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Enzymic synthesis of α- and β-D-glucosides of 1-deoxynojirimycin and their glycosidase inhibitory activities

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Abstract

1-Deoxynojirimycin (**1**) is a potent inhibitor of mammalian and rice α-glucosidase. Several glucosides of **1** were synthesized by use of the native and immobilized enzyme and their effect on various enzymes was investigated. Transglucosylation reactions using rice α-glucosidase, yeast α- and β-glucosidases purified from *Rhodotorula lactosa* were performed with maltose or cellobiose as a glucose donor and *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**2**) as an acceptor. The transglucosylation reaction using native rice α-glucosidase afforded 3-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**4**), 4-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**5**), and 2-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**3**) in yields of 40, 13, and 2%, respectively, after 30 min. The transglucosylation reaction using immobilized rice α-glucosidase was similar to that using the native enzyme. In the system using native yeast α-glucosidase, **3**, **5**, and **4** were formed in yields of 34, 13, and 6%, respectively, after 15 h. The immobilization of yeast α-glucosidase caused a significant decrease in transglucosylation activity. Yeast β-glucosidase showed a high transglucosylation activity and incubation with the reaction system afforded 2-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**6**) and 4-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**7**) in yields of 69 and 3%, respectively, after 3 h. The transglucosylation reaction using immobilized yeast β-glucosidase preferentially afforded **6** in a yield of 73% after 3 h. After removal of *N*-benzyloxycarbonyl group from the product glucosides, their glycosidase inhibitory activities were measured. 3-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**9**) retained the potent inhibition of **1** against rat intestinal sucrase activity and was more effective than **1** against

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rice α-glucosidase. 4-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**10**) retained the potency of **1** against rat intestinal sucrase and isomaltase. 2-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**8**) was more effective than **1** against trehalases.

1. Introduction

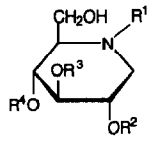
1-Deoxynojirimycin (**1**) has been shown to inhibit mammalian digestive α-glucosidases in vitro and in vivo [1–3], and α-glucosidases I and II involved in *N*-linked oligosaccharide processing as reviewed by Fuhramn et al. [4] and Elbein [5]. Compound **1** and its *N*-alkyl derivatives have been reported to reduce the infectivity of human immunodeficiency virus (HIV) at concentrations non-cytopathic to T-lymphocytes [6,7]. Although many *N*-alkyl derivatives of the alkaloidal glycosidase inhibitors have been synthesized and studied, the inhibition characteristics of their glycosides have not been investigated in detail. 4-*O*-α-D-Glucopyranosylfagomine has been isolated in seeds of *Xanthocericis zambesica* [8] and has been shown to be a weak inhibitor of lactase and β-galactosidase [9]. 4-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**10**) has been prepared by the microbial transglucosylation with *Streptomyces lavendulae* [10] and enzymically by the combination of cyclodextrin glycosyltransferase and glucoamylase [11]. 4-*O*-β-D-Galactopyranosyl-1-deoxynojirimycin has been enzymically synthesized by use of β-1,4-galactosyltransferase [12,13]. Several castanospermine glucosides have been chemically synthesized to investigate the effect of an attached glucopyranosyl residue on the potency and selectivity of castanospermine toward inhibition of intestinal glucosidases [14].

We previously reported the enzymic synthesis of *p*-nitrophenyl α-glucobiosides by use of native and immobilized rice α-glucosidase [15]. Compound **1** has been shown to be a potent inhibitor of rice α-glucosidase ($K_i = 0.01$ μM) [16]. We adopted *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**2**) as a glucosyl acceptor since *N*-benzyloxycarbonylation of **1** caused the complete loss of α-glucosidase inhibitory activity. In addition, when **2** is used as an acceptor, it is easy to separate the transglucosylation products from the mixture by use of an adsorbent such as Amberlite XAD-4 resin, and to assay the individual products quantitatively by HPLC.

In this report, we describe the preparation of the glucosides of **1** by use of native and immobilized rice α-glucosidase, and yeast α- and β-glucosidases purified from *Rhodotorula lactosa*, and their enzyme inhibitory activities.

2. Experimental

Materials.—Baker's yeast and rice α-glucosidase (EC 3.2.1.20), almond and *Caldocellum saccharolyticum* (Recombinant) β-glucosidase (EC 3.2.1.21), and porcine kidney trehalase (EC 3.2.1.28) were purchased from Sigma Chemical Co. Yeast α- and β-glucosidases were purified from *Rhodotorula lactosa* IFO 1424 by



	R^1	R^2	R^3	R^4
1	H	H	H	H
2	Cbz	H	H	H
3	Cbz	α-Glc	H	H
4	Cbz	H	α-Glc	H
5	Cbz	H	H	α-Glc
6	Cbz	β-Glc	H	H
7	Cbz	H	H	β-Glc
8	H	α-Glc	H	H
9	H	H	α-Glc	H
10	H	H	H	α-Glc
11	H	β-Glc	H	H
12	H	H	H	β-Glc

Cbz = benzyloxycarbonyl
α-Glc = α-Glucopyranosyl
β-Glc = β-Glucopyranosyl

the procedure of Furumoto et al. [17]. Brush border membranes prepared from the small intestine of male Wister rats, by the method of Kessler et al. [18], were used as the source of digestive glycosidases. Lysosomal α-glucosidase was partially purified from the liver of male Wister rats according to the method of Jeffrey et al. [19]. *p*-Nitrophenyl glycosides and palatinose were obtained from Sigma Chemical Co. Other saccharides were purchased from Wako Pure Chemical Industries. 1-Deoxynojirimycin (**1**) was purified from the roots of *Morus* sp. by the method of Yagi et al. [20], and *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**2**) was conventionally prepared from **1**.

