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Synthesis and Antimicrobial Activities of Some Derivatives of L-Rhamnose

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Abstract: Selective pivaloylation (trimethyl acetylation) of methyl-α-L-rhamnopyranoside using the dibutyltin oxide method furnished the corresponding 3-*O*-pivaloyl derivative in reasonable yields and 2, 4-di-*O*-pivaloyl derivative in somewhat lower yields. A number of acetyl, methanesulphonyl derivatives of these pivaloyl products were also prepared in order to gather additional information for structure elucidation. Direct pivaloylation of the rhamnopyranoside provided the 3-*O*-substitution products only but in somewhat lower yields. Antibacterial and antifungal activities of the acylated products were also determined *in vitro*.

Key words: Pivaloylation, dibutyltin oxide, methyl-α-L-rhamnopyranoside, pyridine, antibacterial activity, antifungal activity

INTRODUCTION

Protection of a particular functional group of an organic compound is not only necessary for the modification of properties of the remaining functional groups but also the synthesis of newer derivatives of great importance. Various methods for acylation of carbohydrates and nucleosides have so far been developed and employed successfully[1-3]. Of these the dibutyltin oxide method was found to be the most encouraging and serves as a versatile reagent. When this reagent is used[4-6], an intermediate tin complex with oxygen-tin linkage formed first, thereby enhancing the nucleophilicity of the original O-H bonds. The intermediate is then attacked by the acylating agent selectively to give the desired product.

In the last few decades' considerable works have been done in the field of antimicrobial activities[7,8] by chemical compounds. Different classes of chemicals have been screened all over the world. Carbohydrates, especially acylated glycosides, are very important due to their effective biological activity[9,10]. The benzene and substituted benzene nuclei play important role as common denominator for various biological activities. Encouraged by the above results, we deliberately synthesized a number of rhamnopyranoside derivatives containing various substituents and tested their antimicrobial activities.

MATERIALS AND METHODS

All reagents used were commercially available (Aldrich) and were used as received unless otherwise specified. Melting points were determined on an electrothermal melting point apparatus and were uncorrected. Evaporations were performed under reduced pressure on a Büchii rotatory evaporator. IR spectra were recorded on a Perkin-Elmer -298 spectrophotometer. ^{1}H-NMR (200 MHz) and ^{13}C-NMR spectra (50 MHz) were recorded for solutions in deuteriochloroform (internal tetramethyl silane) with a Bruker spectrometer at the Department of Chemistry, University of Dundee, UK. Mass spectra were recorded on a VG 70S (EI) mass spectrometer at the Department of Chemistry, University of Dundee, UK. Analytical thin layer chromatography (t.l.c.) was performed on Kieselgel GF_{254}. Visualization was accomplished by spraying the plates with 1% H_2SO_4 followed by heating the plates at 150-200°C until coloration took place. Column chromatography was performed with silica gel G_{60}. Solvent system employed for t.l.c analyzes was ethyl acetate-hexane.

Methyl α-L-rhamnopyranoside, 2: To a solution of L-rhamnose (1) (2 g, 12.19 mmol) in anhydrous methanol (15 mL) was added Amberlite IR-120 (H^+) resin (2.0 g) and the mixture was heated under reflux for 20 h with constant stirring. T.l.c. (ethyl acetate-chloroform, 20:1) examination showed complete conversion of the starting material into one faster-moving product. The reaction mixture was then filtered off and the filtrate was evaporated under reduced pressure. The residue was purified by passage through a silica gel column, with ethyl acetate-chloroform (20:1) as eluant, to afford methyl α-L-rhamnopyranoside (2) (1.7 g, 78%) as a crystalline solid. This compound was used in the next step without further purification and identification.

