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Synthesis of α-L-Mannopyranosyl-containing Disaccharides and Phenols as Substrates for the α-L-Mannosidase Activity of Commercial Naringinase

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In order to clarify the substrate specificity of the α-L-mannosidase activity of naringinase (Sigma), the following disaccharides and phenol glycosides were freshly prepared: methyl 2-*O*-(α-L-mannopyranosyl)-β-D-glucoside (1), methyl 3-*O*-(α-L-mannopyranosyl)-α-D-glucoside (2), methyl 4-*O*-(α-L-mannopyranosyl)-α-D-glucoside (3), methyl 5-*O*-(α-L-mannopyranosyl)-β-D-glucoside (4), methyl 6-*O*-(α-L-mannopyranosyl)-α-D-glucoside (5), 6-*O*-(α-L-mannpyranosyl)-D-galactose (6), *p*-nitrophenyl α-L-mannoside (7), and 4-methyl umbelliferone α-L-mannoside (8). These compounds, except for 3 and 5, were hydrolyzed with naringinase.

Naringin, whose structure is 4′,5,7-trihydroxyflavanone 7-*O*-[2-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside], is a bitter principle of several citrus juices, and its hydrolyzing enzyme "naringinase" has been used on an industrial scale to remove the bitterness from such juices. The term "naringinase" is given to multiple enzymes which contain α-L-rhamnosidase and β-D-glucosidase activities.

Until now, several α-L-rhamnosidases have been isolated from various microorganisms, *e.g.*, *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium decumbens*. Michon *et al.*[1] have recently reported the substrate specificity of naringinase, a commerical preparation obtained from *Penicillium decumbens* (Sigma), by using a number of synthetic α-L-rhamnooligosides and two disaccharides containing an α-L-mannopyranosidic bond as substrates. They found that both α-L-rhamonosides and, surprisingly, α-L-mannosides were hydrolyzed, indicating that the hydroxylmethyl group at C-5 of the terminal residue of each did not retard the hydrolysis of these oligomeric molecules.

On the other hand, we have reported in a previous paper[2] that α-L-rhamnosidase obtained from *Aspergillus niger* No. 0 was inactive toward disaccharides having an α-L-mannopyranosidic bond. Since we are interested in the α-L-mannosidase activity of the foregoing commercial naringinase, several disaccharides containing non-reducing terminal α-L-mannopyranosyl residues were synthesized, and their susceptibility to enzymatic hydrolysis with naringinase was examined. Additionally, *p*-nitrophenyl α-L-mannopyranoside and umbelliferone α-L-mannopyranoside were prepared and their hydrolysis with naringinase was tested.

The following disaccharides were prepared in order to use as the enzyme substrates: methyl 2-*O*-(α-L-mannopyranosyl)-β-D-glucopyranoside (**1**), methyl 3-*O*-(α-L-mannopyranosyl)-α-D-glucopyranoside (**2**), methyl 4-*O*-(α-L-mannopyranosyl)-α-D-glucopyranoside (**3**), methyl 5-*O*-(α-L-mannopyranosyl)-β-D-glucofuranoside (**4**), methyl 6-*O*-(α-L-mannopyranosyl)-α-D-glucopyranoside (**5**) and 6-*O*-(α-L-mannopyranosyl)-D-galactopyranose (**6**). The syntheses of **1**–**6** do not appear to have been described previously.

Treatment of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (**7**)[3] with chloroacetyl chloride gave 1,3,4,6-tetra-*O*-acetyl-2-*O*-chloroacetyl-α-D-glucopyranose (**8**). The α-glycosyl bromide (**9**) of **8** was treated with methanol in the presence of silver carbonate to give a corresponding methyl glycoside (**10**). De-*O*-chloroacetylation of **10** with thiourea[4] gave methyl 3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (**11**). The reaction of 2,3,4,6-tetra-*O*-acetyl-α-L-mannopyranosyl chloride (**12**)[5] with **11** in the presence of mercury(II) cyanide and mercury(II) bromide[3] in acetonitrile gave coupling product **13** in a 23.5% yield, which, after deprotection, afforded **1**. The reaction of **12** with methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**14**)[6] in the presence of silver triflate and tetramethylurea[7] in dichloromethane gave coupling product **15** in a 26. 1% yield, which, after deprotection, afforded **2**. Coupling 2,3,4,6-tetra-*O*-benzoyl-α-L-mannopyranosyl bromide (**16**)[8] to methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**17**)[9] in the presence of silver triflate and 4A molecular sieves gave reaction product **18** in a 33% yield, which, after de-*O*-benzoylation, gave **3**. Treatment of **12** with 3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**19**)[10] in the presence of mercury(II) cyanide and mercury(II) bromide gave coupling product **20** in a 42.5% yield, which, after deacetylation gave 1,2-*O*-isopropylidene-5-*O*-(α-L-mannopyranosyl)-α-D-glucofuranose (**21**). Compound **21**, after de-*O*-isopropylidenation, was reacetylated with acetic anhydride and pyridine, and the resultant product (**22**) was further treated with hydrogen bromide to yield a bromide (**23**). Methanolysis of **23** in the presence of silver carbonate, gave methyl glycoside **24**, which was deacetylated to yield **4**. Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (**25**)[11] was reacted with **12** in the presence of mercury(II) cyanide