Chromatography.—HPLC was performed under the following conditions: column, Cosmosil $5C_{18}$, 4.6 × 150 mm; solvent system, 1:4 MeCN–H_2O; flow rate, 0.5 mL min^{-1}; detection, UV spectrophotometry at 254 nm. TLC was performed on HPTLC Silica Gel 60 F_{254} (Merck) using the solvent system 4:1:1 PrOH–AcOH–H_2O.

NMR analysis.—^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Jeol JNM-GX 400 spectrometer in D_2O containing sodium 3-(trimethylsilyl)propionate as an internal standard.

Enzyme assays.—The activities of rice α-glucosidase, yeast α- and β-glucosidases from *R. lactosa*, digestive glycosidases, and porcine kidney trehalase were determined using the appropriate disaccharides as substrates at the optimum pH

of each enzyme. The released D-glucose was determined colorimetrically using commercially available Glucose B-test Wako (Wako Pure Chemical Industries). Other enzyme activities were determined using the appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The released *p*-nitrophenol was measured at 400 nm. The activity that will hydrolyze 1 μmol of substrate per min is defined as 1 unit.

Enzyme immobilization.—(a) Rice α-glucosidase. Chitopearl BCW-3570 (Fuji Spinning Co., 1.92 g) washed with 0.05 M acetate buffer (pH 5.0) was mixed with 320 units of enzyme and 9.6 mL of the same buffer. The mixture was gently shaken overnight at 4°C and filtered off. The immobilized enzyme was washed with 0.05 M acetate buffer (pH 5.0) and exhibited an activity of 147 units g^{-1}, which corresponds to 88% of the added activity.

(b) Yeast α- and β-glucosidases. Chitopearl BCW-3507 (0.5 g) was mixed with 10.7 units of purified yeast α-glucosidase in 3 mL of 0.01 M phosphate buffer (pH 6.0) containing 0.5 mM dithiothreitol. The mixture was gently shaken for 2 h at 27°C and treated in the same way as described above. Immobilized yeast α-glucosidase exhibited an activity of 15 units g^{-1}, which corresponds to 71% of the added activity. Purified yeast β-glucosidase (7.2 units) was treated in a manner similar to that described for yeast α-glucosidase. Immobilized yeast β-glucosidase exhibited an activity of 11.2 units g^{-1}, which corresponds to 78% of the added activity.

Transglucosylation reactions.—(a) Using native rice α-glucosidase. A mixture containing 0.1 mL of 2% *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**2**, final 0.2%), 0.1 g of maltose (final 10%), 0.2 mL of 0.2 M acetate buffer (pH 5.0), and 4 units of enzyme solution in a final volume of 1 mL was incubated at 37°C, and intermittently analyzed by HPLC.

(b) Using immobilized rice α-glucosidase. A mixture containing 0.25 mL of 2% **2** solution (final 0.2%), 0.25 g of maltose (final 10%), 0.5 mL of 0.2 M acetate buffer (pH 5.0), and 70 mg of immobilized enzyme (10.3 units) in a final volume of 2.5 mL was incubated at 37°C with shaking, and intermittently analyzed by HPLC.

(c) Using native yeast α- or β-glucosidase. A mixture containing 0.1 mL of 2% **2** solution (final 0.2%), 0.1 g of maltose or cellobiose (final 10%), 0.2 mL of 0.2 M phosphate buffer (pH 6.0), and 1 unit of yeast α-glucosidase or 0.75 unit of yeast β-glucosidase in a final volume of 1 mL was incubated at 37°C, and intermittently analyzed by HPLC.

(d) Using immobilized yeast α- or β-glucosidase. A mixture containing 0.1 mL of 2% **2** solution (final 0.2%), 0.1 g of maltose or cellobiose (final 10%), 0.2 mL of 0.2 M phosphate buffer (pH 6.0), and 70 mg of immobilized yeast α-glucosidase (1.1 units) or 67 mg of immobilized yeast β-glucosidase (0.75 unit) in a final volume of 1 mL was incubated at 37°C, and intermittently analyzed by HPLC.

