Synthesis of Some Disaccharides Containing an \( \alpha \)-L-Rhamnopyranosyl or \( \alpha \)-L-Mannopyranosyl Residue, and the Substrate-specificity of \( \alpha \)-L-Rhamnosidase from *Aspergillus niger* †

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In order to investigate the substrate-specificity of \( \alpha \)-L-rhamnosidase from *Aspergillus niger*, the following disaccharides were synthesized: \( 2-O-\alpha \)-L-rhamnopyranosyl-\( \alpha \)-D-fucopyranose (1), methyl \( 4-O-\alpha \)-L-rhamnopyranosyl-\( \beta \)-L-arabinopyranoside (2), methyl \( 2-O-\alpha \)-L-rhamnopyranosyl-\( \alpha \)-L-rhamnopyranoside (3) and \( 6-O-\alpha \)-L-mannopyranosyl-D-glucopyranose (4).

The action of \( \alpha \)-L-rhamnosidase on compounds 1~4 and another fifteen disaccharides containing \( \alpha \)- or \( \beta \)-L-rhamnopyranosidic bonds or an \( \alpha \)-L-mannopyranosidic bond was examined. As the result, all the disaccharides having an \( \alpha \)-L-rhamnopyranosidic linkage were hydrolyzed well, while the ones having \( \beta \)-L-rhamnopyranosidic or \( \alpha \)-L-mannopyranosidic linkage could not be hydrolyzed at all. Accordingly, this enzyme might be used for the determination of anomeric configurations of the L-rhamnopyranosidic bond, although further studies on the specificity of the enzyme are required.

L-Rhamnose is found as a constituent of many glycosides, glycolipids, plant gums and immunologically important polysaccharides. It is usual to find that naturally occurring L-rhamnose has an \( \alpha \)-L-pyranosidic linkage, but several reports of \( \beta \)-L-rhamnopyranosidic residues in glycosides and polysaccharides have also appeared. In the determination of the anomeric configurations of L-rhamnoses, Klyne’s rule \(^1\) has been applied. \(^1\)H-NMR spectra are of no use, since each anomeric proton signal of \( \alpha \)- and \( \beta \)-L-rhamnosides appears as a singlet. Recently, however, \(^13\)C-NMR spectra \(^2\) have been successfully used for this objective.

We planned to search for well-defined L-rhamnosidas induced in *Aspergillus* species and to use them for the structural analysis of glycosides and complex carbohydrates containing L-rhamnosyl linkages. In the present paper, an attempt was made to prepare \( 2-O-\alpha \)-L-rhamnopyranosyl-\( \alpha \)-D-fucopyranose (1), methyl \( 4-O-\alpha \)-L-rhamnopyranosyl-\( \beta \)-L-arabinopyranoside (2), methyl \( 2-O-\alpha \)-L-rhamnopyranosyl-\( \alpha \)-L-rhamnopyranoside (3) and \( 6-O-\alpha \)-L-mannopyranosyl-D-glucopyranose (4) in order to use them as substrates toward \( \alpha \)-L-rhamnosidase from *Aspergillus niger*. Compound 1 and \( 4-O-\alpha \)-L-rhamnopyranosyl-\( \alpha \)-L-rhamnopyranoside have been found as the sugar components of ophiopogonin B \(^3\) and hederagenin 3,28-Bisglycoside (saponin G), \(^4\) respectively. The syntheses of 1, 2 and 4 have not been previously reported.

Until now, methyl \( 3,4-O\)-benzylidene-\( \beta \)-D-fucopyranoside, \(^5\) methyl \( 3,4-O\)-isopropylidene-\( \alpha \), \(^6\) and -\( \beta \)-D-fucopyranoside \(^7\) have been known as the partially protected D-fucopyranoses, which have a free hydroxyl group only at C-2. We freshly prepared methyl \( 3,4-di-O\)-benzyl-\( \beta \)-D-fucopyranoside (5) as follows. \( 2,3,4\)-Tri-\( O\)-acetyl-\( \alpha \)-D-fucopyranosyl bromide (6) \(^8\) was treated with methanol in the presence of tetra-n-butylammonium bromide to give \( 3,4-di-O\)-acetyl-\( 1,2-O\)-(1-methoxyethylidene)-\( \alpha \)-D-fucopyranoside (7), which was

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benzylated after deacetylation with methanolic ammonia to afford 3,4-di-O-benzyl-1,2-O-(1-methoxyethylidene)-\(\alpha\)-D-fucopyranoside (8).

