equiseti*	*Macrophomina phaseolina*
1	15.4	—	07.7	29.4
3	26.2	11.3	22.5	45.7
4	29.6	22.2	32.2	—
5	09.5	30.5	—	*60.5

6	50.1	16.9	50.2	*78.7
7	*60.5	43.5	42.3	59.4
8	48.2	—	—	43.1
9	04.2	34.2	32.2	*64.3
10	*62.6	*75.1	55.2	22.4
11	28.4	*68.2	48.1	29.5
**Nystatin	55.5	*70.0	45.8	*70.8

"*" shows good inhibition, "—" indicates no inhibition,
"**" indicates standard antibiotic, "dw" means dry weight

Antibacterial potentiality of the acylated rhamnopyranosides

The results of the inhibition zone against the selected bacteria due to the effect of acylated rhamnopyranosides are mentioned in Table 2 and Table 3. The effect of the rhamnopyranosides on each bacterium is discussed below.

Antibacterial potentiality against Gram-positive bacteria

The inhibition growth data of various Gram-positive bacteria for different chemical treatments (Table 2) indicated that most of the acylated rhamnopyranosides were found weak or less toxic against *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Staphylococcus aureus.* But, 4-*O*-acetyl-2,3-di-*O*-octanoate 10 was found to be very toxic against all these Gram-positive bacteria and more active than the standard antibiotic, Ampicillin. This observation indicated that attachment of acetyloxy group at C-4 and octanoyloxy group at C-2, C-3 positions in a rhamnopyranoside molecular frame work is very good for toxicity against Gram-positive organisms.

Antibacterial potentiality against Gram-negative bacteria

The inhibition zone of Gram-negative bacteria for different rhamnopyranosides (1, 3-11) treatment is presented in Table 3. Rhamnopyranoside 1 was found inactive against all these Gram-negative microorganisms as well as against Gram-positive microorganisms. All the acylated rhamnopyranosides exhibited moderate to weak inhibition potentiality against these microorganisms except compound 10 and 11. 4-*O*-Acetyl-2,3-di-*O*-octanoate 10 was found more prone to *Salmonella paratyphi* (25 mm) and *Salmonella paratyphi* (22 mm). 4-*O*-Acetyl-2,3-di-*O*-decanoate 11 showed very good toxicity against *Escherichia coli* (22 mm) and *Pseudomonas* species (21 mm).

TABLE 2: INHIBITION AGAINST GRAM-POSITIVE BACTERIA BY THE RHAMNOPYRANOSIDES (1, 3-11).

Compound no	Diameter of zone of inhibition in mm, 50 µg.dw./disc			
	Bacillus subtilis	*Bacillus cereus*	*Bacillus megaterium*	*Staphylococcus aureus*
1	—	—	—	—
3	—	—	—	08
4	09	—	07	—
5	—	09	—	—
6	07	—	*22	*20
7	—	—	11	07
8	*23	16	—	19
9	08	—	—	09
10	*31	*21	*23	*27
11	*23	12	14	08
**Ampicillin	*25	*22	*19	*21

"*" shows good inhibition, "—" indicates no inhibition,
"**" indicates standard antibiotic 10µg/disc, "dw" = dry weight

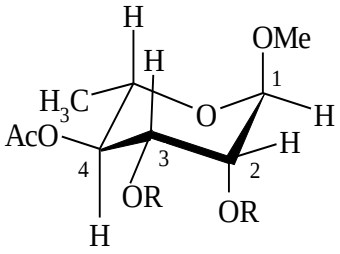
TABLE 3 : INHIBITION AGAINST GRAM - NEGATIVE BACTERIA BY THE RHAMNOPYRANOSIDES (1, 3-11).

Compound no	Diameter of zone of inhibition in mm, 50 µg.dw./disc					
	Escherichia Coli	INABA ET (*Vibrio*)	*Pseudomonas species*	*Salmonella paratyphi*	*Shigella dysenteriae*	*Salmonella typhi*
1	—	—	—	—	—	—
3	11	09	12	14	07	—
4	13	07	—	—	08	08
5	07	—	10	—	10	09
6	—	11	—	12	11	—
7	13	—	14	10	09	06
8	09	14	11	16	—	17
9	08	08	06	09	17	—
10	—	19	—	*25	19	*22
11	*22	11	*21	12	14	16
**Ampicillin	*25	*24	17	*35	*35	13

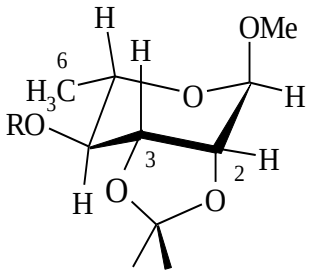
"*" shows good inhibition, "—" indicates no inhibition,
"**" indicates standard antibiotic 10µg/disc, "dw" = dry weight

Structure activity relationship

It has been observed that monosaccharides and their derivatives in furanose form or distorted pyranose form are very weak inhibitor towards antimicrobial functionality (Matin 2006). But, in pyranose form with regular $^4C_1$ or $^1C_4$ conformation these compounds exhibited excellent antimicrobial potentiality (Kabir *et al*. 2004). In this context, our present test chemicals, i.e. most of the acylated rhamnopyranoside derivatives (5-11) possess regular $^1C_4$ conformation (Figure 3) except monoacetonides 3 and 4 (distorted $^1C_4$ conformation as five-membered ring fused with six-membered ring). Thus, the synthesized acylated rhamnopyranosides (5-11) are expected to be potent antimicrobial agents.



Regular $^1C_4$ conformation
R = various acyl groups



3: R = H; **4**: R = Ac
slightly distoted $^1C_4$ conformation

FIGURE: 3. REGULAR AND DISTORTED $^1C_4$ CONFORMATIONS

The results obtained from the present investigation of antifungal studies mentioned in Table 1 clearly demonstrated that incorporation of acetyloxy moiety at C-4 and mesyloxy, pivaloyloxy, 2-chlorobenzoyloxy, benzenesulfonyloxy, octanoyloxy and decanoyloxy groups at C-2, C-3 positions as in 6-11 increased the antifungal potentiality. 4-*O*-Acetyl rhamnopyranoside in combination with 2,3-di-*O*-mesyl (6), 2,3-di-*O*-pivaloyl (7), 2,3-di-*O*-benzenesulfonyl (9), 2,3-di-*O*-octanoyl (10) and 2,3-di-*O*-decanoyl (11) groups increased antifungal potentiality than that of the original rhamnopyranoside 1. On the other hand, incorporation of these groups increased moderate to little toxicity against human pathogenic Gram-positive and Gram-negative bacteria (Table 2 and Table 3). Thus, incorporation of acetyl group at C-4 position and other acyl groups at C-2, C-3 positions made rhamnopyranoside 1 more prone to toxic against fungal pathogens than that of the bacterial pathogens. Monoacetonides 3 and 4 exhibited little or no toxicity against

all the fungal and bacterial pathogens proabably due to the distorted $^1C_4$ conformation as five-membered ring fused with six-membered ring (Figure 3).

CONCLUSION

Incorporation of acetyl group at C-4 and other acyl groups at C-2, C-3 positions of methyl α-L-rhamnopyranoside (1) increased its antimicrobial potentiality and these compounds (5-11) were found to be more prone to fungal pathogens than bacterial strains. Our synthesized acylated rhamnopyranosides (3-11) have not been tested before against the selected bacterial and fungal pathogens. The results of the present investigation showed that some of the newly synthesized acylaled methyl α-L-rhamnopyranoside derivatives may be tested against a wide range of phytopathogenic fungi and bacteria.

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