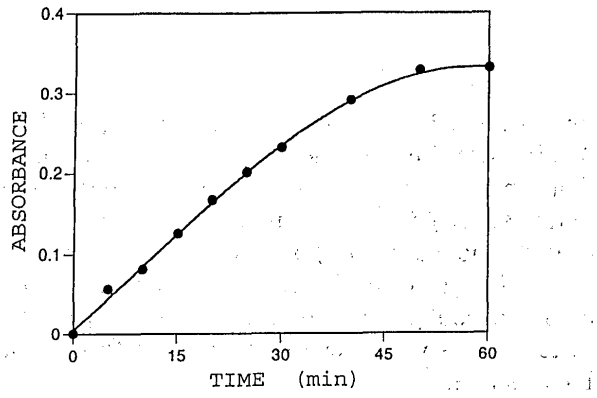

Fig. 1. Relationship between the Reaction Time and the Absorbance of Liberated *p*-Nitrophenol.

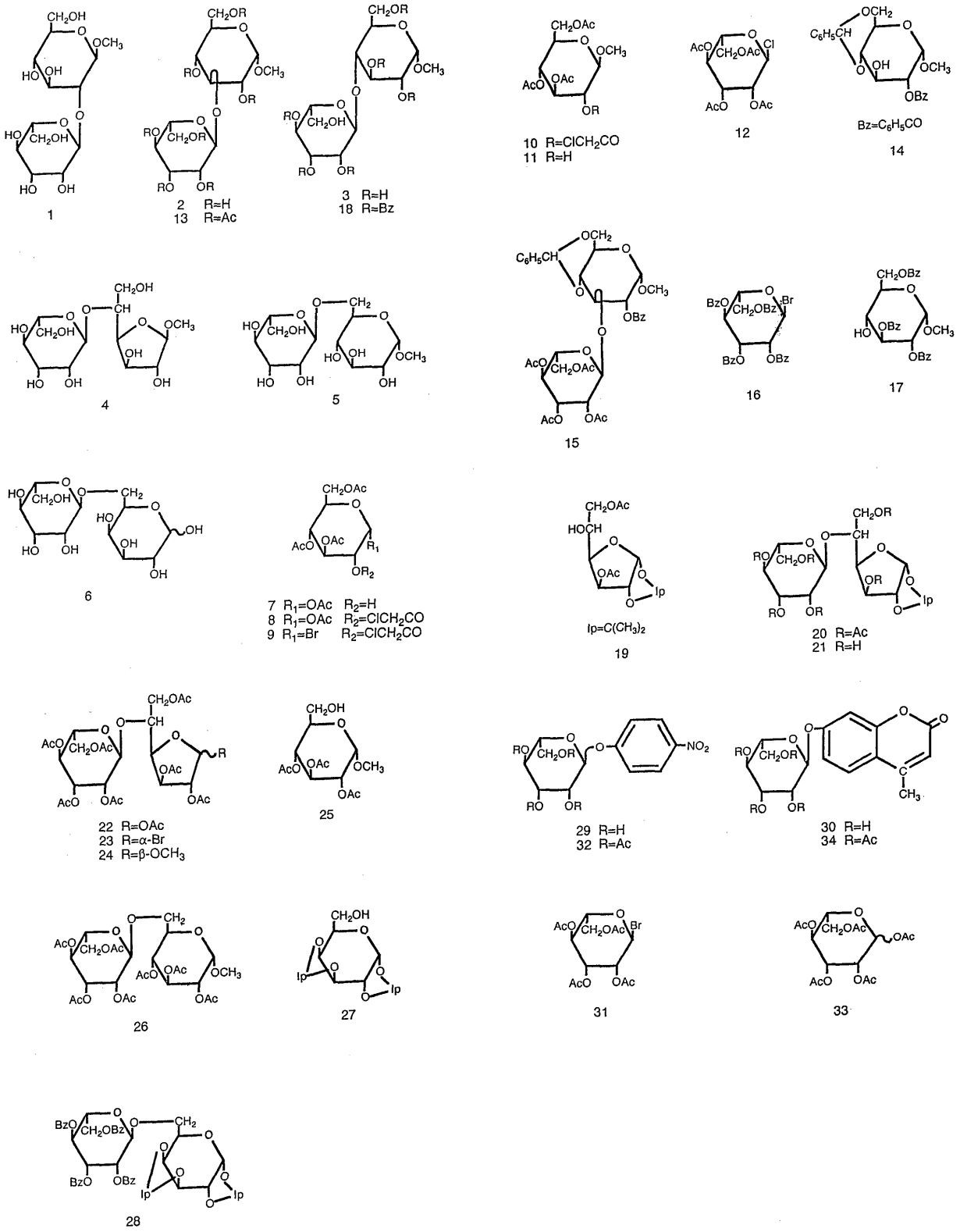

Fig. 2.