Treatment of 8 with stannic chloride\(^9\) gave isomeric methyl 2-O-acetyl-3,4-di-O-benzyl-\(\beta\)-D-fucopyranoside (9) (anomeric proton, \(J = 8\) Hz), which yielded after deacetylation methyl 3,4-di-O-benzyl-\(\beta\)-D-fucopyranoside (5). Condensation of 5 with 2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl bromide (10)\(^10\) according to the Helferich–Zirner method gave methyl 3,4-di-O-benzyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-D-fucopyranoside (11) and methyl 3,4-di-O-benzyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-D-fucopyranoside (12) in 44.87% and 2.47% yields, respectively. Hydrogenolysis followed by acetylation of 11 and 12 yielded methyl 3,4-di-O-acetyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-D-fucopyranoside (13) and methyl 3,4-di-O-acetyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-D-fucopyranoside (14), respectively. Acetolysis of 13 with 1.5% sulfuric acid-acetic anhydride afforded 1,3,4-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-D-fucopyranosyl-\(\alpha\)-L-rhamnopyranosyl) (15), from which 1,2,3-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-D-fucopyranose (16) could be isolated by column chromatography. Deacetylation of 13, 14 and 16 with methanolic sodium methoxide gave methyl 2-O-\(\alpha\)-L-rhamnopyranosyl-3,4-di-O-benzyl-\(\beta\)-D-fucopyranoside (17), methyl 2-O-\(\beta\)-L-rhamnopyranosyl-3,4-di-O-benzyl-\(\beta\)-D-fucopyranoside (18) and 2-O-\(\alpha\)-L-rhamnopyranosyl-3,4-di-O-benzyl-\(\beta\)-D-fucopyranoside (19). The anomeric configuration of the d-fucose residue of 1 was deduced to be \(\alpha\)-form, because its \([\alpha]_D^o\) changed from an initial value of +25.8° to a final value of −4.5° after 24 hr in water.

Coupling of methyl 2,3-di-O-benzoyl-\(\beta\)-L-arabinopyranoside (19)\(^12\) with 2,3,4-tri-O-benzoyl-\(\alpha\)-L-rhamnopyranosyl bromide (20)\(^13\) according to the Hanessian–Banoub method\(^14\) yielded the coupling product, which gave after debenzylation followed by acetylation, methyl2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\beta\)-L-arabinopyranoside (21). Deprotection of 21 with methanolic sodium methoxide gave 2.

The synthesis of 3 has already been reported by Lipták et al.\(^15\) using methyl 3,4-di-O-benzyl-\(\alpha\)-L-rhamnopyranoside (22) and 10 as starting materials. We could prepare 22 by a different method, namely, the stereoselective cleavage of easily accessible 3,4-di-O-benzyl-1,2-O-(1-methoxyethylidene)-\(\beta\)-L-rhamnopyranose (23)\(^16\) with methanolic hydrogen chloride to give 22. Coupling of 22 with 10 by the Helferich–Zirner method\(^11\) yielded methyl 3,4-di-O-benzyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\alpha\)-L-rhamnopyranoside (24) in a 31.59% yield. Hydrogenolysis followed by acetylation of 24 gave methyl 3,4-di-O-acetyl-2-O-(2,3,4-tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl)-\(\alpha\)-L-rhamnopyranoside (25). Deacetylation of 25 afforded 3.

