Enzymatic Synthesis of Glucoside Derivatives of Validamine and Valienamine

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α- And β-glucoside derivatives of validamine and valienamine were prepared by enzymatic transglucosidation using α- and β-glucosidase of *Rhodotorula lactosa*. The structures of these derivatives have been elucidated by ^{13}C- and ^{1}H-nuclear magnetic resonance spectral analysis. Thus, 7-α-glucoside, 7-α-isomaltoside, and 4-α-glucoside of validamine and 7-α-glucoside, 7-α-isomaltoside, 4-α-glucoside, and 4-α-isomaltoside of valienamine were obtained from maltose and validamine or valienamine using α-glucosidase. 7-β-Glucoside, 2-β-glucoside, and 4-β-glucoside of validamine or valienamine were obtained from cellobiose and validamine or valienamine using β-glucosidase. These derivatives were tested for α-glucosidase inhibitory activity on rat small intestinal glycosidases.

Keywords *Rhodotorula lactosa*; α-glucosidase; β-glucosidase; pseudo-aminosugar; transglucosidation; α-glucosidase inhibitory activity

Validamine and valienamine (Chart 1), intermediates formed by the microbial degradation of validamycins by several soil bacteria such as *Pseudomonas denitrificans*[1] and *Flavobacterium saccharophilum*,[2] are pseudo-aminosugar analogs of D-glucose, in which the ring oxygen is replaced with a carbon atom with the same configuration. The names and numbering are used for convenience by analogy with the glucose analog, validamine. These pseudo-aminosugars show strong competitive inhibition selectively, on α-glucosidase, sucrase, and maltase from porcine small intestine.[3] It is therefore interesting to examine the glucoside derivatives of these pseudo-aminosugars as α-glucosidase inhibitors. Thus, we attempted to prepare α- and β-glucoside derivatives of validamine and valienamine. Glucosidase is known to catalyze not only the hydrolysis of the glucoside linkage in various substrates but also transglucosidation reactions. The transglucosidation activity of glucosidase has frequently been used for the synthesis of di- or tri-saccharides.[4] However, the isolation of the products from the transglucosidation mixture is generally very complicated because the incubation solution contains starting materials, reaction products, and hydrolyzed materials. However, when pseudo-aminosugars are used as acceptors, it is easy to separate the transglucosidation products from the reaction mixture by the use of an adsorbent cation exchanger resin such as Dowex 50-X8, and to determine the individual products with thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) of trimethylsilyl (TMS) derivatives. In a previous paper,[5] we reported on the microbial glucosidation formation of α- and β-D-glucosyl validamycin derivatives with the yeast, *Rhodotorula lactosa* IFO 1424. *R. lactosa* has strong α- and β-transglucosidation activities and wide acceptor specificity. Both α- and β-glucosidases isolated by carboxymethyl (CM)-cellulose column are used for the transglucosidation reaction of validamine and valienamine. This report deals with the enzymatic preparation of α- and β-D-glucoside derivatives of validamine and valienamine and their inhibitory activities on α-glucosidases.

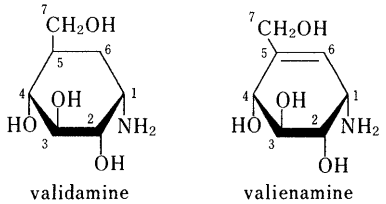

Chart 1

Materials and Methods

Materials Validamine and valienamine were prepared as described in the previous paper.[2] Maltose, cellobiose, sucrose, isomaltose, trehalose, soluble starch used as substrate and Glucose B test were obtained from Wako Chemicals Ltd., and diethylaminoethyl-(DEAE-) and CM-celluloses were products of Braun Company. Sephacryl S-300 was a product of Pharmacia Fine Chemicals. All other chemicals were of reagent grade and were used without further purification.