and mercury(II) bromide to yield coupling product **26** in a 49.2% yield. Zémplen de-*O*-acetylation of **26** then gave **5**. Condensation of 1,2,3,4-di-*O*-isopropylidene-α-D-galacto-pyranose (**27**)[12] with **16** in the presence of mercury(II) cyanide and mercury(II) bromide gave coupling product **28** in a 15.1% yield, which, after deprotection, afforded **6**. The anomeric configuration of the α-L-mannopyranoside bond in each of **1**–**6** was determined to be α-form by applying Klyne's rule.[13]

p-Nitrophenyl α-L-mannopyranoside (**29**), 4-methyl umbelliferone α-L-mannopyranoside (**30**) were also synthesized. Coupling 2,3,4-tri-*O*-acetyl-α-L-mannopyranosyl bromide (**31**)[14] to *p*-nitrophenol in aqueous sodium hydroxide[15] gave the reaction product (**32**) in a 10.8% yield, which was subsequently deacetylated to afford **29**. As an alternative method, well-mixed penta-*O*-acetyl-L-mannopyranose (**33**) and *p*-nitrophenol were heated at 100°C in the presence of *p*-toluenesulfonic acid[16] to give foregoing coupling product **32** in a 24.4% yield.

Heating **33** and 4-methyl umbelliferone in an acetic acid-acetic anhydride mixture in the presence of anhydrous zinc chloride gave coupling product **34** in a 15.4% yield, which

was deacetylated to afford **30**.

A study of the naringinase action toward compounds **1–6** was made by applying HPLC method. As a result, the (1→2)- (1→3)- and (1→5)-linked methyl α-L-mannopyranosyl-D-glucosides and (1→6)-linked α-L-mannopyranosyl-D-galactose were hydrolyzed, while, unexpectedly, the methyl (1→4)- and (1→6)-linked α-L-mannopyranosyl-D-glucosides did not accept the enzyme action at all.

4-Methyl umbelliferone α-L-mannopyranoside and *p*-nitrophenyl α-L-mannopyranoside were hydrolyzed by the α-L-mannosidase activity of naringinase, providing 4-methyl umbelliferone and *p*-nitrophenol, respectively. Therefore, the progress of the reaction can be followed by spectrophotometrically measuring the amount of ether of these aglycones (see Fig. 1).

Experimental

General methods. Reactions were monitored by TLC on silica gel 60 (Merck), using detection by charring with 10% sulfuric acid in ethanol. Analytical high-pressure liquid chromatography (HPLC) was performed with Shimadzu LC-6S apparatus equipped with a Shim-pack (CLC-NH_2) column, using a differential refractometer as a detector and acetontrile–water (7:3) at a flow rate of 1 ml/min. Optical rotation values were measured with a Horiba SEPA-200 digital polarimeter. NMR spectra (^1H at 90 Mz and ^{13}C at 24.5 MHz) were recorded with a Hitachi R-90H spectrometer, using tetramethylsilane as an internal standard for solutions in $CDCl_3$. Microanalyses were performed by the Microanalytical Laboratory of the School of Pharmaceutical Sciences at this university.

1,3,4,6-Tetra-O-acetyl-2-O-chloroacetyl-α-D-glucopyranose (**8**). Compound **7** (10 g) was dissolved in acetonitrile (430 ml), before pyridine (7 ml) was added and the solution cooled to 0°C. A solution of chloroacetyl chloride (3.2 ml) in acetonitrile (35 ml) was added dropwise, the mixture being kept at 0°C for 1 h and then at room temperature for 3 h. After diluting with chloroform, the reaction mixture was successively washed with 5% HCl, aq. $NaHCO_3$ and water, and dried ($CaCl_2$). The filtrate was concentrated, and the residue was crystallized from ethanol to provide **8** (10.8 g, 89.1%), R_f 0.27 (toluene–ethyl acetate 8:2), mp 147.5°C, $[\alpha]_D^{20}$ 91° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.98, 2.00, 2.06, 2.15 (each 3H, s, 4 CH_3CO); 3.93 (2H, s, $ClCH_2CO$); 4.15 (3H, m, H-5, 6); 4.95–5.60 (3H, m, H-2–4); 6.32 (1H, d, $J=4$ Hz, H-1). *Anal.* Found: C, 44.94; H, 4.79. Calcd. for $C_{16}H_{21}O_{11}Cl$: C, 45.23; H, 4.95%.