Preparation of transglucosylation products.—(a) Using immobilized rice α-glucosidase. A mixture containing 80 mg of **2**, 4 g of maltose, 40 mL of 0.05 M acetate buffer (pH 5.0), and 1.09 g of immobilized enzyme (160 units) was incubated for 2 h at 37°C with shaking. After reaction, the mixture was filtered and the immobilized enzyme was washed with 0.05 M acetate buffer (pH 5.0) and reused. The reaction was repeated continuously five times. The pooled filtrate and

washings were applied to a column of Amberlite XAD-4 (20 mL), eluted with MeOH, and concentrated. The concentrate was applied to a column of Toyopearl HW-40S (2.5 × 90 cm) and eluted with 20% MeOH to give 2-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**3**, 24 mg), 4-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**5**, 92 mg), and 3-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**4**, 210 mg) eluted in that order.

Compound **3**: $[\alpha]_D$ + 66.5° (*c* 1.58, H_2O); retention time (t_R) 3.8 min. ^{13}C NMR data (D_2O): δ 42.3 (C-1), 62.1 (C-6′), 62.3 (C-5), 63.3 (C-6), 70.5 (C-4), 70.7 (–CH_2–Ph), 72.3 (C-4′), 74.0 (C-2′), 74.7 (C-3), 74.9 (C-5′), 75.8 (C-3′), 77.5 (C-2), 98.5 (C-1′), 130.7, 131.3, 131.6, 139.1 (C_6H_5–), 160.5 (> N–COO–).

Compound **4**: $[\alpha]_D$ + 67.5° (*c* 1.61, H_2O); t_R 5.3 min. ^{13}C NMR data (D_2O): δ 45.2 (C-1), 61.7 (C-6′), 61.9 (C-5), 63.4 (C-6), 68.9 (C-2), 70.7 (–CH_2–Ph), 70.9 (C-4), 72.4 (C-4′), 74.1 (C-2′), 75.5 (C-5′), 75.7 (C-3′), 80.4 (C-3), 102.1 (C-1′), 130.7, 131.3, 131.7, 139.4 (C_6H_5–), 161.1 (> N–COO–).

Compound **5**: $[\alpha]_D$ +52.9° (*c* 0.89, H_2O); t_R 4.2 min. ^{13}C NMR data (D_2O): δ 44.4 (C-1), 61.1 (C-5), 61.7 (C-6′), 63.3 (C-6), 70.6 (C-2), 70.6 (–CH_2–Ph), 71.2 (C-3), 72.3 (C-4′), 74.2 (C-2′), 75.5 (C-5′), 75.9 (C-3′), 77.1 (C-4), 101.4 (C-1′), 130.6, 131.3, 131.8, 139.5 (C_6H_5–), 161.1 (> N–COO–).

(b) Using native yeast β-glucosidase. A mixture containing 200 mg of **2**, 10 g of cellobiose, 20 mL of 0.2 M phosphate buffer (pH 6.0), and 16 units of yeast β-glucosidase in a total volume of 100 mL was incubated for 3 h at 37°C. The mixture was treated with Amberlite XAD-4 resin and chromatographed on a Toyopearl HW-40S column (2.5 × 90 cm) to give 2-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**6**, 88 mg) and 4-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**7**, 12 mg) eluted in that order.

Compound **6**: $[\alpha]_D$ −6.8° (*c* 1.54, H_2O); t_R 4.1 min. ^{13}C NMR data (D_2O): δ 45.1 (C-1), 62.0 (C-5), 62.5 (C-6′), 63.7 (C-6), 70.5 (C-4), 70.6 (–CH_2–Ph), 72.4 (C-4′), 73.6 (C-3), 76.0 (C-2′), 78.3 (C-3′), 78.8 (C-5′), 80.9 (C-2), 105.0 (C-1′), 130.6, 131.2, 131.6, 139.2 (C_6H_5–)$^-$, 160.6 (> N–COO–).

Compound **7**: $[\alpha]_D$ −11.9° (*c* 0.53, H_2O); t_R 4.6 min. ^{13}C NMR data (D_2O): δ 45.4 (C-1), 62.2 (C-5), 62.6 (C-6′), 63.8 (C-6), 70.6 (–CH_2–Ph), 70.8 (C-2), 72.6 (C-4′), 73.8 (C-3), 76.1 (C-2′), 78.5 (C-3′), 78.9 (C-5′), 81.0 (C-4), 105.1 (C-1′), 130.7, 131.4, 131.7, 139.4 (C_6H_5–), 160.7 (> N–COO–).

(c) Using immobilized yeast β-glucosidase. A mixture containing 20 mg of **2**, 1 g of cellobiose, 2 mL of 0.2 M phosphate buffer (pH 6.0), and 0.67 g of immobilized yeast β-glucosidase (7.5 units) was incubated for 2 h at 37°C with shaking. The reaction was continuously repeated ten times. The pooled filtrate and washings were treated with Amberlite XAD-4 resin and chromatographed on a Toyopearl HW-40S column (2.5 × 90 cm) to give **6** (160 mg) preferentially.

Hydrogenolysis.—A solution of each sample (15 mg) in 50% EtOH (2 mL) and AcOH (0.4 mL) was hydrogenated in the presence of 5% Pd–C (20 mg) at atmospheric pressure for 16 h. The mixture was processed conventionally and purified on a Dowex 1-× 2 (OH^-) short column to give the free base.