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Methyl 2, 4-di-*O*-pivaloyl-α-L-rhamnopyranoside, 4-Dibutyltin oxide method: A suspension of methyl-α-L-rhamnopyranoside, (2) (1 g, 5.61 mmol) and dibutyltin oxide (1.5 g, 6.03 mmol) in anhydrous methanol (30 mL) was heated under gentle reflux until the solution became homogeneous and clear (~ 4 h). The solution was refluxed for an additional hour and then allowed to attain room temperature. Evaporation of the solvent under reduced pressure gave a white solid which was taken in dry 1, 4 dioxane (30 mL) and trimethyl acetyl (pivaloyl) chloride (0.69 mL, 5.61 mmol) was added to it. The reaction mixture was stirred at room temperature overnight when t.l.c. (ethyl acetate-hexane, 1:2) showed the formation of two products. The solvent was removed under reduced pressure and the residue was packed in a silica gel column. Initial elution with hexane removed the contaminating tin compounds. Further elution with ethyl acetate-hexane (1:4) furnished methyl 2, 4-di-*O*-pivaloyl-α-L-rhamnopyranoside, (4) (279 mg, 14%) as prism, m.p. 108-109°C (ethyl acetate -hexane) $[\alpha]_D$ -30° (c1, chloroform).

Anal.calcd. for $C_{17}H_{30}O_7$: C, 58.94%; H, 8.73%. Found: C, 59.18%; H, 8.85%.

IR (Nujol): 1710 cm^{-1}, 1720 cm^{-1} (-CO stretching), 3500 cm^{-1} (-OH stretching).

^{1}H-NMR (200 MHz, $CDCl_3$) data: δ_H5.16 (1H, dd, J = 1.5 and 3.2Hz, H-2), 5.08 (1H, t, J = 9.2 Hz, H-4), 4.57 (1H, d, J = 1.5 Hz, H-1), 3.73 (1H, dd, J = 3.4 and 9.1 Hz, H-3), 3.60 (1H, m, H-5), 3.37 (3H, s, 1-OCH_3), 2.31 (1H, m, 3-OH), 1.37 (3H, d, J = 5.6Hz, 6-CH_3), 1.22, 1.17 (2×9H, 2×s, 2×$(CH_3)_3$ CCO).

^{13}C-NMR (50 MHz, $CDCl_3$) data: δ_C179.35, 178.10, 98.46, 72.24, 72.08, 69.94, 68.65, 54.99, 38.93 (×2), 27.12 (×6), 17.69

Mass spectrum: M/Z 315.180 (M^+ - OCH_3) corresponding to $C_{17}H_{30}O_7$ (M^+) (requires 346.403)

Methyl 3-*O*-pivaloyl-α-L-rhamnopyranoside, 5: Final elution with ethyl acetate-hexane (1:2) afforded methyl-3-*O*-pivaloyl-α-L-rhamnopyranoside (5) (1.12 g, 76%) as needles, m.p. 128-129°C (ethyl acetate -hexane) $[\alpha]_D$ -79° (c1.1, chloroform).

Anal.calcd. for $C_{12}H_{22}O_6$: C, 54.95%; H, 8.45%.

Found: C, 55.26%; H, 8.62%.

IR (Nujol): 1708, (-CO stretching), 3480 cm^{-1} (-OH stretching).

^{1}H-NMR (200 MHz, $CDCl_3$) data: δ_H4.96 (1H, dd, J = 3.2 and 9.3 Hz, H-3), 4.64 (1H, d, J = 1.7 Hz, H-1), 3.98 (1H, dd, J = 1.7 and 3.2 Hz, H-2), 3.70 (1H, m, H-4), 3.62 (1H, m, H-5), 3.37 (3H, s, 1-OCH_3), 1.33 (3H, d, J = 5.8 Hz, 6-CH_3), 1.23 (9H, s, $(CH_3)_3$CCO).

^{13}C-NMR (50 MHz, $CDCl_3$) data: δ_C179.29, 100.54, 74.59, 71.52, 69.67, 66.53, 54.94, 39.12, 27.18 (×3), 17.57.

