Communication to the Editor

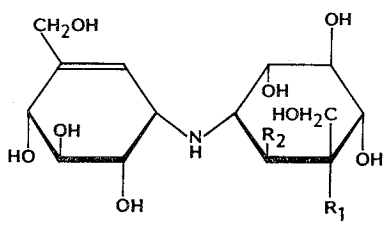
VALIDOXYLAMINES AS TREHALASE INHIBITORS

Sir:

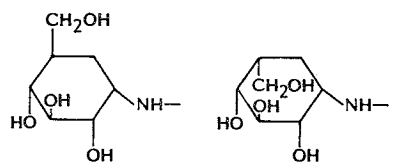
Validamycins, effective fungicides against the sheath blight of rice plants are members of a homologous series of pseduo-oligosaccharides, and contain three types of cores essential for their activity. These are composed of two unique aminocyclitol units and are linked to a varying number and/or a varying linkage of glucose residues. Validoxylamine A is a common core of validamycins A, C, D, E and F. Validoxylamines B and G are cores of validamycins B and G, respectively. (Fig. 1)

Since the validamycins are structurally similar to trehalose, we attempted to elucidate the inhibitory effect of validoxylamines on α,α-trehalase (E.C. 3.2.1.28). This enzyme is widely spread among animals, plants, insects and microorganisms, and its important role becomes particularly apparent in the biological regulation of such mechanisms as the active transport of glucose into intestines, reserve supply of energy, germination of spores, *etc.*

Fig. 1. Structure of validoxylamines.



Validoxylamine A R_1 = H R_2 = H
Validoxylamine B R_1 = H R_2 = OH
Validoxylamine C R_1 = OH R_2 = H



D-*gluco*-Dihydro- L-*ido*-Dihydro-

In the preceding paper[1], we showed that validamycins are potent competitive inhibitors against the trehalase of *Rhizoctonia solani* which causes rice plant disease. This inhibition might disrupt the glucose supply system of the hyphae. In this report, we paid particular attention to the inhibitory effects of validoxylamines and their derivatives on trehalases of various origins.

The preparation of validoxylamines A, B, G were previously described[2] and two dihydrovalidoxylamines A of D-*gluco* and L-*ido* configuration were obtained by the hydrogenation of validoxylamine A with Pt - H_2. Many products were formed by the cleavage of C-N bond and two C-O bonds in the position allylic to the double bond in the hydrogenolysis reaction. In addition, two diastereometric products were also formed by simple saturation of the double bond. The isolation of the saturated products were accomplished by the repetition of chromatography procedure as follows. The reaction mixture was first treated with Amberlite IR-120 (H^+) and eluted with 0.5 N NH_4OH. The eluate concentrate was chromatographed on Amberlite CG-50 (NH_4^+) and developed with water. The D-*gluco*-form was first eluted followed by the L-*ido*-form, which was more retarded owing to its basicity. Both were further chromatographed on Dowex 1-X2 (OH^-) with water, homogeneous D-*gluco*-dihydrovalidoxylamine A (TLC; Silica gel G, PrOH - AcOH - H_2O, 4 : 1 : 1, Rf 0.45) and L-*ido*-form (TLC; Rf 0.63) were obtained. The structures of the derivatives were confirmed by ^1H and ^{13}C NMR spectral studies.

The inhibitory activity of validoxylamines and dihydrovalidoxylamines on various trehalases was assayed as follows; the reaction mixture (each crude trehalase in suitable buffer 0.1 ml, 0.05 M trehalose 0.2 ml, the inhibitor solution 0.2 ml) was incubated at 37°C for 15 minutes. The residual enzyme activity was determined by the glucose oxidase method as previously described. The crude trehalases of porcine intestine[3], rat intestine[4], rabbit kidney[5], baker's yeast[6], *Mycobacterium smegmatis*[7], and insect

Table 1. Inhibitory effect of validoxylamines on various trehalases.

Compound	IC_{50} (M)*					
	Porcine (intestine)	Rat (intestine)	Rabbit (kidney)	Baker's yeast	*M.s.*	*S.l.* (larva)
Validoxylamine A	1.4×10^{-8}	4.9×10^{-9}	1.1×10^{-10}	3.0×10^{-9}	1.1×10^{-8}	4.8×10^{-8}
Validoxylamine B	3.4×10^{-6}	2.0×10^{-6}	4.0×10^{-7}	1.9×10^{-6}	2.6×10^{-6}	6.6×10^{-6}
Validoxylamine G	5.4×10^{-6}	3.0×10^{-6}	1.1×10^{-6}	3.2×10^{-6}	1.0×10^{-6}	5.9×10^{-6}
D-*gluco*-Dihydro-validoxylamine A	3.0×10^{-8}	3.1×10^{-7}	1.9×10^{-8}	7.4×10^{-7}	2.0×10^{-7}	5.3×10^{-8}
L-*ido*-Dihydro-validoxylamine A	1.9×10^{-4}	1.0×10^{-3}	5.3×10^{-4}	8.4×10^{-5}	1.9×10^{-5}	8.4×10^{-4}
Validamycin A	4.2×10^{-7}	2.4×10^{-6}	8.2×10^{-9}	7.4×10^{-6}	1.8×10^{-6}	3.7×10^{-7}
Deoxynojirimycin	2.6×10^{-5}	7.4×10^{-5}	4.0×10^{-5}	2.7×10^{-5}	4.2×10^{-4}	8.7×10^{-5}

