VALIOLAMINE, A NEW α-GLUCOSIDASE INHIBITING AMINOCYCLITOL PRODUCED BY *STREPTOMYCES HYGROSCOPICUS*

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Valiolamine, a new aminocyclitol has been isolated from the fermentation broth of *Streptomyces hygroscopicus* subsp. *limoneus* and its structure has been determined to be (1(OH),2,4,5/1,3)-5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol. Valiolamine has more potent α-glucosidase inhibitory activity against porcine intestinal sucrase, maltase and isomaltase than valienamine, validamine and hydroxyvalidamine which were reported as building blocks of validamycins and microbial oligosaccharide α-glucosidase inhibitors. In addition, valienamine, validamine and hydroxyvalidamine have been isolated from the fermentation broth.

In the course of an intensive search for aminocyclitols in the fermentation broth of *Streptomyces hygroscopicus* subsp. *limoneus* IFO 12703, which is a producer of validamycins, a new aminocyclitol has been isolated, together with valienamine, validamine and hydroxyvalidamine which were first isolated by the chemical or microbial degradation of validamycins[1,2].

This paper deals with the isolation of the new aminocyclitol, named valiolamine, as well as valienamine, validamine and hydroxyvalidamine from the fermentation broth, the structure elucidation of valiolamine and the characteristic of its glucosidase inhibitory activity.

Isolation and Purification

A culture of *S. hygroscopicus* subsp. *limoneus* IFO 12703 was fermented as described in a previous paper[3]. The culture filtrate (70 liters, pH 6.0) was passed through a column of Amberlite IRC-50

Fig. 1.

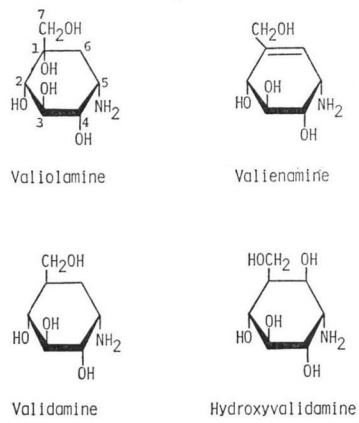


Table 1. Rf values of aminocyclitols.

Aminocyclitols	I	II	III
Validamine	0.35	0.49	0.61
Valienamine	0.41	0.49	0.61
Hydroxyvalidamine	0.41	0.39	0.56
Valiolamine	0.27	0.39	0.41

Solvent system

I: 1-PrOH - AcOH - H_2O (4:1:1).

II: 1-BuOH - MeOH - $CHCl_3$ - concd NH_4OH (4:5:2:5).

III: $CHCl_3$ - MeOH - concd NH_4OH (1:3:2).

Silica gel TLC: Kieselgel 60 F_{254} (Merck Art. 5719).

(NH_4^+, 6 liters) and the column was eluted with 0.5 N ammonium hydroxide. The eluate was concentrated and rechromatographed on a column of Amberlite CG-50 (NH_4^+, 1 liter) with 0.1 N ammonium hydroxide. The eluate was monitored by means of TLC (silica gel; 1-PrOH - AcOH - H_2O, 4: 1: 1; detected with ninhydrin reagent) and fractionated into three fractions in order of elution; fraction I (containing hydroxyvalidamine), fraction II (containing validamine and valienamine) and fraction III (containing valiolamine). Fraction II was chromatographed on Dowex 1X2 (OH^-, 450 ml) with water to separate into validamine (the first fraction) and valienamine (the later fraction). Each fraction was rechromatographed on Dowex 1X2 and finally hydroxyvalidamine (25 mg), validamine (1.1 g), valienamine (45 mg) and valiolamine (0.25 g) were isolated. The Rf values of these aminocyclitols on silica gel TLC are shown in Table 1 and some physico-chemical properties of valiolamine are as follows.

Valiolamine: white powder; $[\alpha]_D^{20}$ +18.8° (*c* 1.0, H_2O); *Anal* Calcd for $C_7H_{15}NO_5 \cdot H_2O$: C 39.80, H 8.11, N 6.63, Found: C 39.35, H 7.82, N 6.59; *pKa'* 8.6 (neutral equiv 200±20); color reaction: positive to ninhydrin, GREIG-LEABACK reactions.