Condensation of 1,2,3,4-tetra-O-acetyl-\(\beta\)-D-glycopyranose (26)\(^17\) with 2,3,4,6-tetra-O-acetyl-\(\alpha\)-L-mannopyranosyl bromide (27)\(^18\) as just described gave the coupling product which was deacetylated with methanolic sodium methoxide to afford 4. In order to characterize 4, it was acetylated with acetic anhydride and sodium acetate to yield crystalline 1,2,3,4-tetra-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-L-mannopyranosyl)-\(\beta\)-D-glucofuranose (28).

The anomeric configurations of the \(\alpha\)-L-rhamnosidic and \(\alpha\)-L-mannopyranosidic linkages of 1, 2 and 4 were deduced to be \(\alpha\)-forms from the results of applying Klyne’s rule to 2, 4, 11, 12, 17, 18 and 28 as described under Experimental.

Until now, the \(\alpha\)-L-rhamnosidases of Rhamnus dahurica,\(^19\) Aspergillus niger,\(^20\) Fagopyrum esculantum,\(^21\) liver of Turbo cornutus,\(^22\) Klebsiella aerogenes,\(^23\) Corticium rolfsii\(^24\) and hog adrenals\(^25\) have been described. We also examined the hydrolytic properties of 1, 2, 3, 4, 17, 18 and the other substrates listed in the table toward \(\alpha\)-L-rhamnosidase obtained by the induction of Aspergillus niger with naringin. As the result, all the disaccharides which have an \(\alpha\)-L-rhamnopyranosidic bond were well hydro-
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Table I. Hydrolysis Test of Disaccharides toward α-L-Rhamnosidase from Aspergillus niger

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Hydrolytic products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-O-α-L-Rhamnopyranosyl-D-fructopyranose</td>
<td>L-Rhamnose, D-fructose</td>
</tr>
<tr>
<td>2-O-α-L-Rhamnopyranosyl-D-glucopyranose</td>
<td>L-Rhamnose, D-glucose</td>
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<tr>
<td>2-O-α-L-Rhamnopyranosyl-D-galactopyranose</td>
<td>L-Rhamnose, D-galactose</td>
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<td>2-O-α-L-Rhamnopyranosyl-D-mannopyranose</td>
<td>L-Rhamnose, D-mannose</td>
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<tr>
<td>2-O-α-L-Rhamnopyranosyl-D-xylopyranose</td>
<td>L-Rhamnose, D-xylene</td>
</tr>
<tr>
<td>2-O-α-L-Rhamnopyranosyl-L-arabinopyranose</td>
<td>L-Rhamnose, L-arabinose</td>
</tr>
<tr>
<td>2-O-α-L-Rhamnopyranosyl-D-fucopyranose</td>
<td>L-Rhamnose, d-fucose</td>
</tr>
<tr>
<td>Methyl 2-O-β-L-Rhamnopyranosyl-D-fucopyranoside</td>
<td>L-Rhamnose, methyl β-D-fucose</td>
</tr>
<tr>
<td>Methyl 2-O-β-L-Rhamnopyranosyl-D-fucopyranoside</td>
<td>L-Rhamnose, methyl β-L-rhamnose</td>
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<tr>
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<td>4-O-α-L-Rhamnopyranosyl-D-glucopyranose</td>
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<td>Methyl 4-O-α-L-Rhamnopyranosyl-L-arabinopyranoside</td>
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<tr>
<td>5-O-α-L-Rhamnopyranosyl-D-glucopyranose</td>
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<td>6-O-β-L-Rhamnopyranosyl-D-glucopyranose</td>
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<td>6-O-α-L-Rhamnopyranosyl-D-mannopyranose</td>
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<tr>
<td>2-O-α-L-Mannopyranosyl-D-glucopyranose</td>
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</tr>
<tr>
<td>6-O-α-L-Mannopyranosyl-D-glucopyranose</td>
<td>L-Rhamnose, D-glucose</td>
</tr>
</tbody>
</table>

All the compounds listed in the above Table were synthesized according to the following literatures.