3,4,6-Tri-O-acetyl-2-O-chloroacetyl-α-D-glucopyranosyl bromide (**9**). Compound **8** (10.87 g) was dissolved in chloroform (42 ml), and 30% hydrogen bromide in acetic acid (56 ml) was added at 0°C. The mixture was kept at 0°C for 1 h and then at room temperature for 1 h. After diluting with chloroform, the reaction mixture was successively washed with water (three times), aq. $NaHCO_3$ and water again, and then dried ($CaCl_2$). The filtrate was concentrated to a syrup which was crystallized from ether–petroleum ether (1:3) to yield **9** (10.5 g, 92%), R_f 0.58 (toluene–ethyl acetate 7:3), mp 107–108°C, $[\alpha]_D^{20}$ 157° ($c=1$, $CHCl_3$). *Anal.* Found: C, 37.49; H, 3.88. Calcd. for $C_{14}H_{18}BrClO_9$: C, 37.71; H, 4.04%.

Methyl 3,4,6-tri-O-acetyl-2-O-chloroacetyl-β-D-glucopyranoside (**10**). Compound **9** (10.49 g) was dissolved in methanol (580 ml), silver carbonate (6.5 g) was added, and the mixture was stirred for 24 h at room temperature in the dark. After filtering through a bed of Celite, the filtrate was concentrated, and the residue was chromatographed in a column of silica gel (toluene–ethyl acetate, 7:3) to afford **10** (0.76 g, 8.2%), after crystallizing from ethanol, R_f 0.37 (toluene–ethyl acetate, 7:3), mp 91–92°C, $[\alpha]_D^{20}$ −9° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.96, 2.00, 2.05 (each 3H, s, 3CH_3CO); 3.46 (3H, s, OCH_3); 3.98 (2H, s, $ClCH_2CO$); 4.15 (2H, t, H-6); 4.40 (1H, d, $J=8$ Hz, H-1); 4.80–5.10 (3H, m, H-2-5). *Anal.* Found: C, 45.34; H, 5.17. Calcd. for $C_{15}H_{21}ClO_{10}$: C, 45.40; H, 5.30%.

Methyl 3,4,6-tri-O-acetyl-β-D-glucopyranoside (**11**). To a solution of **10** (0.76 g) in 75% aq. acetonitrile (11 ml) was added thiourea (1.36 g), and the mixture was kept at room temperature overnight while stirring. After diluting with chloroform, the reaction mixture was sufficiently washed with water and then dried ($CaCl_2$). The filtrate was concentrated, and the residue was crystallized from ether–petroleum ether to afford **11** (0.35 g, 57.4%), R_f 0.22 (toluene–ethyl acetate 7:3), mp 96°C, $[\alpha]_D^{20}$ 14° ($c=1$, $CHCl_3$); lit.[12] 97–99°C, $[\alpha]_D^{20}$ 18° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.00 (3H), 2.04 (6H each s, 3CH_3CO); 2.60 (1H, s, OH); 3.53 (3H, s, OCH_3); 4.00–4.40, 4.90–5.20 (each m, sugar protons).

Methyl 1,3,4,6-tetra-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-L-mannopyranosyl)-β-D-glucopyranoside (**13**). To a solution of **11** (0.25 g) in acetonitrile (3 ml) containing mercury(II) cyanide (0.1 g) and mercury(II) bromide (0.14 g) was added **12** (0.29 g). The mixture was kept at room temperature overnight, diluted with chloroform, and then filtered to remove the mercury(II) salts. The filtrate was washed three times with 1 N KBr solution, and then dried ($CaCl_2$). The filtrate was concentrated, and the residue was chromatographed in a column of silica gel (chloroform–acetone, 15:1) to give **13** (0.12 g, 23.5%), R_f 0.30 (chloroform–acetone 9:1), $[\alpha]_D^{20}$ −7° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.96 (3H), 1.98 (3H), 2.00 (3H), 2.03 (6H), 2.05 (3H), 2.10 (3H each s, 7CH_3CO); 3.50 (3H, s, OCH_3); 4.00–4.40 (7H), 4.80–5.35 (7H, each m, sugar protons). *Anal.* Found: C, 49.95; H, 5.73. Calcd. for $C_{27}H_{38}O_{18}$: C, 49.85; H, 5.85%.

Methyl 2-O-(α-L-mannopyranosyl)-β-D-glucopyranoside (**1**). Deacetylation of **13** (0.12 g) with 0.1 N methanolic sodium methoxide (2.5 ml) in the usual manner yielded **1** (50 mg, 83.3%), $[\alpha]_D^{20}$ −68° ($c=1$, CH_3OH). *Anal.* Found: C, 43.24; H, 6.97. Calcd. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.74%. $[M]_D$ (methyl α-L-mannoside) −15908, $[M]_D$ (methyl β-L-mannoside) 10282, $[M]_D$ (methyl β-D-glucoside) −6790; observed $[M]_D$ (**1**) −24208, Calcd. $[M]_D$ (**1**) −22698, Calcd. $[M]_D$ (β-anomer of **1**) 3492.

Methyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-acetyl-α-L-mannopyranosyl)-α-D-glucopyranoside (**15**). To a stirred solution of **14** (2.79 g, 1 mmol), silver triflate (2.76 g, 1.2 mmol) and 1,1,3,3-tetramethylurea (3 ml) in dichloromethane (35 ml) was added dropwise a solution of **12** (2.97 g, 1.2 mmol) in dichloromethane (8.6 ml). After stirring for 4 h, the reaction mixture was filtered through a bed of Celite, and the filtrate was successively washed with sat. $NaHCO_3$ and water, before being dried ($MgSO_4$). After filtering, the filtrate was evaporated to a syrup, which was crystallized from ethanol to give **15** as colorless needles (1.33 g, 26.1%), mp 167–169°C, R_f 0.30 (toluene–ethyl acetate 8:2), $[\alpha]_D^{20}$ 47° ($c=1$, $CHCl_3$). *Anal.* Found: C, 58.38; H, 5.50. Calcd. for $C_{34}G_{40}O_{16}$: C, 57.95; H, 5.68%.

Methyl 3-O-(α-L-mannopyranosyl)-α-D-glucopyranoside (**2**). A solution of **15** (0.92 g) in 60% acetic acid (9 ml) was heated at 100°C for 30 min. The solvent was evaporated, and the residue was repeatedly distilled with toluene to remove all traces of the volatile compounds. The de-*O*-benzylidene compound (0.73 g) was dissolved in 0.1 N methanolic sodium methoxide (15 ml) and left to stand for 2 h at room temperature. After neutralizing with Amberlite IR-120 (H^+) resin, the filtrate was evaporated to give **2** as a syrup (0.38 g, 92.3%), $[\alpha]_D^{20}$ 27° ($c=1$, CH_3OH). The sum of the $[M]_D$ values for methyl α-L-mannoside (−15908) and for methyl α-D-glucoside (30846) was 14938. The sum of the $[M]_D$ values for methyl β-L-mannoside (10280) and for methyl α-D-glucoside (30840) was 41126. The observed $[M]_D$ value for **2** was 9612; therefore, the configuration of the L-mannosidic bond of **2** was deduced to be α-form. *Anal.* Found: C, 43.82; H, 6.74. Calcd. for $C_{13}H_{24}O_{11}\cdot 1/2CH_3OH$: C, 43.55; H, 6.99%.

Methyl 2,3,4,6-tetra-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-L-mannopyranosyl)-α-D-glucopyranoside (**18**). To a solution of **16** (31.2 g) and **17** (24 g) in dry dichloromethane (800 ml) was added silver triflate (18.92 g) and 4A molecular sieves (105 g) while ice-cooling. The solution was stirred at 0° for 15 min and then at room temperature for 2 h in the dark. After filtering through a bed of Celite, the filtrate was successively washed with sat. $NaHCO_3$ and water, and dried ($CaCl_2$). Removal of the solvent gave a syrup (52 g), from which a 15 g sample was taken and chromatographed in a column of silica gel (toluene–ethyl acetate, 9:1) to give a syrup (**14**), which was crystallized from ethanol as needles (5 g, 33%), R_f 0.41 (toluene–ethyl acetate 9:1), mp 62–65°C, $[\alpha]_D^{20}$ 59° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 3.50 (3H, s, OCH_3); 3.90–6.20 (m, sugar protons); 7.20–7.65 (21H); 7.70–8.20 (14H, each, m, 7C_6H_4CO). *Anal.* Found: C, 66.00; H, 4.77. Calcd. for $C_{62}H_{52}O_{18}\cdot 2H_2O$: C, 66.42; H, 5.00%.

Methyl 4-O-(α-L-mannopyranosyl)-α-D-glucopyranoside (**3**). A solution of **18** (4.14 g) in 0.1 N methanolic sodium methoxide (90 ml) was left overnight at room temperature. After treating the reaction mixture as already mentioned, **3** was obtained as a syrup (1.2 g, 89.8%), $[\alpha]_D^{20}$ 42°

($c=1$. CH_3OH). The observed $[M]_D$ for **3** was 16732. Accordingly, the anomeric configuration of the L-mannosyl bond of **3** was deduced to be α-form. *Anal.* Found: C, 40.12; H, 6.69. Calcd. for $C_{13}H_{24}O_2 \cdot H_2O$: C, 39.79; H, 7.14%.

3,6-Di-O-acetyl-1,2-O-isopropylidene-5-O-(2,3,4,6-tetra-O-acetyl-α-L-mannopyranosyl)-α-D-glucofuranose (**20**). To a solution of $Hg(CN)_2$ (2.88 g) and $HgBr_2$ (4.11 g) in acetonitrile (87 ml) were added **19** (6.94 g) and **12** (8.37 g), and the mixture was left overnight at room temperature. After removing the solvent, the residue was dissolved in chloroform and filtered, and the filtrate was washed with aq. 1 N KBr and dried ($CaCl_2$). The filtrate was then concentrated, and the residual syrup was chromatographed in a column of silica gel, using benzene–ether (2:1) as the eluent, to give **20** (2.14 g, 42.5%), R_f 0.20 (chloroform–acetone 15:1), $[\alpha]_D^{20}$ −30° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.29 (3H), 1.50 (3H each s, $2CH_3$); 1.95 (3H), 2.00 (3H), 2.06 (3H), 2.10 (6H), 2.12 (3H, each s, $6CH_3CO$); 3.80, 4.60 (8H), 4.98 (1H, d, $J=1$ Hz, H-1′); 5.15–5.40 (4H); 6.83 (1H, d, $J=4$ Hz, H-1). *Anal.* Found: C, 51.05; H, 6.02. Calcd. for $C_{27}H_{38}O_{17}$: C, 51.10; H, 5.99%.