^1H NMR and ^{13}C NMR data of 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**10**) were consistent with those given in the literature [11,21].

2-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**8**): $[\alpha]_D$ + 127.6° (*c* 0.61, H_2O); R_f 0.27. ^{1}H NMR data (D_2O): δ 2.47 (dd, 1 H, $J_{1ax,2}$ 10.6, $J_{1ax,1eq}$ 12.5 Hz, H-1*ax*), 2.58 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 6.2, $J_{5,6b}$ 2.9 Hz, H-5), 3.29 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.31 (dd, 1 H, $J_{1eq,2}$ 5.1, $J_{1ax,1eq}$ 12.5 Hz, H-1*eq*), 3.45 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H-4'), 3.48 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.56 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 9.5 Hz, H-2'), 3.57 (ddd, 1 H, $J_{1ax,2}$ 10.6, $J_{1eq,2}$ 5.1, $J_{2,3}$ 9.5 Hz, H-2), 3.65 (dd, 1 H, $J_{5,6a}$ 6.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.75 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3'), 3.79 (dd, 1 H, $J_{5',6'a}$ 4.8, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 3.85 (dd, 1 H, $J_{5,6b}$ 2.9, $J_{6a,6b}$ 11.7 Hz, H-6b), 3.86 (dd, 1 H, $J_{5',6'b}$ 2.5, $J_{6'a,6'b}$ 12.5 Hz, H-6'b), 3.96 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'a}$ 4.8, $J_{5',6'b}$ 2.5 Hz, H-5'), 5.06 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1'). ^{13}C NMR data (D_2O): δ 48.4 (C-1), 63.2 (C-6'), 63.3 (C-5), 64.1 (C-6), 72.3 (C-4'), 74.0 (C-2'), 74.3 (C-4), 74.5 (C-5'), 75.7 (C-3'), 78.3 (C-2), 79.2 (C-3), 98.6 (C-1').

3-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**9**): $[\alpha]_D$ + 137.5° (*c* 0.52, H_2O); R_f 0.30. ^{1}H NMR data (D_2O): δ 2.49 (dd, 1 H, $J_{1ax,2}$ 10.5, $J_{1ax,1eq}$ 12.5 Hz, H-1*ax*, 2.58 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 5.9, $J_{5,6b}$ 2.9 Hz, H-5), 3.15 (dd, 1 H, $J_{1eq,2}$ 5.1, $J_{1ax,1eq}$ 12.5 Hz, H-1*eq*), 3.46 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.47 (t, 1 H, *J* 9.5 Hz, H-4 or H-4'), 3.48 (t, 1 H, *J* 9.5 Hz, H-4' or H-4), 3.58 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 9.5 Hz, H-2'), 3.59 (ddd, 1 H, $J_{1ax,2}$ 10.5, $J_{1eq,2}$ 5.1, $J_{2,3}$ 9.5 Hz, H-2), 3.65 (dd, 1 H, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.77 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3'), 3.80 (dd, 1 H, $J_{5',6'a}$ 4.5, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 3.81 (dd, 1 H, $J_{5',6'b}$ 2.5, $J_{6'a,6'b}$ 12.5 Hz, H-6'b), 3.83 (dd, 1 H, $J_{5,6b}$ 2.9, $J_{6a,6b}$ 11.7 Hz, H-6b), 4.02 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'a}$ 4.5, $J_{5',6'b}$ 2.5 Hz, H-5'), 5.32 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1'). ^{13}C NMR data (D_2O): δ 51.7 (C-1), 63.1 (C-6'), 63.3 (C-5), 63.9 (C-6), 72.2 (C-4'), 72.3 (C-2), 74.6 (C-4,2',5'), 75.8 (C-3'), 88.6 (C-3), 102.2 (C-1').

2-*O*-β-D-Glucopyranosyl-1-deoxynojirimycin (**11**): $[\alpha]_D$ −0.8° (*c* 0.51, H_2O); R_f 0.32. ^{1}H NMR data (D_2O): δ 2.53 (dd, 1 H, $J_{1ax,2}$ 11.0, $J_{1ax,1eq}$ 12.5 Hz, H-1*ax*), 2.55 (ddd, 1 H, $J_{4,5}$ 10.2, $J_{5,6a}$ 6.2, $J_{5,6b}$ 2.9 Hz, H-5), 3.29 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 10.2 Hz, H-4), 3.31 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.2 Hz, H-2'), 3.36 (dd, 1 H, $J_{1eq,2}$ 5.1, $J_{1ax,1eq}$ 12.5 Hz, H-1*eq*), 3.38 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.9 Hz, H-4'), 3.48 (ddd, 1 H, $J_{4',5'}$ 9.9, $J_{5',6'a}$ 5.9, $J_{5',6'b}$ 2.2 Hz, H-5'), 3.509 (t, 1 H, *J* 9.2 Hz, H-3 or H-3'), 3.513 (t, 1 H, *J* 9.2 Hz, H-3' or H-3), 3.63 (ddd, 1 H, $J_{1ax,2}$ 11.0, $J_{1eq,2}$ 5.1, $J_{2,3}$ 9.2 Hz, H-2), 3.65 (dd, 1 H, $J_{5,6a}$ 6.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.72 (dd, 1 H, $J_{5',6'a}$ 5.9, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 3.83 (dd, 1 H, $J_{5,6b}$ 2.9, $J_{6a,6b}$ 11.7 Hz, H-6b), 3.92 (dd, 1 H, $J_{5',6'b}$ 2.2, $J_{6'a,6'b}$ 12.5 Hz, H-6'b), 4.66 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'). ^{13}C NMR data (D_2O): δ 50.8 (C-1), 63.0 (C-5), 63.5 (C-6'), 64.0 (C-6), 72.5 (C-4'), 74.1 (C-4), 76.3 (C-2'), 78.5 (C-3'), 78.7 (C-5'), 79.9 (C-3), 83.6 (C-2), 106.3 (C-1').