lyzed while the ones which have a β-L-rhamnopyranosidic bond were not hydrolyzed at all. The disaccharides which have an α-L-mannopyranosidic linkage were not hydrolyzed either, so this enzyme might be useful for the elucidation of the anomic character of natural L-rhamnopyranosides, although further studies are required in order to draw general conclusions as to the nature of L-rhamnosidic bonds susceptible to the enzyme. Barker et al.<sup>23</sup>) have reported that α-L-rhamnosidase and β-L-rhamnosidase were inducted in the culture of Klebsiella aerogenes with methyl α-L-rhamnopyranoside and with methyl β-L-rhamnopyranoside, respectively, and these enzymes were shown to be stereospecific in their ability to hydrolyze α- and β-L-rhamnopyranosides in the *Diplococcus pneumoniae* Type II polysaccharide. Accordingly, it is very interesting to investigate that the *Aspergillus niger* used in this paper has or has not the ability of producing β-L-rhamnosidase with such inducers as compounds having a β-L-rhamnopyranosidic bond.

Okada et al.<sup>20</sup>) have reported that there are two regiospecific types of α-L-rhamnosidases from *Aspergillus niger*, namely, one hydrolyzed rutinose (6-O-α-L-rhamnopyranosyl-D-glucose) and the other neohesperidose (2-O-α-L-rhamnopyranosyl-D-glucose), these being
our laboratory.

EXPERIMENTAL

All melting points are uncorrected. NMR spectra were recorded in CDCl₃ with TMS as an internal standard at 60 MHz on a Varian EM-360 spectrometer. Optical rotations were measured on a Horiba SPA-200 digital polarimeter. Thin-layer chromatography was performed on Merck Kieselgel 60 F₂₅₄ using the following solvents. Solvent A: benzene-ethyl acetate (9:1); solvent B: n-hexane-ethyl acetate (2:1); solvent C: benzene-ether (3:1); solvent D: ethyl acetate-isopropyl alcohol-water (5:7:3); solvent E: benzene-ether (1:1); solvent F: chloroform-ethyl acetate (1:1); solvent G: benzene-ether (2:1).

Microorganism and its cultural method. Aspergillus niger No. 0, stocked at the Laboratory of Applied Microbiology, Faculty of Agriculture, Shizuoka University, was surface-cultured at 30°C for 3 days in a Erlenmeyer flask containing 20 ml of a medium composed of naringin (0.5%), peptone (2%), K₂HPO₄ (0.5%) and KH₂PO₄ (0.5%) and adjusted to pH 6.5.

Crude enzyme (α-L-rhamnosidase) preparation. The mycelia from seven flasks were harvested by filtration, washed with tap water and the excess water was removed from the mycelia by pressing them between dry filter papers. The yield of the mycelia was approximately 1.95 g on wet weight basis. The mycelia were homogenized in a mortar and to this, 30 ml of water was added. After the suspension had been incubated at 30°C for 24 hr, the mycelial debris was removed by centrifugation, and the supernatant was used as a crude enzyme solution.

Hydrolysis test of various disaccharides toward α-L-rhamnosidase. The reaction mixture containing 5 ml of enzyme solution, 100 mg of the substrate in 5 ml of water, and 5 ml of McIlvaine buffer (pH 6.8) was incubated at 30°C for 24 hr and the reaction was stopped by heating for 5 min in a boiling water bath. The reaction mixture was evaporated to a small quantity, which was spotted on a Toyo No. 2 filter paper, and the paper was developed by the ascending method with H-butanol-acetic acid-water (4:1:1). After drying, the hydrolytic products were detected by anilinehydrophthalate.