1,2-O-Isopropylidene-5-O-(α-L-mannopyranosyl)-α-D-glucofuranose (**21**). Compound **20** (2.0 g) was deacetylated with 0.1 N methanolic sodium methoxide (40 ml) as already mentioned to afford syrupy **21** (0.84 g, 69.4%), $[\alpha]_D^{20}$ −48° ($c=1$, CH_3OH). NMR δ_H (DMSO-d_6): 1.20 (3H), 1.33 (3H, each s, $2CH_3$); 3.10–5.10 (m, sugar protons); 5.73 (1H, d, $J=4$ Hz, H-1). *Anal.* Found: C, 45.12; H, 6.81. Calcd. for $C_{15}H_{26}O_{11} \cdot H_2O$: C, 45.00; H, 7.00%.

Octa-O-acetyl-5-O-(α-L-mannopyranosyl)-D-glucofuranose (**22**). A solution of **21** (0.84 g) in 1 N HCl (40 ml) was heated at 65°C for 2 h while stirring. After neutralizying with Amberlite IRA-400 (OH^- form), the solution was evaporated, and 5-O-(α-L-mannopyranosyl)-D-glucofuranose was obtained in a quantitative yield, R_f 0.4 (butanol–acetic acid–water 4:1:2), $[\alpha]_D^{20}$ −49° ($c=1$, CH_3OH). *Anal.* Found: C, 39.05; H, 6.75. Calcd. for $C_{12}H_{22}O_{11} \cdot 2 \cdot 1/2H_2O$: C, 39.02; H, 6.77%. Acetylation of the free sugar (0.89 g) with a mixture of acetic acid (10 ml) and pyridine (10 ml) in the usual manner gave **22** (1.1 g, 62.7%), R_f 0.38 (chloroform–acetone 9:1), $[\alpha]_D^{20}$ −7° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.93 (3H), 2.00 (3H), 2.05 (9H), 2.10 (9H, each s, $8CH_3CO$); 3.80–6.50 (m, sugar protons); 6.03 (1H, $J=4$ Hz, H-1α), 6.37 (1H, d, $J=8$ Hz, H-1β). *Anal.* Found: C, 49.36; H, 5.71. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.60%.

2,3,6-Tri-O-acetyl-5-O-(2,3,4,6-tetra-O-acetyl-α-L-mannopyranosyl)-β-D-glucofuranoyl bromide (**23**). To a solution of **22** (1.1 g) in a small amount of chloroform was added a saturated solution of hydrogen bromide in acetic acid (4.4 ml) at 0°C, and the mixture was kept at the same temperature for 2 h. After diluting with chloroform, the reaction mixture was treated as already described and then concentrated to afford **23** (0.95 g, 84.4%), $[\alpha]_D^{20}$ 21° ($c=1$, $CHCl_3$). This compound was unstable, and was used immediately in the next step.

Methyl 2,3,6-tri-O-acetyl-5-O-(2,3,4,6-tetra-O-acetyl-α-L-mannopyranosyl)-β-D-glucofuranoside (**24**). Compound **23** (0.95 g) was dissolved in methanol (20 ml), siliver carbonate (0.38 g) was added, and the mixture was stirred at room temperature for 1 h in the dark. After filtering through a bed of Celite, the filtrate was concentrated and the residue was chromatographed in a column of silica gel (chloroform–acetone 20:1) to yield **24** (0.1 g, 11.3%), R_f 0.30 (chloroform–acetone 9:1), $[\alpha]_D^{20}$ −39° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.96 (3H), 2.00 (3H), 2.07 (3H), 2.09 (3H), 2.10 (6H), 2.12 (3H, each s, $7CH_3CO$); 3.50 (3H, s, OCH_3): 3.85–4.40 (m, sugar protons); 4.65 (1H, d, $J=8$ Hz, H-1β); 4.80 (1H, d, $J=1$ Hz, H-1′α); 4.90–5.40 (m, sugar protons). *Anal.* Found: C, 49.53; H, 5.78. Calcd. for $C_{27}H_{38}O_{18}$: C, 49.53; H, 5.78%.

Methyl 5-O-(α-L-mannopyranosyl)-β-D-glucofuranoside (**4**). Deacetylation of **24** (0.1 g) with methanolic sodium methoxide (2 ml) in the usual manner gave **4** in a quantitative yield, $[\alpha]_D^{20}$ −95° ($c=1$, CH_3OH). *Anal.* Found: C, 39.84; H, 7.20. Calcd. for $C_{13}H_{24}O_{11} \cdot 2H_2O$: C, 39.79; H, 7.14%.