* Molar concentration required to 50% inhibition.
M.s.: *Mycobacterium smegmatis*, *S.l.*: *Spodoptera litura*.

Table 2. Inhibition constants (*Ki*) of validoxylamine A for various trehalases.

Origin	*Km* (M)	*Ki* (M)
Porcine (intestine)	4.0×10^{-3}	7.8×10^{-10}
Rat (intestine)	1.0×10^{-2}	3.1×10^{-7}
Rabbit (kidney)	1.3×10^{-3}	1.2×10^{-10}
Baker's yeast	5.3×10^{-3}	2.7×10^{-10}
Mycobacterium smegmatis	9.0×10^{-4}	4.9×10^{-9}
Spodoptera litura (larva)	2.0×10^{-3}	1.0×10^{-9}

Substrate: Trehalose.

(*Spodoptera litura*)[8] were prepared as described in the literature.

As shown in Table 1, the inhibitory effect of validoxylamines was found to be potent against the various trehalases, especially validoxylamine A and D-*gluco*-dihydrovalidoxylamine A.

The general proposition, that the inhibitory activity of competitive analogues can be expected to increase as the configurational structure approaches similarity to the substrate, was again substantiated in this experiment. However, D-*gluco*-validoxylamine A is less active than validoxylamine A and there is some difference in the activity between them. Generally, it may be seen that the double bond in validoxylamine A is not as essential to the activity as the configuration of the hydroxyl and hydroxymethyl groups. In other experiments, not illustrated, similar behavior was observed on the inhibitory activity against *Rhizoctonia solani* trehalase and the activity assayed by the "dendroid-test method"[9].

Lineweaver-Burk plots showed competitive inhibition on each trehalase by validoxylamine A. The *Ki* values were found to be over 10,000 times smaller than the *Km*s, respectively, as shown in Table 2, and their affinities are among the highest currently reported in the literature.

YUKIHIKO KAMEDA
NAOKI ASANO
TAKUJI YAMAGUCHI
KATSUHIKO MATSUI

School of Pharmacy,
University of Hokuriku,
Kanazawa 920-11, Japan

(Received August 6, 1986)

References

1) ASANO, N.; T. YAMAGUCHI, Y. KAMEDA & K. MATSUI: Effect of validamycins on glycohydrolases of *Rhizoctonia solani*. J. Antibiotics 40: 526~532, 1987
2) KAMEDA, Y.; N. ASANO, T. YAMAGUCHI, K. MATSUI, S. HORII & H. FUKASE: Validamycin G and validoxylamine G, new members of the validamycins. J. Antibiotics 39: 1491~1494, 1986
3) DAHLQVIST, A.: Method for assay of intestinal disaccharidase. Anal. Biochem. 7: 18~25, 1964
4) NAKANO, M.; Y. SUMI & M. MIYAKAWA: Purification and properties of trehalase from rat intestinal mucosal cells. J. Biochem. 81: 1041~1049, 1977
5) HEHRE, E. J.; T. SAWAI, C. F. BREWER, M. NAKANO & T. KANDA: Trehalase: Stereocomplementary hydrolytic and glucosyl transfer reactions with α- and β-D-glucosyl fluoride. Biochemistry 21: 3090~3097, 1982

6) PENEK, A. & N. O. SOUZA: Purification and properties of baker's yeast trehalase. J. Biol. Chem. 239: 1671~1673, 1964

7) PATTERSON, B. W.; A. H. FERGNSON, M. MATULA & A. D. ELBEIN: Trehalase from *Streptomyces hygroscopicus* and *Mycobacterium smegmatis*. Methods Enzymol. 28: 996~1000, 1972

8) KALF, G. F. & S. V. RIEDER: The purification and properties of trehalase. J. Biol. Chem. 230: 691~698, 1958

9) IWASA, T.; E. HIGASHIDE, H. YAMAMOTO & M. SHIBATA: Studies on validamycins, new antibiotics. II. Production and biological properties of validamycins A and B. J. Antibiotics 24: 107~113, 1971