General Procedure The optical rotations were measured with a digital polarimeter DIP 370 (Japan Spectroscopic Co., Ltd.). Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded with a JEOL JNM-GSX-400 spectrometer in D_2O solution and chemical shifts are given on a δ (ppm) scale with sodium 2,2-dimethyl-2-silapentane sulfonate (DSS) as an internal standard. TLC were carried out with silica gel plates using a solvent system $CHCl_3$: MeOH : 29%NH_4OH = 1 : 3 : 2. Spots were visualized by spraying chlorine reagents.[6] GLC were carried out on a Shimadzu GC-6A gas chromatograph equipped with an hydrogen flame ionization detector (7% OV-17 on chromosorb AW, 3 mm i.d. × 2000 mm, 280 °C, at a nitrogen flow of 60 ml per min).

Microorganisms and Growth *R. lactosa* IFO 1424 was grown in a 5 l shaking flask containing 1 l of nutrient medium (1% glucose, 0.5% polypepton, 0.3% yeast extract, pH 6.0) at 27 °C with reciprocal shaking for 24 h. After growth, cells (approximately 32 g wet weight/1 culture) were harvested by continuous centrifugation at 25000 × *g* and washed with 10 mM phosphate buffer (pH 6.0).

Preparation of Cell-Free Extract The washed cells (320 g obtained from 10 l culture) were suspended in 960 ml of 10 mM phosphate buffer (pH 6.0) and ruptured with a sonic oscillator (Tomy model UR2000P). The suspension was subjected to sonication for 10 min under ice cooling. The cell debris was removed by centrifugation at 25000 × *g* for 15 min. The reddish supernatant fluid was used as a cell-free extract.

Purification of α- and β-Glucosidases Ammonium sulfate was added to the cell-free extract and the resulting precipitate at 0.9 saturation was dissolved in 10 mM phosphate buffer (pH 6.0). After dialysis, the solution was put onto a DEAE-cellulose column (2.6 × 95 cm) equilibrated with the same buffer. The column was washed with the buffer and eluted with 800 ml of the buffer containing 50 mM NaCl. The solution containing α- and β-glucosidases was dialyzed against 10 mM phosphate buffer (pH 6.0) and put onto a CM-cellulose column (2.6 × 95 cm) equilibrated with the same buffer. Nonadsorbable α-glucosidase was washed out with the buffer and concentrated by ultrafiltration and put onto a DEAE-cellulose column (2.1 × 29 cm) and eluted with 400 ml of a linear gradient of NaCl from 0 to 100 mM. The active fractions were combined and concentrated by ultrafiltration and put onto a Sephacryl S-300 column (1.6 × 95 cm). The active fractions were combined and used for the α-glucosidation. β-Glucosidase was eluted with 2000 ml of a linear gradient from 0 to 500 mM NaCl from the CM cellulose column. The active fractions were

combined and concentrated by ultrafiltration and put onto a Sephacryl S-300 column (1.6×95 cm). The active fractions were combined and used for the β-glucosidation.

Assay for α-(β-)Glucosidase Activity The reaction mixture, consisting of 0.04 ml of 25 mM maltose (10 mM cellobiose), 0.04 ml of 100 mM acetate buffer (pH 4.5) (100 mM phosphate buffer pH 6.0), and 0.02 ml of appropriately diluted enzyme solution was incubated at 37 °C for 30 min. The reaction was stopped by heating at 100 °C for 5 min. The liberated glucose was determined by the glucose oxidase–peroxidase method using a Glucose B test.[7] One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed 1 μM of maltose (cellobiose) per min under the above conditions.

Protein Determination Protein was determined as described by Lowry *et al.*[8] with bovine serum albumin as a standard.

Transglucosidation Reaction with α-Glucosidase Reaction mixture containing validamine or valienamine (2.5 g), maltose (100 g), and α-glucosidase (50 U) in 500 ml of 40 mM acetate buffer (pH 5.0) was incubated at 27 °C for 72 h. After the mixture was heated in a boiling water bath for 10 min to inactivate the enzyme, the proteinaceous precipitate was removed by centrifugation and put onto a column of Dowex 50W-X8 (H^+ form, 1.6×60 cm), washed with water, eluted with 0.5 N NH_4OH, and concentrated under reduced pressure. The syrup thus obtained was purified by the chromatography on a Dowex 1-X2 column. In a typical case for the separation of validamine glucosides, the syrup put onto a Dowex 1-X2 column (OH^- form, 2.5×90 cm), the column was developed with water and fractions of 20 ml were collected. The fractions were checked by TLC and GLC to give four components, validamine (fractions 10—14), Va-α-1 (fractions 16—18), Va-α-2 (fractions 26—30), and Va-α-3 (fractions 46—58). These chromatographic separations were repeated on the small column. As a result, three sugar derivatives of Va-α-1 (106 mg), Va-α-2 (22 mg), and Va-α-3 (44 mg) were obtained from validamine, and four sugar derivatives of Ve-α-1 (301 mg), Ve-α-2 (90 mg), Ve-α-3 (32 mg), and Ve-α-4 (22 mg) were obtained from valienamine as white amorphous powder, respectively.