Methyl 2,3,4-tri-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-α-L-mannopyranosyl)-α-D-glucopyranoside (**26**). A solution of **25** (2.34 g) and **12** (2.7 g) containing mercury(II) cyanide (0.92 g) and mercury(II) bromide (1.33 g) in acetonitrile (28 ml) was kept overnight at room temperature. After processing as already described, the residual syrup was chromatographed in a column of silica gel (chloroform–acetone, 15:1) to afford **26** (1.28 g, 49.2%), R_f 0.57 (chloroform–acetone, 9:1), $[\alpha]_D^{20}$ 26° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.95 (3H); 2.10 (3H), 2.03 (6H), 2.06 (6H), 2.10 (3H), 2.12 (3H, each s, $8CH_3CO$); 3.36 (3H, s, CH_3O); 3.60–4.40 (m), 4.60–4.58 (m, each sugar protons). *Anal.* Found: C, 49.33; H, 5.75. Calcd. for $C_{27}H_{38}O_{18}$: C, 49.85; H, 5.85%.

Methyl 6-O-(α-L-mannopyranosyl)-α-D-glucopyranoside (**5**). Deacetylation of **26** (1.28 g) with 0.1 N methanolic sodium methoxide (25 ml) in the usual manner gave **5** (0.59 g, 83.8%), $[\alpha]_D^{20}$ 36° ($c=1$, CH_3OH). *Anal.* Found: C, 43.92; H, 7.06. Calcd. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.74%. $[M]_D$ (methyl α-L-mannoside) −15908, $[M]_D$ (methyl β-L-mannoside) 10289, $[M]_D$ (methyl α-D-glucoside) 30458; observed $[M]_D$ (**5**) 12816, calcd. $[M]_D$ (**5**) 14550, calcd. $[M]_D$ (β-L-mannosyl anomer of **5**) 40740.

6-O-(2,3,4,6-Tetra-O-benzoyl-α-L-mannopyranosyl)-1,2,3,4-di-O-isopropylidene-α-D-galactopyranose (**28**). To a solution of **27** (4.57 g) containing mercury(II) cyanide (2.2 g) and mercury bromide (3.16 g) was added **16** (11.58 g), and the mixture was kept overnight at room temperature. After processing as already described, the residual syrup was chromatographed in a column of silica gel (toluene–ethyl acetate, 9:1) to give **28** (2.21 g, 15.1%), R_f 0.25 (toluene–ethyl acetate, 8:1), $[\alpha]_D^{20}$ 8° ($c=1$, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.32 (3H), 1.36 (3H), 1.40 (3H), 1.58 (3H, each s, $4CH_3$); 3.60–6.20 (m, sugar protons); 7.10–7.60 (12H), 7.70–8.15 (8H, each m, $4C_6H_5CO$). *Anal.* Found: C, 65.35; H, 5.56. Calcd. for $C_{46}H_{42}O_{15} \cdot 1/2H_2O$: C, 65.50; H, 5.10.

6-O-(α-L-Mannopyranosyl)-α-D-galactopyranose (**6**). A solution of **28** (2.2 g) in 0.1 N methanolic sodium methoxide (110 ml) was left overnight at room temperature. After neutralizing with Amberlite IR-120 resin (H^+ form), the solution was concentrated, and the residue was dissolved in water, washed with ether to remove the benzoic acid, and then concentrated. The residue was dissolved in 0.1 N H_2SO_4, and the solution was heated at 70°C for 2 h. The reaction mixture was neutralized with Amberlite IRA-400 resin (OH^- form), filtered and evaporated to afford **6** (0.5 g, 55.6%), R_f 0.1 (butanol–acetic acid–water 4:1:2), $[\alpha]_D^{20}$ −29° ($c=1$, CH_3OH). *Anal.* Found: C, 38.47; H, 6.34. Calcd. for $C_{12}H_{22}O_{11} \cdot 2H_2O$: C, 38.10; H, 6.88%. $[M]_D$ (methyl α-L-mannoside) −15908, $[M]_D$ (methyl β-L-mannoside) 10289, $[M]_d$ (D-galactose) 14400. Observed $[M]_D$ (**6**) 10962, calcd. $[M]_D$ (**6**) 1508, calcd. $[M]_D$ (β-L-mannosyl anomer of **6**) 24689.

p-Nitrophenyl α-L-mannopyranoside (**29**). To a stirred solution of *p*-nitrophenol (5.8 g) in aq. alkali (2.34 g of KOH in 50 ml of water), a solution of **31** (13.8 g) in acetone (90 ml) was added dropwise at 0°C. The mixture was stirred at room temperature for 5 h, and the acetone was distilled off *in vacuo*. The aqueous solution was extracted with chloroform, and the chloroform layer was washed with a 5% NaOH solution to remove the unreacted *p*-nitrophenol, and then with water. After drying with $CaCl_2$, the filtrate was concentrated, and the residue (**32**, 1.7 g, 10.8%) was deacetylated with 0.1 N methanolic sodium methoxide to afford **29** (0.1 g, 10%), R_f 0.58 (ethyl acetate–methanol–water 80:14:10), mp 177°C, $[\alpha]_D^{20}$ −160° ($c=1$, CH_3OH); lit.[17] mp 180°C.