Structure of Valiolamine

The molecular formula was established by elementary analysis, mass spectrometry and ^{13}C and ^1H NMR spectrometry as $C_7H_{15}NO_5$. The $(M+1)^+$ peak of valiolamine was observed as *m/z* 194 in the mass spectrum (SIMS). The ^{13}C NMR spectrum of valiolamine revealed 7 signals ($CH_2 \times 2$, $CH \times 4$ and $C \times 1$) and is summarized as shown in Table 2. Assignment of the chemical shifts was performed by off-resonance and selective proton decoupling technique. The 100 MHz ^1H NMR spectrum is shown in Fig. 2 and 400 MHz ^1H NMR spectral data are summarized in Table 3. In this report, the position number of carbon atoms of valiolamine are assigned as shown in Fig. 1.

Acetylation of valiolamine with acetic anhydride in pyridine afforded an *N,O*-pentaacetate and the ^1H NMR spectral data (100 MHz, in $CHCl_3$) of the *N,O*-pentaacetate are summarized in Table 4. The broad singlet at δ 3.24 and the broad doublet at δ 7.00 disappeared by deuterium oxide exchange and the former was assigned to tertiary alcoholic OH which remains unacetylated under the condition used and the latter to -NH-CO-. The multiplet at δ 4.75 was changed to a quartet by deuterium oxide exchange and assigned to the methine proton at the carbon atom bearing the acetamido group.

The ^1H NMR spectral studies of valiolamine including decoupling experiments showed the presence of a six-membered ring and the side chain methylene as a partial structure. The splitting patterns of

Table 2. ^{13}C NMR spectral data of valiolamine in D_2O.

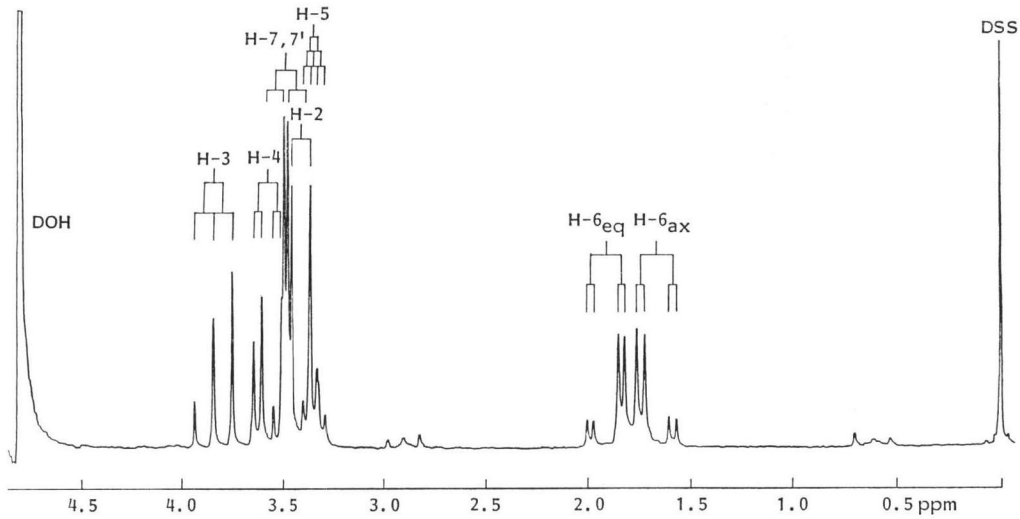
Carbon	Chemical shift δ (ppm)*	Multiplicity
C-1	78.7	s
C-2	76.4	d
C-3	73.8	d
C-4	76.3	d
C-5	52.9	d
C-6	35.0	t
C-7	68.2	t

* δ (ppm) from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

Table 3. Assignment of ^1H NMR signals of valiolamine in D_2O (400 MHz).