Mass spectrum: M/Z 263.149 (M^+ +1) corresponding to $C_{12}H_{22}O_6$ (M^+) (requires 262.290).

Direct method: A solution of methyl-α-L-rhamnopyranoside (2) (1.0 g, 5.61 mmol) in anhydrous pyridine (10 mL) was cooled to -15°C when pivaloyl chloride (0.69 mL, 5.61 mmol) was added to it. The solution was stirred at -15°C for 6hrs and then allowed to stand in the refrigerator overnight. The solution was then poured into ice water with constant stirring and the product was extracted with chloroform. The chloroform layer was washed with dilute HCl, followed by saturated aqueous $NaHCO_3$ solution and water.

The organic layer was dried ($MgSO_4$) filtered and concentrated under reduced pressure to leave a solid mass. T.l.c. (ethyl acetate-hexane, 1:1) showed formation of one faster-moving product with some starting material remaining unreacted. Recrystallization of the solid mass from ethyl acetate-hexane gave compound (5) (0.85 g, 58%) as needles, m.p and mixture m.p. 128-129°C; $[\alpha]_D$ -79.4° (c 1.1, chloroform). The ^{1}H-NMR spectrum of this compound was indistinguishable from that of the same sample prepared by the dibutyltin oxide method.

Methyl 3-*O*-acetyl- 2, 4-di-*O* -pivaloyl-α-L-rhamnopyranoside,6: A cooled (0°C) and stirred solution of the di-*O*-pivaloyl derivative (4) (70 mg, 0.2 mmol) in dry pyridine (1.5 mL) was treated with acetic anhydride (0.1 mL, 1.06 mmol) and stirring was continued at 0°C for 6 h. T.l.c. (ethyl acetate-hexane, 1:3) showed complete conversion of starting material into one faster-moving product. A few pieces of ice were added to the flask with constant shaking and the content in the flask was extracted with chloroform. The chloroform layer was washed successively with dilute HCl, followed by saturated aqueous $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$) filtered and the filtrate was evaporated off under reduced pressure. Percolation of the residue through a silica gel column, with ethyl acetate-hexane (1:4) as eluant, provided the 3-*O*-acetate (6), (67 mg, 85%) as needles, m.p. 88-89°C (ethyl acetate-hexane); $[\alpha]_D$ -39.3° (c1.1, chloroform).

Anal.calcd. for $C_{19}H_{32}O_8$: C, 58.75%; H, 8.30%.

Found: C, 58.92%; H, 8.42%.

IR (Nujol): 1736 cm^{-1}, (-CO stretching).

^{1}H-NMR (200 MHz, $CDCl_3$) data: δ_H 5.28 (1H, dd, J = 3.3 and10.0Hz, H-3), 5.19 (1H, d, J = 3.3 Hz, H-2), 5.10 (1H, t, J = 9.9 Hz, H-4), 4.59 (1H, s, H-1), 3.86 (1H, dd, J = 6.2 and 9.3 Hz, H-5), 3.37 (3H, s, 1-OCH_3), 2.00 (3H, s, CH_3CO), 1.42(3H, d, J = 5.9 Hz, 6-CH_3), 1.24, 1.20 (2×9H, 2×s, 2×$(CH_3)_3CCO$).

Methyl 2, 4-di-*O*-pivaloyl-3-*O*-methanesulphonyl-α-L-rhamnopyranoside, 7: A solution of the di-*O*-pivaloyl derivative (4) (155 mg, 0.45 mmol) in dry pyridine (3 mL) was cooled to -5°C when methanesulphonyl chloride (0.1 mL, 1.35 mmol) was added to it. The solution was stirred at -5°C for 5 h. when T.l.c. (ethyl acetate-hexane, 1:3) showed formation of one product. Work-up as described earlier and silica gel column chromatography (with ethyl acetate-hexane, 1:4 as eluant) afforded the 3-*O*-mesylate (7) (156 mg, 82%) as chromatographically homogeneous syrup $[\alpha]_D$ -24° (c1.2, chloroform).

