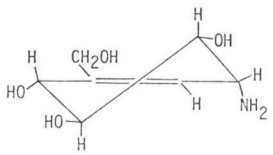
VALIENAMINE AS AN α-GLUCOSIDASE INHIBITOR

Sir:

Valienamine (Fig. 1), an intermediate formed by the microbial degradation of validamycins by several soil bacteria such as *Pseudomonas denitrificans*[1] and *Flavobacterium saccharophilum*[2], is a pseudo-sugar analog of D-glucose with the same configuration. In aqueous solution it is expected to assume a half-chair conformation, as shown in Fig. 1.

Fig. 1.



Valienamine

Glucono-δ-lactone and nojirimycin are powerful, competitive inhibitors of β-glucosidases and some α-glucosidases. It is generally understood that the inhibitory effect depends on their structural similarity to the D-glycosyl cation forming a half-chair conformation on the transition state in the course of enzyme-catalyzed pyranoside hydrolysis[3]. In this regard, we attempted to elucidate the inhibitory effect of valienamine on α- and β-glucosidases.

We now report on the characterization of valienamine as a new α-glucosidase inhibitor.

For the preparation of valienamine, *F. saccharophilum* was shaked-cultured at 27°C for 4 days in the medium consisting of validamycin A 1%, $(NH_4)_2SO_4$ 1%, K_2HPO_4 0.7%, KH_2PO_4 0.3% and $MgSO_4 \cdot 7H_2O$ 0.01% (pH 7.1). The cultured broth (2 liters) was passed through a column of Amberlite IRC-50 (NH_4 form, 500 ml), which was eluted with 0.5 N aqueous ammonia. The concentrate of the eluate was chromatographed on a column of Dowex 1×2 (OH form, 500 ml) and developed with water to give valienamine (3 g).

The inhibitory activity of valienamine on glucosidases was assayed as follows; the reaction mixture (0.25 ml of α(β)-glucosidase dissolved in 0.02 M phosphate buffer, pH 6.8 (acetate buffer, pH 5.5), 0.25 ml of 0.01 M *p*-nitrophenyl-α(β)-D-glucoside and 0.5 ml of inhibitor solution) was incubated at 40°C for 15 minutes. The residual enzyme activity was determined by the colorimetric method. The activity on invertase was assayed by the NELSON-SOMOGYI method and on amylases by the dinitrosalicylate-method reported by NOELTING and BERNFELD[4].

As shown in Table 1, the inhibitory effect of valienamine was found to be strong on yeast-α-glucosidase, and α-glucoamylase from *Rhizopus* sp. using *p*-nitrophenyl-α-D-glucoside as substrate, moderate on β-glucosidase from almonds, whereas weak or negligible on invertase from *Candida utilis* and on α- and β-amylases. These findings indicate that valienamine is a specific inhibitor for α-D-glucoside hydrolase which liberates glucose from the substrate.

The LINEWEAVER-BURK plots in Fig. 2 illustrate the competitive inhibition of yeast-α-glucosidase by valienamine using *p*-nitrophenyl-α-D-glucoside as substrate. The *Ki* value of valienamine found to be *ca.* 6.0×10^{-6} mol/liter which is 100 times smaller than the *Km* value (*ca.* 3.0×10^{-4} mol/liter) for *p*-nitrophenyl-α-D-glucoside.

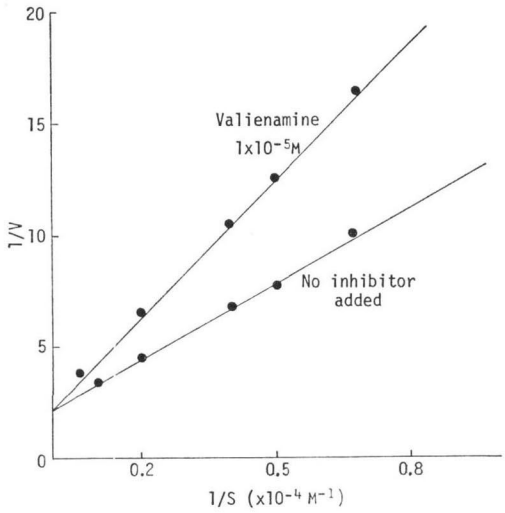
NAMIKI *et al.*[5] reported on the antibiotic activity of amylase inhibitors TAI-A and B against a

Table 1. Inhibition effects of valienamine on various glucoside hydrolases.

Enzyme (Origin)	Substrate	Molar concentration required to give 50% inhibition
α-Glucosidase (Yeast)	*p*-Nitrophenyl-α-D-glucoside	1.7×10^{-4} (M)
β-Glucosidase (Almond)	*p*-Nitrophenyl-β-D-glucoside	2.2×10^{-3}
Invertase (Yeast)	Sucrose	2.0×10^{-2}
α-Glucoamylase (*Rhizopus* sp.)	*p*-Nitrophenyl-α-D-glucoside	3.5×10^{-4}
〃	Starch	6.8×10^{-3}
α-Amylase (Porcine pancreas)	Starch	None*
β-Amylase (Sweet potato)	Starch	1.8×10^{-2}
β-Amylase (Barley)	Starch	1.7×10^{-2}

* None; No inhibition was observed at a concentration of 2×10^{-2} M.

Fig. 2. Effect of valienamine on hydrolysis of *p*-nitrophenyl-α-D-glucoside by yeast-α-glucosidase.



certain *Enterobacteriaceae*, such as *Escherichia coli, Shigella sonnei* and *Salmonella paratyphi.* As to valienamine, it showed antibiotic activity against *Bacillus* sp. such as *B. subtilis* and *B. cereus* on bouillon medium by the cylinder-agar plate method as shown in Table 2. However, the activity was not observed with D-glucose, D-fructose, D-mannitol and D-glucosamine. The addition of D-galactose and lactose had no effect on the activity, and that of maltose, sucrose, cellobiose and D-sorbitol had some effects, as shown in Table 3.

The phenomenon may be due to the antagonism of inhibition on the sugar metabolism. Findings of the relationships between the glucosidase inhibition and the antibiotic activity will require further studies.

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Table 2. Antibiotic activity of valienamine against *Bacillus subtilis* by the cylinder-agar plate method.

Substance	Concentration	Diameter of inhibition zone
Valienamine	16 mg/ml	20 mm
	8	16
	4	12
Validamycin A	16	0

Table 3. Reversal of valienamine inhibition by various sugars.

Sugar (1 mg/ml)	Diameter of inhibition zone* (mm)
None	19
D-Glucose	0
D-Fructose	0
D-Galactose	19
Maltose	(13)**
Cellobiose	14
Lactose	20
Sucrose	(12)**
D-Mannitol	0
D-Sorbitol	(16)**

* Valienamine 10 mg/ml.
** inhibited incompletely.

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