**Communication to the Editor**

# ENZYMIC SYNTHESIS OF VALIENAMINE GLUCOSIDES AND THEIR ANTIBIOTIC ACTIVITY

Sir:

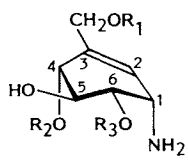
Although glycosidases have long been known to catalyse the stereospecific formation of glycosidic bonds by reversed hydrolysis or transglycosidation, the predominant formation of (1~6) linkages and the difficulties in the isolation of products have prevented their wider use for synthesis of biologically active carbohydrates. However, the regioselectivity of glycosidase-catalysed formation of oligosaccharides can be manipulated in certain situations by using the appropriate glycosidases and acceptors, and the isolation of products simplified in many reports[1~3]. We also have reported on microbial glycosidation to form validamycin α- and β-D-glucoside analogs with the yeast, *Rhodotorula lactosa* IFO 1424[4,5]. Thus, we attempted to prepare α- and β-D-glucosides of valienamine with glucosidases.

Valienamine (Fig. 1), a pseudo-aminosugar produced by the microbial degradation of validamycins by several soil bacteria[6,7], is a competitive α-glucosidase inhibitor, active against yeast-α-glucosidase, invertase and *Rhizopus*-α-glucoamylase, and it shows some antibiotic activity against *Bacillus* sp.[8]

This report deals with the enzymic preparation of α- and β-D-glucoside analogs of valienamine and their antibiotic activity.

For the preparation of partially purified α- and β-glucosidases of *R. lactosa*, cell suspension (400 g) in 20 mM sodium phosphate buffer (pH 6.0) was ruptured with a sonic oscillator and the cell debris were removed by centrifugation at 13,000 × *g* for 10 minutes. Ammonium sulfate was added to the supernatant and the precipitate at 0.9 saturation was dissolved in 5 mM phosphate buffer. The solution was put on a DEAE-cellulose column (2.6 × 95 cm)

Fig. 1. Structure of α- and β-D-glucosides of valienamine.




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Valienamine | H | H | H |
| VE-α-1 | H | α-Glc | H |
| VE-α-2 | H | α-Isomal | H |
| VE-α-3 | α-Glc | H | H |
| VE-α-4 | α-Isomal | H | H |
| VE-β-1 | β-Glc | H | H |
| VE-β-2 | H | H | β-Glc |
| VE-β-3 | H | β-Glc | H |

Glc: D-Glucosyl, Isomal: D-isomaltosyl.

Table 1. Selected physico-chemical properties of α- and β-D-glucosides of valienamine.

| Compound | $[\alpha]_D^{25}$ ($H_2O$) | TLC Rf[a] | GLC Rt[b] (minutes) | $^1H$ NMR, in $D_2O$[c] anomeric proton ($\delta$, $J$=Hz) |
|---|---|---|---|---|
| VE-α-1 | +126.4° | 0.55 | 2.6 | 5.41 (3.9) |
| VE-α-2 | +160.6° | 0.33 | 27.2 | 5.43 (3.7), 4.96 (3.7) |
| VE-α-3 | +144.4° | 0.48 | 3.7 | 4.91 (3.7) |
| VE-α-4 | +137.6° | 0.27 | 33.9 | 4.94 (3.8), 4.92 (3.7) |
| VE-β-1 | +31.8° | 0.46 | 3.7 | 4.9 (8.1) |
| VE-β-2 | +30.2° | 0.57 | 2.5 | 4.64 (7.7) |
| VE-β-3 | +28.4° | 0.57 | 2.7 | 4.64 (8.3) |
| Valienamine | +81.6° | 0.68 | | |

[a] Solvent: $CHCl_3$-MeOH-29% $NH_4OH$ (1:3:2), Silica gel G.
[b] 7% OV-17, 2 m, 280°C TMS-derivatives.
[c] At 400 MHz with TMS standard.

Table 2. $^{13}C$ NMR data of α- and β-D-glucosides of valienamine.

| Carbon | VE | α-Glucosides | | | | β-Glucosides | | |
|---|---|---|---|---|---|---|---|---|
| | | VE-α-1 | VE-α-2 | VE-α-3 | VE-α-4 | VE-β-1 | VE-β-2 | VE-β-3 |
| C-1 | 51.7 | 51.0 | 51.0 | 51.8 | 51.8 | 51.8 | 51.1 | 51.2 |
| C-2 | 127.2 | 128.0 | 128.7 | 129.6 | 128.8 | 129.2 | 126.0 | 128.5 |
| C-3 | 142.0 | 140.6 | 140.2 | 139.5 | 140.0 | 140.0 | 142.6 | 140.5 |
| C-4 | 74.7 | 79.5 | 79.8 | 74.1 | 73.8 | 74.3 | 73.7 | 84.7 |
| C-5 | 74.9 | 73.5 | 74.2 | 74.8 | 74.7 | 74.7 | 74.0 | 73.5 |
| C-6 | 72.9 | 72.2 | 73.6 | 72.4 | 72.2 | 72.3 | 82.7 | 72.2 |
| C-7 | 64.1 | 64.5 | 64.6 | 68.9 | 68.8 | 72.6 | 64.1 | 64.3 |
| C-1′ | | 100.6 | 100.8 | 99.1 | 99.1 | 104.9 | 106.2 | 106.3 |
| C-2′ | | 74.1 | 74.2 | 73.9 | 74.2 | 75.9 | 76.0 | 76.1 |
| C-3′ | | 75.7 | 75.8 | 75.8 | 76.0 | 78.4 | 78.3 | 78.4 |
| C-4′ | | 72.1 | 72.1 | 72.3 | 72.2 | 72.4 | 72.1 | 72.1 |
| C-5′ | | 75.4 | 72.4 | 74.6 | 73.0 | 78.6 | 78.5 | 78.7 |
| C-6′ | | 63.2 | 68.5 | 63.2 | 68.2 | 63.4 | 63.2 | 63.4 |
| C-1″ | | | 100.6 | | 100.6 | | | |
| C-2″ | | | 74.0 | | 74.0 | | | |
| C-3″ | | | 75.9 | | 75.8 | | | |
| C-4″ | | | 72.2 | | 72.0 | | | |
| C-5″ | | | 74.5 | | 74.6 | | | |
| C-6″ | | | 63.2 | | 63.2 | | | |