4-*O*-β-D-Glucopyranosyl-1-deoxynojirimycin (**12**): $[\alpha]_D$ + 25.0° (*c* 0.42, H_2O); R_f 0.30. ^{1}H NMR data (D_2O): δ 2.48 (dd, 1 H, $J_{1ax,2}$ 10.6, $J_{1ax,1eq}$ 12.5 Hz, H-1*ax*), 2.71 (m, 1 H, H-5), 3.12 (dd, 1 H, $J_{1eq,2}$ 4.8, $J_{1ax,1eq}$ 12.8 Hz, H-1*eq*), 3.34 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.2 Hz, H-2'), 3.43 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5 Hz, H-4'), 3.47 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.49 (t, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), ~ 3.50 (m, 1 H, H-5'), 3.52 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.2$ Hz, H-3'), ~ 3.56 (m, 1 H, H-2), 3.75 (dd, 1 H, $J_{5',6'a}$ 5.8, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 3.77 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 11.0 Hz, H-6a), 3.89 (dd, 1 H, $J_{5,6b}$ 2.6, $J_{6a,6b}$ 11.0 Hz, H-6b), 3.93 (dd, 1 H, $J_{5',6'b}$ 2.4, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), 4.52 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'). ^{13}C NMR data (D_2O): δ 51.1 (C-1), 62.4

(C-5), 63.4 (C-6,6'), 72.3 (C-4'), 73.6 (C-2), 76.1 (C-2'), 78.4 (C-3'), 78.9 (C-5'), 79.6 (C-3), 84.1 (C-4), 105.6 (C-1').

3. Results

Transglucosylation reaction.—The time course of the transglucosylation reaction of native rice α-glucosidase acting on maltose and *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**2**) is shown in Fig. 1a. The concentration of 3-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**4**) rapidly reached a maximum after 30 min and its yield was 40% of the total composition calculated from HPLC data. Compound **4** then rapidly decreased. 4-*O*-α-D-Glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**5**) reached maximal yield (25%) after 3 h and then gradually decreased. A small amount of 2-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**3**) was formed with time. The time course of the transglucosylation reaction with immobilized rice α-glucosidase, which was immobilized on Chitopearl BCW-3507 at the immobilization rate of 88%, was similar to that seen in the native enzyme, except that the maximal yield of **5** was observed after 3–9 h of incubation (Fig. 1b).

The time course of the transglucosylation reaction with yeast α-glucosidase purified from *Rhodotorula lactosa* by the method of Furumoto et al. [17] is shown in Fig. 2. In the reaction using native yeast α-glucosidase, products **3**, **5**, and **4** reached in their maximal yields (34, 13, and 6%, respectively) after 15 h of incubation and then decreased (Fig. 2a). We could efficiently immobilize yeast α-glucosidase on Chitopearl BCW-3507, which exhibited a high activity (71%). However, as shown in Fig. 2b, the immobilization of yeast α-glucosidase caused a marked decrease in transglucosylation activity.

The time course of the transglucosylation reaction of yeast β-glucosidase acting on cellobiose and **2** is shown in Fig. 3. Yeast β-glucosidase showed a high transglucosylation activity. In the reaction with native yeast β-glucosidase (Fig. 3a), the formation of 2-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**6**) reached a maximum in 3 h and its yield was 69%. In addition, 4-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**7**) was formed with time. Yeast β-glucosidase, as well as yeast α-glucosidase, was immobilized on Chitopearl BCW-3507 at the immobilization rate of 78% and exhibited a high transglucosylation activity. Incubation of the system with immobilized yeast β-glucosidase afforded **6** preferentially, and **6** reached its maximal yield (73%) in 3–9 h of incubation (Fig. 3b).

Glycosidase inhibitory activities.—*N*-Benzyloxycarbonylation of 1-deoxynojirimycin (**1**) causes the complete loss of its inhibitory activity, because the electrostatic interactions of the protonated inhibitor and the negatively charged active site of the enzyme are indispensable for enzyme inhibition. The glycosidase inhibitory activity was measured after removal of the benzyloxycarbonyl group from the enzymically synthesized glucosides.