Proton	Chemical shift δ (ppm)	Multiplicity and coupling constant (*J* in Hz)
H-2	3.425	1H, d, J=9.5
H-3	3.847	1H, dd, J=9.5, J=9.8
H-4	3.584	1H, dd, J=9.8, J=4.2
H-5	3.342	1H, ddd, J=4.2, J=2.9, J=3.9
H-6_{axial}	1.699	1H, dd, J=3.9, J=15.1
H-$6_{equatorial}$	1.893	1H, dd, J=15.1, J=2.9
H-7	3.464	each 1H, ABq, J=11.2
H-7'	3.530	

Fig. 2. ^{1}H NMR spectrum of valiolamine (100 MHz, in D_2O).



ring methylene protons H-6 and H-6′ suggest that ring methine H-5 is equatorial and the splitting patterns of ring methines H-2, H-3 and H-4 indicate that these three methine protons are all *trans*-axial. The doublet splitting of H-2 shows that C-1 carbon atom bears no proton.

The structure of valiolamine, except the stereochemistry of C-1, was elucidated to be (2, 4,5/3)-5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol from the above results. The configuration of the hydroxyl-bearing tertiary carbon atom (C-1) was elucidated by the following evidence. If the hydroxyl group at C-1 is axial, valiolamine is expected to form the cyclic carbamate between the C-1 hydroxyl and the C-5 amino group (1,3 diaxial configuration) as well as between the C-4 hydroxyl and the C-5 amino group (vicinal axial-equatorial configuration). When *N*-(benzyloxycarbonyl)valiolamine was refluxed with sodium hydrogen carbonate in methanol, two cyclic carbamates of valiolamine were produced as expected. One showed IR carbonyl absorption at 1730 cm^{-1}, which is characteristic of a five membered cyclic carbamate, and the other showed carbonyl absorption at 1660 cm^{-1}, which suggests the presence of a six-membered cyclic carbamate ring[4].

Table 4. Assignment of ^{1}H NMR signals of *N,O*-pentaacetylvaliolamine in $CDCl_3$ (100 MHz).

Proton	Chemical shift δ (ppm)	Multiplicity and coupling constant (J in Hz)
H-2	5.05	1H, d, J=10
H-3	5.50	1H, t, J=10
H-4	4.90	1H, dd, J=10, J=3.7
H-5	4.75	1H, m
H-7	3.82	each 1H, ABq, J=11.4
H-7′	3.98	
–OH	3.24	1H, br s
–CONH–	7.00	1H, br d, J=8.5
$-COCH_3$	1.99	6H, s
	2.01	3H, s
	2.08	3H, s
	2.09	3H, s

* H-6 and H-6′ (δ 1.85~2.15) are overlapped with Ac.

Acetylation of six-membered cyclic carbamate with acetic anhydride and pyridine afforded a tetraacetate, and no hydroxyl proton signal was observed in the ^{1}H NMR spectrum (in $CDCl_3$) of the tetraacetate. Therefore the structure of the latter cyclic carbamate was elucidated to be the 1,5-cyclic carbamate of 5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol and the former cyclic carbamate to be the 4,5-cyclic carbamate. Thus the structure of valiolamine was elucidated to be (1(OH),2,4, 5/1,3)-5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol. The absolute stereochemistry at C-1

was determined to be 1*S* by the chemical conversion of valienamine into valiolamine[5], which will be reported elsewhere in detail.

Inhibitory Effects on D-Glucose Hydrolases

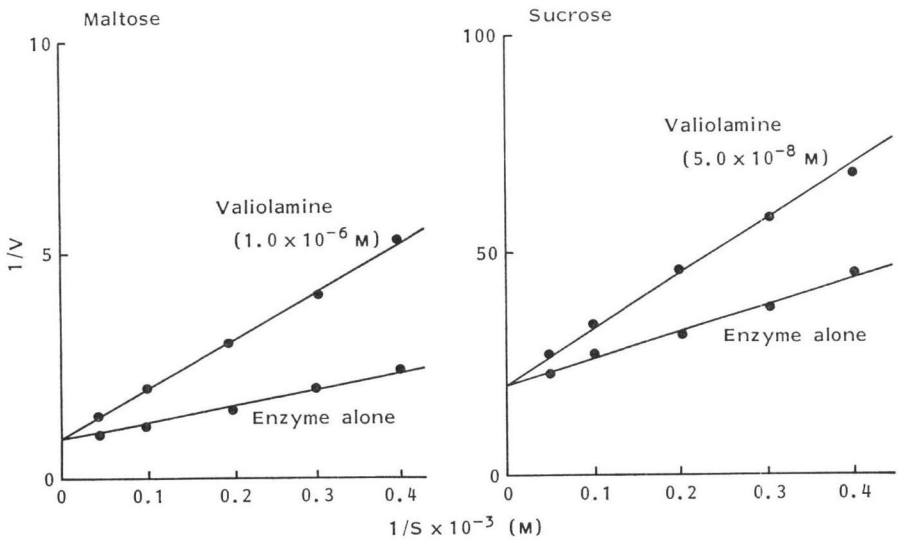
The inhibitory activities (IC_{50}: molar concentration required to give 50% inhibition) against D-glucose hydrolases of valiolamine, valienamine, validamine and hydroxyvalidamine were examined and the results appear in Table 5. Valiolamine, with IC_{50}s in the range of 10^{-6} to 10^{-8} mol/liter against porcine intestinal maltase, sucrase and isomaltase, was considerably more active than the others.

