INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–5–760–763
http://www.ijab.org

Antimicrobial Evaluation of Some Decanoyl Derivatives of Methyl α –D –Glucopyranoside

ABUL K.M.S. KABIR[1], PIJUSH DUTTA AND M.N. ANWAR†
Department of Chemistry and †Microbiology, University of Chittagong, Chittagong –4331, Bangladesh.
[1]Corresponding author's e–mail: kabir562000@yahoo.com

ABSTRACT

Methyl 4,6 –*O* –benzylidene –α –D –glucopyranoside and eleven of its decanoylation derivatives were evaluated for *in vitro* antibacterial screening studies against a number of Gram –positive and Gram –negative micro –organisms. The same series of compounds were examined for *in vitro* antifungal activity against six fungal phytopathogens. For comparative studies, two standard antibiotics, Ampicillin and Nystatin, were also examined. From the antimicrobial screening results, it was revealed that a good number the tested D –glucose derivatives exhibited promising antibacterial and antifungal activity. A reasonable number of test chemicals showed remarkable antimicrobial activity comparable to and in some cases, even higher than the reference antibiotics employed.

Key Words: Glucopyranoside; Antibiotics; Phytopathogens; Nutrient agar; Potato dextrose

INTRODUCTION

A considerable number of heterocyclic compounds are known to be bioactive. They display antibacterial (Hin *et al.*, 2002) anti–inflammatory (Ram *et al*., 2002) and antimicrobial (Patel *et al.,* 2002) activities. Compounds having amino acid and sulfonamide moieties are also known to possess a wide range of antibacterial and antifungal activities (Ghorab *et al*., 2004). In the field of carbohydrate chemistry, acylated glycosides were considered as very important test chemicals due to their effective biological activity (Andary *et al.,* 1982). Literature survey revealed that a wide variety of biologically active substances contain aromatic, heteroaromatic and acyl substituents (Gupta *et al.,* 1997). It is also known that the combination of two or more potent acyl substituents in a single molecular framework enhances the biological profile many –fold than its parent nuclei (Kabir *et al.*, 1998; Kabir *et al.*, 2000). The benzene and substituted benzene nuclei play important role as common denominator for various biological activities. In the context of our studies, we observed that some acylated derivatives of L–lyxose ((Kabir *et al.*, 2001), D–glucose (Kabir *et al.*, 2003), D–mannose (Kabir *et al.*, 2004) and L–Rhamnose (Kabir *et al.*, 2003) also exhibited effective antibacterial and antifungal activities. In view of the above mentioned facts and in continuation of our work on synthesis of biologically important monosaccharide derivatives, we report herein the results of antibacterial and antifungal activities of a series of D–glucose derivatives containing various prospective biologically potent acyl substituents in a single molecular framework.

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*, ii) *Fusarium equiseti* (corda) Sacc., iii) *Alternaria alternata* (Savulescu and Sandu ville), iv) *Curvularia lunata* (wakker boedijin), v) *Botryodiplodia theobromae* (pat.) and vi) *Maccrophomina phaseolina* (Maubi) ashby.

Used test chemicals. Some partially protected derivatives of D–glucose (1–12) (Fig. 1) were used as test chemicals. The chemicals were synthesized, isolated, purified and characterized in the Organic Research Laboratory, Department of Chemistry, University of Chittagong and reported earlier (Kabir *et al*., 2004). In all the cases, a 1% solution (W/V) in chloroform of the chemicals was used.

Preparation of Nutrient Agar (NA) medium, stock culture, bacterial suspension and preservation of stock culture. A suspension of beef extract (3.0 g), peptone (5.0 g), NaCl (3.0 g) and agar (15.0 g) in distilled water (1000 mL) was boiled and mixed thoroughly with a glass rod. After complete dissolution of agar, the medium was

dispensed into several conical flasks, closed with cotton plug and rapped with aluminum foil. Then the medium was autoclaved for 15 minutes 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 (Bauer *et al*., 1966) as described bellow:

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 200 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 hrs 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 D–glucose derivatives (1–12) were investigated against six plant pathogenic fungi. The investigation was based on food poisoned technique (Grover and Moore, 1962) and the technique in some modified condition (Miah *et al*., 1990). The antifungal results were compared with that of the reference antibiotic, Nystatin. 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. The materials and methods of the present investigation are described in below**:**

Preparation of the medium. 200 g 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 dextrose (20.0 g) and 16 g of agar was added in portions to the solution with gentle heating and constant stirring with a glass rod. After boiling for an additional 15 minutes, the medium was transferred to four 250 mL capacity conical flasks whereupon the flasks were closed with cotton plug and autoclaved for half an hour at 121°C and 15 psi. The sterilized medium was then used for culturing different micro –organisms under investigation.