The IC_{50} values of the deprotected glucosides against intestinal glycosidases are shown in Table 1. All glucosides of **1** were weaker inhibitors than **1** of all intestinal

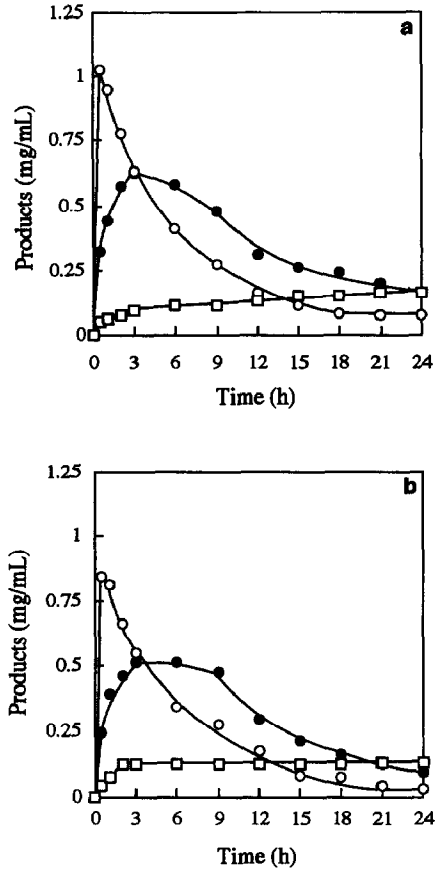


Fig. 1. Time course of the transglucosylation reaction of native and immobilized rice α-glucosidase with maltose as a glucosyl donor and *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (**2**) as an acceptor. The mixtures were incubated at 37°C with the native enzyme (a), or immobilized enzyme (b). Symbols: □ 2-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin; ○, 3-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin; ●, 4-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin.

glycosidase activities, except for the activity of 2-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**8**) against trehalase. However, 3-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**9**) and 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**10**) retained potent inhibitory activity for sucrase. Compound **10** retained a potent inhibitory activity for isomaltase in addition to sucrase. Compound **8** was more effective against trehalase than the parent compound **1**, but **8** was less active against maltase, sucrase, and isomaltase than 2-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**11**) and 4-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**12**). The addition of an α-glucosyl residue to **1** destroyed the inhibitory activities against cellobiase and lactase. The IC_{50} values of the glucosides against various α-,β-glucosidases and trehalase are shown in Table 2. All glucosides exhibited no significant inhibitory

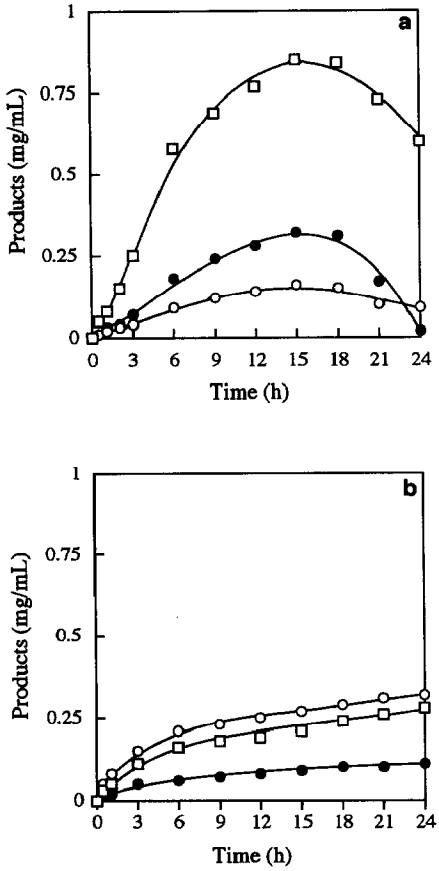


Fig. 2. Time course of the transglucosylation reaction of native and immobilized yeast α-glucosidase with maltose and **2**. The mixtures were incubated at 37°C with the native enzyme (a), or immobilized enzyme (b). Symbols: □, 2-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin; ○, 3-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin; ●, 4-*O*-α-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin.

activity against Baker's yeast α-glucosidase. However, compound **9** was a very powerful inhibitor of rice α-glucosidase, with 50% inhibition at only 0.034 μM. The addition of a glucosyl residue to **1** resulted in a significant decrease of inhibitory activity against rat liver lysosomal α-glucosidase, but that of the α-glucosyl residue at C-2 (**8**) and C-4 (**10**) of **1** led to an increase of inhibitory activity against porcine kidney trehalase and β-glucosidases, respectively.