Compound **29** was also prepared according to Helferich's method. A mixture of **33** (6.1 g), *p*-nitrophenol (9 g) and *p*-toluenesulfonic acid (100 mg) was heated at 100°C for 30 min *in vacuo*. The reaction mixture was then diluted with benzene, washed with 1 N NaOH and sufficient water, and dried ($CaCl_2$). The filtrate was concentrated, and the residual syrup was crystallized from ethanol to give *p*-nitrophenyl 2,3,4,6-tetra-O-acetyl-α-L-mannopyranoside (**32**, 2.1 g, 28.8%), R_f 0.36 (toluene–ethyl acetate 7:3), mp 158°C; lit.[17] D-enantiomer, mp 156–157°C. Deacetylation of **32** gave **29** (0.59 g, 52.7%), mp 180–183°C, $[\alpha]_D^{20}$ −165° ($c=1$, CH_3OH); lit.[17] D-enantiomer, $[\alpha]_D^{20}$ 161°.

4-Methyl umbelliferone 2,3,4,6-tetra-O-acetyl-α-L-mannopyranoside (**34**). 4-Methyl umbelliferone (2.6 g) and **33** (1.59 g) were well-mixed and melted by heating. To this melt, a mixture of acetic acid–acetic anhydride (95:5, 5 ml) and anhydrous zinc chloride (0.6 g) were added, and the resulting mixture was heated at 125–135°C for 1 h *in vacuo*, after cooling, the reaction mixture was dissolved in chloroform and filtered, and the resulting solution was successively washed with 1.5 N HCl, 1 N NaOH and water, and finally dried ($MgSO_4$). The filtrate was concentrated, and the residue was crystallized from ethanol to yield **34** (0.39 g, 15.4%), R_f 0.30 (toluene–ethyl acetate 7:3), mp 161°C, $[\alpha]_D^{20}$ −136° ($c=1$, $CHCl_3$); lit.[18] D-enantiomer, mp 160–161°C, $[\alpha]_D^{20}$ 136°. NMR δ_H ($CDCl_3$): 2.00 (9H), 2.19 (3H, each s, $4CH_3CO$); 2.38 (3H, d, $J=1$ Hz, C4-CH_3); 3.87–4.32 (3H, m, H-5-6);

5.15–5.55 (3H, m, sugar H-2-4); 5.38 (1H, d, $J=1$ Hz, sugar H-1); 6.07 (1H, d, $J=1$ Hz, H-3); 6.80–7.00 (2H, m, H-6, 8); 7.40 (1H, d, $J=10$ Hz, H-5).

4-Methyl umbelliferone α-L-mannopyranoside (**30**). Deacetylation of **34** (200 mg) with 0.1 N methanolic sodium methoxide (10 ml) yielded **30** (40 mg, 30%), mp 160.8°C, $[\alpha]_D^{20}$ −113° ($c=1$, CH_3OH). *Anal.* Found; C, 56.18; H, 5.08. Calcd. for $C_{16}H_{18}O_8$: C, 56.80; H, 5.33%.

Hydrolysis test on the disaccharides and phenol glycosides toward naringinase from Penicillium decumbens. To a sample (200 mg) in water (10 ml) was added an enzyme solution (1 ml), which had been prepared by dissolving 100 units of naringinase (Sigma) in water (10 ml). After 2 days of incubation at 40 °C, the reaction mixture was filtered and subjected to an HPLC analysis. Rt: methyl glucoside, 6.086; L-mannose, 8.036; D-galactose, 8.747; D-glucose, 8.275.

α-L-Mannosidase activity assay. Naringinase (Sigma) was used in this experiment. One unit of this enzyme liberates 1 mmol of reducing sugar (measured as glucose) from naringin per min at pH 4.0 and 40°C. An enzyme solution was prepared by dissolving 1.8 units of the enzyme in 20 ml of a 0.2 M sodium acetate buffer at pH 4, *p*-nitrophenyl-α-L-mannopyranoside (PNM) being used as the substrate. The reaction mixture contained 0.8 ml of a sodium acetate buffer (pH 4) and 0.1 ml of 0.05 M PNM in a sodium acetate buffer (pH 4). After 5 min of preincubation at 40°C, the reaction was started by adding 0.05 ml of the enzyme solution. After a suitable time of incubation (5–60 min) at 40°C, the reaction was terminated by adding 4 ml of 1 M sodium carbonate, and the amount of liberated *p*-nitrophenol was measured spectrometrically at 400 nm.

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