Table 5. Inhibitory effects of valiolamine and related aminocyclitols against various glucose hydrolases.

Enzyme (Origin)	Substrate	IC_{50} (M)*			
		Valiolamine	Valienamine	Validamine	Hydroxy-validamine
α-Glucosidase (Yeast)	Maltose	1.9×10^{-4}	1.8×10^{-5}	5.8×10^{-4}	3.6×10^{-4}
Maltase (Porcine)	Maltose	2.2×10^{-6}	3.4×10^{-4}	1.1×10^{-4}	8.3×10^{-3}
Isomaltase (Porcine)	Isomaltose	2.7×10^{-6}	1.0×10^{-3}	1.3×10^{-4}	$>1.0\times10^{-2}$
Sucrase (Yeast)	Sucrose	$>1.0\times10^{-2}$	1.8×10^{-3}	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$
(Porcine)	Sucrose	4.9×10^{-5}	5.3×10^{-5}	7.5×10^{-6}	4.2×10^{-4}
β-Glucosidase (Almond)	Cellobiose	8.1×10^{-3}	8.8×10^{-3}	1.5×10^{-3}	7.4×10^{-3}
Glucoamylase (*Rhizopus* sp.)	Starch	$>1.0\times10^{-2}$	6.8×10^{-3}	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$
α-Amylase (Porcine)	Starch	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$
β-Amylase (Sweet potato)	Starch	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$	$>1.0\times10^{-2}$

* IC_{50}: Molar concentration required to give 50% inhibition.

Fig. 3. Effects of valiolamine on hydrolysis of maltose and sucrose by porcine intestinal α-glucosidases.



However, valienamine showed somewhat stronger activity than valiolamine against yeast α-glucosidase and *Rhizopus* glucoamylase.

As shown by LINEWEAVER-BURK plots in Fig. 3, valiolamine is competitive with maltose and sucrose, and such a relationship had been seen previously with valienamine[6]. The *Ki* values of valiolamine for maltase and sucrase were found to be 3.5×10^{-7} mol/liter and 3.0×10^{-5} mol/liter respectively, which are 10^{-4} to 10^{-5} times smaller than the *Km* values (2.9×10^{-3} mol/liter for maltose and 3.0×10^{-3} mol/liter for sucrose) as shown in Table 6.

Table 6. *Ki* values of valiolamine, validamine and valienamine for porcine intestinal maltase and sucrase.

Inhibitors	*Ki* (M)	
	Maltase	Sucrase
Valiolamine	3.5×10^{-7}	3.0×10^{-5}
Validamine	2.3×10^{-5}	2.3×10^{-6}
Valienamine	1.8×10^{-4}	5.0×10^{-5}

* *Km* value; 2.9×10^{-3} M (for maltose), 3.0×10^{-3} M (for sucrose).

Experimental

Melting points were measured with a Yamato MP-1 capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco digital polarimeter DIP-4. ^{1}H NMR spectra were recorded with a Jeol JNM-FX100 spectrometer (at 100 MHz) or a Jeol JNM-GX400 spectrometer (at 400 MHz). Chemical shifts are reported in ppm from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate standard (DSS, in D_2O) or TMS (in $CDCl_3$). ^{13}C NMR spectra were recorded with a Jeol JNM-FX100 spectrometer (at 25.2 MHz). Chemical shifts are reported in ppm using DSS as an internal reference in D_2O. IR spectra were recorded with a Jasco IRA-2 spectrometer. Mass spectra were recorded with Hitachi RMU-6D (EI) and M-80A (SIMS) spectrometers.