3,4-Di-O-acetyl-1,2-O-(1-methoxyethylidene)-α-D-fucopyranose (7). To a mixture of lutidine (30 ml) and methanol (1.2 ml) were added tetra-n-butylammonium bromide (3 g) and 6 (9.8 g), and the solution was kept at 50°C overnight. The reaction mixture was diluted with chloroform, washed with just sufficient hydrochloric acid to neutralize the lutidine, then washed withaq. sodium hydrogen carbonate and finally with water. The chlo-
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roform layer was dried (CaCl₂). After filtration, the filtrate was evaporated and the residual syrup (7 g) was purified on a silica gel column (160 g) with benzene-ethyl acetate (9:1). 7, colorless syrup, 4.86 g (57.4%), [α]D + 138.3° (c = 1.8, MeOH), Rf 0.36 (A). NMR δ[H] at CCl₃: 1.10 (3H, d, J = 6 Hz, CH-Me); 1.50, 1.56 (3H, each s, C-Me and exo); 1.96 (3H), 2.03 (3H, each s, 2Ac); 3.16, 3.23 (3H, each s, OMe and exo); 3.86~4.30 (2H, m, H-2,5); 4.73~5.13 (2H, m, H-3,4); 5.43, 5.56 (1H, each d, J = 4.5 Hz, H-1end and exo). Anal. Found: C, 51.05; H, 6.42. Calcld. for C₁₃H₂₀O₈: C, 51.32; H, 6.58%. 3,4-Di-O-benzyl-1,2-O-(1-methoxyethylidene)-α-D-fucopyranoside (8). To a solution of 7 (4.8 g) in methanol (30 ml) was added methanol saturated with ammonia (30 ml), and the solution was left for 48 hr at room temperature. After evaporation of the solvent, the syrupy residue (4 g, [α]D + 72° (c = 1.0, CHCl₃)) was dissolved in dry dimethylformamide (30 ml). Benzyl bromide (5.9 ml) was added dropwise to the suspension with ice-cooling and stirring. The mixture was stirred for 48 hr at room temperature and methanol was added. After being kept for 1 hr, the solution was poured into ice-cooled water (500 ml) and brought to pH 8 with 1 N hydrochloric acid. The solution was extracted with ethyl acetate three times, and the extract was washed with water and dried (CaCl₂). After filtration, the filtrate was evaporated and the syrupy residue (7.08 g) was chromatographed on a silica gel column (160 g) with benzene-ethyl acetate (9:1) as the eluant. 9, needles (1.54 g) in acetonitrile (40 ml) was added 10% Pd-C (2 g), and the mixture was left for 24 hr at room temperature. The solution was treated according to the literature and the final syrup (5.93 g) was fractionated on a silica gel column (170 g) using n-hexane-ethyl acetate (2:1) as the eluant. 11, syrup, 2.4 g (44.8%), Rf 0.49 (B), [α]D + 38° (c = 1.26, CHCl₃). NMR δ[H] in CDCl₃: 1.16 (3H, d, J = 6 Hz, H-1); 1.25 (3H, each s, J = 6 Hz, 2Me); 1.96 (3H, 2.00 (3H, 2.03 (3H, each s, 3Ac); 3.23~4.73 (10H, m, H-1~5); 3.16 (3H, s, OMe); 4.73~5.20 (4H, m, 2 PhCH₂). 6.93 (10H, 2Ph). Anal. Found: C, 62.75; H, 6.58. Calcd. for C₁₃H₂₀O₈: C, 62.86; H, 6.67%. 12, plates (130 mg, 2.42%), mp 151~153°C, Rf 0.81 (B), [α]D + 9° (c = 1.87, CHCl₃). NMR δ[H] in CDCl₃: 1.14 (3H, d, J = 6 Hz, H-1); 1.20 (3H, each s, J = 6 Hz, 2Me); 1.90 (3H), 1.96 (6H, each s, 3Ac); 3.03~4.66 (9H, m, H-1~5); 3.16 (3H, s, OMe); 4.67~4.87 (4H, m, 2 PhCH₂); 5.16 (1H, s, H-1'); 6.66~7.13 (10H, m, 2Ph). Anal. Found: C, 62.73; H, 6.61. Calcd. for C₁₃H₂₀O₈: C, 62.86; H, 6.67%. Calcld. for [M]D (methyl triacetyl-α-L-rhamnoside) = −16324+ [M]D (5, −3340) = +19664. The glycosidic linkages of 11 and 12 were, therefore, of α- and β-configurations.