^{1}H-NMR (200 MHz, $CDCl_3$) data: δ_H 5.18 (1H, dd, J = 3.3 and 10.0 Hz, H-3), 5.13 (1H, d, J = 3.4 Hz, H-2), 5.05 (1H, t, J = 9.3 Hz, H-4), 4.62 (1H, s, H-1), 3.80 (1H, m, H-5), 3.42 (3H, s, 1-OCH_3), 3.12 (3H, s, CH_3SO_2), 1.41 (3H, d, J = 6.1 Hz, 6-CH_3), 1.23, 1.16 (2×9H, 2×s, 2×$(CH_3)_3CCO$).

Methyl 2, 4-di-*O*-acetyl-3-*O*-pivaloyl-α-L-rhamnopyranoside, 8: Acetic anhydride (0.4 mL, 4.24 mmol) was added to a cooled (0°C) and well-stirred solution of the 2, 4-diol (5) (210 mg, 0.8 mmol) in dry pyridine (4 mL) and stirring at this temperature was continued for 6 h. T.l.c. (ethyl acetate-hexane, 1:3) showed completion of reaction with the formation of one faster-moving product. Excess reagent was destroyed by the addition of a few pieces of ice to flask with constant shaking and the product was extracted with chloroform. The chloroform layer was processed as described earlier and then purified by silica gel column chromatography (ethyl acetate-hexane, 1:4) to afford the 2,4-di-*O*-acetate (8) (205 mg, 74%) as a crystalline solid. Recrystallization from ethyl acetate-hexane provided compound (8) in analytically pure form. m. p. 145-146°C (plates); $[\alpha]_D$ -56.9°(c1, chloroform).

Anal.calcd. for $C_{16}H_{26}O_8$: C, 55.48%; H, 7.57%.

Found: C, 55.72%; H, 5.59%.

IR (Nujol): 1735 cm^{-1}, 1740 cm^{-1}(-CO stretching).

^{1}H-NMR (200 MHz, $CDCl_3$) data: δ_H 5.26 (1H, d, J = 3.4 Hz, H-2), 5.21 (1H, dd, J = 3.4 and 9.7 Hz, H-3), 5.08 (1H, t, J = 9.5 Hz, H-4), 4.59 (1H, s, H-1), 3.84 (1H, dd, J = 6.2 and 9.3 Hz, H-5), 3.35 (3H, s, 1-OCH_3), 2.10, 1.99 (2×3H, 2×s, 2×CH_3CO), 1.23 (3H, d, J = 6.1 Hz, 6-CH_3), 1.16 (9H, s, $(CH_3)_3$ CCO).

Methyl 2, 4-di-*O*-methanesulphonyl-3-*O*-pivaloyl-α-L-rhamnopyranoside, 9: A solution of the 3-*O*-pivaloyl derivative (5) (110 mg, 0.42 mmol) in dry pyridine (2 mL) was cooled to -5°C when methanesulphonyl chloride (0.15 mL, 1.93 mmol) was added. The mixture was stirred at this temperature for 4 h and then left in the refrigerator overnight. T.l.C. (ethyl acetate-hexane, 1:2) showed formation of one product and complete disappearance of the diol. A few pieces of ice were added to the flask to decompose excess reagent and the product was extracted with chloroform. The chloroform layer was washed with dilute HCl, followed by saturated aqueous $NaHCO_3$ solution and water.

The organic layer was dried ($MgSO_4$) filtered and concentrated under reduced pressure to a syrup. Purification by passage through a silica gel column, with ethyl acetate-hexane (1:3) as eluant, provided the di-*O*-mesylate (9) (151 mg, 86%) as a solid. Recrystallization from ethyl acetate-hexane afforded compound (9) as needles, m. p. 124-125°C; $[\alpha]_D$ -15° (c1, chloroform).