4. Discussion

We reported previously that incubation of a reaction system containing maltose as a glucosyl donor and *p*-nitrophenyl (PNP) α-D-glucoside as an acceptor with

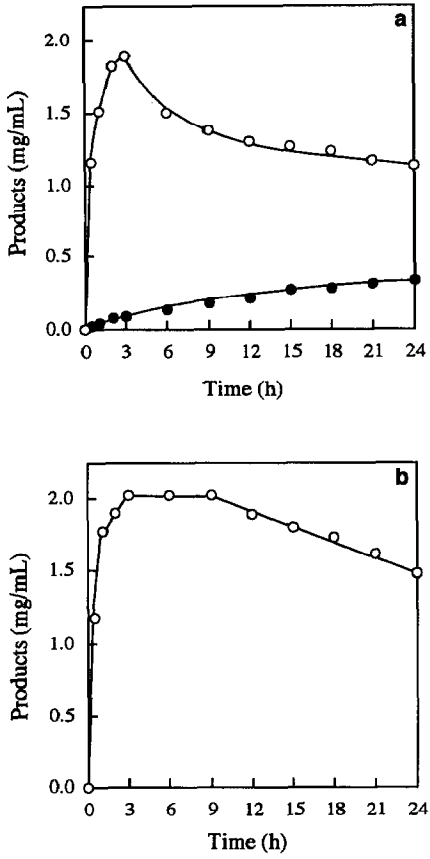


Fig. 3. Time course of the transglucosylation reaction of native and immobilized yeast β-glucosidase with cellobiose and 2. The mixtures were incubated at 37°C with the native enzyme (a), or immobilized enzyme (b). Symbols: ○, 2-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin; ●, 4-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)-1-deoxynojirimycin.

Table 1
Concentrations of the glucosides of 1-deoxynojirimycin (molar) giving 50% inhibition of glycosidase activities in brush border membranes of rat small intestine

Substrate	IC_{50} (M)					
	1	**8**	**9**	**10**	**11**	**12**
Maltose	3.6×10^{-7}	2.4×10^{-5}	5.2×10^{-6}	2.3×10^{-6}	4.0×10^{-6}	2.4×10^{-5}
Sucrose	2.1×10^{-7}	2.4×10^{-6}	3.5×10^{-7}	4.0×10^{-7}	7.9×10^{-7}	2.5×10^{-6}
Palatinose	3.0×10^{-7}	6.2×10^{-6}	9.0×10^{-7}	4.7×10^{-7}	1.4×10^{-6}	2.7×10^{-6}
Trehalose	4.2×10^{-5}	2.2×10^{-5}	NI	NI	NI	NI
Cellobiose	5.2×10^{-4}	NI [a]	NI	NI	NI	NI
Lactose	3.4×10^{-5}	NI	NI	NI	5.2×10^{-4}	4.6×10^{-4}

[a] Less than 50% inhibition at 1.0×10^{-3} (M)

Table 2
Concentrations of the glucosides of 1-deoxynojirimycin (molar) giving 50% inhibition of various glycosidase activities [a]

Enzyme	IC_{50} (M)					
	1	**8**	**9**	**10**	**11**	**12**
α-Glucosidase						
Baker's yeast	3.3×10^{-4}	NI	NI	NI	NI	NI
Rice	5.0×10^{-8}	1.6×10^{-6}	3.4×10^{-8}	6.1×10^{-7}	2.3×10^{-4}	2.2×10^{-5}
Rat liver lysosome	4.0×10^{-7}	1.0×10^{-3}	2.5×10^{-5}	4.4×10^{-4}	NI	1.0×10^{-3}
β-Glucosidase						
Almond	2.0×10^{-4}	1.0×10^{-3}	NI	8.0×10^{-5}	NI	NI
Caldocellum saccharolyticum	1.0×10^{-4}	2.3×10^{-4}	NI	5.0×10^{-5}	NI	5.6×10^{-4}
Trehalase						
Porcine kidney	4.1×10^{-5}	5.6×10^{-6}	NI	NI	NI	6.0×10^{-4}

[a] Rice α-glucosidase and trehalase activities were measured with maltose and trehalose, respectively, by the D-glucose oxidase–peroxidase method. Other enzyme activities were measured using the corresponding *p*-nitrophenyl glycoside. The released *p*-nitrophenol was measured spectrophotometrically at 400 nm.

native and immobilized rice α-glucosidase affords PNP α-isomaltoside and a mixture of PNP α-isomaltoside, PNP α-maltoside, and PNP α-nigeroside, respectively [15]. In this work, we prepared the glucosides of 1-deoxynojirimycin (**1**), which is a powerful inhibitor of α-glucosidase, using native and immobilized α- and β-glucosidases by introducing the benzyloxycarbonyl group to its imino group and masking its inhibitory activity. Incubation of the system with native and immobilized rice α-glucosidase afforded the 3-*O*-, 4-*O*-, and 2-*O*-α-D-glucosides (in order of yields in 30 min of incubation). The absence of the 6-*O*-α-D-glucoside appears to be due to the steric hindrance caused by the introduction of the *N*-benzyloxycarbonyl group. Furumoto et al. [17] isolated the products of maltose and isomaltose types by incubation of a system containing maltose as a glucosyl donor and pseudo α-glucosylamine, validamine, as an acceptor with α-glucosidase purified from *R. lactosa*. Incubation of a system containing maltose and **2** with the same purified enzyme afforded the 2-*O*-, 4-*O*-, and 3-*O*-α-D-glucosides of **2** (in order of yields in a 15 h incubation). Furumoto et al. [17] also isolated the products of sophorose [β-(1 → 2) linkage], cellobiose [β-(1 → 4)], and gentiobiose [β-(1 → 6)] types by incubation of a system containing cellobiose and validamine with β-glucosidase purified from *R. lactosa*. The transglucosylation reaction using the same enzyme gave the 2-*O*- and 4-*O*-β-D-glucosides of **2** in yields of 69 and 3%, respectively, after 3 h of incubation. The transglucosylation reaction using immobilized β-glucosidase preferentially gave the 2-*O*-β-D-glucoside of **2** in a maximal yield of 73%.