Methyl 3,4-di-O-benzyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-fucopyranoside (13) and Methyl 3,4-di-O-benzyl-2-O-(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)-β-D-fucopyranoside (14). To a solution of 11 (2.2 g) in ethanol (40 ml) was added 10% Pd-C (2 g), and the mixture was shaken with hydrogen (3.5 kg/cm²) at 50°C for 48 hr. After the reaction was complete, the mixture was filtered, and the filtrate was evaporated. The residual syrup was dissolved in pyridine (20 ml), mixed with acetic anhydride (20 ml) and left to stand for 24 hr at 30°C. The solution was poured into ice-cooled water with stirring and left overnight. The precipitate was collected by filtration and dried (890 mg). The filtrate was extracted with chloroform, and the extract was washed with acq. 5% sulfuric acid,aq. sodium hydrogen carbonate and finally with water, and (100 ml) was left for 4 hr at 30°C. After neutralization with acetic acid, the solution was evaporated to a small quantity, and the crystals deposited were collected by filtration and recrystallized from aq. ethanol as needles in a 2.03 g (90.62%) yield, mp 109°C, Rf/0.23 (A), [α]D + 12° (c = 2.25, CHCl₃). NMR δ[H] at CCl₃: 1.20 (3H, d, J = 6 Hz, Me); 2.23 (1H, bs, OH); 3.20~4.13 (5H, m, H-1~5); 3.13 (3H, s, OMe); 4.60 (2H, s, PhCH₂); 4.70 (2H, q, J = 12 Hz, PhCH₂); 7.40 (10H, bs, 2Ph). Anal. Found: C, 70.45; H, 7.28. Calcd. for C₂₁H₂₆O₈: C, 70.39; H, 7.26%.
then dried (CaCl₂). After filtration, the filtrate was evaporated, and the solid (1.2 g) was combined with the aforementioned precipitate and purified on a silica gel column (160 g) with benzene–ether (3:1) as the eluant. 13, needles (EtOH), 1.83 g (98.3%), mp 125°C, [α]D 19° (c = 1, CHC1₃), Rf 0.18 (C). NMR δ(Me, D): 1.10 (6H, d, J = 6 Hz, 2Me); 1.85 (3H), 1.90 (6H), 1.96 (6H, each s, 5Ac); 3.30 (3H, s, Me); 3.16 ~ 4.16 (4H, m, H-1,2,5,5') 4.40 ~ 4.90 (7H, H-1,3,4,1'-4'). Anal. Found: C, 51.60; H, 6.28.

Calcd. for C₂₃H₃₄O₁₄: C, 51.69; H, 6.67%.

1,3,4-Tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-fucopyranose (15) and 1,3,4-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-fucopyranose (16). A solution of 13 (0.7 g) in acetic anhydride (100 ml) was mixed with 1.5% (v/v) sulfuric acid–acetic anhydride (12 ml) and stirred for 3.5 hr at room temperature. The mixture was poured into ice-cooled water containing sodium hydrogencarbonate. The usual work-up gave a syrupy free sugar which was debenzoylated with 0.1 n methanolic sodium methoxide (20 mL) and left to stand for 3 hr at room temperature. The reaction mixture was treated as described above to give 1 as needles (acetone) in a 142 mg (50.0%) yield, mp 145°C (lit.,27) 145°C). Rf 0.74 (D), [α]D+55.5° (c = 1.1, H₂O). Anal. Found: C, 51.69; H, 6.37%

1,3,4-Tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-fucopyranose (15) and 1,3,4-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-fucopyranose (16). A solution of 13 (0.7 g) in acetic anhydride (6 ml) was mixed with 1.5% sulfuric acid–acetic anhydride (12 ml) and stirred for 3.5 hr at room temperature. The reaction mixture was poured into ice-cooled water containing sodium hydrogen carbonate. The mixture was extracted with chloroform, and the extract was washed with water and dried (CaCl₂). After filtration, the filtrate was evaporated and the residual syrup, 80 mg (94.3%), [α]D 50° (c = 1, CHCl₃), Rf 0.10 (C). NMR δ(Me, D): 1.14 (3H), 1.24 (3H, each s, J = 6 Hz, 2Me); 2.01 (3H), 2.08 (6H), 2.18 (6H, each s, 5Ac); 3.51 (3H, s, Me); 3.31 ~ 4.44 (3H, m, H-1,3,4,1'-4'). Anal. Found: C, 51.69; H, 6.67%.