Anal.calcd. for $C_{14}H_{26}O_{10}S_2$: C, 40.18%; H, 6.26%.

Found: C, 40.25%; H, 6.35%.

IR (Nujol): 1712 cm^{-1}, (-CO stretching).

^{1}H-NMR (200 MHz, $CDCl_3$) data: δ_H 5.31 (1H, dd, J = 3.2 and 9.9 Hz, H-3), 4.99 (1H, d, J = 3.1 Hz, H-2), 4.82 (1H, s, H-1), 4.68 (1H, t, J = 9.7 Hz, H-4), 3.98 (1H, m, H-5), 3.40 (3H, s, 1-OCH_3), 3.11, 3.03 (2×3H, 2×s, 2× CH_3SO_2), 1.40 (3H, d, J = 6.2 Hz, 6-CH_3), 1.23 (9H, s, $(CH_3)_3CCO$).

Antimicrobial screening experiments: The antimicrobial activities of rhamnopyranoside derivatives were screened *in vitro* for their antibacterial activities by disc diffusion method[11] against four human pathogenic bacteria, viz., *Bacillus cereus* BTCC 19, *Escherichia coli* ATCC 25922, *Shigella dysenteriae* AC 14396 and *Staphylococcus aureus* ATCC 6538. The derivatives were also screened for their antifungal activities by poisoned food technique[12] against four phytopathogenic fungi, viz., *Macrophomina phaseolina* (Maubl) Ashby, *Alternaria alternata* (Fr.) Kedissler, *Curvularia lunata* Wakker Boedjin and *Fusarium equiseti* (Corda) Sacc. Nutrient agar and potato dextrose agar were used as basal media for antibacterial and antifungal activities, respectively.

Pyridine was used as solvent for preparation of solution of desired concentration of the derivatives.

RESULTS AND DISCUSSION

The main view of the present work was to study selective pivaloylation of methyl-α-L- rhamnopyranoside (2) using the dibutyltin oxide method. Direct method of pivaloylation was also applied on this substrate in order to compare the result with those obtained by using the dibutyltin oxide method.

Our initial effort was to prepare methyl α-L-rhamnopyranoside (2) which involved reaction of L-rhamnose (1) with methyl alcohol and Amberlite IR-120 (H^+) resin under reflux condition, followed by chromatographic purification. The rhamnopyranoside (2) obtained as a crystalline solid in 78% yield was used in the next stage without further purification and analysis. The rhamnopyranoside (2) on treatment with dibutyltin oxide in dry methanol under reflux condition followed by evaporation of the solvent under reduced pressure furnished the intermediate tin ethers (3a and 3b) as a white solid. This white solid, when reacted with trimethyl acetyl (pivaloyl) chloride (one molar equivalent) in anhydrous 1, 4-dioxane at room temperature overnight, it afforded a mixture of compounds 4 (14%) and 5 (76%) after removal of solvent and silica gel column chromatography. The IR spectrum of compound 4 showed absorption peaks at 1710, 1720 cm^{-1} (-CO stretching) and at 3500 cm^{-1} (-OH stretching), thereby suggesting the presence of two pivaloyl groups and one hydroxyl group. In its ^{1}H-NMR spectrum, two nine - proton singlets at δ 1.22 and 1.17 indicated the presence of two pivaloyl groups in the molecule. The downfield shift of H - 2 to δ 5.16 (as dd, J= 1.5 and 3.2 Hz) and H - 4 to δ 5.08 (as, t, J= 9.2 Hz) from their usual values (~ 3.50 ppm) showed the attachment of the two pivaloyl groups at position 2 and 4. The ^{13}C-NMR spectrum showed the presence of seventeen carbon atoms corresponding to the molecular formula $C_{17}H_{30}O_7$, thereby suggesting the formation of a di-*O*-pivaloyl derivative (4). The mass spectrum showed a molecular ion at m/z 315.180 corresponding to the ion (M^+-OCH_3).