Transglucosidation Reaction with β-Glucosidase A reaction mixture containing validamine or valienamine (2.5 g), cellobiose (50 g), and β-glucosidase (50 U) in 500 ml of 40 mM phosphate buffer (pH 6.0) was incubated at 27 °C for 72 h. The incubation mixture was treated in a similar manner as that described for transglucosidation with α-glucosidase. The transglucosidation products were purified on a column of Dowex 1-X2 (OH^- form, 2.5×90 cm) and eluted with water to give Va-β-1 (125 mg), Va-β-2 (51 mg), and Va-β-3 (48 mg) from validamine and Ve-β-1 (231 mg), Ve-β-2 (189 mg), and Ve-β-3 (64 mg) from valienamine.

Preparation of Rat Intestinal Brush Border Membranes Brush border membranes prepared from the small intestine of male Wistar rats (250—300 g), by the method of Kessler *et al.*,[9] were used as the source of intestinal glycosidases.

Assay of Glycosidase Activity and Inhibition Activity The sucrase, maltase, isomaltase, glucoamylase, and trehalase activities were assayed by the method of Dahlquist.[10] The enzyme (rat intestinal brush border membrane) was preincubated at 37 °C for 10 min in the absence or presence of the inhibitors in 100 mM maleate buffer (pH 5.8). The reaction was started by the addition of respective substrates (100 mM) at 37 °C for 30 min. The reaction was stopped by heating in a boiling water bath for 5 min. The liberated glucose was determined by the glucose oxidase–peroxidase method using a Glucose B test.[7] The concentration producing 50% inhibition (IC_{50}) was determined from a plot of percent inhibition versus the concentration.

Results and Discussion

Purification of α- and β-Glucosidase Both α- and β-glucosidases were found in the cell free extract of *R. lactosa*. Then we isolated both activities by ion exchange chromatography. The cell free extract was concentrated by ammonium sulfate precipitation and dialysed enzyme solution was passed through a DEAE cellulose column. Active fractions were combined and concentrated by ultrafiltration. Then, α-glucosidase and β-glucosidase were isolated by CM-cellulose chromatography. Nonadsorbable α-glucosidase was washed out with the buffer and then β-glucosidase was the eluted linear gradient of NaCl. A typical purification procedure is summarized in Table I. The final enzyme preparation gave a single band by polyacrylamide gel electrophoresis (data not shown).

Preparation and Separation of α-Glucoside Derivatives of Validamine or Valienamine The mixture of maltose as a sugar donor and validamine or valienamine as a sugar acceptor was incubated in admixture with the α-glucosidase at 27 °C for 72 h. The mixture was then immersed into a boiling water bath to heat for 10 min to terminate the reaction, and a centrifugal separation was carried out to remove the sediment. The obtained supernatant was let into a Dowex 50-X8 cation exchanger resin column. The adsorbed validamine or valienamine derivatives were eluted by aqueous ammonia solution. After the eluted liquid was concentrated to dryness under reduced pressure, it was dissolved into a small amount of water, and let into a Dowex 1-X2 anion exchanger resin column. The eluted liquid was separated to yield individual sugar derivatives of validamine or valienamine, accompanied with the identification procedure by the use of TLC, and GLC. As a result, three sugar derivatives of Va-α-1, Va-α-2, and Va-α-3 were obtained from validamine, and four sugar derivatives of Ve-α-1, Ve-α-2, Ve-α-3, and Ve-α-4 were obtained from valienamine as white amorphous powder, respectively.