Acetylation of Valiolamine

A suspension of valiolamine (30 mg) in pyridine (1.5 ml) and acetic anhydride (0.8 ml) was stirred at room temp for 20 hours. The resulting solution was concentrated. The residue was dissolved in EtOAc and washed with 1 N HCl and satd $NaHCO_3$. The organic layer was concentrated to give colorless crystals (16 mg); mp 137~138°C; $[\alpha]_D^{25}$ −14.8° (*c* 1, $CHCl_3$); MS(EI) *m/z* 404 $(M+1)^+$, 385 $(M-H_2O)$.

Anal Calcd for $C_{17}H_{25}NO_{10}$: C 50.62, H 6.25, N 3.47.
Found: C 50.11, H 6.00, N 3.40.

N-(Benzyloxycarbonyl)valiolamine

A solution of carbobenzyloxy chloride (3 ml) in acetone (10 ml) was added dropwise to an ice-cold solution of valiolamine (1.5 g) and $NaHCO_3$ (1.2 g) in H_2O (30 ml) with stirring. The mixture was stirred at room temp for additional 3 hours and then adjusted to pH 5 with 2 N HCl. The precipitate was collected by filtration, washed with cold H_2O and ethyl ether, and crystallized from H_2O to give colorless needles (2.1 g); mp 181~184°C; IR (KBr) cm^{-1} 1710, 1520.

Anal Calcd for $C_{15}H_{21}NO_7$: C 55.04, H 6.47, N 4.28.
Found: C 54.56, H 6.39, N 4.14.

Preparation of Cyclic Carbamate Derivatives of Valiolamine

To a solution of *N*-(benzyloxycarbonyl)valiolamine (1.2 g) in MeOH (30 ml) was added $NaHCO_3$ (1.0 g). The mixture was refluxed with stirring for 5 hours and then evaporated to dryness. The residue was dissolved in H_2O (10 ml), neutralized with 2 N HCl, and passed through a column of activated carbon (50 ml). The column was washed with H_2O and then eluted with 50% aq MeOH. The eluate was concentrated to dryness and the residue (350 mg) was chromatographed on a silica gel column with 1-BuOH - MeOH - $CHCl_3$ - H_2O (4: 4: 2: 1). Valiolamine 4,5-carbamate was first eluted, followed by valiolamine 1,5-carbamate. After evaporation of solvents, each residue was chromatographed on activated carbon column (50 ml). The columns were washed with H_2O and eluted with

50% aq MeOH. Evaporation of the solvents gave valiolamine 4,5-carbamate (110 mg) and valiolamine 1,5-carbamate (95 mg) as white powder.

Valiolamine 4,5-carbamate: $[\alpha]_D^{25}$ +54.9° (*c* 1, H_2O); IR (KBr) cm^{-1} 1730 (five membered cyclic carbamate C=O); ^1H NMR (100 MHz, D_2O) δ 2.00 (2H, m, H-6, 6'), 3.50 (1H, d, *J*=10.5 Hz, H-2), 3.61 and 3.46 (each 1H, ABq, *J*=12 Hz, CH_2O), 4.07 (1H, dd, *J*=7.7 Hz, 10.5 Hz, H-3), 4.30 (1H, ddd, *J*=7.7 Hz, *J*=3.7 Hz, *J*=4.6 Hz, H-5), 4.60 (1H, t, *J*=7.7 Hz, H-4).

Valiolamine 1,5-carbamate: $[\alpha]_D^{25}$ +37.6° (*c* 1, H_2O); IR (KBr) cm^{-1} 1660 (six membered cyclic carbamate C=O); ^1H NMR (100 MHz, D_2O) δ 1.92 (1H, dd, *J*=2.0 Hz, *J*=14.2 Hz, H-6), 2.18 (1H, dd, *J*=4.0 Hz, *J*=14.2, H-6'), 3.59~3.92 (6H, m).