Complete analysis of the IR, ^{1}H - NMR, ^{13}C - NMR and mass spectra of this compound enabled us to assign its structure as methyl 2, 4-di-*O*-pivaloyl-α-L-rhamnopyranoside (4). The formation of the di-*O*-pivaloyl derivative (4) from the rhamnopyranoside (2) may be explained by assuming the formation of the intermediate tin complex (3a) and preferential attack by pivaloyl chloride at the more reactive hydroxyl groups at C-2 and C-4. However this $^4C_1$ chair conformation is particularly less stable than the $^1C_4$ chair conformation, the rhamnopyranoside (2) usually occupies. For this reason compound (4), the formation of which is only possible via the tin complex (3a), is obtained as a minor product (14%). The structure of compound 4 was finally confirmed by preparation of its acetyl and methanesulphonyl derivatives (6 and 7) and analyzing their ^{1}H-NMR spectra. Thus reaction of compound 4 with acetic anhydride in pyridine followed by conventional work-up procedure and chromatographic purification furnished the acetyl derivative (6) as needles, m. p. 88-89°C; $[\alpha]_D$ -39.3° (c 1.1, chloroform). In the ^{1}H-NMR spectrum of compound 6, a three-proton singlet at δ 2.00 was due to methyl protons of one acetyl group. Also, H-3 resonance shifted downfield to δ 5.28 (as dd, J = 3.3 and 10.0 Hz) as compared to the precursor compound 4 (δ 3.73, dd, J = 3.4 and 9.1 Hz), indicating the attachment of the acetyl group at position-3. By complete analysis of the ^{1}H-NMR spectrum of this acetyl derivative, it was possible to assign its structure as methyl 3-*O*-acetyl-2, 4-di-*O*-pivaloyl-α-L-rhamnopyranoside (6). The methanesulphonyl derivative (7) of compound 4 was also prepared which involved reaction with methanesulphonyl chloride in pyridine followed by usual work-up and chromatographic purification. In the ^{1}H-NMR spectrum of the mesyl derivative (7), a three-proton singlet at δ 3.12 corresponded to the methyl protons of the mesyloxy group. The downfield shift of the C-3 proton to δ 5.18 (as dd, J = 3.3 and 10.0 Hz), as compared to the 3-hydroxy compound (4) (δ 3.73, dd, J=3.4 and 9.1 Hz), indicated the attachment of the mesyloxy group at C-3. The rest of the ^{1}H-NMR spectrum was in complete agreement with the structure of the mesylate accorded as methyl 2, 4-di-*O*-pivaloyl-3-*O*-methanesulphonyl-α-L-rhamno- pyranoside (7).

Selective pivaloylation of the rhamnopyranoside (2) using the dibutyltin oxide method provided compound 5 in 76% yield as needles, m. p. 128-129°C; $[\alpha]_D$ -79° (c 1.1, chloroform). The IR spectrum of compound 5 showed absorption bands at 1708 and 3480 cm^{-1} corresponding to carbonyl and hydroxyl stretchings, respectively. In its ^{1}H-NMR spectrum, a nine-proton singlet at δ 1.23 indicated the formation of a mono-*O*-pivaloyl derivative. The deshielding of the C-3 proton to δ 4.96 (as dd, J = 3.2 and 9.3 Hz) from its usual value (~4.00 ppm) suggested the introduction of the pivaloyl group at C-3. The ^{13}C-NMR spectrum showed the presence of twelve carbons corresponding to $C_{12}H_{22}O_6$ and this molecular formula was also supported by C, H analyses. The mass spectrum showed a molecular ion peak at m/z to 263.149 (M^++1). Complete analysis of the IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrum of this compound was consistent with the structure accorded as methyl 3-*O*- pivaloyl-α-L-