Preparation and Separation of β-Glucoside Derivatives of Validamine or Valienamine The mixture of cellobiose as a

Table I. Purification of α- and β-Glucosidases from *R. lactosa*

Fraction	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)
α-Glucosidase				
Sonication sup.	2680	132	0.0493	100
90%-$(NH_4)_2SO_4$	616	74.1	0.120	56
1st-DEAE-cellulose	16.2	46.7	4.37	35
2nd-DEAE-cellulose	9.6	47.0	6.93	35
CM-cellulose	2.31	43.1	18.6	33
Sephacryl S-300	1.49	36.3	24.3	27
β-Glucosidase				
Sonication sup.	2680	50.8	0.0190	100
90%-$(NH_4)_2SO_4$	616	32.4	0.0526	64
DEAE-cellulose	14.6	27.2	1.86	54
CM-cellulose	0.480	12.2	25.4	24
Sephacryl S-300	0.167	3.6	21.4	7

Table II. Selective Physico-Chemical Properties of α-Glucoside Derivatives of Validamine and Valienamine

	$[\alpha]_D^{25}$, H_2O	TLC Rf[a]	GLC t_R[b] (min)	^{1}H-NMR, anomeric H[c]
Va-α-1	+128.0	0.40	3.3	δ 4.90 (J=3.7)
Va-α-2	+139.2	0.23	33.3	4.94 (J=3.3), 4.90 (J=3.3)
Va-α-3	+115.2	0.48	2.5	5.30 (J=3.7)
Ve-α-1	+126.4	0.55	2.6	5.41 (J=3.9)
Ve-α-2	+160.6	0.33	27.2	5.43 (J=3.7), 4.96 (J=3.7)
Ve-α-3	+144.4	0.48	3.7	4.91 (J=3.7)
Ve-α-4	+137.6	0.27	33.9	4.94 (J=3.8), 4.92 (J=3.7)
Validamine	+66.2	0.60		
Valienamine	+81.6	0.68		

a) Solvent; $CHCl_3$: MeOH : NH_4OH = 1 : 3 : 2, Silica gel G. b) 7% OV-17, 2 m, 280 °C, TMS-derivatives. c) At 400 MHz with DSS standard.

TABLE III. ^{13}C-NMR Data of α-Glucoside Derivatives of Validamine and Valienamine

	Validamine	Va-α-1	Va-α-2	Va-α-3	Valienamine	Ve-α-1	Ve-α-2	Ve-α-3	Ve-α-4
C-1	52.3	52.4	52.6	52.2	51.7	51.0	51.0	51.8	51.8
C-2	76.5	76.2	76.1	75.6	72.9	72.2	73.6	72.4	72.2
C-3	76.0	76.8	76.7	77.0	74.9	73.5	74.1	74.8	74.7
C-4	76.1	76.1	75.9	85.0	74.7	79.5	79.8	74.1	73.8
C-5	40.6	38.8	38.7	39.5	142.0	140.6	140.2	139.5	140.0
C-6	32.3	32.5	32.1	31.8	127.2	128.0	127.7	129.6	128.8
C-7	65.2	71.0	70.9	64.7	64.1	64.5	64.6	68.9	68.8
C-1′		100.7	100.7	102.8		100.6	100.8	99.1	99.1
C-2′		74.1	74.1	74.5		74.1	74.2	73.9	74.2
C-3′		75.8	75.8	75.7		75.7	75.8	75.8	76.0
C-4′		72.3	72.3	72.0		72.1	72.1	72.3	72.2
C-5′		74.5	74.0	75.3		75.4	72.4	74.6	73.0
C-6′		63.2	68.3	63.2		63.2	68.5	63.2	68.2
C-1″			100.5				100.6		100.6
C-2″			74.1				74.0		74.0
C-3″			75.7				75.9		75.8
C-4″			72.2				72.2		72.0
C-5″			74.5				74.5		74.6
C-6″			63.2				63.2		63.2

ppm from DSS in D_2O. Underline shows the signal of carbon in α-glucoside bond.

sugar donor and validamine or valienamine as a sugar acceptor was incubated in admixture with β-glucosidase at 27 °C for 72 h. Transglucosidation products of amino cyclitol were isolated by cation exchanger resin column from cellobiose and glucose, and separated by Dowex 1-X2 anion exchanger resin column. As a result, three sugar derivatives of Va-β-1, Va-β-2, and Va-β-3 were obtained from validamine and three sugar derivatives of Ve-β-1, Ve-β-2, and Ve-β-3 were obtained from valienamine as white amorphous powder, respectively.