Acetylation of Valiolamine 1,5-Carbamate

A suspension of valiolamine 1,5-carbamate (70 mg) in pyridine (0.5 ml) and acetic anhydride (0.2 ml) was stirred overnight at room temp. The resulting solution was concentrated to dryness and the residue was extracted with EtOAc. The extract was washed with 1 N HCl and saturated $NaHCO_3$ and then concentrated. Ethyl ether was added to the residue and the mixture was allowed to stand overnight in a refrigerator to give the tetraacetate of valiolamine 1,5-carbamate (81 mg) as colorless crystals: mp 173~174°C; $[\alpha]_D^{25}$ +49.0° (*c* 1, $CHCl_3$); IR (KBr) cm^{-1} 1735, 1708; MS(EI) *m/z* 388 $(M+1)^+$; ^1H NMR (100 MHz, $CDCl_3$) δ 1.97 (3H, s, Ac), 2.03 (3H, s, Ac), 2.08 (3H, s, Ac), 2.24 (3H, s, Ac), 2.0~2.3 (2H, H-6 and H-6', overlapped with Ac), 3.88 (1H, m, H-5), 3.94 and 4.23 (each 1H, ABq, *J*=11.7 Hz, CH_2O), 5.00 (1H, dd, *J*=2.8 Hz, *J*=9.1 Hz, H-4), 5.19 (1H, d, *J*=9.1 Hz, H-2), 5.40 (1H, t, *J*=9.1 Hz, H-3), 7.53 (1H, d, *J*=4.8 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 20.4 (q, $COCH_3$), 20.6 (q, $COCH_3\times3$), 27.0 (t, C_6), 47.7 (d, C_5), 63.9 (t, C_7), 71.0, 73.1, 74.2 (each d, C_2~C_4), 80.2 (s, C_1), 153.1 (s, CONH), 169.7 (s, $COCH_3$), 170.1 (s, $COCH_3\times2$), 171.0 (s, $COCH_3$).

Anal Calcd for $C_{16}H_{21}NO_{10}$: C 49.61, H 5.46, N 3.62.
Found: C 49.53, H 5.46, N 3.46.

Acetylation of Valiolamine 4,5-Carbamate

Valiolamine 4,5-carbamate formed a triacetate when treated as described above to give colorless powder: mp 171~173°C; $[\alpha]_D^{25}$ +79.1° (*c* 1, $CHCl_3$); MS(EI) *m/z* 346 $(M+1)^+$; ^1H NMR (100 MHz, $CDCl_3$) δ 2.06 (6H, s, 2×Ac), 2.07 (3H, s, Ac), 2.00~2.20 (2H, H-6 and H-6', overlapped with Ac), 3.80 and 4.16 (each 1H, ABq, *J*=11.4 Hz, CH_2O), 3.95 (1H, s, OH), 4.36 (1H, m, H-5), 4.65 (1H, t, *J*=7.4 Hz, H-4), 5.04 (1H, d, *J*=10.2 Hz, H-2), 5.70 (1H, dd, *J*=7.4 Hz, *J*=10.2 Hz, H-3), 6.56 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ 20.4 (q, $COCH_3$), 20.7 (q, $COCH_3\times2$), 32.3 (t, C_6), 50.8 (d, C_5), 66.3 (t, C_7), 70.9, 71.9, 72.9 (each d, C_2~C_4), 78.6 (s, C_1), 159.1 (s, CONH), 170.1 (s, $COCH_3$), 170.4 (s, $COCH_3$), 170.6 (s, $COCH_3$).

Anal Calcd for $C_{14}H_{19}NO_9$: C 48.69, H 5.54, N 4.05.
Found: C 48.31, H 5.62, N 3.88.

Assay of Glucoside Hydrolase Inhibition Activity

Sucrase, maltase and isomaltase were prepared from porcine small intestine mucosa according to the method of BORGSTRÖM and DAHLQVIST[7]. The inhibitory activity was determined by incubating a solution (0.25 ml) of glucose hydrolase (5~50 μg) with a 0.2 M substrate solution (0.25 ml) and a solution (0.5 ml) of inhibitor (at several different concentrations) in 0.02 M phosphate buffer (pH 6.8, in a final volume of 1.0 ml) at 37°C for 10 minutes and then by measuring the amount of released D-glucose by glucose oxidase method[8]. The concentration producing 50% inhibition (IC_{50}) was determined from a plot of percent inhibition vs. the concentration.

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