rhamnopyranoside (5). The formation of the 3-*O*-pivaloyl derivative (5) from the rhamnopyranoside (2) may be explained by assuming that the intermediate tin complex (3b) is formed between the cis-glycol system (2-OH and 3-OH) and that the reactivity of the equatorial 3-OH is enhanced much more than the axial 2-OH and therefore substitution taking place preferentially at the more reactive position -3 .Further support for the structure of the 3-*O*-pivaloyl derivative (5) was obtained by its conversion to the acetyl derivative (8), using acetic anhydride in pyridine followed by conventional work-up procedure and chromatographic purification. The structure of the acetyl derivative (8), isolated in 74% yield as plates (m.p.145-146°C; $[\alpha]_D$ - 56.9°), was ascertained by IR and ^{1}H-NMR spectroscopy . In its IR spectrum, the absorption bands at 1735 and 1740 cm^{-1} corresponded to carbonyl stretching. In the ^{1}H-NMR spectrum, two three-proton singlets at δ 2.10 and 1.99 suggested the presence of the two acetyl groups in the molecule. Furthermore, the deshielding of C-2 and C-4 protons to δ 5.26 (as d, J=3.4 Hz) and δ 5.08 (as t, J=9.5 Hz) as compared to the 2, 4-diol (5) (δ 3.98, dd, J=1.7 and 3.2 Hz, H-2 and δ 3.70, m, H-4), indicated the attachment of the acetyl groups at positions 2 and 4. Thus the structure of the diacetate was confidently assigned as methyl 2, 4-di-*O*-acetyl -3-*O*-pivaloyl -α-L rhamnopyranoside (8).

Additional information towards the assignment of the structure of the 3-*O*-pivaloyl derivative (5) was achieved by its conversion to the mesyl derivative (9) with methanesulphonyl chloride in pyridine, followed by usual work-up and chromatography. The mesylate (9) was obtained in 86% yield as needles, m. p. 124-125°C; $[\alpha]_D$ - 15° (c 1, chloroform). The IR spectrum of this mesylate (9) showed carbonyl stretching band at 1712 cm^{-1} and absence of hydroxyl stretching band. The ^{1}H-NMR spectrum of the mesyl derivative (9) showed two three-proton singlets at δ 3.11 and 3.03 corresponding to the methyl protons of two mesyloxy groups. The introduction of the two mesyloxy groups at positions 2 and 4 was ascertained by observing the downfield shift of the C-2 and C-4 protons to δ 4.99 (as d, J = 3.1Hz) and δ 4.68 (as t, J = 9.7 Hz) as compared to the 3-*O*-pivaloyl derivative (5) (δ 3.98, dd, J = 1.7 and 3.2 Hz, H-2; δ 3.70, m, H-4). Complete analysis of the ^{1}H-NMR spectrum of this compound corresponded to the structure assigned as methyl 2, 4-di-*O*-methanesulphonyl-3-*O*-pivaloyl -α-L rhamnopyranoside (9).

The rhamnopyranoside (2) when reacted with pivaloyl chloride under direct acylation condition (with one molar equivalent of pivaloyl chloride in pyridine at -15°C) followed by usual work-up procedure furnished a solid mass. This on recrystallization from ethyl acetate-hexane afforded the 3-*O*-pivaloyl derivative (5) in 58% yield as needles, m. p. and mixture m. p. 128-129°C; $[\alpha]_D$ -79.4° (c 1.1 , chloroform). The ^{1}H-NMR spectrum of this compound was identical with the same sample prepared by the dibutyltin oxide method. Although the yield of the 3-*O*-pivaloyl derivative (5) is lower by this method, as compared to the dibutyltin oxide method, direct acylation method has the advantage in that it gives a single product and also no column chromatography is needed. The formation of the 3-*O*-pivaloyl derivative (5) by the direct method may be explained by assuming that the pivaloyl chloride preferentially attacks the most reactive and less sterically hindered hydroxyl group at position-3. Structures of these compounds are given in Fig. 1.