Chemical Structure of α-Glucoside Derivatives of Validamine and Valienamine Some physico-chemical properties of α-glucoside derivatives of validamine and valienamine were shown in Table II. α-Glucoside derivatives of validamine and valienamine showed such optical rotation and they could be discriminated by using the silica gel TLC and GLC by the use of TMS derivatives. Structural determinations are done mainly by ^{13}C- and ^{1}H-NMR spectral analysis for these derivatives. Each signal was attributed by comparing it with such signals as those of glucobiose and glucotriose.[11] With ^{1}H-NMR, the anomeric proton of glucoside has been known to be the coupling constant with an adjacent proton of 2—4 Hz in the case of α-type.[12] As the coupling constant of anomeric proton in these derivatives has been known to be 3.3—3.9 Hz, it has been found that all the derivatives above synthesized using α-glucosidase are compounds which link with α-glucose. Further, Va-α-2, Ve-α-2, and Ve-α-4 were an α-derivative that was composed of a rearrangement product of two molecules of glucose. ^{13}C-NMR chemical shifts of α-glucoside derivatives of validamine and valienamine are listed in Table III. The signal of carbons which participated in α-glucoside bondage shifts to downfield by 3—5 ppm due to the glycosidation effect.[13] In the case of validamine α-glucoside derivatives, the signals of C-7 of Va-α-1 and Va-α-2 have shifted to downfield by 5.8 ppm and 5.7 ppm, respectively. From these results, it has been found that Va-α-1 is a derivative which links with α-glucose at C-7 of validamine, while Va-α-2 is a derivative which links with α-isomaltose at C-7 of validamine. In the case of Va-α-3, chemical shifts of C-2, C-3, and C-4 of validamine are close together, and it is difficult to discriminate the signal of which carbon has shifted 85.0 ppm to downfield. Therefore, the identification has been accomplished by the use of ^{13}C–^{1}H correlation spectroscopy experiments. It has been ascertained that the carbon whose signal shifted to downfield has a bond with H-4. From these results, Va-α-3 is ascribed to the derivative that linked with α-glucose at C-4 of validamine. Similarly, it has been found that Ve-α-1 and Ve-α-2 are derivatives which link with α-glucose and α-isomaltose at C-4 of valienamine, while Ve-α-3 and Ve-α-4 are derivatives which link with α-glucose and α-isomaltose at C-7 of valienamine.

TABLE IV. Selective Physico-Chemical Properties of β-Glucoside Derivatives of Validamine and Valienamine

	$[\alpha]_D^{25}$, H_2O	TLC Rf[a]	GLC t_R[b] (min)	^{1}H-NMR, anomeric H[c]
Va-β-1	+24.0	0.42	3.3	δ 4.44 (J=8.0)
Va-β-2	+6.4	0.49	2.6	4.61 (J=8.1)
Va-β-3	+40.4	0.49	2.3	4.51 (J=8.1)
Ve-β-1	+31.8	0.46	3.7	4.49 (J=8.1)
Ve-β-2	+30.2	0.57	2.5	4.64 (J=7.7)
Ve-β-3	+28.4	0.57	2.7	4.64 (J=8.3)
Validamine	+66.2	0.60		
Valienamine	+81.6	0.68		

a) Solvent; $CHCl_3$: MeOH : NH_4OH = 1 : 3 : 2, Silica gel G. *b*) 7% OV-17, 2 m, 280 °C, TMS-derivatives. *c*) At 400 MHz with DSS standard.