Methyl 4-O-α-L-rhamnopyranosyl-β-D-fucopyranoside (2). Deacetylation of 21 (0.2 g) with 0.1 n methanolic sodium methoxide (20 ml) for 3 hr at room temperature in the usual way gave 2 as an amorphous powder (0.1 g,
83.33\%, Rf 0.68 (D), [a]_{D}^{20} + 120° (c=0.4, H_{2}O), mp 200~203°C. Anal. Found: C, 45.85; H, 7.06. Calcd. for C_{36}H_{59}O_{19}C, 46.45; H, 7.06\%. Calcd. for [M]_{D} (methyl \alpha-L-rhamnopyranoside), -11100 + [M]_{D} (methyl \beta-L-arabinoside), +39688 = +28768. Calcd. for [M]_{D} (methyl \beta-L-rhamnopyranoside), +17000 + [M]_{D} (methyl \beta-L-arabinoside), +39688 = +56868. Found for [M]_{D} (2) = +37200. Therefore, the rhamnopyranosidic linkage of 2 is likely to be of z configuration.

**Methyl 3,4-di-O-benzyl-\alpha-L-rhamnopyranoside** (22). To a stirred and boiling solution of 23 (6 g) in methanol (120 ml) was added dropwise acetyl chloride (3.6 ml) and the solution was refluxed for 18 hr. The methanol was removed from the solution and the resulting syrup was dissolved in chloroform, washed with aqueous sodium hydrogencarbonate and water, and dried (CaCl_{2}). After filtration, the filtrate was evaporated to give a syrup 22 (4.1 g, 76.35\%). This was contaminated with a slight amount of the \beta-isomer but could be used in the next reaction without further purification. Some of this syrup was subjected to silica gel column chromatography using benzene-ethyl acetate (9:1) and the pure 22 was obtained. Rf 0.77 (F). [M]_{D}^{20} - 46° (c=1, CHCl_{3}) (lit., 15) -46.4°. Its NMR data was identical with that reported in the literature.15

**Methyl 3,4-di-O-benzyl-2-O-(\alpha-L-rhamnopyranosyl)-(\alpha-L-rhamnopyranoside)** (24). Compound 24 has already been prepared by Lipták et al.15 by coupling 22 with 10 in benzene-nitromethane solvent and the mixture was allowed to stand overnight at room temperature. We carried out as follows. To a solution of 24 (1 g) in ethanol (20 ml) was added 10% Pd-C (10%) (1 g) and the mixture was shaken under hydrogen (3.5 kg/cm²) for 48 hr at 50°C. After removal of the catalyst by filtration, the filtrate was evaporated, and the residue (0.8 g) was acetylated with a mixture of acetic anhydride (10 ml) and pyridine (10 ml) as described above. 25 was obtained as needles (aq. ethanol) in a 0.5 g (59.03\%) yield, mp 151~152°C. [a]_{D}^{20} - 45° (c=1, CHCl_{3}) (lit., 15) mp 151~152°C, [a]_{D} - 44.8°. No NMR data were given in the literature.15 \delta_{\text{CDCl}_{3}}: 1.12 (3H), 1.15 (3H, each d, /=8Hz, 2Me); 1.93 (3H), 1.97 (3H, 2.00 (6H), 2.07 (3H, each s, 5Ac); 2.30 (3H, s, OMe); 3.56~4.16 (3H, m, H-2,5,5'); 4.50~5.42 (7H, m, 31.59% (2.3 g), mp 125~126°C (lit., 15). Compound 25 was deacetylated according to the literature15 to give 3 as a syrup in almost a quantitative yield. [a]_{D}^{20} - 95° (c=1, H_{2}O) (lit.) [a]_{D} - 92°.