Table 1: Antibacterial screening studies of some rhamnopyranoside derivatives

	Diameter of Zone of Inhibition in mm, sample 5 µg dw/disc			
Compound No.	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Shigella dysenteriae*
2	2.40	5.20	-	10.00
4	15.00	12.00	10.60	12.90
5	7.50	5.00	3.22	3.76
6	4.65	3.3.	-	3.00
7	3.00	15.00	4.00	10.11
8	27.34**	16.00	11.20	18.00
9	10.00	5.12	5.00	25.20**
Ampicillin*	25.10**	21.00**	15.00	30.00**

-indicates no inhibition, * indicates standard antibiotic, **indicates good inhibition and dw = dry weight

Table 2: Antifungal screening studies of some rhamnopyranoside derivatives

	% inhibition of fungal mycelial growth, sample 100 µg dw. mL^{-1} PDA			
Compound No.	*Macrophomina phaseolina*	*Curvularia lunata*	*Fusarium equiseti*	*Alternaria alternata*
2	18.00	4.33	25.40	10.20
4	52.23**	25.50	22.00	30.00
5	40.22	51.50**	24.25	45.50
6	55.60**	23.10	38.20	28.35
7	20.20	25.12	32.66	30.75
8	75.40**	35.20	50.40**	30.30
9	72.50**	34.00	32.00	36.70
Nystatin*	70.20**	68.20**	56.70**	45.50

*indicates standard antibiotic, **indicates good inhibition and dw = dry weight

Antibacterial activities: Antibacterial screening results of the rhamnopyranoside derivatives are presented in Table 1. In the present study most of the selective acylated derivatives were found less effective against the tested human pathogenic bacteria except compound 8 and 9. The compound 8 was found more effective against *Bacillus cereus* than Ampicillin and compound 9 was found significantly effective against *Shigella dysenteriae*.

Antifungal activities: The *in vitro* result of percent inhibition of mycelial growth (in mm) of plant pathogenic

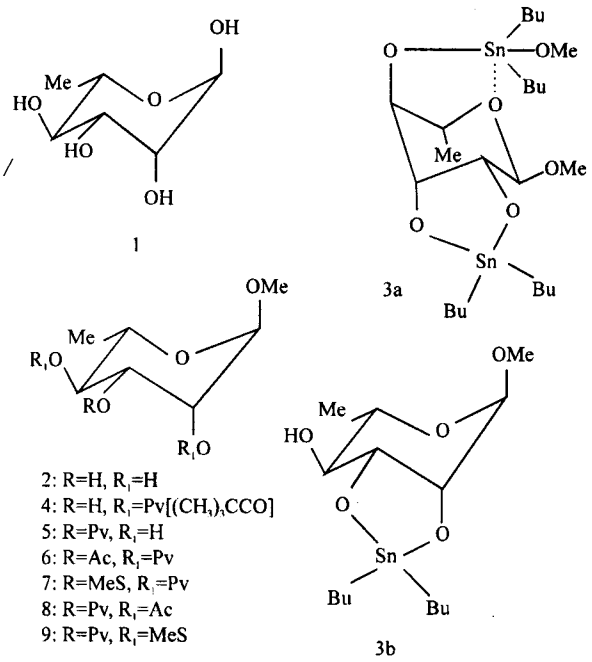

Fig. 1: Structures of compounds 1-9

fungal strains is listed in Table 2. The overall result indicated that out of four fungi tested against newly developed seven test chemicals, *Macrophomina phaseolina* was more sensitive than those of other organisms. All the tested chemicals showed moderate antifungal activities. The di-*O*-acetyl derivative 8 and di-*O*-methane-sulphonyl derivative 9 were found more effective against *Macrophomina phaseolina* than that of standard antibiotic, Nystatin.

In present study it was evident that some test chemicals showed marked inhibition against certain human pathogenic bacteria and phytopathogenic fungi. So it is hoped that these rhamnopyranoside derivatives may be commercially potential antibacterial and antifungal chemicals which will be evaluated later along with their toxicity (LD_{50}).

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