Chemical Structure of β-Glucoside Derivatives of Validamine and Valienamine Some physico-chemical properties of β-glucoside derivatives of validamine and valienamine were shown in Table IV. β-Glucoside derivatives of validamine and valienamine showed such optical rotation and they could be discriminated by using the silica gel TLC and GLC by the use of TMS derivatives. With ^{1}H-NMR, the anomeric proton of glucoside has been known to be the coupling constant with an adjacent proton of 7—9 Hz in the case of β-type.[12] As the coupling constant of anomeric proton in these derivatives has been known to be 7.7—

TABLE V. ^{13}C-NMR Data of β-Glucoside Derivatives of Validamine and Valienamine

	Validamine	Va-β-1	Va-β-2	Va-β-3	Valienamine	Ve-β-1	Ve-β-2	Ve-β-3
C-1	52.3	52.5	51.6	52.1	51.7	51.8	51.1	51.2
C-2	76.5	76.1	86.8	76.1	72.9	72.3	82.7	72.3
C-3	76.7	76.7	76.1	74.5	74.9	74.7	74.0	73.5
C-4	76.1	75.6	75.7	81.7	74.7	74.3	73.7	84.7
C-5	40.6	39.5	40.4	40.1	142.0	140.0	142.6	140.5
C-6	32.3	32.1	31.6	32.0	127.2	129.2	126.0	128.5
C-7	65.2	73.9	65.0	64.4	64.1	72.6	64.1	64.3
C-1′		105.8	106.3	105.6		104.9	106.2	106.3
C-2′		75.9	75.9	76.1		75.9	76.0	76.1
C-3′		78.4	78.3	78.3		78.4	78.3	78.4
C-4′		72.4	72.1	72.1		72.4	72.1	72.1
C-5′		78.7	78.5	78.7		78.6	78.5	78.7
C-6′		63.5	63.2	63.2		63.4	63.2	63.4

ppm from DSS in D_2O. Underline shows the signal of carbon in β-glucoside bond.

TABLE VI. ^{13}C-Chemical Shifts of Compounds Va-β-2 and Ve-β-2 at pD 1 Together with Deuteronation

	Validamine		Va-β-2		Valienamine		Ve-β-2	
	pD>8	pD<1	pD>8	pD<1	pD>8	pD<1	pD>8	pD<1
C-1	52.3	54.1	51.6	53.5	51.7	52.1	51.1	51.5
C-2	76.5	72.8 (β, −3.7)	86.8	82.6 (−4.2)	72.9	69.4 (β, −3.5)	82.7	78.1 (−4.6)
C-3	76.7	76.6	76.1	74.5	74.9	74.4	74.0	73.1
C-4	76.1	74.9	75.7	75.6	74.7	73.6	73.7	72.9
C-5	40.6	40.6	40.4	40.4	142.0	148.7 (γ, +6.7)	142.6	148.0 (+5.4)
C-6	32.3	28.6 (β, −3.7)	31.6	28.6 (−3.0)	127.2	118.4 (β, −8.8)	126.0	118.1 (−7.9)
C-7	65.2	64.3	65.0	64.2	64.1	63.8	64.1	63.7
C-1′			106.3	106.5			106.2	106.2
C-2′			75.9	75.6			76.0	75.8
C-3′			78.3	78.1			78.3	78.6
C-4′			72.1	71.9			72.1	71.9
C-5′			78.5	78.4			78.5	78.7
C-6′			63.2	63.0			63.2	63.1

ppm from DSS in D_2O. Underline shows the signal of carbon in β-glucoside bond.