Chemical shifts are in ppm downfield of DSS. $^{13}C$ NMR spectra were taken in $D_2O$ on a Jeol JNM-GX 400 spectrometer.

equilibrated with the buffer. The column was washed with 10 mM buffer and eluted with the buffer containing 50 mM NaCl. The α-glucosidase fraction (72.6 U/maltose, 0.12 U/mg·protein) and β-glucosidase fraction (63.5 U/cellobiose, 0.22 U/mg·protein) were obtained.

α-(or β-)Glucosidation reaction of valienamine was accomplished as follows. Reaction mixture (500 ml) containing valienamine (2.5 g), maltose (100 g, or cellobiose 50 g) and the α-glucosidase fraction (50 U, or the β-glucosidase fraction) in 40 mM acetate buffer (pH 5.0) was incubated at 27°C for 72 hours. The glycosidation process was followed by TLC (silica gel, $CHCl_3$-MeOH-29% $NH_4OH$, 1:3:2) and gas-liquid chromatography (GLC, trimethylsilyl derivatives, 7% OV-17 on Chromosorb AW, 280°C)[9]. The products were isolated by column chromatography on Dowex 50WX8 (H form, 1.6×60 cm), eluted with 0.5 N $NH_4OH$ and then on Dowex 1X2 (OH from, 1.0×70 cm) and eluted with water. The reaction of α-glucosidation gave four components, VE-α-1 (301 mg), VE-α-2 (90 mg), VE-α-3 (32 mg) and VE-α-4 (22 mg) as amorphous materials which are distinguishable from each other and homogeneous by the TLC and GLC. β-Glucosidation gave three components, VE-β-1 (180 mg), VE-β-2 (138 mg) and VE-β-3 (60 mg). Selected physico-chemical properties and NMR chemical shifts of the valienamine glucosides are listed in Tables 1 and 2. Complete signal assignment of $^{13}C$ NMR spectra was almost made by comparing each spectrum with corresponding α-glucotrioses and α-glucobioses[10,11]. Their shift patterns were consistent with the known effects of glucosidation (underlined in Table 2), including β- and γ-effects of the olefinic carbons at the allylic position[12]. The $^{13}C$ resonance shifts produced by changes of pD were useful for the assignment. Deuteronation of

Table 3. Antibacterial activity of valienamine glucosides in agar diffusion assay with nutrient broth.

| Compound | Diameter of inhibition zones (10 mg/ml)[a] | | |
|---|---|---|---|
| | *Bacillus subtilis* | *B. cereus* | *B. megaterium* |
| VE-α-1 | 25 mm | 21 mm | 23 mm |
| VE-α-2 | 20 | 19 | 20 |
| VE-α-3 | 26 | 19 | 24 |
| VE-α-4 | 13 | + | 12 |
| VE-β-1 | 0 | 0 | 0 |
| VE-β-2 | 0 | 0 | 0 |
| VE-β-3 | 0 | 0 | 0 |
| Valienamine | (26)[b] | (21)[b] | (24)[b] |

[a] Nutrient agar (Nissui), 24 hours incubation at 37°C, compound 500 μg/disc (diameter 8 mm).
[b] Indicates inhibitory zones were not clear.

amino groups caused large upfield shifts of the signals which are $\beta$- to the amino groups[13]. For example, the chemical shifts of C-2 and C-6 in VE-$\beta$-2 were shifted upfield by 7.9 and 4.6 ppm, respectively by protonation. Further confirmation of spectral assignment was achieved from $^{13}C$-$^{1}H$ COSY experiments. The structures are depicted in Fig. 1.

As shown in Table 3, the $\alpha$-D-glucosides of valienamine have some antibacterial activity against *Bacillus* sp. on nutrient agar medium by the paper-disc method (concentration 10 mg/ml, 500 $\mu$g/disc) as well as valienamine and showed clear inhibition zones, while valienamine showed hazy zones, incomplete inhibition. However, the $\beta$-D-glucosides showed no inhibitory zone.

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