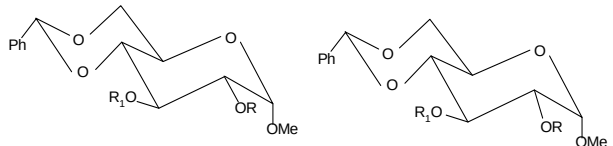
Maintenance and preparation of fungal cultures. Test tube slants of PDA medium were prepared for the maintenance of cultures. Small portions of mycelia of the test pathogens were transferred to the test tubes separately from old cultures with the help of sterilized needles. A number of test tubes were 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.

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 petriplates. After a few days, the mycelium grown in the whole petriplate and these are ready for antifungal evaluation tests of the test chemicals.

Mycelial growth test. A required amount of medium (PDA) was taken in conical flasks separately and was sterilized in autoclave (at 121°C & 15 psi) for 15 minutes. 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 on the centre of the petriplates at inverted position.

All the plates were incubated at room temperature on the laboratory desk for three days and the inoculated plates were then incubated at 25±2°C. The experiment was replicated three times. After three to five days of incubation,

Fig. 1. The structure of compound 1-12



1 : R=R_1=H
2 : R=Decanoyl; R_1=H
3 : R=Decanoyl ; R_1=Ac
4 : R=Decanoyl ; R_1=Ms
5 : R=Decanoyl ; R_1=Bs
6 : R=Decanoyl ; R_1=Bz
7 : R=Decanoyl ; R_1=2-Cl.Bz
8 : R=Decanoyl ; R_1=4-Cl.Bz
9 : R=Decanoyl ; R_1=Pivaloyl
10 : R=Decanoyl ; R_1=Octanoyl
11: R=Decanoyl ; R_1=Lauroyl
12: R=Decanoyl ; R_1=2.CO_2H.Bz

Table I. Antibacterial screening studies against some Gram-positive bacteria diameter of zone of inhibition in mm. sample 200µg.dw./disc

Compound no.	*B. cereus*	*B. subtilis*	*Stap. aureus*	*B. megaterium*
1	-	-	-	-
2	* 19	* 15	* 11	* 19
3	-	-	-	-
4	-	-	-	-
5	* 15	* 11	9	* 12
6	* 15	* 14	* 15	* 18
7	-	-	-	-
8	7	-	-	7
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	* 11	-	-	* 12
**Ampicillin (20µg.dw./disc)	16	16	20	15

* = Marked inhibition; ** = Standard antibiotic; - = No inhibition; dw =Dry weight

the diameters of fungal mycelial growth were measured and the average of two 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:

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

Where, I = Percentage of inhibition.
C = Diameter of the fungal colony in control
(CHCl3)T = Diameter of the fungal colony in treatment.

RESULTS AND DISCUSSION

Eleven acylated derivatives (2–12) and their precursor compound methyl 4,6 –O –benzylidene –α –D –glucopyranoside (1) were the test chemicals of the present investigation. For comparative study, two standard antibiotic substances were also screened against eleven human pathogenic bacteria and six plant pathogenic fungi.

The antibacterial evaluation results of the test chemicals and the standard antibiotic, Ampicillin against Gram –positive bacteria and Gram –negative bacteria are listed in Table I and II, respectively. From the results, we observed that compound 2 and 6 were very sensitive towards all of both Gram –positive and Gram –negative bacterial organisms. In case of 2, *B. cereus* (19 mm), *B. megaterium* (19 mm), Pseudomonus species (17 mm), *S. sonnei* (17mm) and in case of 6, *B. megaterium* (18 mm), Pseudomonus species (19 mm), *S. sonnei* (20 mm) were found very sensitive. Compound 5 was found very effective only against the Gram –positive bacteria and had no effect upon the Gram –negative bacteria. The test chemical 12 was also found to have potential antibacterial power except in case of *B. subtilis, Stap. aureus, E. coli* and *S. sonnei*. The inhibition (18 mm) of the growth of *V. cholerae* by compound 12 was remarkable. Compounds 1, 4, 9, 10 and 11 were quite insensitive towards any of the Gram –positive or Gram –negative bacterial strains.