TABLE VII. Inhibitory Effects on Glucoside Hydrolases of Rat Small Intestine

	IC_{50} (M)[a]				
	Sucrase	Maltase	Glucoamylase	Isomaltase	Trehalase
Validamine	8.2×10^{-5}	2.0×10^{-3}	2.1×10^{-4}	8.0×10^{-4}	4.5×10^{-4}
Va-α-1	6.4×10^{-4}	1.9×10^{-2}	2.7×10^{-3}	5.0×10^{-3}	4.1×10^{-4}
Va-α-2	3.8×10^{-3}	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	1×10^{-2}
Va-α-3	9.1×10^{-4}	7.2×10^{-3}	1.3×10^{-3}	$(>2\times10^{-2})$	4.3×10^{-4}
Va-β-1	1.7×10^{-4}	7.7×10^{-3}	1.3×10^{-3}	8.8×10^{-3}	5.3×10^{-5}
Va-β-2	2×10^{-2}	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	8.1×10^{-4}
Va-β-3	3.9×10^{-3}	$(>2\times10^{-2})$	4.9×10^{-3}	$(>2\times10^{-2})$	6.2×10^{-3}
Valienamine	2.3×10^{-4}	3.7×10^{-3}	5.9×10^{-4}	1.4×10^{-3}	2.4×10^{-5}
Ve-α-1	6.2×10^{-3}	$(>2\times10^{-2})$	4.1×10^{-3}	$(>2\times10^{-2})$	9.1×10^{-4}
Ve-α-2	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	5.6×10^{-4}
Ve-α-3	2.3×10^{-3}	7.6×10^{-3}	1.5×10^{-3}	1.2×10^{-2}	1.9×10^{-4}
Ve-α-4	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$
Ve-β-1	1.4×10^{-3}	1.1×10^{-2}	2.9×10^{-3}	5.3×10^{-3}	4.2×10^{-3}
Ve-β-2	$(>2\times10^{-2})$	2×10^{-2}	$(>2\times10^{-2})$	$(>2\times10^{-2})$	4.9×10^{-3}
Ve-β-3	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	$(>2\times10^{-2})$	9.8×10^{-3}

a) Molar concentration required to give 50% inhibition.

8.3 Hz, it has been found that all the derivatives above synthesized using β-glucosidase are compounds which link with β-glucose. ^{13}C-NMR was measured to determine the respective structures of these derivatives and are shown in Table V. The signal of carbon which participated in β-glucoside bondage shifts to downfield by 8—10 ppm due to the glycosidation effect. In the case of β-glucosyl validamine derivatives, the signals of C-7 of Va-β-1, C-2 of Va-β-2, and C-4 of Va-β-3 have shifted to downfield by 8.7 ppm, 10.2 ppm, and 10.6 ppm, respectively. From

these results, its has been found that Va-β-1, Va-β-2, and Va-β-3 are derivatives which link with β-glucose at C-7, C-2, and C-4 of validamine, respectively. Similarly, it has been found that Ve-β-1, Ve-β-2, and Ve-β-3 are derivatives which link with β-glucose at C-7, C-2, and C-4 of valienamine, respectively. The binding position of derivatives to which β-glucose was linked at C-2 was confirmed by examining the protonation effect. As to amino sugars, it has been known that signals of the carbon, to which the amino group is attached, shift slightly to downfield, while signals of the adjacent carbon shift to upfield under acidic conditions.[14] In the case of valienamine, it was observed that a signal of γ-carbon shifted to downfield by the allyl system.[15] Signals of C-2 of Va-β-2 and Ve-β-2 have shifted to upfield by 4.2 ppm and 4.6 ppm, respectively, as shown in Table VI. From these data, it has been found that Va-β-2 and Ve-β-2 are derivatives which link with β-glucose at C-2 of validamine and valienamine, respectively.

Inhibitory Effect on Glucoside Hydrolase of Rat Small Intestine Glucoside derivatives of validamine and valienamine were tested for α-glucosidase inhibitory activity and the inhibition data *in vitro* (molar concentrations required for a 50% inhibition: IC_{50} (M)) against rat intestinal sucrase, maltase, glucoamylase, and trehalase are shown in Table VII. Glucoside derivatives showed generally lower inhibitory activity than the parent compounds. Especially, isomaltoside derivatives (Va-α-2, Ve-α-2, and Ve-α-4) showed lower inhibitory activity than corresponding glucoside derivatives. However, 7-*O*-β-D-glucosyl validamine (Va-β-1) showed more potent inhibitory activity than the parent validamine against trehalase. We prepared α- and β-glucoside derivatives of validamine and valienamine for the purpose of obtaining more potent α-glucosidase inhibitors. However, these compounds showed generally lower inhibitory activity than the parent compounds. From these results, it is suggested that validamine or valienamine bind to the glycone (the non-reducing-end part of the substrate)-binding subsite of the enzyme. Then, the *N*-binding derivative, such as validamine-(1→4)-D-glucose, may have more potent inhibitory activity on the rat intestinal sucrase and maltase.

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