Yoshikuni et al. [22] reported that 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**10**) showed selection for sucrase relative to maltase in comparison to 1-deoxynojirimycin (**1**). Compound **1** shares a structural similarity to castanospermine. Rhinehart et al. [14] reported that 7-*O*-α-D-glucopyranosylcastanospermine (corresponding to **9**) and 8-*O*-α-D-glucopyranosylcastanospermine (corresponding to **10**)

retained castanospermine's potent inhibition of sucrase activity, lost some potency against maltase, glucoamylase, and isomaltase, and lost much more potency against trehalase and lactase. In our work, **9** and **10**, by analogy with the corresponding castanospermine glucoside, retained the potent inhibition of **1** against intestinal sucrase activity. Compound **10**, differing from the corresponding castanospermine glucoside, retained its potency against isomaltase. It is very interesting that **8** and **9** were more effective than the parent **1** against trehalases and rice α-glucosidase, respectively.

References

[1] D.D. Schmidt, W. Frommer, L. Muller, and E. Truscheit, *Naturwissenschaften*, 66 (1979) 584–585.
[2] W. Frommer, B. Junge, L. Muller, and E. Truscheit, *J. Med. Plant Res.*, 35 (1979) 195–217.
[3] Y. Yoshikuni, *Agric. Biol. Chem.*, 52 (1988) 121–128.
[4] U. Fuhrmann, E. Bause, and H. Ploegh, *Biochim. Biophys. Acta*, 825 (1985) 95–110.
[5] A.D. Elbein, *EASEB J.*, 5 (1991) 3055–3063.
[6] R.A. Gruters, J.J. Neefjes, M. Tersmette, R.E.Y. de Goede, A. Tulp, H.G. Huisman, F. Miedema, and H.L. Ploegh, *Nature*, 330 (1987) 74–77.
[7] A. Karpas, G.W.J. Fleet, R.A. Dwek, S. Peturson, S.K. Namgoong, N.G. Ramsden, G.S. Jacob, and T.W. Rademacher, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 9229–9233.
[8] S.V. Evans, A.R. Hayaman, L.E. Fellows, T.K.M. Shing, A.E. Derome, and G.W.J. Fleet, *Tetrahedron Lett.*, 26 (1985) 1465–1468.
[9] A.M. Scofield, L.E. Fellows, R.J. Nash, and G.W.J. Fleet, *Life Sci.*, 39 (1986) 645–650.
[10] M. Arai, M. Sumida, S. Nakatani, and S. Murao, *Agric. Biol. Chem.*, 47 (1983) 183–185.
[11] Y. Ezure, *Agric. Biol. Chem.*, 49 (1985) 2159–2165.
[12] C.-H. Wong, Y. Ichikawa, T. Krach, C.G.-Le Narvor, D.P. Dumas, and G.C. Look, *J. Am. Chem. Soc.*, 113 (1991) 8137–8145.
[13] C.G.-Le Narvor and C.-H. Wong, *J. Chem. Soc. Chem. Commun.*, 1991 (1991) 1130–1131.
[14] B.L. Rhinehart, K.M. Robinson, C.R. King, and P.S. Liu, *Biochem. Pharmacol.*, 39 (1990) 1537–1543.
[15] N. Asano, K. Tanaka, and K. Matsui, *Carbohydr. Res.*, 217 (1991) 255–261.
[16] G. Hanozet, H.P. Pircher, P. Vanni, B. Oesch, and G. Semenza, *J. Biol. Chem.*, 256 (1981) 3703–3711.
[17] T. Furumoto, Y. Kameda, and K. Matsui, *Chem. Pharm. Bull.*, 40 (1992) 1871–1875.
[18] M. Kessler, O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Semenza, *Biochim. Biophys. Acta*, 506 (1978) 136–154.
[19] P.L. Jeffrey, D.H. Brown, and B.I. Brown, *Biochemistry*, 9 (1970) 1403–1415.
[20] M. Yagi, T. Kouno, Y. Aoyagi, and H. Murai, *Nippon Nogei Kagaku Kaishi*, 50 (1976) 571–572.
[21] M. Arai, M. Sumida, K. Fukuhara, M. Kainosho, and S. Murao, *Agric. Biol. Chem.*, 50 (1986) 639–644.
[22] Y. Yoshikuni, Y. Ezure, T. Seto, K. Mori, M. Watanabe, and H. Enomoto, *Chem. Pharm. Bull.*, 37 (1989) 106–109.