The results of *in vitro* antifungal screening studies of the test chemicals (1 –12) and the standard antibiotic, Nystatin is presented in Table III. From these results we observed that compounds 5 and 6 were very sensitive towards the mycelial growth of all the fungal test organisms and in most of the cases, the inhibition was greater than the reference antibiotic, Nystatin. Furthermore, the growth of *Fusarium equiseti* was found to be inhibited (63.64%) by the test chemical 8 which was higher than that of Nystatin. Again *Botryodiplodia theobromae* in case of chemicals 2, 4, 12 and *Macrophomina phaseolina* in case of chemicals 2, 11, 12 were found very sensitive. Rest of the test chemicals showed their antifungal activities by varying degrees. Interestingly, chemical 4 in case *Colletotrichum corchori* (+5.45%), chemical 9 in case of *Macrophomina phaseolina* (+20.00) and chemical 11 in case of *Alternaria alternata* (+5.41%) exhibited stimulation rather than inhibition.

From the results placed in Tables I, II and III, it is evident that the presence of some particular groups in the test chemicals enhanced their sensitivities towards the growth of bacteria and fungi. The incorporation of decanoyl, benzoyl and 2 –carboxybenzoyl groups in the precursor molecule (1) seems to enhance the sensitivity towards the growth of both Gram–positive and Gram –negative bacteria. It is interesting to find that the presence of benzenesulphonyl group made the test chemical (5) very effective against Gram –positive bacteria whereas the same compound was found to be quite insensitive towards the gram –negative organisms. The test chemicals containing benzoyl and benzenesulphonyl group were found to show very high antifungal activity which was in accordance with our previous work. Thus, a good number of test chemicals reported herein exhibited promising antibacterial and antifungal activity. This piece of work, in our opinion, has created an opportunity for further work with these test chemicals, ultimately leading to develop new pesticides/medicines for human disease control with less environmental hazards.

Table II. Antibacterial screening studies against some Gram-negative bacteria diameter of zone of inhibition in mm. sample 200μg.dw./disc

Compound no.	*E. coli*	*V. cholerae*	*S. typhi*	*S. paratyphi*	*Pseudomonus species*	*S. sonnei*	*S. dysenterei*
1	-	-	-	-	-	-	-
2	* 12	8	* 15	* 12	* 17	* 17	* 13
3	-	-	10	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
6	* 15	10	* 15	* 14	* 19	* 20	* 13
7	-	-	8	-	-	-	-
8	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
12	-	* 18	10	* 12	7	-	* 11
**Ampicillin (20μg. dw./disc)	28	24	25	12	19	24	13

* = Marked inhibition; ** = Standard antibiotic; - = No inhibition; dw =Dry weight

Table III. Percent inhibition of fungal mycelial growth, sample 100 μg dw./mL PDA

Sample	*Colletotrichum corchori*	*Fusarium equiseti*	*Alternaria alternata*	*Curvularia lunata*	*Botryodiplodia theobromae*	*Macrophomina phaseolina*
1	4.00	-	3.70	22.86	-	15.38
2	18.18	20.45	13.51	17.78	* 53.01	* 46.67
3	5.45	6.82	2.70	4.44	19.28	-
4	+5.45	9.09	5.41	2.22	* 55.42	21.33
5	* 60.00	* 50.00	32.43	* 44.44	* 50.60	* 45.33
6	* 85.45	* 81.82	* 67.57	* 60.00	* 73.49	* 46.67
7	5.45	15.91	-	6.67	13.25	2.67
8	10.91	* 63.64	8.11	37.78	30.12	-
9	9.09	13.64	2.70	-	6.02	+20.00
10	3.64	9.09	5.41	8.89	10.67	17.33
11	18.18	4.55	+5.41	4.44	10.67	* 44.00
12	21.82	25.00	10.81	4.44	* 57.83	* 46.67
**Nystatin 100 μg dw./disc	41.00	45.00	51.00	70.00	70.00	76.00

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

Acknowledgements. P. Dutta wishes to thank the University of Chittagong for a post –graduate scholarship and the Ministry of Education, Government of Bangladesh, for granting him deputation.