**6-O-\alpha-L-Mannopyranosyl-\alpha-D-glucopyranose** (4). To a solution of mercuric cyanide (2.76 g) and mercuric bromide (3.91 g) were added 26 (7.59 g) and 27 (8.8 g), and the mixture was allowed to stand overnight at room temperature. The reaction mixture was treated according to the literature,11 and the final syrup (17 g) was dissolved in 0.05 n nethanolic sodium methoxide (90 ml) and the solution was kept at 0°C overnight. The solution was neutralized with Amberlite IR-120 (H⁺) resin and filtered. The filtrate was evaporated to a syrup (6.29 g) which were composed of L-mannose, D-glucose and 4. The syrup was dissolved in a small quantity of water and chromatographed on a carbon-cellulose (1:1) column (200 g). Elution with water (3000 ml) gave a mixture of D-glucose and D-mannose. The next elution with 10% ethanol (1500 ml) gave an amorphous 4, after evaporation of the solvent, in a yield of 28.85\% (2.19 g), [a]_{D}^{20} + 13° (c=7.78, H_{2}O), Rf 0.37 (D). Anal. Found: C, 38.26; H, 6.45. Calcd. for C_{12}H_{22}O_{11}·H_{2}O: C, 38.09; H, 6.88\%. Calcd. for [M]_{D} (methyl \alpha-L-mannoside), -15908 + [M]_{D} (methyl \alpha-D-glucose, +9450) = -4658. Calcd. for [M]_{D} (methyl \beta-L-rhamnopyranoside), +10282 + [M]_{D} (methyl \beta-D-glucopyranose, +19732) + [M]_{D} (\alpha-D-glucopyranose, +9450) = -6458. Calcd. for [M]_{D} (methyl \beta-L-mannoside), +15908 + [M]_{D} (\alpha-D-glucopyranose, +9450) = +19732. Found for [M]_{D} (4) = -4680. Therefore, the configuration of L-mannopyranosidic linkage of 4 was deduced to be of \alpha form.

1,2,3,4-Tetra-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-\alpha-L-rhamnopyranosyl)-\beta-D-glucopyranose (28). Compound 4 (1.5 g) was mixed with acetic anhydride (15 ml) and anhydrous sodium acetate (1.5 g), and heated in a boiling water bath for 2hr. After cooling, the reaction mixture was poured into ice-cooled water and left to stand overnight. The precipitate was collected, dried and crystallized from ethanol to give 28 as needles (1.43 g, 48.15\%), mp 177°C, [a]_{D}^{20} - 17° (c=1, CHCl_{3}) NMR \delta_{\text{CDCl}_{3}}: 1.89, 1.96 (24H, each s, 8Ac); 3.56~3.96 (6H, H-5,6,6,5',6',6'); 4.48 (1H, s, H-1'); 4.56~5.06 (6H, H-2~4, 2~4'); 5.29 (1H, d, /=8Hz, H-1). Calcd. for [M]_{D} (26, +4176) + [M]_{D} (methyl 2,3,4,6-tetraacetyl-\alpha-L-rhamnopyranoside).
l-mannopyranoside,\(^{31}\) \(-17378\times 10^3\) = \(-13562\). Calcd. for (26, +4176) + [M]\(_D\) (methyl 2,3,4,6-tetraacetyl-\(\beta\)-l-mannopyranoside,\(^{31}\) +15322) = +19498. Found for [M]\(_D\) (28) = -11256. Therefore, the l-mannopyranoside linkage of 28 was of \(\alpha\) configuration. Anal. Found: C, 49.36; H, 5.06. Calcd. for C\(_{28}\)H\(_{38}\)O\(_{19}\): C, 49.55; H, 5.60%.

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