REFERENCES

Andary, C., R. Wylde, C. Laffite, G. Privat and I. Winternitz, 1982. Structures of verbascoside and orobancoside caffeic acid sugar esters from Orobanche rapumgenistae. *Phytochem.* 21: 1123–7

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standard single disk methods. *American J. Clinic. Pathol.,* 45: 493–6

Ghorab, M.M., Z.H. Ismail, S.M.A. Gaward and A.A. Aziem, 2004. Antimicrobial activity of amino acid, imidazole and sulfonamide derivatives of pyrazolo[3,4 –d]pyrimidine. *Heteroatom Chem.,* 15: 57–62

Grover, R.K. and J.D. Moore, 1962. Toximetric studies of fungicides against the brown rot organisms *Sclerotin fructicola* and *S. laxa*, *Phytopathol.,* 52: 876–80

Gupta, R, S. Paul, A.K. Gupta, P.L. Kachroo and S. Bani, 1997. Synthesis and biological activities of some 2 –substituted phenyl –3 –(3 –alkyl/aryl –5,6 –dihydro –s –triazolo[3,4 –b] [1, 3, 4] thiazol –6 –yl) indoles. *Indian J. Chem.,* 36: 707–10

Hui, X.P, C.H. Chu and Z.Y. Zhang, 2002. Synthesis and antibacterial activities of 1, 3, 4 –triazole derivatives containing 5 –methylisoxazole moiety. *Indian J. Chem.,* 41B: 2176–9

Kabir, A.K.M.S. and P. Dutta, 2004. Regioselective decanoylation of methyl 4,6 –O –benzylidene –α –D –glucopyranoside and related studies. *Bull. Pure and Applied Sci., [Section –C, Chemistry] (India)*, 23C (2): 131-138

Kabir, A.K.M.S., P. Dutta and M.N. Anwar, 2004. Biological evaluation of some acylated derivatives of D –mannose. *Pakistan J. Biol. .Sci.,* 7: 1730–34

Kabir, A.K.M.S., M.M. Matin, A. Hossain and M.A. Sattar, 2003. Synthesis and antimicrobial activities of some rhamnopyranoside derivatives. *J. Bangladesh Chem. Soc.,* 16: 85–93

Kabir, A.K.M.S., M.M. Matin, A.F.M. Sanaullah, M.A. Sattar and M.S. Rahman, 2001. Antimicrobial activities of some lyxoside derivatives. *Bangladesh J. Microb.,* 18: 89–95

Kabir, A.K.M.S., M.M. Matin, M.A.U. Mridha and S.M. Shahed, 1998. Antifungal activities of some methyl 6 –O –trityl –α –D –mannopyranoside. *Chittagong Univ. J. Sci.,* 22: 40–6

Kabir, A.K.M.S., M.M. Matin, M.J. Alam and M.A. Manchur, 1999. Synthesis and antimicrobial activities of some D –glucose derivatives, *J. Bangladesh Chem. Soc.,* 13: 107–15

Kabir, A.K.M.S., M.M.R. Bhuiyan, S.M.A. Kawsar, M.M. Munshi and M.S. Rahman, 2003. Antimicrobial evaluation of some derivatives of D –glucose. *Chittagong Univ. J. Sci.,* 27: 113–8

Miah, M.A.T, H.U. Ahmed, N.R. Sharma, A. Ali and S.A. Miah, 1990.Antifungal activity of some plant extracts. *Bangladesh J. Bot.,* 19: 5–10

Patel, K.D., B.D. Mistry and K.R. Desai, 2002. Synthesis and antimicrobial activity of 1, 2, 4 –triazoles. *J. Indian Chem. Soc.,* 79: 964–5

Rani, P., A.V.K. Srivastav and A. Kumar, 2002. Synthesis and anti –inflammatory activity of some new 2,3 –disubstituted–6 –monosubstituted –quinazolin–4(3H) –ones. *Indian J. Chem.,* 41B: 2642–6

(Received 18 March 2005; Accepted 22 June 2005)